Context-related drivers, occurrence and human exposure to antibiotic resistant *Campylobacter* in selected river systems in the Eastern Cape, South Africa

A PhD thesis submitted in fulfilment of the requirements

of
Doctor of Philosophy

in
Water Resources Science

at Rhodes University

by

Mary Chibwe

Orcid: 0000-0003-3879-8479

Supervisor: Dr Chika F. Nnadozie
Co-supervisor: Prof. Oghenekaro N. Odume

February 2024

ABSTRACT

The increasing prevalence of antibiotic-resistant *Campylobacter* species in river systems is a growing public health concern globally. This challenge presents the need to understand the drivers influencing the occurrence of these antibiotic-resistant strains to inform effective mitigation strategies. Identifying local anthropogenic pressures/activities will enable tailored intervention and ensure that management efforts are targeted and effective in mitigating actual risks faced by local water resources. In particular, the occurrence of antibiotic-resistant *Campylobacter* in river systems causes concerns regarding potential human exposure. *Campylobacter* species are regularly implicated in both animal and human diarrhoea, and have gained recognition as one of the main causes of waterborne diseases. This study investigated context-related drivers to the presence of antibiotic-resistant *Campylobacter*, its occurrence, and the risk of human exposure in the Bloukrans and Swartkops rivers in the Eastern Cape, South Africa. The study utilised a questionnaire survey and focus group discussions to identify context-related sources and drivers of antibiotic-resistant bacteria and genes in the Bloukrans and Swartkops rivers. Additionally, physico-chemical analysis of water samples was done, and polymerase chain reaction (PCR) was employed to investigate occurrences of *Campylobacter* in both rivers, as well as the presence of virulent and antibiotic-resistant genes. Two control sites, one for each river, and a total of nine other sampling sites were selected for this study, four along the Bloukrans River and five along the Swartkops River. Water samples were collected from all eleven sites over a period of one year, covering all the seasons (winter, spring, summer and autumn). Exposure assessment was investigated by conducting propidium monoazide-quantitative polymerase chain reaction (PMA-qPCR) to quantify the concentration of viable *Campylobacter* and its antibiotic-resistance genes (ARGs) in the river water. The calculated concentration of viable *Campylobacter* and ARGs was then used to estimate human exposure dose of antibiotic-resistant *Campylobacter* via unintentional ingestion during swimming in the Bloukrans or Swartkops rivers. The questionnaire surveys and focus group discussions identified the factors of socio-demographic characteristics of the study catchments, lack of knowledge regarding antibiotic resistance, inappropriate antibiotic and disposal practices as well as anthropogenic activities (improper solid waste management, discharge of poorly treated wastewater, poor animal husbandry practices, urban run-off and storm water, industrial effluent and agricultural run-off) as context-related sources and drivers contributing to the emergence and of continuing occurrence antibiotic-resistant bacteria and genes in the Bloukrans and Swartkops rivers.

The results obtained in this study suggest that the physiochemical properties of the water in the Bloukrans and Swartkops rivers are conducive for *Campylobacter* survival. The prevalence of *Campylobacter* was high at sites that recorded high turbidity, low dissolved oxygen and high conductivity. This study also highlighted the widespread and continual presence of *Campylobacter spp.* in the Bloukrans and Swartkops rivers, which are potentially antibiotic-resistant. The prevalence of *Campylobacter* was 60% virulence genes were detected at 15% (*cadF),* 10% (*htrB),* 10% (*clpP),* 5% (*ciaB)* and 5% (*ctdC)* for water samples from the Bloukrans River. *Campylobacter* was detected

in 58% of water samples from the Swartkops River, while *cadF, htrB, ciaB, clpP* and *ctdB* were detected at 24%, 0%, 9.5%, 14.8% and 4.8%, respectively. Of the *Campylobacter*-positive water samples, the virulence genes *cmeA, cmeB* and *cmeC* were detected in 20%, 65% and 10% of the water samples from Bloukrans River, respectively. Similarly, the genes *cmeA*, *cmeB* and *cmeC* were detected at 14.3%, 52% and 9.5%, respectively, for the Swartkops River. Tetracycline resistance genes (*tetO*) were detected in 70% and 76% of the water samples collected from the Bloukrans and Swartkops rivers, respectively. It was also observed that the sampling site had a significant effect on the detection of *Campylobacter* for both rivers ($p < 0.05$). The prevalence of *Campylobacter* was higher for sites impacted by anthropogenic activities. The results show that sampling season had no significant effect on the occurrence of *Campylobacter* for both rivers. The analysis through PMA-qPCR revealed that the mean concentrations of the *Campylobacter* 16S rRNA gene from viable cells ranged from 2.1×10^4 to 1.75×10^5 copies/ml for the Bloukrans River, and from 0 (no detection) to 1.11×10^4 copies/ml (viable concentration) for the Swartkops River. The concentration of *tetO* from viable cells ranged from 4.75×10^1 to 2.71×10^3 copies/ml and 115.96 to 325.12 copies/ml, for the Bloukrans and Swartkops Rivers, respectively. Similarly, the concentration of *cmeB* genes from viable cells ranged from 7.8×10^1 to 2.76×10^4 copies/ml for the Bloukrans River and from 1.71×10^1 to 7.39×10^3 copies/ml for the Swartkops River. We utilized the calculated concentrations of viable *Campylobacter* 16S rRNA genes and ARGs to estimate the human intake burden of viable *Campylobacter* 16S rRNA copies per one-hour of swimming to range from $9.9 \times 10^1 - 3.7 \times 10^6$ copies/h across the two rivers. The human intake burden of *Campylobacter* ARGs ranged from $3.6 \times 10^2 - 5.8 \times 10^5$ copies/h for *cmeB*, and $9.98 \times 10^2 - 5.7 \times 10^4$ copies/h for *tetO*.

This study highlighted the intricate interplay of socio-demographic characteristics in the study catchments, such as insufficient awareness of antibiotic resistance, inappropriate antibiotic disposal practices and anthropogenic activities, which all contribute to the prevalence of antibiotic-resistant *Campylobacter* in the Bloukrans and Swartkops rivers, emphasizing the need for targeted interventions to address these specific influences. There is a potential risk of *Campylobacter* infections associated with human exposure to the rivers.

This work contributes towards the body of knowledge in closing the current research gap on possible human exposure to antibiotic-resistant *Campylobacter*, and in general, exposure to ARBs through freshwater sources. The findings have practical implications for designing targeted interventions to mitigate these risks, to enhance the overall environmental and public health management in the region.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my Lord and Saviour Jesus Christ for giving me the opportunity, grace and strength to successfully complete this PhD. To Jesus be all the glory.

I would also like to acknowledge the following people for the vital role they played in the success of this PhD. Thank you to my mother, Mrs Violet Chikonoko Chibwe and all my siblings for their emotional and spiritual support. I would also like to specifically thank my two young sisters. Rachel Mwape Chibwe and Christabel Bwalya Chibwe for their support and encouragement. I would also like to honour the memory of my late father, Mr. Oliver Bwalya Chibwe for the encouragement and support he gave me throughout my education journey. Thank you Tata and continue resting in peace!

This work would not have been successful without the support, dedication and input of my two supervisors, Dr Chika Felicitas Nnadozie and Prof Ogenekharo Nelson Odume. Working under your guidance for the past three years has been a great learning experience. I am grateful that you allowed me to have working experience beyond my PhD project. Thank you for embracing and nurturing my gifts, talents and strength and supporting me in my weaknesses. Above all, thank you for being my first family during my stay in South Africa.

Special thanks to Nandipha Ngoni, Khaya Mgaba and Dr Frank Akamagwuna for their assistance during data collection and lab work. I would also like to thank the staff, students and postdocs at IWR especially Ms Juanita Mclean, Ms Margaret Wolff and Mr. David Forsyth.

I would also like to thank my "African family" i.e. the AWARMN scholars at Rhodes University and the other partner universities across Africa. Specifically I would like to thank Peter Wasswa, Enahoro Owawenu and Harriette Okal for their "extra" support.

I would like to thank the Southern African Institute for Aquatic Biodiversity (SAIAB) for allowing us to use their Aquatic genomics Laboratory. I specifically thank Dr. Gwyneth Matcher for her practical assistance, support, and encouragement during the time this work was being conducted at SAIAB. Special thanks to Ms. Hellen Hoffman for teaching me how to write manuscripts and for her work with River rescue. I would also like to thank Ms. Jenny Rump and the environmental activists from the Zwartkops conservancy.

I am also grateful to my employer Evelyn Hone College back in Zambia, for granting study leave for the duration of my studies.

My church family in South Africa, Pastor Innocent Masepo, Mrs Masepo and the entire River of Life Church. Many times I walked through the doors of church discouraged, tired and with difficult situations before me. Through prayers, encouragement and support I came out stronger like the "Springboks".

Lastly and most importantly, I would like to thank the African Water Resources Mobility Network (AWaRMN), which has received funding from the Intra-African Academic Mobility Scheme of the European Union, Grant No. 2019-1973/004-001 for awarding me this PhD scholarship.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AMR	Antimicrobial resistance
ARB	Antibiotic resistant bacteria
ARGs	Antibiotic resistance genes
AU	African Union
CDC	Centers for Disease Control and Prevention
DAFF	Department of Agriculture, Forestry and Fisheries
DEA	Department of Environment Affairs
DNA	Deoxyribonucleic Acid
DOH	Department of Health
DWAF	Department of Water Affairs
ESBL	Extended-spectrum beta-lactamases
FAO	Food and Agriculture Organization
FIB	Faecal indicator bacteria
GAP	Global Action Plan
GIT	Gastrointestinal tract
HGT	Horizontal gene transfer
HIB	Human Intake Burden
HIC	High Income Countries
HIV	Human Immunodeficiency Virus
HMRGs	Heavy metal resistance genes
LMICs	Low- and medium-income countries
MDR	Multi-drug resistant
MGEs	Mobile genetic elements
MST	Microbial source tracking
NAPs	National action plans
NHA	National Health Act
NMMP	National Microbial Monitoring Programme for surface water
OH	One Health
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
PMA	Propidium Monoaxide

PMA-qPCR	Propidium Monoaxide quantitative Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
QRDR	Quinolone resistance-determining region
RNA	Ribonucleic acid
SSA	Sub-Saharan Africa (SSA)
UNEP	United Nations Environment Programme (UNEP)
VBNC	Viable but non-culturable (VBNC)
WASH	Water, sanitation, and hygiene (WASH)
WHO	World Health Organization (WHO)
WMAs	Water Management Areas
WWTW	Wastewater treatment Works

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Publications based on this chapter:
Chibwe, M., Odume, O. N. and Nnadozie, C. F. (2023) 'A review of antibiotic resistance among *Campylobacter* species in human , animal , and water sources in South Africa : a One Health Approach', *Journal of Water and Health*, 00(0), pp. 1–18. https://doi.org/10.2166/wh.2022.146.

Chibwe, M., Odume, O. N. and Nnadozie, C. F. '*Campylobacter*; an emerging pollutant of aquatic environments' chapter in the book *Emerging Pollutants: Protecting water quality for the health of people and the environment. UNESCO* (Edited by : Sarantuyaa Zandaryaa, Ali Fares and Gabriel Eckstein) (in the final review stage)

1.1 Introduction

Antibiotic misuse has led to the emergence of antibiotic resistance, reducing the effectiveness of antibiotics in the treatment of infectious diseases. Globally, it is estimated that antibiotic resistance-related infections cause about 700,000 deaths annually. By 2050, it is estimated that the annual death rate due to antibiotic resistance may reach 10 million deaths if there is no effective global response to address antibiotic resistance (Wilbers *et al.*, 2014; Schreiber *et al.*, 2015; Hoven *et al.*, 2017).

An increase in antibiotic resistance among bacterial species has been reported in South Africa (van den Honert, Gouws and Hoffman, 2018; Kariuki *et al.*, 2022). In clinical settings, medical practitioners are encountering increasingly prevalent antibiotic-resistant infections of the urinary tract and abdomen, often requiring the use of last resort-antibiotics such as colistin. Additionally, there is a rise in pan-drug-resistant infections among patients (Sekyere, 2016). Antibiotic resistance among bacteria from non-clinical sources such as animals and the environment has also been reported in South Africa (Igwaran and Anthony Ifeanyi Okoh, 2020; Pillay *et al.*, 2020).

Antibiotic resistance emerges at the human, animal and the environment interface, as it can be transmitted between these three domains. This is an indication of the interconnectedness of resistance across these domains. Antibiotic resistance is a One Health problem. One Health (OH) is an approach that recognises the linkages between humans, animals and the environment. Environmental transmission of antibiotic resistance has recently attracted attention as the remaining piece of the One Health concept (Berendonk *et al.*, 2015; Robinson *et al.*, 2016). Transmission of antibiotic resistance through the environment has received less attention than antibiotic resistance in humans and animals despite evidence of increasing antibiotic resistance from environmental sources. Antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) have been detected in various environmental sources. The major environmental sources of ARB and ARGs are solid waste, livestock, wastewater, urban run-off, municipal wastewater, aquaculture, hospital wastewater, and industrial wastewater. The ARB and ARGs from these sources are often discharged into rivers (Xiong *et al.*, 2015; Zhang *et al.*, 2016; Al Salah, Laffite and Poté, 2019; Bengtsson-Palme *et al.*, 2019; Tapela and Rahube, 2019; Wang *et al.*, 2022).

Aquatic sources such as rivers are recognised primary reservoirs of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Globally, rivers face pollution from biological and chemical sources, making them hotspots for antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Abraham, 2011; Molobela and Pramod, 2011). The presence of these ARB and ARGs in aquatic sources has been attributed to various anthropogenic activities such as the discharge of treated or untreated effluent from wastewater treatment plant (WWTP), indiscriminate disposal of solid waste, and agricultural runoffs (Abraham, 2011; Molobela and Pramod, 2011; Rajasekar *et al.*, 2023). As an example, approximately 46% of antibiotics ingested by humans and animals are discharged into the rivers through wastewater treatment plant (WWTP) effluents, solid waste and agricultural runoffs (Abraham, 2011; Molobela and Pramod, 2011; Rajasekar *et al.*, 2023). Discharge of high concentrations of residual antibiotics into aquatic sources promotes selective pressure on environmental bacteria, resulting in the development of antibiotic resistance and the expression of ARGs (Ateba *et al.*, 2020; Guo *et al.*, 2022; Larsson and Flach, 2022). Bacteria in river water may be harbouring genes which confer resistance to antibiotics, hence they are increasingly showing resistance to clinically relevant antibiotics.

This global phenomenon has also been observed in South Africa. Antibiotic-resistant bacteria belonging to the family Enterobacteriaceae (*Escherichia coli*, *Enterobacter*, *Klebsiella*, *Citrobacter*), other ARB including (*Aeromonas spp*., *Salmonella spp*., *Shigella*, *Citrobacter spp*., *Escherichia coli*, *Pseudomonas spp*., *Klebsiella spp., Campylobacter spp*., *Enterococcus spp., Staphylococcus spp., Listeria spp*., *Bacillus spp*), protozoa (*Cryptosporidium*- and *Giardia spp*.) and enteric viruses (Rota-, Noro-, Entero-, and Adenoviruses) have been reported frequently in South African rivers (Obi *et al*., 2004; Bessong *et al*., 2009; Otigbu *et al*., 2018; Potgieter *et al*., 2020; Mpondo, Ebomah and Okoh, 2021; Ogunlaja *et al*., 2022). In studies of isolates, colistin resistance genes mcr-1, mcr-5.1, and many variants of mcr-3 have been reported in *Aeromonas* strains from rivers receiving waste from nearby communities and storm waters in South Africa (Snyman *et al.*, 2021). Furthermore, in a South African study, isolates from three wastewater treatment plants showed high resistance to sulphamethoxazole (90%), penicillins (90–100%), rifampin (90%), and the cephems (70%) (Odjadjare *et al.*, 2012). Moreover, *Aeromonas spp* and *Listeria spp*, with notable resistance to ampicillin, penicillin erythromycin, clindamycin, and vancomycin, were recently detected in surface water in the Eastern Cape, South Africa (Mpondo, Ebomah and Okoh, 2021). *Pseudomonas* isolates resistant to cephems, rifampin, penicillins, and sulphamethoxazole have also been isolated from surface water (Odjadjare *et al.*, 2012). Similarly, Adefisoye and Okoh, (2017) reported imipenem and penicillin G resistant *Vibrio* species in treated wastewater. The presence of these bacterial pathogens harbouring ARGs in the treated wastewater suggest that ARGs are discharged into environments where their proliferation could be perpetuated (Hamiwe *et al.*, 2019). Even more disturbing, diverse sets of ARGs, which are considered emerging contaminants, have been reported in aquatic environments in South Africa, and constitute a growing concern (Otigbu *et al.*, 2018; Iwu, Korsten and Okoh, 2020; Mpondo, Ebomah and Okoh, 2021). The presence of ARB and ARGs in aquatic environments poses a threat to human and animal health by contributing to antibiotic resistance transmission, waterborne diseases, and other infections (Gothwal and Shashidhar, 2015). The remaining part of this chapter will explore existing relevant literature, starting with legislative framework for water resources protection in South Africa, followed by *Campylobacter* as an emerging waterborne zoonotic pathogen, epidemiology of waterborne *Campylobacter* infections in humans, assessment of human exposure to *Campylobacter* in South African rivers, the problem statement, and the aim and objectives of the study. This chapter ends with the thesis structure.

1.2 Legislative framework for water resources management and protection in South Africa

As explained in the introduction, it is evident that water resources problems associated to pollution are posing a threat to freshwater resources in South Africa (Adewumi, Ilemobade and Zyl, 2010; Phungela *et al.*, 2022). The reported decline of water quality is attributed to anthropogenic activities, such as the discharge of wastewater, solid waste, agriculture and industrial effluent into rivers. Discharge of wastewater effluent, which often does not meet acceptable standards, may lead to microbial and chemical pollution of rivers (Ngqwala and Muchesa, 2020; Gqomfa, Maphanga and Shale, 2022). Bacteria in rivers are continuously exposed to sub-therapeutic doses of antibiotics that are discharged with WWTWs effluents, and this is leading to the emergence of antibiotic resistance (Ngqwala and Muchesa, 2020). Indiscriminate disposal of solid waste in drainage systems, storm water canals and rivers are also contributing to the microbial pollution of rivers (Adams, Pretorius and Snow, 2019). In addition, run-off from livestock farms introduce bacteria and antibiotics into rivers (Ramaite, Ekwanzala and Momba, 2022).

The Department of Water and Sanitation (DWS) holds the responsibility for sustainable management of the nation's water resources. This department formulates regulatory and policy frameworks for the water sector and ensures efficient protection, utilisation, development, conservation, management and control of water resources. South Africa has various policies and strategies for water quality monitoring and the protection of water resources, including the National Water Act 36 of 1998 (NWA, 1998), the National Health Act 61 of 2003 (NHA, 2003) the Environment Conservation Act 73 of 1989 (ECA, 1989), the National Environmental Management Act 107 of 1998 (NEMA, 1998), Municipal Structures Act 117 of 1998 (MSA, 1998), Municipal Systems Act 32 of 2000 (MSA, 2000) and the National Environmental Management: Waste Act 59 of 2008 (NEMWA). The National Water Act (No. 36 of 1998) is the primary legislative tool in South Africa, providing comprehensive regulations on the integrated use, protection, development, conservation, management and control of groundwater, dams, rivers and streams (Nkosi, Mathivha and Odiyo, 2021). Under the National Water Act (NWA) is the National Water Resource Strategy (NWRS), which outlines the strategies, objectives, plans, guidelines, procedures, and institutional arrangements for safeguarding , utilising, developing, conserving, managing and controlling water resources in South Africa (DWA, 2013). Within the framework of NWA and NWRS are two primary strategies for water resource protection, i.e., resource-directed measures and source-directed controls. Resource-directed measures focus on the quality of water resources, assessing the overall health and condition of the resource and gauging its ecological status (DWAF, 2006) Resource quality encompasses water quantity, the character of in-stream and riparian habitats, and protection for each resource. These objectives aim to prevent irreversible damage to each aspect of the reserve. Source-directed controls manage water use activities and sources of impact, employing tools such as standards, incentives and specific conditions to achieve protection objectives (DWAF, 2006).

Despite the existing strategies to prevent irreversible damages on water resources, South Africa's water resources are at risk of pollution from anthropogenic activities (Potgieter *et al.*, 2020). Indications suggest that the continuing threat is due to a failure to identify local anthropogenic pressures and devise localised water resource management strategies, undermining the effectiveness of safeguarding measures. In South Africa, there is a general knowledge about anthropogenic pressures (Odiyo and Makungo, 2012; Traoré *et al.*, 2016; Weaver *et al.*, 2017; Adams, Pretorius and Snow, 2019; Masiya *et al.*, 2019). However, different regions and catchments may have unique land uses and human activities. Therefore, identifying local anthropogenic pressures/activities will enable tailored intervention and ensure that management efforts are targeted and effective in mitigating actual risks the local water resources face.

Pollution of water resources not only impacts water quality, but also has negative implications for public health, particularly in communities which are heavily reliant on freshwater for domestic, agricultural and recreational purposes. The connection between poor microbial water quality and the prevalence of infectious diarrhoea or gastroenteritis is widely acknowledged (Osiemo, Ogendi and Erimba, 2019). Diarrhoea ranks as a primary cause of morbidity and mortality in South Africa, particularly in children (Chukwu *et al.*, 2020). Approximately 46% of households in South Africa lack access to piped water at home, compelling some to depend on alternative water sources, including untreated freshwater, for domestic use (Potgieter *et al.*, 2020). South African rivers are used for various purposes, including domestic, industrial and irrigation (Obi *et al.*, 2004; Bessong *et al.*, 2009; Otigbu *et al.*, 2018; Potgieter *et al.*, 2020; Mpondo, Ebomah and Okoh, 2021; Ogunlaja *et al.*, 2022). This reliance on untreated freshwater introduces a risk of diarrhoea or gastroenteritis due to the presence of human enteric pathogens (bacteria, viruses, and parasites) and antibiotic resistance genes in rivers. Assessment of the occurrence of these pathogens and their antibiotic resistant genes in aquatic sources is crucial for establishing the potential risks of waterborne infections, and consequently implementing timely interventions to protect public health.

Of significant concern among these pathogens are *Campylobacter* species, zoonotic bacterial pathogens which inhabit the intestines of mammals and aves, and can be transmitted to humans through contaminated food and water (Magana-Arachchi and Wanigatunge, 2020). *Campylobacter* species are increasingly implicated in both animal (zoonotic) and human diarrhoea or gastroenteritis infections, and a major cause of bacterial foodborne and waterborne infections. The rise in the incidence of *Campylobacter* infections globally, and their tendency to withstand the effects of antibiotics, underscores their serious threat to public health (Kaakoush *et al.*, 2015; Igwaran and Okoh, 2019). Locally, there is a paucity of information regarding the risk of *Campylobacter* infection through river transmissions. For accurate risk estimates, comprehending the extent of human exposure to antibiotic resistant bacteria and genes in aquatic environment is crucial.

1.3 Description of *Campylobacter* species

The genus *Campylobacter* falls under the phylum Proteobacteria, class Epsilonproteobacteria, order Campylobacterales, and family Campylobacteraceae (Kaakoush *et al.*, 2015). The genus *Campylobacter* has 16 species and six subspecies (Silva *et al.*, 2011). The species, *Campylobacter jejuni* and *Campylobacter coli* are the main etiological agents for human campylobacteriosis (Asuming-Bediako *et al.*, 2019). Among other *Campylobacter* species are *Campylobacter concisus*, *Campylobacter ureolyticus*, *Campylobacter upsaliensis*, and *Campylobacter lari*, which are said to be ‘emerging *Campylobacter species*’, due to their poorly understood roles in human and animal diseases (Kaakoush *et al.*, 2015).

Regarding their morphological and biochemical characteristics, *Campylobacter* species are gram-negative, non-spore forming, curved or spiral bacteria. Most species have flagella (polar or non-polar) while a few lack flagella (Kaakoush *et al.*, 2015; On *et al.*, 2017; Asuming-Bediako *et al.*, 2019). They are non-spore-forming, with an average size of 0.2 to 0.8 by 0.5 to 5 um (Kaakoush *et al.*, 2015). *Campylobacter* species are motile except for *Campylobacter gracilis*, which is non-motile (Silva *et al.*, 2011). With the exception of *C. gracillis* and some strain of *C. showae*, most *Campylobacter* species produce oxidase (Silva *et al.*, 2011; On *et al.*, 2017). Additionally, they neither ferment nor oxidize carbohydrates, but are chemoorganotrophs which obtain energy from amino acids, or tricarboxylic acid cycle intermediates. *Campylobacter jejuni* hydrolyzes hippurate, indoxyl acetate and reduces nitrate (Silva *et al.*, 2011; Kaakoush *et al.*, 2015). *Campylobacter* species grow well under micro-aerobic conditions (less than 8% oxygen) but some strains are also able to grow under aerobic and anaerobic conditions. *Campylobacter* species such as *C. concisus, C. curvus, C. rectus, C. mucosalis, C. showae, C. gracilis and C. hyointestinalis* utilise hydrogen or formate as an electron donor for micro-aerobic growth (Kaakoush *et al.*, 2015; Asuming-Bediako *et al.*, 2019). Other species such as *Campylobacter jejuni* are obligate microaerophilic (Shagieva, Demnerova and Michova, 2021). The growth temperature ranges from 31 to 36°C, with the optimum temperature at 42°C. *Campylobacter jejuni* and *Campylobacter coli* are thermotolerant (Khan *et al.*, 2014; Shagieva, Demnerova and Michova, 2021). *Campylobacter* species are sensitive to saline conditions and cannot withstand sodium chloride concentrations greater than 2% w/v. Furthermore, *Campylobacter* do not grow in environments with water activity (a_w) lower than 0.987 but their optimal growth occurs at $a_w = 0.997$ (approximately 0.5% w/v NaCl). *Campylobacter* species can survive between the pH range of 4.9 - 9.0 but the pH for their optimum growth 6.5 – 7.5 (Silva *et al.*, 2011).

1.4 Epidemiology of waterborne *Campylobacter* infections in humans

This section discusses the epidemiology of *Campylobacter* infections, pathogenesis, virulence factors, survival in water, reservoirs and transmission pathways, seasonality variations, its incidence, and outbreaks.

1.4.1 Pathogenesis

The mechanisms by which *Campylobacter* can cause infection are poorly understood (Janssen *et al.*, 2008; Igwaran and Okoh, 2019). Ingested *Campylobacter* cells which survive the acidic environment of the stomach are able to adhere to intestinal epithelial cells or to the mucus layer of epithelial cells (Janssen *et al.*, 2008). This results in colonisation of the ileum, jejunum and colon in infected individuals. Colonisation can either be asymptomatic or symptomatic (Janssen *et al.*, 2008; Igwaran and Okoh, 2019). Colonisation of the intestine may result in disease by one of two main mechanisms, either adherence of *Campylobacter* to the intestinal wall or bacterial invasion and replication. Adherence to the intestinal walls leads to the production of toxins. These toxins interfere with the fluid resorption capacity of the intestine, resulting in secretory diarrhoea. In contrast, bacterial invasion and replication within the intestinal mucosa induces an inflammatory response. This causes bloody inflammatory diarrhoea (Janssen *et al.*, 2008). The inflammation of the gastrointestinal tract involving both the small intestine and stomach is referred to as Gastrointestinal Infection (GI). Generally, GI is characterized by diarrhoea, but other common gastrointestinal symptoms associated with *Campylobacter* species include abdominal pain, gastroenteritis and septicaemia (Kaakoush *et al.*, 2015; Igwaran and Okoh, 2019). Acute cholecystitis, Barrett esophagitis, liver abscess, ulcerative colitis and Crohn's disease and celiac disease are also other GI infections caused by *Campylobacter* species (Igwaran and Okoh, 2019). The *Campylobacter* species responsible for GI infections include *C. coli, C. concisus, C. curvus, C. fetus, C. helveticus, C. hominis, C. hyointestinalis, C. jejuni, C. insulaenigrae, C. lari, C. mucosalis, C. rectus, C. showae, C. sputorum, C. upsaliensis,* and *C. ureolyticus* (Igwaran and Okoh, 2019).

In addition to GI, *Campylobacter* species are also responsible for infections outside the intestines, but with symptoms linked to a problem within the intestine. These infections are referred to as Extragastrointestinal Infections (EI). Species responsible for EI include *C. coli, C. concisus C. curvus, C. fetus, C. hominis, C. hyointestinalis, C. jejuni, C. insulaenigrae, C. lari, C. rectus, C. showae, C. sputorum, C. ureolyticus* and *C. upsaliensis* (Igwaran and Okoh, 2019).

Extragastrointestinal infections caused by these *Campylobacter* species include Guillain-Barre syndrome (GBS), septicaemia, reactive arthritis, bacteraemia, septic arthritis, osteomyelitis, endocarditis, neonatal sepsis and meningitis (Wilson *et al.*, 2008; Sahin *et al.*, 2012; Mendonça *et al.*, 2015; Igwaran and Okoh, 2019). Generally, diseases caused by *Campylobacter spp.* are collectively referred to as campylobacteriosis. *Campylobacter* infection is determined by the amount of *Campylobacter* cells ingested by an individual. The infectious dose of *Campylobacter* is low, and ranges from 500−800 cells (Schielke, Rosner and Stark, 2014; Thobela, 2017). In children below 5 years old, the infection dose is lower because their immunity is not well developed (Wilson *et al.*, 2008; Sahin *et al.*, 2012).

1.4.2 Virulence genes

The pathogenicity of *Campylobacter species* is determined by a number of virulence genes (factors). The specific virulence mechanisms are not fully understood because of the differences between virulence factors among *Campylobacter* species and strains. Generally, virulence genes are associated with flagella-mediated motility, adherence to intestinal mucosa, invasiveness, toxin production and environmental stresses, as shown in Table 1.1 (Silva *et al.*, 2011; Reddy and Zishiri, 2018).

Table 1.1. Examples of virulence factors expressed by *Campylobacter spp.*

Main function	Description	Examples
Motility	*Campylobacter* possess flagella which plays a role in its survival in the gastrointestinal tract (GIT). The flagella aids in motility and subsequent colonisation of the small intestine and the colon (Silva *et al.*, 2011).	Major flagellin (*flaA*) Minor flagellin (*flaB*) Sensor (*flgS*) Response regulator (*flgR*) (Silva *et al.*, 2011)
Adherence	Aids the bacteria in adherence to intestinal epithelial cells by the binding activity to the extracellular fibronectin. This helps the bacteria to invade host cells during infection (Otigbu *et al.*, 2018; Reddy and Zishiri, 2018).	*Campylobacter* adhesin to fibronectin *(cadF*) (Otigbu *et al.*, 2018; Reddy and Zishiri, 2018).
Toxin production	Gram-negative bacteria including *Campylobacter* species express the Cytolethal distending toxin (*cdt*) virulence factor. This virulence factor is an apoptosis triggering toxin (Silva *et al.*, 2011; Otigbu *et al.*, 2018; Reddy and Zishiri, 2018).	*cdtA, cdtB* and *cdtC* (Silva *et al.*, 2011; Otigbu *et al.*, 2018; Reddy and Zishiri, 2018).
Invasion and colonisation	Significant for colonisation and invasion of epithelial cells of the GIT. They play a role in modifying the host cell regulatory pathways to promote pathogenicity in *Campylobacter jejuni* species (Ghunaim *et al.*, 2015; Otigbu *et al.*, 2018; Reddy and Zishiri, 2018).	*Campylobacter* invasion protein B (*ciaB*) Phospholipase A (*pldA*) *dnaJ* genes (Ghunaim *et al.*, 2015; Otigbu *et al.*, 2018; Reddy and Zishiri, 2018)
Environmental stress	*Campylobacter* species express virulence factors responsible for surviving under high temperature, hyperosmotic shock, carbon starvation, oxidative and aerobic (O_2) stress	Caseinolytic proteases (*clpP*) *dnaJ*, Carbon starvation regulator gene (*csrA*) High-temperature requirement gene B (*htrB*) (Hung *et al.*, 2011; Kim *et al.*, 2015; Otigbu *et al.*, 2018; Reddy and Zishiri, 2018)

The virulence factors which enable *Campylobacter spp.* to survive and resist physiological stress enhance its pathogenicity (Igwaran and Okoh, 2019). Additionally, virulence factors also complement its resistance to antibiotics. This is because the virulence factors enhance the ability of the bacterium to invade host immune defences, enhance survival in the presence of antibiotics and aid the bacteria in biofilm formation (Beceiro, Tomás and Bou, 2013; Derakhshan, Navidinia and Haghi, 2021). It is also suggested that virulence and antibiotic resistance are said to arise at the same time, but this relationship between virulence genes and antibiotic resistant genes is highly intricate (Beceiro, Tomás and Bou, 2013; Balta *et al.*, 2023). Expression of virulence genes and antibiotic resistant genes is also believed to be influenced by environmental factors.

1.4.3 *Campylobacter* survival in water

The physicochemical quality of the water and the presence of faecal contamination influence the survival of pathogens such as *Campylobacter spp*. in environmental sources such as rivers. Physicochemical variables that affect the survival of *Campylobacter* in aquatic environments include temperature, turbidity, dissolved oxygen concentration and the electrical conductivity of the water (Pitkänen, 2013; Shagieva, Demnerova and Michova, 2021). For example, low temperature favours the survival of *Campylobacter* species, but some species such as *C. jejuni* are able to survive under unfavourable temperature conditions. *Campylobacter jejuni* can stay viable for a few days under ambient temperature and in some cases up to four months at about 4°C (Pitkänen, 2013; Schang *et al.*, 2016; Shagieva, Demnerova and Michova, 2021). However, even under higher temperatures, *Campylobacter* species have been known to express virulence factors which enable them to withstand harsh conditions (Otigbu *et al.*, 2018).

Additionally, the survival of *Campylobacter* in rivers is influenced by oxygen levels. Although *Campylobacter* species such as *C. jejuni* are microaerophilic, they have the ability to survive under atmospheric concentrations of oxygen. When *Campylobacter* is exposed to aerobic atmosphere, reactive oxygen species (ROS) are formed in their cells. This results in oxidative stress, which leads to the destruction of the Fe-S proteins. *C. jejuni* expresses ROS detoxification enzymes, making it aero-tolerant. This allows *C. jejuni* to survive under aerobic conditions. Additionally, the presence of biofilms and free-living amoebae-harbouring bacteria intracellularly promotes the survival of *Campylobacter* in water. Studies show that *C. jejuni* can survive longer in the presence of *Acanthamoeba species*, which is a soil and water based amoeba (Pitkänen, 2013; Shagieva, Demnerova and Michova, 2021). Co-incubation of *C. jejuni* with *Acanthamoeba* is reported to resuscitate *C. jejuni* cultures, increase its acidic resistance and protect it from the action of chemical disinfectants (Pitkänen, 2013; Shagieva, Demnerova and Michova, 2021). *Campylobacter* species such as *C. jejuni* can also survive in low-nutrient, low-temperature and aerobic conditions.

It is important to note that *Campylobacter* species exhibit different survival patterns, ascribed to their high genetic diversity, and this accounts for interspecies differences in genes for stress response (Elmonir *et al.*, 2022). For example*, Campylobacter jejuni* can survive longer in the viable but non-culturable (VBNC) state. In VBNC form, in freshwater, *Campylobacter jejuni* retains infectivity (Pitkänen, 2013). *Campylobacter jejuni* is also more tolerant to stress conditions than *Campylobacter coli* (Pitkänen, 2013; Karikari *et al.*, 2016).

1.4.4 Reservoirs and transmission pathways

The transmission cycle begins with an animal reservoir such as cattle, poultry, wildlife and waterfowl, which contaminate water sources. *Campylobacter* has been detected in swine samples Hull *et al.*, (2021); Sithole *et al.*, (2021) and poultry (Hull *et al.*, (2021); Gharbi *et al.*, (2022). Similarly, studies have reported the occurrence of *Campylobacter* in wildlife (Sippy *et al.*, (2012); Navarro-Gonzalez *et al.*, (2014), birds Weis *et al.*, (2016)), and waterfowl (Abulreesh, Paget and Goulder, (2006); Van Dyke *et al.*, (2010)).

Campylobacter in surface water bodies can originate from anthropogenic activities within the watershed. These activities may include wastewater discharge, runoff from nearby agricultural farms treated with manure from livestock and faecal shedding from domestic and wild animals (Van Dyke *et al.*, 2010; Park *et al.*, 2011; Mughini-Gras *et al.*, 2016).

The *Campylobacter* in water may then be transmitted to humans in the form of drinking water, through consumption of contaminated water, crops irrigated with contaminated water or by contact with water through recreation activities (Evers *et al.*, 2008; Hokajärvi *et al.*, 2013; Gorham and Lee, 2016; Pitkanen and Hanninen, 2017). The link between contact to environmental water and *Campylobacter* infection has been well documented (Schönberg-Norio *et al.*, 2004; Mughini Gras *et al.*, 2012; Ravel *et al.*, 2016), mainly in outbreak situations (Dale *et al.*, 2010; Harder-Lauridsen *et al.*, 2013). Both freshwater and sea water can harbour *Campylobacter spp.* (Moore *et al.*, 2001; Kovanen *et al.*, 2016). In Denmark, up to 4% of sporadic infections is caused by recreational water (Gaardbo Kuhn *et al.*, 2018). Humans become infected by the bacteria and then transfer it to each other. The presence of *Campylobacter* in river water indicates recent contamination with faecal matter of human or animal origin, and its fate in the water environment depends on its ability to survive its environmental conditions (Mughini-Gras *et al.*, 2016). Humans infected with *Campylobacter* may excrete the bacteria through faeces back into the environment in the form of sewage. Human to human transmission rarely occurs, and has only been described in young children (Kaakoush *et al.*, 2015; Pitkanen and Hanninen, 2017).

1.4.5 Seasonal variations in human Campylobacteriosis and *Campylobacter* occurrence in water sources

Seasonality is evident in human *Campylobacter* infections, with distinct patterns based on geographical location. In temperate countries, a peak occurs during spring, while regions with milder winters experience earlier peaks. The timing of these peaks in temperate conditions is associated with the highest temperature, sunlight, and rainfall, highlighting the complex interplay of environmental factors in infection (Louis *et al.*, 2005). In tropical countries there is less variation throughout the year (Strachan *et al.*, 2013). In South Africa, there is very little epidemic surveillance, and this may have contributed to a lack of documented seasonal peaks in *Campylobacter* infections (Thobela 2017).

Similarly, *Campylobacter* in surface water shows seasonal variability, with higher recovery during the colder months of autumn and winter due to lower water temperatures, while it declines in spring and summer (Strakova *et al.*, 2022). Low river water temperatures below 18°C support the occurrence of *Campylobacter spp.* in rivers (Wilkes *et al.*, 2011; Strakova *et al.*, 2022). In addition, seasonal variations in *Campylobacter* occurrence in surface water may result from factors such as contamination, carrier animal prevalence, migratory patterns, rainfall and runoff. *Campylobacter* occurrence in rivers is reported to be positively associated with an increase in rainfall (Vereen *et al.*, 2013). Due to lack of environmental surveillance, significant knowledge gaps also persist regarding *Campylobacter* in surface water and its correlation with environmental parameters and seasons in South Africa.

1.4.6 Incidence

An increase in the incidence of campylobacteriosis in recent years has been reported in many parts of the world. Most of the epidemiological data is from North America, Europe and Australia, where there are efficient surveillance systems (Liu *et al.*, 2022). For example, *Campylobacter* has been linked to both sporadic cases and outbreaks through contaminated water in Finland and the USA (Revez *et al.*, 2014; Liu *et al.*, 2022). The incidences are said to be underreported in Africa, Asia, and the Middle East, but local prevalence studies suggest that *Campylobacter* infections are endemic in these regions (Kaakoush *et al.*, 2015). Although there are no national surveillance data currently available in African countries, several studies show that campylobacteriosis is prevalent in children (Kaakoush *et al.*, 2015; Liu *et al.*, 2022). For example, in South Africa, an infection rate of 38.9% was recorded in children aged 0−2 years (Shobo *et al.*, 2016). *Campylobacter* has been detected in stool samples of both adult and child diarrhoea patients (Kaakoush *et al.*, 2015; Chukwu *et al.*, 2020). *Campylobacter* has been linked to both sporadic cases and outbreaks in South Africa, but in most instances cases are usually sporadic episodes (Sahin *et al.*, 2012; Igwaran and Okoh, 2019). Outbreaks of *Campylobacter* have also been reported in High Income (HC) countries. For example, in Finland, *Campylobacter jejuni* was linked to 19% of the recorded waterborne outbreaks between 1998 and 2011 (Revez *et al.*, 2014). Similarly, in Norway, an outbreak of campylobacteriosis in 2019 resulted in over 2000 sick people, 76 hospitalised cases and two deaths. Consumption of faecal-contaminated drinking water was the cause of this outbreak, and *Campylobacter* was isolated from associated water samples (Paruch, Paruch and Sørheim, 2020). The occurrence was linked to cases of hospitalisation, death and Guillain-Barré syndrome (Gilpin *et al.*, 2020).

Waterborne outbreaks due to *Campylobacter* have been poorly reported in Africa. Poor surveillance, a lack of technology and resources for detection, and a lack of national epidemiological data for campylobacteriosis have led to poor documentation and underestimation of *Campylobacter* infections in low and middle income countries (Ouko *et al.*, 2021; Liu *et al.*, 2022). However the little information available shows that *Campylobacter* infections are endemic in most parts of Africa, as well as in South Africa (Shobo *et al.*, 2016; Chukwu *et al.*, 2019; Igwaran and Okoh, 2019). Despite this, these studies show that campylobacteriosis is especially prevalent in children (Kaakoush *et al.*, 2015; Liu *et al.*, 2022). The prevalence of *Campylobacter* in children has been reported at 21% in Malawi, 8.9% in Madagascar and 34.8% in Tanzania (Kaakoush *et al.*, 2015; Igwaran and Okoh, 2019; Gahamanyi *et al.*, 2020). *Campylobacter* has also been detected in adults at 5.8% in Kenya (Kaakoush *et al.*, 2015; Igwaran and Okoh, 2019; Gahamanyi *et al.*, 2020).

In South Africa, campylobacteriosis is not a notifiable disease and there is limited surveillance at national level (Kaakoush *et al.*, 2015; Shobo *et al.*, 2016; Liu *et al.*, 2022). However, local studies have detected *Campylobacter* in human samples. *Campylobacter* was isolated from stool samples for patients aged one month to 72 years at a private hospital in Durban, South Africa. The infection rate among males was higher (63.9%) than among females (36.1%). The highest infection rate (38.9%) was recorded in children aged between 0−2 year(s) (Shobo *et* *al.*, 2016). *Campylobacter jejuni* (40%), (32.3% *C. jejuni subsp. Jejun*i (32.3%), *C. jejuni subsp. doylei* (7.7%) and *C. concisus* (24.6%) were isolated from stool samples of children with diarrhoea in Cape Town from 2005 to 2009 (Kaakoush *et al.*, 2015). *Campylobacter* spp. was also detected in 24.6% and 29.7% of paediatric stool samples in the Madibeng District (Chukwu *et al.*, 2020). Add a joining sentence on antibiotic resistance

1.4.7 Antibiotic resistance mechanisms among *Campylobacter* species

Campylobacter infections in humans are usually self-limiting, but cases can be severe in immunocompromised individuals, children, elderly people and pregnant women (Kashoma *et al.*, 2016; Bolinger and Kathariou, 2017). When symptoms persist, and in severe cases, antibiotics that are often prescribed include tetracycline, macrolides (erythromycin) and fluoroquinolones (ciprofloxacin). However, *Campylobacter* species have demonstrated resistance to all these antibiotics of choice (Ghunaim *et al.*, 2015; Kashoma *et al.*, 2016; Pillay *et al.*, 2020). *Campylobacter* species are becoming more resistant to clinically relevant antibiotics such flouroquinolones (ciprofloxacin and enrofloxacin), macrolides and tetracyclines (Kovač *et al.*, 2014). The ability of *Campylobacter spp.* to acquire resistance to antibiotics is an issue of concern. Due to high antibiotic resistance among *Campylobacter* species, the World Health Organization (WHO) named *Campylobacter* as one of the 12 bacterial species that pose the greatest threat to human health (Sproston, Wimalarathna and Sheppard, 2018; Veltcheva *et al.*, 2022).

A high resistance to fluoroquinolones has been observed in *Campylobacter jejuni, Campylobacter coli* and other *Campylobacter* species (Sierra-arguello *et al.*, 2018; Sproston, Wimalarathna and Sheppard, 2018; Goulart *et al.*, 2022; Veltcheva *et al.*, 2022). Consequently, in many countries, fluoroquinolones have already become of limited use for treating *Campylobacter* infections (Nelson *et al.*, 2007; Ghunaim *et al.*, 2015; Sproston, Wimalarathna and Sheppard, 2018; Veltcheva *et al.*, 2022). The high resistance to fluoroquinolones among *Campylobacter* species has led to the use of macrolides such as erythromycin and azithromycin in the treatment of *Campylobacter* infections in humans. However, *Campylobacter* species have also been showing resistance to macrolides. Macrolide-resistant *Campylobacter* was listed as one of the critical antibiotic resistance threats to public health by the Centers for Disease Control and Prevention (CDC) in 2013 (Bolinger and Kathariou, 2017; Dabbousi *et al.*, 2022). Common types of macrolides include erythromycin, clarithromycin and azithromycin. Erythromycin is a macrolide commonly used in the treatment of human campylobacteriosis (Iovine, 2013). Unfortunately, resistance to erythromycin and other macrolides has also been observed among *Campylobacter* species. Tetracyclines are another alternative choice in the treatment of clinical campylobacteriosis, but resistance to antibiotics under this class (e.g., tetracycline, doxycycline and minocycline) has also been reported among *Campylobacter* species (Moore *et al.*, 2006; Iovine, 2013).

In *Campylobacter* species, resistance to antibiotics is achieved through different mechanisms (Table 1.2) which include point mutations, target modification, change in permeability of the membrane, antibiotic inactivation and efflux pumps (Iovine, 2013). The fluoroquinolones inhibit the synthesis of bacterial DNA, causing necrosis. They achieve this by targeting two large bacterial enzymes. DNA gyrase and topoisomerase IV. These enzymes act mutually in *Campylobacter* bacterial DNA replication, transcription, recombination and repairing of DNA. The DNA gyrase is made up of subunits *gyrA* and *gyrB*, while the topoisomerase IV gene consists of the subunits ParC and ParE. A single point mutation in the quinolone-resistance-determining region (QRDR) of DNA gyrase or topoisomerase IV in *Campylobacter* causes resistance to fluoroquinolones. This point mutation is caused by the substitution of the amino acid threonine at position 86 with isoleucine (Thr-86-to-Ile), which consequently leads to the modification of the target (Zirnstein *et al.*, 1999; Iovine, 2013; Wieczorek and Osek, 2013; Sierra-arguello *et al.*, 2018; Sproston, Wimalarathna and Sheppard, 2018). Other mechanisms of fluoroquinolone resistance are decreased outer membrane permeability and an efflux pump. In addition, fluoroquinolone resistance due to spontaneous single point mutation is also accelerated by the horizontal transfer of resistance-conferring DNA among strains of the genus *Campylobacter* (Table 1.2).

Table 1.2. Summary of basic resistance mechanisms for resistance among *Campylobacter spp.* (from Wegener, Aarestrup and McDermott, 2008; Mikysková, 2018)

Antibiotics	Mechanism of resistance	Resistance genes or mutation
Aminoglycosides	Antibiotic modification by enzymes (AphA AadE, Sat)	Apha-3, Ant-6, Ant-3, Apha-7
β-lactams	Enzymatic inactivation of antibiotics with β-lactamases Reduced membrane permeability due to MOMP Efflux via CmeABC	- ND Cat
Fluoroquinolones	Modification of DNA gyrase targets Efflux via CmeABC	*gyrA*; Ala-70's Thr; Thr-86's Ile, Lys, Ala, Wall; Asp90 to Ala, Asn, Tyr Mfd
Macrolides	Mutations at 23S rRNA site Mutation in ribosomal proteins L4 / L22 Efflux via CmeABC Reduced membrane permeability due to MOMP	23S rDNA; A to G at position 2.075; AND to C to position2.074
Tetracyclines	Modification of Target Ribosomal Site A by TetO binding Efflux via CmeABC	*tetO*

There are a number of mechanisms of resistance to macrolides in *Campylobacter*. These include target modification, efflux, and a change in permeability of the membrane (Iovine, 2013). Target modifications involves point mutations of the 23S rRNA or ribosomal proteins (Bolinger and Kathariou, 2017; Lluque *et al.*, 2017). High macrolide resistance among *Campylobacter* species has mainly been attributed to mutations in 23S rRNA genes. The mutation is a single nucleotide substitution in the peptidyl encoding region in domain V of the 23S rRNA sequence at position 2074 or 2075. This mutation disrupts the ribosomal structure, which interferes with the macrolide binding sites on the 50S rRNA, thereby inhibiting protein elongation (Bolinger and Kathariou, 2017; Hull *et al.*, 2021). Substitutions in the 23S rRNA gene is the most common mechanism for high macrolide resistance in *Campylobacter* species. The mutation at point A2075G is the most frequently occurring one, while other mutations such as A2074C/G and A2074G/T are less common (Bolinger and Kathariou, 2017; Lluque *et al.*, 2017; Hull *et al.*, 2021). It is worth noting that the ability of these substitutions (A2074C/G, A2074G/T and A2075G) to confer resistance is closely linked to the copy number of the 23S rRNA gene. *Campylobacter* species have three copies of the 23S rRNA gene, and substitutions in all the three copies of the gene results in high-level resistance to macrolides such as erythromycin and azithromycin (Bolinger and Kathariou, 2017; Lluque *et al.*, 2017; Hull *et al.*, 2021). In addition to base substitutions in the 23S rRNA sequence, mutations in the 50S ribosomal proteins L4 (rplD) and L22 (rplV) are also responsible for macrolide resistance. Resistance to macrolides due to L4 and L22 mutations are lower than those of the base substitutions in the 23S rRNA (Bolinger and Kathariou, 2017; Hull *et al.*, 2021). These mechanisms for macrolide resistance are different from those exhibited by tetracycline.

The mechanisms of tetracycline resistance is mainly due to alteration of tetracycline's ribosomal target, antibiotic efflux pumps and antibiotic inactivation (Iovine, 2013; Makowska, Koczura and Mokracka, 2016). The *tetO* gene is the main mediator for tetracycline resistance in *Campylobacter* species. This gene (*tetO*) is usually located on a plasmid or chromosomal DNA of bacteria (Kayman *et al.*, 2019). The *tetO* gene encodes the TetO protein, a ribosome protection protein. The Tet(O) protein protects the ribosome from the inhibitory effect of tetracycline (Pratt and Korolik, 2005). The TetO protein causes a decrease in the ability of tetracyclines to bind to the 30S subunit of the ribosome, leading to lack of inhibition of polypeptide chain synthesis in the bacterial cell. This promotes the release of tetracycline from its site of inhibition on the ribosome, leading to tetracycline resistance (Kayman *et al.*, 2019; Andrzejewska *et al.*, 2022; Benites *et al.*, 2022).

In addition to resistance to specific antibiotics, *Campylobacter spp*. have evolved mechanisms for resistance to multiple antibiotics and toxic compounds such as bile salts and detergents. The antibiotic efflux systems pump structurally diverse antibiotics out of bacterial cells, resulting in resistance to a wide range of antibiotics (Lin, Michel and Zhang, 2002). The increase in multi-drug resistant (MDR) strains among bacteria such as *Campylobacter spp*. is compromising the effectiveness of numerous antibiotics (Uddin *et al.*, 2021). The *cmeABC* genes contribute to high resistance to multiple antibiotics and have been detected in many *Campylobacter* species (García, Fox and Besser, 2010; Karikari *et al.*, 2016). The *cmeABC* complex codes for the multidrug efflux complex located in the bacterial membrane. This efflux pump is an energy-dependent efflux system which aids *Campylobacter* in colonisation of animal intestines, as well as resistance to structurally diverse compounds such as antibiotics, ethidium bromide and various detergents (Hull *et al.*, 2021). The *cmeABC* complex plays a role in resistance to antibiotics such as erythromycin, ciprofloxacin and tetracycline, including multidrug resistance (MDR). The efflux pumps prevent tetracycline from reaching its target by pumping it out of the cell (Kurincic, Klancnik and Mozina, 2012). The multidrug efflux pump cmeABC functions synergistically with the other mechanisms for flouroquinolones, macrolides and tetracyclines. Inactivation of the cmeABC gene leads to a decrease in the ciprofloxacin, erythromycin and tetracycline resistance in *Campylobacter species* (Kurincic, Klancnik and Mozina, 2012; Iovine, 2013; Bolinger and Kathariou, 2017; Sproston, Wimalarathna and Sheppard, 2018; Hull *et al.*, 2021).

1.4.8 Environmental monitoring and surveillance for antibiotic-resistant *Campylobacter* in South Africa

In South Africa, some research has been conducted on different environmental reservoirs to investigate the prevalence, patterns in antibiotic resistance and potential risks to humans. Environmental reservoirs that have been investigated include water sources, poultry, cattle and pig herds. This section presents the findings and implications of South African research from studies related to environmental surveillance of *Campylobacter*, illuminating the progress and areas that require further investigation. It also acknowledges the significance and implications of *Campylobacter* presence in various environmental reservoirs, as this can impact their occurrence in water bodies.

Examination of antibiotic susceptibility profiles has shown that *Campylobacter* isolates from human, animal and water sources exhibited resistance against antibiotics of the classes macrolides, carbapenems, penicillin, fluoroquinolones, aminoglycoside, lincosamide, tetracycline and cephalosporins (Table 1.3). The highest level of antibiotic resistance reported was resistance against macrolides, fluoroquinolones and tetracyclines. Among human isolates, the highest level of resistance of *Campylobacter* was to azithromycin (69.7%), whereas the lowest level of resistance was to gatifloxacin (8.3%).

In animal samples, high resistance to erythromycin (95.06%), clindamycin (95.68%), doxycycline (87.65%),, tetracycline (84.3%), streptomycin (88%), and ampicillin (73%) is reported. Furthermore, resistance to tetracycline (100%), imipenem (100%), clarithromycin (95%), azithromycin (92%), clindamycin (84.2%), doxycycline (80%), and ciprofloxacin (77.8%) has been reported among *Campylobacter spp.* from water samples.

Table 1.3. Antibiotic resistance among *Campylobacter* species from human, animal and water sources in South Africa

Reservoir	Macrolide	Carbapenems	Penicillin	Flouroquinolones	Aminoglycoside	Lincosamide	Tetracycline	Cephalosporins
Human	Cla 29.3% Ery 26.7% Chukwu *et al*., (2019) Ery 33.3% Azi 69.4% Shobo *et al*., (2016)	Imi 15.3% Mer 19.3% Chukwu *et al*., (2019)	Amo 64.7% Amp 60.7% Chukwu *et al*., (2019)	Cip 18% Nor 13.3% Chukwu *et al*., (2019) Cip 23.6% Gat 8.3% Shobo *et al*., (2016)	Ami 18% Gen 15.3% Chukwu *et al*., (2019)		Tet 32% Tig 30% Chukwu *et al*., (2019) Tet 33.3% Shobo *et al*., (2016)	Cep 60% Cef 54% Chukwu *et al*., (2019)
Chicken	Ery – 0 - 87.9%, Bester and Essack, (2012) Ery- 45% Pillay *et al*., (2020)		Amp 33% Pillay *et al*., (2020)	Cip 7.9 - 95.4%, Bester and Essack, (2012) Nal 18% Pillay *et al*., (2020) Nal 98% Cip 21% Mileng, (2021)	Stre 5.4% Gen 1.6 – 12.9% Str 11.5- 40% Bester and Essack, (2012) Pillay *et al*., (2020)	Cli 36% Pillay *et al*., (2020)	Tet 21.6 - 100% Bester and Essack, (2012) Tet 4% Pillay *et al*., (2020)	Cef 16% Pillay *et al*., (2020)
Meat samples (mutton, chicken, turkey, beef, and pork)		Imi 23% Igwaran and Anthony I. Okoh, (2020)	Amp 97% Igwaran and Anthony I. Okoh, (2020)	Cip 76% Lev 55% Igwaran and Anthony I. Okoh, (2020)	Gen 65% Igwaran and Anthony I. Okoh, (2020)	Cli 100% Igwaran and Anthony I. Okoh, (2020)	Dox 94% Tetracycline - 94% Igwaran and Anthony I. Okoh, (2020)	Cep 84% Cef 84% Igwaran and Anthony I. Okoh, (2020)

Clar = Clarithromycin Ery = Erythromycin Azi =Azithromycin Tel = Telithromycin Str = Streptomycin Imi = Imipenem Mer = Meropenem Amox = Amoxicillin/clavulanic acid Amp = Ampicillin Nal =Nalidixic acid Lev = Levofloxacin Cipro = Ciprofloxacin Nor = Norfloxacin Gat = Gatifloxacin Ami = Amikacin Gent = Gentamicin Strep = Streptomycin Cli = Clindamycin Tet = Tetracycline Tig = Tigecycline Dox = Doxycycline Cep = Cephazolin Cef = Ceftriaxone Cefu = Cefuroxime

Table 1.3 (continued): Antibiotic resistance among *Campylobacter* species from human, animal and water sources in South Africa

Reservoir	Macrolide	Carbapenems	Penicillin	Flouroquinolones	Aminoglycoside	Lincosamide	Tetracycline	Cephalosporins
Cow milk	Ery 95.1% Azi 87% Igwaran and Anthony Ifeanyi Okoh, (2020)	Imi 21.47% Igwaran and Anthony Ifeanyi Okoh, (2020)	Amp 87% Igwaran and Anthony Ifeanyi Okoh, (2020)	Cip 77.8% Lev 59.8% Igwaran and Anthony Ifeanyi Okoh, (2020)	Gen 56.2% Igwaran and Anthony Ifeanyi Okoh, (2020)	Cli 95.7% Igwaran and Anthony Ifeanyi Okoh, (2020)	Dox 87.5% Tet 83.3% Igwaran and Anthony Ifeanyi Okoh, (2020)	Cef 93.2% Igwaran and Anthony Ifeanyi Okoh, (2020)
Pig	Ery 90% Stre 88% Sithole *et al.*, (2021)		Amp 73% Sithole *et al.*, (2021)	Cip 57.1% Nal 27.2% Sithole *et al.*, (2021)	Gen 11.6% Sithole *et al.*, (2021)		Tet 84.3% Sithole *et al.*, (2021)	
Water	Clar 95% Ery 85% Chukwu *et al.*, (2019) Azi 87% Igwaran and Anthony Ifeanyi Okoh, (2020) Cla - 80% Ery -70% Otigbu *et al.*, (2018)	Mer 15% Chukwu *et al.*, (2019) Imi 21.7% Igwaran and Anthony Ifeanyi Okoh, (2020)	Amo 30% Amp 70% Chukwu *et al.*, (2019) Amp 87% Igwaran and Anthony Ifeanyi Okoh, (2020)	Cip 25% Nor 40% Chukwu *et al.*, (2019) Cip 77.8% Lev- 59.8% Igwaran and Anthony Ifeanyi Okoh, (2020) Cip 77.8% Nalidixic acid 30.5% Otigbu *et al.*, (2018)	Gen 45% Ami 40% Chukwu *et al.*, (2019) Gen 56.2% Igwaran and Anthony Ifeanyi Okoh, (2020)	Cli 95.7% Igwaran and Anthony Ifeanyi Okoh, (2020) Cli 84.2% Otigbu *et al.*, (2018)	Tet 55% Tig 45% Chukwu *et al.*, (2019) Tet 83.3% Dox 87.7% Igwaran and Anthony Ifeanyi Okoh, (2020) Dox 80% Otigbu *et al.*, (2018)	Cep 50% Cef 35% Chukwu *et al.*, (2019) Cef 93.2% Igwaran and Anthony Ifeanyi Okoh, (2020)

Clar = Clarithromycin Ery = Erythromycin Azi =Azithromycin Tel = Telithromycin Str = Streptomycin Imi = Imipenem Mer = Meropenem Amox = Amoxicillin/clavulanic acid Amp = Ampicillin Nal =Nalidixic acid Lev = Levofloxacin Cipro = Ciprofloxacin Nor = Norfloxacin Gat = Gatifloxacin Ami = Amikacin Gent = Gentamicin Strep = Streptomycin Cli = Clindamycin Tet = Tetracycline Tig = Tigecycline Dox = Doxycycline Cep = Cephazolin Cef = Ceftriaxone Cefu = Cefuroxime Chl = Chloramphenicol Flo = Florfenicol

Genes conferring resistance to clinically relevant antibiotics have been detected in *Campylobacter* isolates from human, animal and water sources in South Africa (Table 1.4). The most prevalent ARGs in human isolates are those encoding resistance to tetracycline (*tetO* 64%), beta-lactams (blaOXA-61 53%) and ciprofloxacin (*gyrA* 49%) (Shobo *et al.*, 2016; Reddy and Zishiri, 2017, 2018; Chukwu *et al.*, 2019). Similarly, *tetO* (75%) and blaOXA-61 (100%) are the most prevalent in chicken samples. High occurrence *tetO* has also been reported in swine samples. Other ARGs that have been detected in animal samples include chloramphenicol resistance genes (*catII*, 95%) and erythromycin resistance genes (*erm*, 89%) in pigs, and gentamycin resistance genes (*aac(3)-IIa-(aacC2)a)* 85%) in cow's milk. The most prevalent ARGs among *Campylobacter* species from water samples are the genes conferring resistance to chloramphenicol (*catII*, 95%), erythromycin (*ermB* 89%), and gentamicin (*aac(3)-IIa-(aacC2)a*, 85%). High occurrences of multidrug-resistant genes (*cmeB*, 90%, *cmeC*, 70%) have also been reported among *Campylobacter* isolates from water, and this suggests that these isolates may also be resistant to other antibiotics not considered in these studies (Otigbu *et al.*, 2018).

Table 1.4. Antibiotic resistant genes detected in human, animal and water samples in South Africa

Samples	Antibiotic Resistant Genes detected
Human	*gyrA*- 18%, *tetO*- 28.6%, Chukwu *et al.*, (2019) *gyrA* 235 bp 49%, *gyrA* 270 bp 36%, *blaOXA-61* 53% *tetO* 64% Reddy and Zishiri, (2017) *tetO* 33%, *gyrA* 34%, V23S rRNA 11%, *cmeA*- 18% Shobo *et al.*, (2016)
Chicken	235 bp *gyrA* 51%, 270 bp *gyrA* 36%, *bla*$_{OXA-61}$ 58% *tetO* 68% Reddy and Zishiri, (2017)
Meat samples (mutton, chicken, turkey, beef and pork)	*catll* 91.78%, *tetA* 68.82%, *gyrA* 61.76%, *ampC* 55%, *aac(3)-IIa (aacC2)*^{a} 40.98%, *tetM* 38.71%, *ermB* 18.29%, *tetB* 12.90%, *tetK* 2.15% Igwaran and Anthony I. Okoh, (2020)
Cow's milk samples	*catII* 95%, *ermB* 89%, *tetA* 27%, *tetM* 32%, *ampC* 81% *aac(3)-IIa-(aacC2)*^{a} 85%, *gyrA* 39% Igwaran and Anthony Ifeanyi Okoh, (2020)

Pig faecal matter and carcasses	*gyrA* 100%, *tetO* 95%, 23SrRNA 100%, *bla*$_{OXA\text{-}61}$ 3% *cmeB* 11% Sithole *et al.*, (2021)
Water	*gryA* 25%, *tetO* 40%, *23S rRNA 2075G* 75%, *23S rRNA 2074C* Chukwu *et al.*, (2019)
	gyrA 39%, *catII* 95%, *ermB* 89%, *tetA* 27%, *tetM* 32% *ampC* 81%, *aac(3)-IIa-(aacC2)*^{a} 85% Igwaran and Anthony Ifeanyi Okoh, (2020)
	cmeA 55% , *cmeB* 90%, *cmeC* 70% Otigbu *et al.*, (2018)

The extensive surveillance across the different environmental reservoirs, water sources, poultry, cattle and pigs reveal similarities in antibiotic resistance patterns to those observed in human populations. *Campylobacter* isolates from human samples and water samples in a study conducted in Northwest Province of South Africa showed genetic similarity (Chukwu *et al.*, 2019). The similarity of resistance profiles across these environmental reservoirs and humans accentuates the interconnected form of antibiotic-resistance dynamics. This finding reinforces the fact that environmental reservoirs play a critical role in determining the antibiotic resistance landscape that can impact both animal and human health. The presence of antibiotic-resistant *Campylobacter* and ARGs in river water suggests that these organisms can be transmitted to humans through this reservoir. Therefore, there is potential for sharing of antibiotic resistance bacteria and genes between animals, humans and the environment (Bengtsson-Palme, 2017). Indeed, the risk of dissemination of antibiotic-resistant *Campylobacter* species between humans, animals and the environment is anticipated. However, thorough exploration of the risk of dissemination of antibiotic-resistant *Campylobacter* species between humans, animals, and the environment has been limited in local research. Water sources serve as crucial pathways for the transmission of antibiotic resistance to humans. Estimating the risk of exposure to antibiotic-resistant *Campylobacter* through water reservoirs is indispensable for public health considerations.

1.4.9 Assessment of human exposure to antibiotic-resistant bacteria

Exposure assessment can be defined as the quantitative and / or qualitative evaluation of the potential intake of chemical, biological or physical agents through food or any other relevant source (CODEX, 2006). It combines consumption data with the concentration of the chemical, biological or physical agents in food. A few studies have provided reliable approaches to assess human exposure to ARB and ARGs in water (O'Flaherty *et al.*, 2018, 2019; X. Li *et al.*, 2020; Shuai *et al.*, 2021). To analyse the potential human exposure to ARGs and ARB in water, the event intake (EI) burden of ARGs and ARB through different routes are calculated. Human exposure routes are through ingestion, inhalation and dermal absorption/contact of pollutants present in water (Shuai *et al.*, 2021). The potential contribution of ingestion and inhalation to human exposure of ARGs or ARB in water is estimated by multiplying the concentration/ abundance of target ARGs or ARB by ingestion rate and inhalation rates, respectively. The potential dermal contact exposure dose of ARGs is calculated by multiplying the concentration/ abundance of target ARGs or ARB by the dermal surface area (Shuai *et al.*, 2021). The concentration of ARB in water in the water samples can be measured by both culture-based and non-culture-based methods.

In detecting *Campylobacter* from environmental water samples, a pre-enrichment in broth is recommended. This step provide nutrients and a favourable environment to promote the growth of *Campylobacter spp.* (Williams *et al.*, 2009). Commonly used enrichment broths include Preston, Exeter, and Bolton broths (Carter *et al.*, 2009). These enrichment media depend on the antibiotics added to the medium's selective supplements. The antibiotics supress growth of all other competing organisms, hence selectively promoting the growth of *Campylobacter* (Kashappanavar *et al.*, 2018). Additionally, microaerophilic conditions during incubation of the broth hinders the growth of other bacteria and favours the growth of *Campylobacter* (Meurer, Payne and Guffey, 2020). Culturing often requires incubation at 37°C and/or 42°C under microaerophilic conditions (Porte *et al.*, 2016). Most *Campylobacter* have been cultured from river water samples at an incubation temperature of 42°C (Van Dyke *et al.*, 2010; Lévesque *et al.*, 2011; Vereen *et al.*, 2013; Kim *et al.*, 2016; Mughini-Gras *et al.*, 2016).

Unfortunately, culture-based methods are unable to detect viable but non-culturable (VBNC) forms of *Campylobacter*. Molecular quantitative (qPCR) can overcome the limitations of culture-based methods, and even detect the presence of VBNC (Cangelosi and Meschke, 2014; Yuan *et al.*, 2018). Some of the studies investigating human exposure of ARG and ARB in water have applied qPCR to detect and quantify ARB and ARG. The copy number of 16S rRNA gene or the ARG is quantified with qPCR. Additionally, a viability PCR assay is employed to distinguish between live and deceased cells (Cangelosi and Meschke, 2014). This method entails incubating samples with a DNA-binding dye, such as propidium monoazide (PMA). PMA selectively binds to free DNA, including DNA from dead cells with damaged membranes, ensuring that only live cells are identified and amplified during the PCR process

(Nocker *et al.*, 2007; Bankier *et al.*, 2018). Viability PCR offers the advantage of excluding the detection of DNA from dead bacteria, focusing instead on the infectious potential of the Viable but Non-Culturable (VBNC) state. Alternatively, exposure data related to concentration values of ARB and ARGs can be collected from literature available from epidemiological studies (Tyagi and Kumar, 2021). Data obtained through these methods are invaluable for assessing the risk of infection.

Regarding the ingestion rate, inhalation rates and dermal surface area, literature-based epidemiological studies are used to estimate these parameters. The ingestion rates of 21 mL per hour and 32 mL per hour, which are most commonly used, are derived from studies by Dorevitch *et al.* (2011) and Dufour *et al.* (2017). Dorevitch *et al.* (2011) estimated that the average quantity of water consumed through a sip, a mouthful or from swimming, is of about 10 mL of water (arithmetic mean), with an upper confidence limit of 35 mL of water. A large-scale study by Dufour *et al.* (2017) estimated an arithmetic mean of 32 mL. The arithmetic mean of 32 mL from the study by Dufour falls within the confidence limits of the Dorevitch mean, and was applied in the study by Tyagi and Kumar (2021). The difference in values obtained in Dorevitch *et al.* (2011) and Dufour *et al.* (2017) is due to the number of people sampled in each study. The 16 mL/45 min (or 21 mL/h) applied in Shuai *et al.*, (2021) is intermediate between the mean estimate of 10 mL and the upper confidence estimate of 35 mL. These volumes are applied for ingestion per hour or per dip. An average inhaled air rate 20 m3/day per person is used for inhalation rate (L. Li *et al.*, 2020), while a skin surface area of 16,000 cm^2, derived from data from the Ministry of Environment Protection in China is typically used (Shuai *et al.*, 2021). The exposure dose of $2.67 \times 10^3 - 4.18 \times 10^6$ copies/h, $7.47 \times 10^2 - 1.41 \times 10^7$ copies/h, $3.81\times10^1 - 7.46\times10^4$ copies/h, and $9.88 - 5.87\times10^3$ copies/h via swimming in a pool has been reported for total antibiotic-resistant opportunistic pathogens and *Mycobacterium*, *Pseudomonas*, and *Acinetobacter*, respectively (Shuai *et al.*, 2021). In the same study, the exposure dose of ingestion $2.08–2.17\times10^2$ for the ARG *bla*$_{TEM}$ for 1 h through accidental ingestion of water was measured. Two other studies estimated exposure doses of 10^4 –10^5 ARG copies/day via ingestion of drinking water, and 10^6–10^7 ARG copies/day via inhalation (L. Li *et al.*, 2020; N. Li *et al.*, 2020). These studies contribute to the body of knowledge regarding the potential health risks of antibiotic microbial resistance (AMR) in human-related environments. In South Africa, there are no existing studies which estimate potential human exposure (dose) to antibiotic-resistant pathogens, including *Campylobacter* and associated ARGs in human-related environments.

1.5 Problem Statement

Campylobacter species are increasingly implicated in both animal (zoonotic) and human cases of diarrhoea and gastroenteritis infections. Diarrhoea is a primary cause of morbidity and mortality in South Africa, particularly among children (Chukwu *et al.*, 2020). Adding to the concern is the propensity of *Campylobacter* species to resist the effects of antibiotics. While the etiological agent has been known for a long time, it has recently been recognized as one of the main causes of waterborne diseases (Chukwu *et al.*, 2019; Igwaran and Okoh, 2019). These bacteria exhibit the ability to survive and multiply in aquatic environments, and exposure to contaminated water poses a potential source of infection. The occurrence of antibiotic-resistant *Campylobacter* species has been on the rise in rivers worldwide (Kaakoush *et al.*, 2015). Locally, there have been frequent reports of the increasing presence of antibiotic-resistant bacteria (ARB) and antibiotics in source waters (Otigbu *et al.*, 2018; Chukwu *et al.*, 2019; Mpondo, Ebomah and Okoh, 2021).

The presence of antibiotic-resistant bacteria and their resistance genes in freshwater used for domestic, industrial, irrigation and agricultural purposes indicates a threat to a potentially exposed population. South Africa, being a water-scarce country, relies directly on available freshwater systems, including rivers, for irrigation, domestic purposes and recreational activities. It is widely acknowledged that environmental bacteria serve as reservoirs of antibiotic-resistant bacteria and resistance genes, which can potentially be incorporated into humans and pathogens over time. The multifaceted factors influencing the occurrence and emergence of such strains in aquatic environments are not fully understood. In the context of this study, emergence means rapidly increasing in incidence in aquatic environments (Larsson and Flach, 2022). Furthermore, the contribution of local rivers to human infections with *Campylobacter* is unknown, and warrants further study. Understanding the contribution of local rivers to human infections with *Campylobacter* is crucial, as transmission through water from these rivers to humans is a possibility (Igwaran and Okoh, 2019). The relative contribution of various river water transmission routes to *Campylobacter* and its ARG transmission is unknown, and quantitative data on human exposure through these routes are needed. Significant knowledge gaps also persist regarding the presence of *Campylobacter* in surface water, and its correlation with environmental parameters and seasons in South Africa. *Campylobacter*'s survival in surface water is influenced by environmental conditions and seasonal variations. Lastly, the risk of emergence and transmission of ARB and ARGs in rivers is multidimensional, requiring a holistic approach that integrates physicochemical conditions and seasons with social dimensions. However, holistic risk assessment frameworks are currently lacking.

1.6 Aim

The overall aim of the study is to investigate the context-related drivers, occurrence and human exposure to antibiotic resistant *Campylobacter* in selected river systems.

1.7 Objectives

1. To assess context-related drivers of antibiotic resistant bacteria and genes in selected river systems in the Eastern Cape, South Africa.
2. To relate selected physicochemical variables to spatiotemporal occurrence of antibiotic-resistant *Campylobacter* in the selected river systems.
3. To assess human exposure to antibiotic-resistant *Campylobacter* species and genes in the selected river systems.
4. To develop a holistic framework for assessing the risk of emergence and transmission of antibiotic-resistant bacteria and genes in rivers.

1.8 Thesis structure

The work is presented in various published and unpublished chapters. Chapter 1 presents the general introduction and literature review while Chapter 2 reveals the study areas and methods employed in the study. Chapters 3, 4, 5 and 6 present the key results of the study as they relate to the specific objectives. Chapter 3 discusses the context-related drivers of antibiotic-resistant bacteria and genes in selected river systems. In Chapter 4, investigations regarding the spatiotemporal occurrence of antibiotic-resistant *Campylobacter* species are presented. Chapter 5 presents results on the detection of *Campylobacter, ARGs* and virulence genes. It also presents information on the quantification of the concentration of *Campylobacter* and selected ARGs in water. In Chapter 6 the results of an estimation of human exposure to antibiotic resistant *Campylobacter* species are presented, while Chapter 7 presents the framework for assessing the risk of emergence and transmission of antibiotic-resistant bacteria and genes in rivers. Chapter 8 is a general discussion of the findings of this study, with a conclusion and recommendations for this study.

CHAPTER 2: STUDY AREA, GENERAL MATERIALS AND METHODS

2.1 Introduction

In this chapter, the study area and the general methods employed throughout this study are presented. Understanding the geographical context is important as it lays the foundation for the entire study. The aim of this chapter is to provide clarity on the geographic setting and to highlight the significance of the selected study areas. Furthermore, the water sampling and processing methods are detailed. These common experiments are crucial study components that contribute significantly to the integrity of a study and form the basis for subsequent analyses in this study.

2.2 Study Area

This study was conducted in the Bloukrans and Swartkops river catchments in the Eastern Cape, South Africa. The Bloukrans River has a length of approximately 40 km, and flows in a south-easterly direction through the town of Makhanda (33°18′33.0″ S and 26°31′36.0″ E) and farms in the Belmont valley. The Bloukrans River catchment area is approximately 230 km^2, and the population of Makhanda is roughly 70,000 (Mangadze *et al.*, 2019). The major economic activities in the catchment are education, agriculture, tourism and the legal industry (Hoefnagels, Margaret and Sinenhlanhla, 2022). Figure 2.1 shows a map of the Bloukrans River.

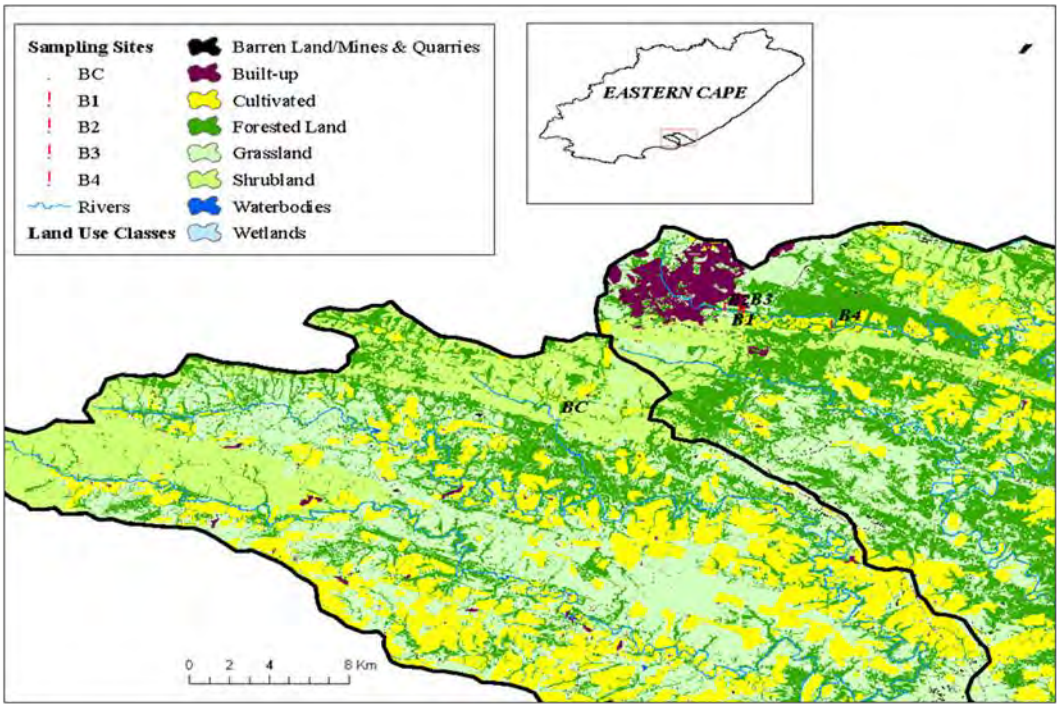

Figure 2.1. Map of the Bloukrans catchment and location of the sampling sites (sites BC, B1, B2, B3 and B4) on the Bloukrans River, Eastern Cape, South Africa and land use within the catchment

The Swartkops River is approximately 155 km long, has a mean annual runoff of 84.2 x 10^6 m^3 and flows through the highly urbanised and industrialised areas of the Nelson Mandela Bay Municipality (NMBM) (Bate, Smailes and Adams, 2004; Adams, Pretorius and Snow, 2019). The NMBM has a population of approximately 1.2 million people (Zingela *et al.*, 2022). The Swartkops River catchment area is about 1,354 km^2 and lies in the Groot Winterhoek Mountains. Swartkops River also flows through urban areas such as Uitenhage, Kwanobuhle, Despatch, Motherwell, Zwide and Blue Water Bay. The main tributaries of the Swartkops River are the Elands and KwaZunga rivers, while the Brak and Chatty rivers are considered to be subsidiary tributaries (Adams, Pretorius and Snow, 2019; Odume *et al.*, 2022). This municipality is a major seaport, automotive manufacturing centre and holiday hot spot, and is the economic powerhouse of the Eastern Cape Province. Other economic activities within the catchment include manufacturing, salt mining, tourism, community services, finance, trade, agriculture and transport (Adams, Pretorius and Snow, 2019). Figure 2.2 shows a map of the Swartkops catchment.

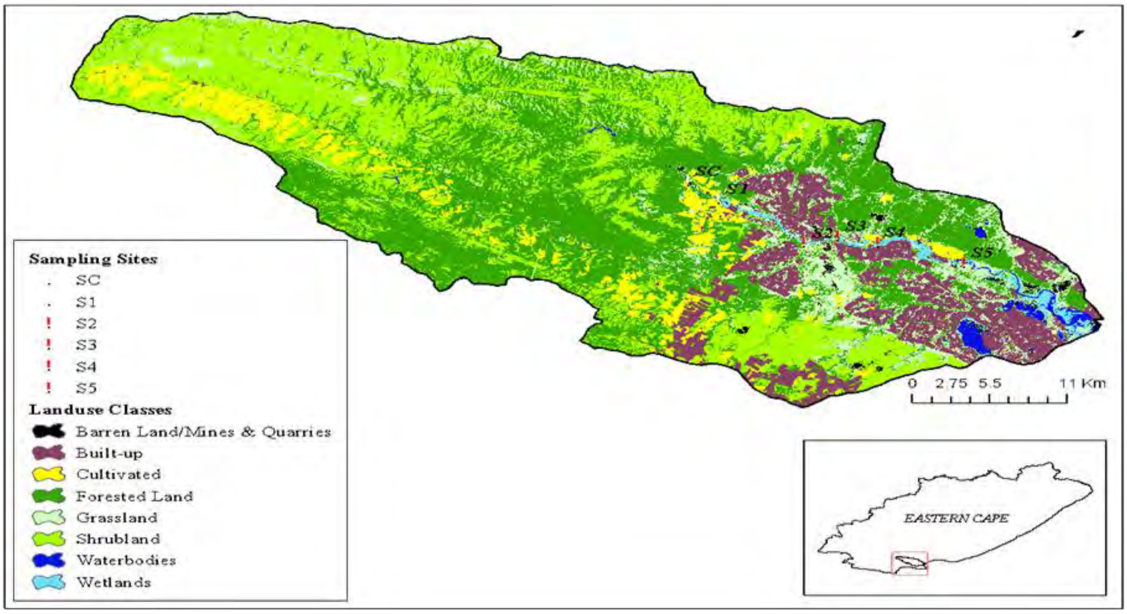

Figure 2.2. Map of Swartkops catchment and location of sampling points (sites SC, S1, S2, S3, S4 and S5) on the Swartkops River, Eastern Cape, South Africa and land-use within the catchment

2.2.1 Climate of the Bloukrans and Swartkops catchments

The climate of the Bloukrans catchment in Makhanda, Eastern Cape, South Africa is characterised by a warm temperate climate with a mean annual precipitation of about 600–800 mm. Winter temperatures can drop to 1.5 °C, while summer temperatures can reach up to 43°C (Dalu *et al.*, 2018; Mangadze *et al.*, 2019). The Swartkops catchment in Gqeberha (the city which the NMBM encompasses)., Eastern Cape South Africa experiences an annual rainfall of approximately 636 mm, with variable temperatures ranging from 10 to 22°C (Bate, Smailes and Adams, 2004; Odume *et al.*, 2012; Hamandawana, Atyosi and Bornman, 2020).

In general, South Africa's heaviest rainfall occurs in summer across the country (MacKellar, New and Jack, 2014). The Eastern Cape province of South Africa, including Makhanda and Gqeberha, has experienced severe drought conditions since 2015 (Mahlalela *et al.*, 2020). Despite experiencing droughts, these two catchments support communities along the rivers. The land use for the Bloukrans catchment and Swartkops catchments are shown in Figures 2.1 and 2.2, respectively, and are described briefly in the next section.

2.2.2 Land usage

The main land use activity within the Bloukrans River catchment is mainly limited to urban settlements, livestock production, irrigation farming and private game reserves (Dalu *et al.*, 2018; Mangadze *et al.*, 2019). The Bloukrans River is prone to pollution from agriculture run-off, wastewater and poorly disposed solid waste from the community (Dalu *et al.*, 2018). The Bloukrans catchment is less urbanised and industrialised than the Swartkops catchment.
The Swartkops catchment lies in the industrial hub of the Eastern Cape. Industrial activities include carbon black manufacturing (brick, carbon, motor vehicles), sand and clay mining, salt processing, tanneries, wool industries and fishing. Other land use activities include game reserves, crop farming and livestock production (cattle, sheep, poultry and ostriches) (Hamandawana, Atyosi and Bornman, 2020). The Swartkops River is influenced by pollution from wastewater, urban run-off, industrial effluent, agriculture run-off, solid waste and storm water (Adams, Pretorius and Snow, 2019; Taljaard *et al.*, 2023). These anthropogenic activities may introduce pollutants such as chemicals, plastics, antibiotics and bacteria into the rivers.

2.3 Water sampling

2.3.1 Sampling sites

The sampling site selection was based on an assessment of land use types surrounding the rivers, to include the various anthropogenic activities associated with each land use category. This method was employed to capture diverse sources of microbial pollution, acknowledging the unique influences caused by anthropogenic activities such as human settlements, wastewater treatment works (WWTW), crop farming, livestock farms, traditional/cultural rituals and recreation. The selected sampling sites are spatially distributed along the rivers from the upper and middle reaches of the rivers. Five sampling sites were selected for the Bloukrans River, whereas six sampling sites were selected for the Swartkops River, due to their accessibility. The characteristics of the sampling sites and their proximity to potential sources of contamination are presented in Tables 2.1 and 2.2 for the Bloukrans and Swartkops rivers, respectively. For each of the rivers, a control site, which is a site least impacted by human activities and therefore relatively pristine, was selected.

For the Bloukrans River, the first site is a control site (BC), and this site is least impacted by human activities except for traditional/cultural rituals that take place. The second site (B1) is influenced by storm water, and human settlements, and is a place where animals (livestock) graze and drink water, while the third site (B2) is influenced by wastewater effluent. The fourth site (B3) lies in an area where the predominant activity is agriculture while the last site (B4) is a point of human exposure (recreation and cultural activities) and is mainly influenced by upstream activities.

Table 2.1. Details of the sampling sites along Bloukrans River

Site	Description	Coordinates
BC	Control site: Less impact by anthropogenic pollution, except for traditional/cultural rituals that takes place.	33°22'08.4"S 26°28'30.0"E
B1	Considerable contamination is expected from runoffs from the informal settlement and sewerage from leaking sewer and potable water pipes of the urban area of Grahamstown.	33°18'51.4"S 26°33'06.0"E
B2	Substantial contamination expected with insufficiently treated effluents from a nearby Wastewater Treatment Plant (WWTP).	33°18'56.2"S 26°33'30.8"E
B3	Considerable contamination expected with wastewater and livestock excreta. It also lies in an area of predominantly agricultural activities.	33°18'55.5"S 26°33'36.5"E
B4	This site is expected to be least contaminated because it is situated approximately 36 kilometres downstream of the WWTP effluent discharge point along the Bloukrans River system.	33°19'24.0"S 26°36'00.4"E

For the Swartkops River, six sampling sites were selected using the same criteria employed for the Bloukrans River. The control site (SC), for the Swartkops River is relatively pristine, and has lower anthropogenic influence. The second site (S1) lies within an agriculture settlement area immediately downstream of the control site. The third site lies in an industrial area (S2) while the fourth site is immediately after a wastewater discharge point (S3). The fifth site (S4) lies downstream of a WWTW discharge point and is in an agricultural area, while the last site

(S5) is in an area influenced by storm water and industrial waste, human settlements and animals. The details of sampling sites are described in Table 2.2.

Table 2.2. Details of the sampling sites along Swartkops River

Site	Description	Coordinates
SC	Control site: Less impacted by anthropogenic pollution.	33°44'10.7"S 25°19'11.0"E
S1	Agriculture and point of human exposure through traditional/cultural rituals that take place.	33°45'06.9"S 25°20'34.6"E
S2	Influenced by storm water, industrial waste, human settlements and animals.	33°47’11.66”S, 25°26’00.46”E
S3	Substantial contamination with insufficiently treated effluents from a nearby wastewater treatment plant (WWTP).	33°47’11.66”S, 25°26’00.46”E
S4	Considerable contamination with domestic sewage and livestock excreta. It also lies in a predominantly agricultural area.	33°47'31.5"S 25°27'51.5"E
S5	Urban settlement and point of exposure.	33°48'40.2"S 25°31'49.1"E

2.3.2 Sample collection

Water samples were collected at the sampling points from June 2021 to April 2022, spanning all four seasons: winter, spring, summer, and autumn. The approaches used to define seasonal margins in South Africa vary. According to the South African Weather Service (SAWS), there is no existing official designation and definition of seasons. The most common meteorological classification is based on temperature seasonality and rainfall. However, it is notable that these approaches also have disparities, and are affected by distance from the Indian and Atlantic Oceans (Van der Walt and Fitchett, 2020).This study used the seasonal boundaries as reported in the study by Kruger and Nxumalo (2017). Therefore, the seasons were classified as follows:

summer (December/January/February), autumn (March/April/May), winter (June/July/August) and spring (September/October/November) (Kruger and Nxumalo, 2017). This study used the seasonal boundaries as reported in the study by Kruger and Nxumalo, (2017). Water samples were collected from the Bloukrans River on 17 and 18 June 2021 (winter), 25 and 26 October 2021 (spring), 7, 23, and 24 February 2022 (summer), and 5, 20, and 21 April 2022 (autumn). For the Swartkops River, sampling took place on 14 and 15 June 2021 (winter), 25 and 26 October 2021 (spring), 3, 4, and 28 February 2022 (summer), and 4, 22, and 23 April 2022 (autumn). Sampling across seasons was done to capture potential variations in water quality influenced by temperature, humidity, atmospheric pressure, and precipitation, identifying seasonal patterns and trends in *Campylobacter* occurrence. Selected meteorological conditions (temperature, humidity, atmospheric pressure, and precipitation) for each day of sampling were obtained from the South African Weather Services (SAWS).

In total 30 and 36 samples were collected from the Bloukrans, and Swartkops rivers, respectively. Two litres of water samples were collected at each sampling site. Water samples were collected using sterile sampling bottles. The water samples were then transported in a cooler box to the laboratory at the Institute for Water Research, Rhodes University, and kept at 4°C before processing.

Onsite analysis of selected physicochemical parameters (pH, turbidity (NTU), dissolved oxygen (mg/L), temperature (°C) and electrical conductivity parameter (µs/cm) was conducted. Turbidity of the water was measured in Nephelometric Turbidity Units (NTU) using a Turbidimeter (Eutech Instruments, USA), while dissolved oxygen (mg/L), temperature (°C), electrical conductivity (µs/cm), and pH measurements were taken using Hanna Instruments, South Africa. These measurements were conducted following the standard protocols and methods of the American Public Health Association (APHA). The physicochemical variables were measured because these variables play a critical role in the occurrence and survival of *Campylobacter* species in the aquatic environment. Before measuring the parameters, the instruments were calibrated according to the manufacturer's instructions. Measurements were obtained by submerging the probe in the river water and steadily holding it for a few minutes to achieve a reliable reading. The probes were then rinsed with de-ionized water to avoid cross-contamination among different sampling sites (Luvhimbi *et al.*, 2022).

2.3.3 Sample processing

Water samples were processed using the combined centrifugation and membrane filtration method described by Nnadozie and Ngoni (2023). This method was chosen because the river water samples might have contained bacterial cells as well as solid particles that may have bacterial cells trapped or attached to them. Therefore, through centrifugation, the free bacterial cells are pelleted together with the bacteria trapped in the solid particles. With this method, some bacteria may still remain in the liquid phase of the sample, but this can be collected as a residue on the filter membrane using filtration. Therefore, to capture cells that may have remained in the supernatant, the supernatant was filtered and cells were collected as a residue on a filter membrane. *Campylobacter spp.* occur in very low numbers in environmental waters, relative to background flora., and by pooling cells in the pellets and residue, the number of recoverable cells is increased.

Briefly, each 2 L water sample was processed by centrifuging at a maximum of 14000 g for 60 minutes (Avanti® J-E Centrifuge Beckman Coulter, Inc. USA). The supernatant was carefully poured into a sterile container while the pellets were scrapped off and placed into sterile Eppendorf tubes. The supernatant was then filtered through a sterile 47 mm/0.45 µm cellulose nitrate membrane filter (Whatman, plc, UK), using a membrane filter assembly. The filter was added to the pellet and mixed.

2.4 Propidium monoazide (PMA) treatment for quantitative PCR

The nucleic acid intercalating dye PMA, in contrast to ethidium monoazide (EMA), is now the preferred method for studying viable cell populations in risk assessment studies. This preference arises because PMA, when used at low concentrations, is not known to enter cells with intact membranes, according to a report by Nocker, Cheung and Camper (2006). Therefore, the filter and pellet mixes from each sampling site were suspended in PBS and then divided into two portions. One portion was subjected to PMA treatment by adding PMAxx (Biotium, Hayward, USA) at a final concentration of 15 µmol/L, as recommended by Banihashemi, Van Dyke and Huck (2012). It has been reported that optimal PMA concentrations which resulted in a maximum reduction in the dead cell signal without affecting the live cells) are below 20 µmol/L, and that PMA concentrations higher than 20 µmol/L are toxic to live cells (Banihashemi, Van Dyke and Huck, 2012). The micro-centrifuge tubes were then placed on ice and incubated in the dark for 5 min. The micro-centrifuge tubes were then exposed to a 650W halogen light source for 10 minutes. The tubes were placed at a distance of 20 cm from the light source and were inverted occasionally to ensure thorough mixing (Li *et al.*, 2014). As the control group, the other portion was not treated with PMAxx, but was also incubated in the dark and exposed to light. The PMA-treated and untreated portions were subjected to DNA extraction as described in the following section.

2.5 DNA extraction

DNA extraction was conducted using a commercial genomic DNA isolation kit (Qiagen Kit, DNeasy PowerSoil Pro Kit, Thermo Fisher Scientific, Germany). The DNeasy PowerSoil Pro Kit is effective at removing PCR inhibitors associated with environmental samples. DNA was extracted according to the manufacturer's instructions. Briefly, water samples were added to a bead beating tube and subjected to homogenisation and cell lysis. Homogenisation was achieved through vortexing, while cell lysis was achieved by mechanical and chemical methods. Total genomic DNA was captured on a silica membrane in a spin column format. DNA was then washed and 100 µL was eluted from the membrane, ready for downstream applications. The concentration and quality of the extracted DNA were determined using a Nanodrop 2000 UV-Vis Spectrophotometer (ThermoFisher Scientific, Lenexa KS, USA). Samples that had a 260/280 ratio between 1.7 and 2.1 were regarded as good-quality DNA and used for downstream applications.

2.6 Polymerase Chain Reaction (PCR)

2.6.1 Conventional Polymerase Chain reaction

Conventional PCR was exclusively conducted on the extracted DNA from PMA untreated samples. This was done to ensure the specificity and sensitivity of the PCR primers and assay conditions. Conventional PCR was also used to obtain qualitative information about the presence or absence of the *Campylobacter* 16S rRNA gene, as well as *Campylobacter* virulence genes involved in adherence (*cadF*), invasion (*ciaB*), the production of cytholethal distending toxin (*ctdB*), response to environmental stress (*htrB*), stress tolerance (*clpP*), multidrug efflux system (*cmeA*, *cmeB,* and *cmeC*), and antibiotic resistance genes (ARGs). Tetracycline-resistant genes (*tetO*) were also considered for detection. The *Campylobacter* multidrug efflux pump (*cmeABC*) genes are responsible for resistance to a wide range of structurally unrelated antibiotics, and therefore it was selected to cover a wider range of other antibiotics (Otigbu *et al.*, 2018).

The PCR was carried out using the primer sets and optimized protocols as described in Tables 2.3 and 2.4, respectively. All the primers were synthesised by Inqaba Biotech, South Africa. The DNA was amplified using a Bio-Rad T100 thermocycler, and the thermocycling conditions are indicated in Table 2.4. Each PCR reaction consisted of a total reaction volume of 50 µL containing 25 µL EmeraldAmp GT PCR master mix (Takara Bio Inc, China), 2 µL forward, 2 µL reverse primers, 2 µL template DNA, and 19 µL molecular grade water. To confirm positive amplification, electrophoresis was conducted on the amplicons using a 1% (w/v) agarose gel (CSL-AG100, Cleaver Scientific Ltd. Warwickshire, UK) pre-stained with ethidium bromide. The resulting products were visualized using a gel documentation unit (Molecular imager ChemiDocTM XRS+, BIO-RAD). The purified PCR amplicons were subsequently submitted to Inqaba Biotechnical Industries (Pty), South Africa, for further confirmation using sequencing and analysis.

Table 2.3. Primers for detecting the *Campylobacter* 16S rRNA gene, and selected ARGs and virulence genes

Gene Target	**Primer sequence (5'- 3')**	**Expected amplicon size (base pair)**	**Reference**
Campylobacter 16S rRNA (Primer name: C412F/ C1228R)	F-GGATGACACTTTTCGGAGC R- CATTGTAGCACGTGTGTC	816	Linton, Owen and Stanley, (1996)
cdtB	F- AATGCAAGCTGAAGAAGTGATTGT R- AGCATCATTTCCATTGCGAAT	85	Ghunaim *et al.*, (2015)
htrB	F- CGCACCCAATTTGACATAGAAC R- TTTTTAGAGCGCTTAGCATTTGTCT	70	Ghunaim *et al.*, (2015)
cadF	F-TTGAAGGTAATTTAGATATG R-CTAATACCTAAAGTTGAAAC	400	Konkel *et al.*, (1999)
ciaB	F-TGCGAGATTTTTCGAGAATG R-TGCCCGCCTTAGAACTTACA	527	Zheng *et al.*, (2006)
clpP	F-TGGGAGCATTTTTGCTTAGTTG R-CTCCACCTAAAGGTTGATGAATCAT	90	Ghunaim *et al.*, (2015)

Table 2.3 (continued). Primers for detecting the *Campylobacter* 16S rRNA gene, and selected ARGs and virulence genes

Gene Target	**Primer sequence (5'- 3')**	**Expected amplicon size (base pair)**	**Reference**
tetO	F-GGCGTTTTGTTTATGTGCG R-ATGGACAACCCGACAGAAGC	559	Gibreel *et al.*, (2004)
cmeA	F-TAGCGGCGTAATAGTAAATAAAC R- ATAAAGAAATCTGCGTAAATAGGA	435	De Vries *et al.*, (2015)
cmeB	F-AGGCGGTTTTGAAATGTATGTT R-TGTGCCGCTGGGAAAAG	444	
cmeC	F-CAAGTTGGCGCTGTAGGTGAA R-CCCCAATGAAAAATAGGCAGAGTA	431	
23S rRNA at position 2074	F-TTAGCTAATGTTGCCCGTACCG R-AGTAAAGGTCCACGGGGTCTCG	485	Alonso *et al.*, (2005)
23S rRNA at position 2075	F-TTAGCTAATGTTGCCCGTACCG R-TAGTAAAGGTCCACGGGGTCGC	485	Alonso *et al.*, (2005)

Table 2.4. Primers and the amplification conditions for the genes detected in the study

Primer/Target gene	Initial Denaturation		Cycles	Denaturation		Annealing		Elongation		Final elongation		Reference
	ºC	Time		ºC	Time	ºC	Time	ºC	Time	ºC	Time	
C412F/C1228R	94	1 min	35	94	1 min	58	1 min	72	4 min	72	5 min	Linton, Owen and Stanley, (1996)
cmeA, *cmeB*,	94	7 min	30	94	1 min	50	1.5 min	72	3 min	72	5 min	De Vries *et al*., (2015)
cmeC	94	7 min	30	94	1 min	52	1.5 min	72	3 min	72	5 min	De Vries *et al*., (2015)
tetO	95	1 min	35	95	15s	52	1 min	72	1 min	72	4 min	Gibreel *et al*., (2004)
23S rRNA (at position 2074/2075)	95	5 min	35	95	1 min	59	30s	72	30s	72	4 min	Alonso *et al*., (2005)
cdtB, htrB and *clpP*	94	5 min		94	30s	52	5 min	72	50s	72	4 min	Ghunaim *et al*., (2015)
cadF	95	10 min	40	95	10s	43	15s	72	20s	72	5 min	Konkel *et al*., (1999)
ciaB	95	3 min	45	94	30s	54	30s	72	1 min	72	5 min	Zheng *et al*., (2006)

2.6.2 Quantitative Polymerase Chain Reaction (qPCR) analysis of *Campylobacter* and Antibiotic Resistance Genes (ARGs) in river water samples

The qPCR assay was carried out with the primer sets and optimized protocols described in Tables 2.3 and 2.4, respectively. The reactions were performed on a QuantStudio 5 Real-Time PCR System (ThermoFisher Scientific, Lenexa KS, USA). The qPCR reaction mixture was made up of 4 µL HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis Biodyne, Tartu, Estonia), 0.4 µL forward primer, 0.4 µL reverse primer, 2 µL template DNA (10ng/µL) and 13 µL molecular grade water to a final volume of 20 µL. In each experimental configuration, suitable negative controls devoid of genomic DNA were subjected to the same amplification conditions. Melting curve analysis was used to confirm the specificity of each qPCR assay. The conditions for the melt curve cycle were 95°C for 15s, 60°C for 60 seconds, and 95°C for 1 second. The qPCR results were analysed using the QuantStudio Design and Analysis software version 1.5.2 (ThermoFisher Scientific, Lenexa KS, USA). Only wells with a slope of -3.1 to -3.6, $r^2 = 0.980$ to 1.00, amplification efficiencies of 90% to 110%, and melting curves with single peaks were considered (Bustin *et al.*, 2009). Cycle threshold (C_T) values and the concentration of DNA were obtained after analysis using the QuantStudio Design and Analysis software version 1.5.2 (ThermoFisher Scientific, Lenexa KS, USA). The number of copies of DNA were estimated using Equation 2.1 (Morpeth *et al.*, 2014).

$$\text{Copies of DNA template per µl} = \frac{DNA\ concentration\ (ng/\mu l) \times 6.023 \times 10^{23}}{DNA\ size\ (base\ pairs) \times 660 g/mole} \quad \text{(Equation 2.1)}$$

2.7 Assessment of human exposure to *Campylobacter* 16s rRNA and ARGs in water from Bloukrans and Swartkops rivers

The assessment of exposure to viable infectious concentrations of *Campylobacter* and its Antibiotic Resistance Genes (ARGs) in water is crucial as it directly influences the risk of human infection. Section 2.6.2 explained how the concentrations of viable *Campylobacter* (16S rRNA genes) and its ARGs (genomic copies/mL) in the PMA treated samples were quantified. The qPCR-derived concentration of viable *Campylobacter* and *Campylobacter* ARGs was used to estimate human exposure risk to *Campylobacter* and *Campylobacter* ARGs in the Bloukrans and Swartkops rivers. A detailed description of qPCR reaction and conditions used was presented in Section 2.6.2.

2.8 Data analysis

To analyse the qualitative data on the context-related drivers of antibiotic resistant bacteria and genes in the Bloukrans and Swartkops river catchments, the collected data was entered in a Microsoft Excel spreadsheet and analysed using the R software version 4.2.0. Descriptive statistics such as frequency and percentages for categorical variables were determined to generate and summarize the results in tables and figures. Furthermore, information on knowledge and practices regarding antibiotic resistance/antibiotic use was analysed using Chi-square to determine its statistical association with selected demographic characteristics (gender, age, education, and household income). The value of $p < 0.05$ was considered statistically significant.

Analysis of collected data was also conducted to investigate the spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers. The mean physicochemical variables were presented in the form of box plots using the R software version 4.2.0. To investigate the temporal dynamics, logistic regression was conducted to investigate the influence of meteorological conditions on the sampling days (independent variables) on the detection of *Campylobacter* (dependable variable). Additionally, logistic regression was conducted to determine the influence of physicochemical parameters, season of the year, and sampling site (independent variables) on *Campylobacter* detection (dependable variable). All statistical analyses were performed using R software version 4.2.0.

Additionally, statistical analysis was performed using R software version 4.2.0 using the post-ANOVA Tukey HSD test. The post-ANOVA Tukey HSD test was used to determine whether there was a significant difference between the concentration of the *Campylobacter* 16S rRNA, *cmeB* and *tetO* genes in PMA treated and non-PMA treated samples at 95% CI (Golpayegani *et al.*, 2019). Two sample t-tests were used to determine whether the sampling sites had an impact on the mean copy number of *Campylobacter* 16S rRNA, *cmeB* and *tetO* genes at 95% CI.

CHAPTER 3: ASSESSMENT OF THE CONTEXT-RELATED DRIVERS OF ANTIBIOTIC-RESISTANT BACTERIA (ARB) AND GENES (ARGs) IN THE BLOUKRANS AND SWARTKOPS RIVERS IN THE EASTERN CAPE, SOUTH AFRICA

3.1 Introduction

Antibiotic resistance is a serious emerging public health challenge (Larsson and Flach, 2022). Diseases which were previously easily treated with antibiotics are becoming resistant to antibiotics. Antibiotic-resistant bacteria (ARB) have developed mechanisms to persist or grow in the presence of antibiotics designed to inhibit or kill them (Kariuki *et al.*, 2022). In recent years, there has been research focus on antibiotic resistance genes (ARGs) due to their capacity to persist in the environment, even if the bacterial cells carrying these genes are eliminated (Koch *et al.*, 2021) This persistence is leading to higher morbidity, longer hospitalisation, increased mortality, loss of income, and economic losses and social implications (Truter, 2015; Iwu and Patrick, 2021).

Approximately 1.27 million deaths were attributed to antimicrobial resistance (AMR) globally in 2019 (Allel *et al.*, 2020). In Sub-Saharan Africa (SSA) it is estimated that in the year 2019, 1.1 million deaths were associated with AMR, with 23.5 deaths per 100,000 deaths attributable to AMR (Kariuki *et al.*, 2022; Moyo *et al.*, 2023). South Africa also has a high burden of deaths attributed to AMR. According to a previous study, there were 9,500 deaths attributable to AMR and 39,000 deaths linked to AMR in South Africa in 2019 (Collaborators, 2022). This public concern calls for global, regional and national efforts to mitigate this problem.

In 2016, the World Health Organization (WHO), the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the UN jointly released global strategies for the control of antimicrobial resistance (AMR) and established a formal tripartite collaboration. The One Health tripartite also recognised the importance of the environmental dimension of One Health and in 2021; the United Nations Environment Programme (UNEP) was requested to join the Tripartite. The four organizations formed a new quadripartite partnership. The Quadripartite recognises UNEP as an equal partner and acknowledges that the health of the environment is a cornerstone for human, animal and plant health. The inclusion of the UN Environment Programme (UNEP) ensures holistic, sustained and effective global action against AMR. One of the action plans of the UN agencies involves reinforcing measures to restrict the transmission of AMR among humans, animals, the environment and plants.

Aligning with the UN's consensus on priorities, the African Union (AU) took a significant step in 2018 by forming a Task Force on AMR. This task force is dedicated to enhancing AMR control efforts within the AU, delaying the emergence of AMR and restricting its transmission. The African Union advocates for the implementation of policies that support long-term prevention and control of AMR (Musa *et al.*, 2023). South Africa has made progress in combating AMR through the development and implementation of the National Action Plan (NAP) on AMR. The South African National AMR strategic framework has adopted a One Health approach (incorporating animal health and environment components in addition to human health). However, the environment has not been adequately addressed within this strategic framework.

Although the spread and transmission of ARGs and ARB occur mainly in healthcare facilities, environmental sources such as urban rivers play a significant role as vectors in the spread of ARB and ARGs (Maugeri *et al.*, 2023). The primary reason for this is the direct contact of both humans and animals with extensively polluted river water, coupled with the increased movement of organisms such as pathogenic and non-pathogenic bacteria, aquatic animals, as well as genetic elements, including antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) within the river. Therefore, it is increasingly critical to address AMR in urban rivers to control emerging infections and resistance in humans and animals. By identifying the causes and sources of antibiotic-resistant bacteria and genes in rivers, policymakers can develop evidence-based policies that are targeted and effective, to prevent and reduce ARGs and ARB pollution of rivers and contribute to the broader global effort to control AMR. This proactive approach is essential for safeguarding public health and preserving the effectiveness of antibiotics. Furthermore, it ensures that interventions are tailored to the specific challenges and conditions of each region, contributing to the overall global effort to address this critical public health issue.

There have been recent calls for comprehensive research initiatives to explore the drivers of antimicrobial resistance (AMR) in urban rivers worldwide. Literature indicates that often ARGs and ARB in rivers originate from discharges of partially treated and untreated wastewater from domestic and industrial sources. Other sources of ARGs and ARB in rivers include run-off from unplanned settlements, recreational and domestic activities (bathing, washing clothes and religious rituals (Cho, Jackson and Frye, 2023). Many of these studies have focussed on detecting the presence of ARB/ARGs and attributing them to specific sources based on sites of detection in the river or use of microbiological/molecular methods such as microbial source tracking and metagenomics (Table 3.1).

Table 3.1. Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Yang *et al.*, (2022)	The dynamic distribution of ARGs in a human-intensive watershed over different seasons was investigated through source tracker analysis and molecular detection of ARGs.	Wastewater, Human/Animal faecal matter	No
Thornber *et al.*, (2022)	The Driver- Pressure-State-Impact-Response (DPSIR) conceptual framework to an aquaculture case study site by analysis of distinct social, microbiological, and metagenomic data. Socioeconomic data were collected through interviews while antibiotic susceptibility testing, resistome profiling and metagenomics were used to detect ARB/ARGs from pond water and fish samples.	Increased farm productivity, poor water and sanitation, poor wastewater treatment, climate change, lack of knowledge on AMR, inappropriate use of antimicrobials, easy access to antibiotics	Yes
Banu *et al.*, (2021)	Detected ESBL *E. coli* in river water	Lack of sewer drainage systems in human settlements, animal excreta	No

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Kusi, Ojewole and Ojewole, (2022)	The pathways and drivers of antimicrobial resistance (AR) in surface waters were investigated by analysing antibiotic resistance healthcare-associated infection (HAI) data and literature research.	Healthcare facilities, wastewater, agricultural settings, wildlife, antibiotic residues, heavy metals, natural processes and climate change.	Yes
Yamashita, Katakawa and Tanaka, (2017)	This study profiled ARGs and mobile genetic elements (MGEs) in water and sediment samples from an urban river.	WWTP effluent and hospital Wastewater samples	No
Baniga and Hounmanou, (2020)	Characterised extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* in lake water samples. The sequence types of the isolates were detected using in silico Multilocus sequence typing (MLST).	Sewage, Agriculture runoff	No
Leila *et al.*, (2016)	Analysed the occurrence of ESBL and plasmid-mediated AmpC beta-lactamase producing Enterobacteriaceae (pAmpC-Eb) in surface water samples.	WWTP, hospital effluent	No
Nakayama *et al.*, (2017)	Evaluated the occurrence of antimicrobial residues, ARGs, and microbiota in the freshwater systems.	Aquaculture effluent	No

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Kimera *et al.*, (2020)	The drivers of antimicrobial use and resistance in livestock production and the environment in a selected river basin were investigated. Data on demographic characteristics, knowledge, practices, attitudes, and perceptions of the drivers of antimicrobial use and resistance in livestock production and the environment was collected using questionnaires, in-depth interviews, and focus group discussions.	Solid wastes, Agricultural activities, Poor disposal antibiotics, irrigation	Yes
Hassen *et al.*, (2020)	Investigated the occurrence and the genetic characterisation of cefotaxime-resistant (CTXR) Enterobacteriaceae river water samples. The genetic relatedness of isolates was studied by MLST.	Human, environment sources, animal excreta and WWTP	No
Ortega-Paredes *et al.*, (2020)	Quantifying and characterizing Cefotaxime-resistant and MDR *E. coli* in river water. REP-PCR analysis and MLST were used to show relatedness between the isolates.	Animal rearing, irrigation, recreational, domestic and industrial activities	No
Assawatheptawee *et al.*, (2017)	Detected cefotaxime-resistant *E coli* from water sources	Livestock, Aquaculture and Agriculture	No

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Vivas, Caicedo and Castillo, (2019)	Investigated the presence of antibiotic resistant Gram-negative bacteria in rivers.	Domestic, hospital and industrial effluent	No However, the study acknowledged that socio-economic pressure may contribute to the development and evolution of ARGs these genes along the selected river system.
Conte *et al.*, (2017)	Characterised ESBL and quinolone-resistant Enterobacteriaceae from river water	WWTP, hospital effluent	No However, the study acknowledged the multifactorial nature of antimicrobial resistance in rivers systems and stated that antimicrobial use in several human activities is a driver of AMR in rivers.
Bartley *et al.*, (2019)	Investigated the prevalence of faecal contamination, ARGs, and Enterobacteriaceae in a lake and river.	Wastewater treatment and poor sanitation in the community	No
Freitas *et al.*, (2019)	Evaluated the occurrence and diversity of third generation cephalosporin-resistant gram-negative bacteria in water samples from a lake.	Discharge of raw wastewater from unplanned human settlements nearby the lake	No

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Haque *et al.*, (2014)	Isolated ESBL-producing *E coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* from river water and identified the clones within the isolates using MLST.	Anthropogenic activities waste water effluent	No
Tissera and Lee, (2013)	Detected multiple antibiotic-resistant ESBL producing bacteria in surface waters.	Agriculture activities, industrial effluent, poor Hygiene and sanitation in human settings, domestic and hospital waste.	No. However, the study stated that socio-economic effects are associated with occurrence of multi-drug resistant pathogens in rivers.
Guzman-otazo *et al.*, (2019)	Determined the quantity and occurrence of antibiotic resistant diarrheagenic *E coli*, *Salmonella enterica*, *Klebsiella pneumoniae, Shigella spp.* and total enterobacteria in river water. The relatedness of the isolates was determined by MLST.	Industrial, hospital and sewage effluent. Run-off from human settlements.	No
Runcharoen *et al.*, (2017)	Investigated ESBL-producing *E. coli* isolated from water and human sources. Isolates were analysed using whole genome sequencing and MSLT. There *E. coli* from clinical and environmental samples showed some genetic relationship.	Hospital effluent patients, canals and livestock wastewater	No

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Zhong *et al.*, (2021)	Characterised and sequenced ESBL-producing bacteria from water samples in a reservoir.	Contamination due to human activities	No
Ahsan *et al.*, (2022)	Investigated ESBL *E. coli* contamination in river water.	Wet markets and wastewater effluent	No
Díaz-Gavidia *et al.*, (2021)	Detected colistin resistant and extended spectrum β-lactamases (ESBL) enterobacterales in river water samples.	Anthropogenic activities (agricultural)	Yes Using physical observations (results indicated as Yes or No), this study evaluated environmental factors on the day of sampling at each sampling point. Factors evaluated include presence of faecal matter, domestic animals and garbage. These factors were linked to the presence of antibiotic resistant enterobacterales in river water.

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Chaturvedi *et al.*, (2021)	Investigated MDR, ESBL, AmpC, efflux genes, and heavy metals resistance genes (HMRGs) in bacterial isolates from river water samples	Effluents from health care facilities, industrial effluent and domestic sewage.	No
Johnson *et al.*, (2020)	Determined the prevalence of ARB from open canals, drains, and rivers.	Poor sanitation facilities in human settlements, run-off from swine farms	No
Hu *et al.*, (2013)	Determined the prevalence of ARB in an urban aquatic environment.	Hospital effluent and effluent from pig farms	No
Zou *et al.*, (2019)	Isolated *Escherichia coli* from river water and analysing the genetic relatedness of isolates from different sources.	Anthropogenic activities, wild birds, pig faecal matter	No
Haberecht *et al.*, (2019)	Quantified antibiotic resistant *E. coli* in surface water	WWTP	No
Muraleedharan *et al.*, (2019)	Antibiotic resistant bacteria were enumerated from river water and found to be closely related to clinical isolates from the local hospitals	Agriculture and urban activities.	No
Cho *et al.*, (2019)	Detection and characterisation of antibiotic resistant *E. coli* from river water.	Agricultural run-off, effluent from industrial, and medical facilities	No

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Atta *et al*., (2022)	Isolated ESBL genes in *Escherichia coli* and *Klebsiella pneumoniae* from recreational water.	Run-off from human settlements and farmlands, human/animal excreta, hospital effluent	No
Chen *et al*., (2014)	Identified the ESBL-producing *E. coli* strains in river water samples and determined the sequence types using MLST	Runoffs from livestock and poultry farms	No
Siddiqui *et al*., (2019)	The prevalence of ESBLs producing bacteria and co-occurrence of ESBLs and silver resistance genes in river water was investigated.	Domestic sewage, hospital and industrial effluent and agricultural waste	No
Fuentes *et al*., (2019)	Detected antibiotic residues and ARB from river water samples.	Run off from dairy farms and horse breeding facilities, recreational activities, wastewater and migratory birds	No
Fernando *et al*., (2016)	Analysed raw and drinking water samples to detect the presence of coliform bacteria and antibiotic resistance genes.	Animal faecal matter	No

Table 3.1. (Continued) Compilation of current studies analysing driver of antibiotic resistance in rivers: extending Cho, Jackson and Frye (2023)

Reference	Description and Methods	Drivers attributed to increase in ARB and ARGs in rivers	Did the study involve assessment of context-related drivers of ARB/ARGs at river catchment level (Yes or No)
Nolan *et al.*, (2023)	The study investigated the impact of different land uses on the ARB concentration in riverine systems.	Agricultural and urban activities	No
Tafoukt, Touati and Leangapichart, (2017)	Detected carbapenemase-producing Enterobacteriaceae (CPE) in river water and analysed the clonal relatedness between isolates using MLST.	Agricultural, domestic, industrial, aquaculture, wastewater effluent	No
Jang *et al.*, (2013)	Investigated the association between antibiotic-resistant pathogens in river water and their public health risks.	Anthropogenic activities in urban settlements	No
Nzima *et al.*, (2020)	Investigated the patterns of antibiotic resistance and ESBL genes among *E. coli* isolates from surface water.	WWTP	No

Drawing on existing studies that have identified various drivers of ARGs occurrence and emergence in freshwater environments (anthropogenic activities, recreational activities, knowledge and practices), this study employed a questionnaire survey to identify the context-related sources and drivers of occurrence and emergence of antibiotic resistant bacteria and genes in local river systems (Bloukrans and Swartkops rivers). The drivers considered in this study are the demographic characteristics, knowledge and attitudes regarding antibiotic resistance and their link to river pollution, antibiotic disposal practices and anthropogenic and recreational activities within the catchments. Identifying the context-related drivers of ARB/ARGs pollution in rivers at catchment level will enable tailored intervention and ensure that management efforts are targeted and effective in mitigating actual risks faced by the local water resources.

3.2 Materials and Methods

3.2.1. Study Strategy and Sample Size

The sampling frame for this survey included residents from communities along the Bloukrans and Swartkops rivers. The sample size was determined using the Slovin's formula (Equation 2.1). This formula assumes a degree of variability (proportion) of 0.05 and a confidence level of 95% (Haziq and Panezai, 2017).

$$n = \frac{N}{1 + N(e)^2}$$

Where: n = sample size; N = population size; e = the level of precision (Equation 2.1). The level of precision used in this study was 0.05.

Sample size determination for Bloukrans River Catchment:

$$n = \frac{70{,}000}{1 + 70{,}000(0.05)^2}$$

$$n = 398$$

The survey was conducted in Makhanda East among households in 2022 and due to time constraints data were only collected from 357 respondents.

Sample size determination for Swartkops River Catchment:

$$n = \frac{1200000}{1 + 1200000(0.05)^2}$$

$$n = 400$$

The survey was conducted in communities living along the Swartkops River in 2021. Due to restrictions imposed by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic at the time, data was collected from only 181 respondents.

3.2.2. Ethical clearance

The research required institutional ethics clearance as it involved interviews with participants through a semi-structured questionnaire. Ethical clearance was obtained from the Rhodes University Ethics Committee with the approval number 2021-4864-6101.

3.2.3. Data Collection

Pre-tested questionnaires (Appendix A) were utilised, and interviews were conducted at the household level. The heads of the households were the main respondents. In the absence of the head of households, information was sought from any other adult (>18 years old). The questionnaire contained closed and open-ended questions covering aspects of demographic characteristics, social factors, health-seeking behaviours, knowledge and practices regarding antibiotic resistance and antibiotic use, and anthropogenic activities and its link to river pollution. The closed-ended questions were coded into categorical variables and the open-end questions and observed information was organised into subcategories before analysis. The information collected through the questionnaire survey was also validated by discussion in focus groups during a workshop (Appendix B) which involved 60 stakeholders, including officials from the municipalities, catchment management forum, environmental activists and community leaders. The stakeholders were selected based on their expertise. The suitable interventions that can be undertaken by individuals, stakeholders, and policy makers for reducing ARG and ARB pollution of rivers were suggested during the focussed discussion groups.

3.2.3. Data analyses

To analyse the qualitative data on the context-related drivers of antibiotic-resistant bacteria and genes in the Bloukrans and Swartkops river catchments, the collected data were entered in a Microsoft Excel spreadsheet and analysed using the R software version 4.2.0. Descriptive statistics such as frequency and percentages for categorical variables were determined to generate and summarize the results in tables and figures. Information on knowledge and practices regarding antibiotic resistance/antibiotic use was analysed using Chi-square to determine its statistical association with selected demographic characteristics (gender, age, education and household income). The value of $p < 0.05$ was considered statistically significant.

3.3 Results

This section presents the results that were obtained through the questionnaire survey and focussed discussion groups. The context factors were presented under five thematic areas, sociodemographic characteristics, anthropogenic and recreational activities within the catchments, and knowledge and practices regarding antibiotic resistance/ antibiotic use.

3.3.1 Sociodemographic characteristics of the Bloukrans and Swartkops river catchments

Demographic characteristics of the community are one of the key drivers of the spread of antibiotic resistance from the community to rivers. Demographic characteristics mirror people's living conditions, which may influence the spread and exposure to antibiotic resistance in rivers (Allel *et al.*, 2020; Larsson and Flach, 2022). In this study, 162 (45.4%) of the participants were males, while 195 (54.6%) were females for the Bloukrans River catchment. For the Swartkops catchment, 107 (59.1%) were males while 74 (40.9%) were females. The highest number of respondents fell within the age range between 45 and 54 years (33.6%) for the Bloukrans River catchment, while the highest number of respondents fell within the age range between 35 and 44 years (35.4%) for the Swartkops River catchment (Table 3.2).

In terms education levels, the highest recorded level of education, 60.8%, was Matriculation (High School graduation) among the respondents from the Bloukrans River catchment (Table 3.2). For the Swartkops River catchment, the highest recorded level of education, 41.4%, was Diploma level (tertiary level). This study shows that a high percentage of respondents from the Bloukrans River catchment had not attained education beyond high school. Additionally, the monthly household income for many of the respondents (65.1%) in the Bloukrans River catchment was less than R2000. In contrast, the monthly household income for the respondents in the Swartkops River catchment mainly fell within the range of R2000 to R5000 (32.7%) and R5000 to R10000 (33.7%) (Table 3.2).

Among the respondents from the Swartkops River catchment, 70.7% of them resided in formal houses with municipal services, while 16% resided in informal houses with municipal services and 13.3% resided in informal houses without municipal services. For the Bloukrans River catchment, 41.2% of the respondents resided in formal settlements with municipal services, 45.1% lived in informal houses with municipal services and 13.7% lived in informal houses with no municipal services (Table 3.2). Municipal services provided include water supply, sewage collection and disposal, refuse removal, electricity supply, municipal health services, road maintenance and storm/drainage water management.

Table 3.2. Socio-demographic characteristics of participants in the Bloukrans River and Swartkops River catchments

Characteristic		**Bloukrans River**		**Swartkops River**	
		Frequency	**Percent**	**Frequency**	**Percent**
Gender	Male	162	45.4	107	59.1
	Female	195	54.6	74	40.9
	Total	**357**	**100**	**181**	**100**
Age	Less than 25	14	3.9	9	5.0
	25-34	60	16.8	50	27.6
	35-44	87	24.4	64	35.4
	45-above	196	54.9	58	32.0
	Total	**357**	**100**	**181**	**100**
Level of Education	Primary school	86	24.1	5	2.8
	High school	217	60.8	50	27.6
	Diploma	36	10.0	75	41.4
	Bachelor/Master/PhD	12	3.4	32	17.7
	Other	6	1.7	19	10.5
	Total	**357**	**100**	**181**	**100**
Level of Income	Less than R2000	231	65.1	37	20.4
	R2000-R5000	70	19.7	58	32.0
	R5000-R10000	39	11.0	61	33.7
	R10000-R20000	9	2.5	18	9.9
	Greater than R20000	6	1.7	7	3.9
	Total	**357**	**100**	**181**	**100**

Type of housing	Formal with municipal services	147	41.2	128	70.7
	Informal with municipal services	161	45.1	29	16.0
	Informal without municipal services	49	13.7	24	13.3
	Total	**357**	**100**	**181**	**100**

3.3.2 Anthropogenic activities associated with the emergence and occurrence of antibiotic resistant bacteria and genes in the selected catchments

Both the Swartkops and Bloukrans rivers hold cultural significance and significant importance in the lives of the respondents. Examining their varied interactions with these rivers reveals the anthropogenic drivers responsible for its pollution. Firstly, the significance of these rivers to the community was highlighted. This was followed by uncovering the anthropogenic activities that may lead to the occurrence and emergence of ARB and ARGs in the Bloukrans and Swartkops river catchments.

In these communities, rivers are important for recreation, fishing, irrigation and as a source of water for animals. Additionally, rivers are used for cultural purposes such as communicating with ancestors, spiritual cleansing, healing and other rituals. They are also used for religious purposes such as baptisms.

For the Bloukrans catchment, the major use of the river water is irrigation (Figure 3.1). Farms located along the river cultivate crops such as cabbage, lettuce, spinach, tomatoes and onions. The second most common use of water from the Bloukrans River is for livestock watering. Despite the low volume and poor state of the river, it serves as a primary source of drinking water for livestock from the community. Use of the Bloukrans River for spiritual/cultural/recreation activities was ranked third. Spiritual/cultural activities are conducted mainly at the control site (BC) and the Bloukrans pool (site B4). Recreation activities are only conducted at the Bloukrans pool (site B4) where there is a high depth of clear water. The perceived relative importance of the rivers was ranked from 1 (lowest) to 6 (highest) as shown in Figure 3.1.

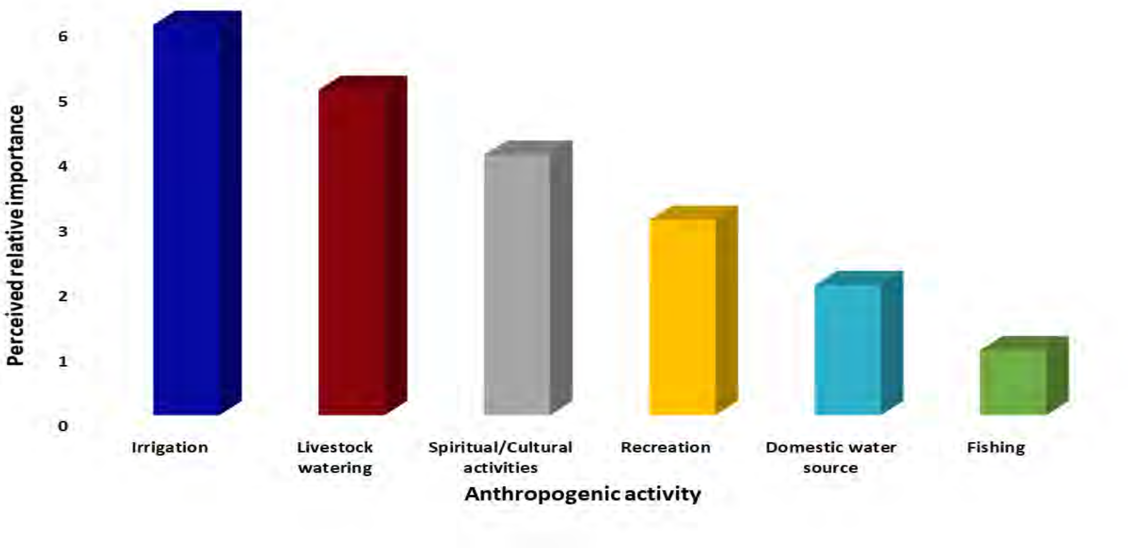

Figure 3.1. Perceived relative importance of the river to the community in the Bloukrans River catchment

For the Swartkops River catchment, the major use of river water is for irrigation of crops and this was ranked as the most important use of water by the respondents. This was followed by the use of the river for spiritual activities such as baptism, cleaning or communicating with the ancestors. Several sites along the Swartkops River are camps used by the local traditional healers (Sangomas) for rituals which involve contact with water. The third highest ranked use of river water was for watering livestock, while some respondents (2.7%) who lived in informal settlements near the river stated that they sometimes used the river as a source of water for domestic purposes. Water supply in informal settlements is erratic, and so residents use the river as an alternative source of water. As opposed to the Bloukrans catchment where no fishing activities took place (recorded as the least important in Figure 3.1), the respondents from the Swartkops catchment stated that some community members were involved in subsistence fishing along the Swartkops River and the estuary. The respondents (5%) also stated that the Swartkops River was used for recreation activities (swimming, boating) at some sites, especially towards the estuary (Figure 3.2).

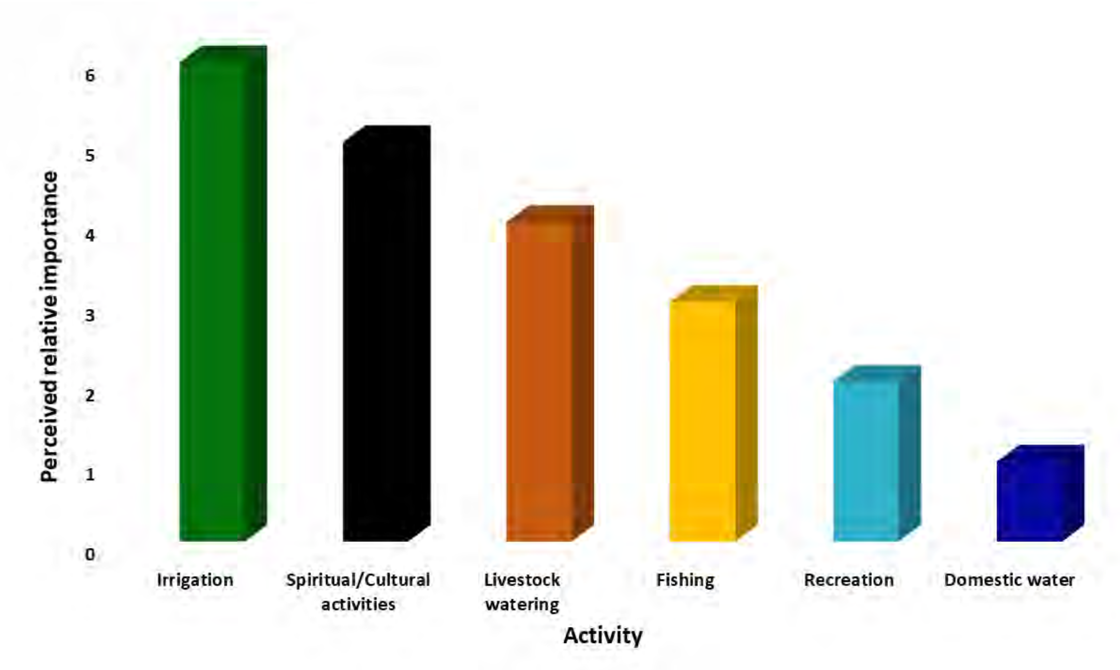

Figure 3.2. Perceived relative importance of the river to the community in the Swartkops River catchment

Having described the significance of these rivers to the community, the following section presents the anthropogenic activities that may lead to the occurrence and emergence of ARB and ARGs in the Bloukrans and Swartkops river catchments. The respondents from both catchments in this study attributed key potential anthropogenic drivers of the occurrence and spread of ARB and ARGs in the selected rivers.

Poor solid waste management was identified as one of the key drivers of microbial pollution of the river. Indiscriminate disposal of solid waste at illegal dumpsites, and using the rivers and streams as dumping sites for solid waste is common practice in both catchments (Figure 3.3).

Figure 3.3. Solid waste disposal at Vukani bridge on the Bloukrans River, Makhanda (right) and along the banks of the Swartkops river in Uitenhage (left), South Africa.

Additionally, wastewater effluent from wastewater treatment works (WWTW) was identified as a leading cause of microbial pollution for both catchments. The respondents stated that the WWTW in both communities discharge poorly treated effluent into the rivers. The Belmont WWTW discharges effluent into the Bloukrans River, while the Kelvin Jones WWTW in Uitenhage, KwaNobuhle WWTW, and Despatch WWTW discharge wastewater into the Swartkops River. The respondents also stated that vandalism and theft of electricity cables at WWTWs is a common practice and this results in the discharge of raw or poorly treated wastewater into the rivers.

Another identified key driver is livestock production. In these two catchments, livestock production occurs both on a commercial and small scale. Commercial livestock production is practiced by farmers usually located along the river banks. Small scale livestock production is often practised by a majority of the Black community. Due to the historical apartheid land allocation, traditional livestock keepers have limited access to land, and so most of the local people in the townships keep livestock within their backyards. This is more prominent in the Bloukrans River catchment where cattle, goats and donkeys are kept in backyards with limited space, and the faecal matter is usually disposed-off within the premises or at illegal dumpsites in the community. The livestock also freely move around the town and graze at solid waste dumpsites and along the river banks (Figure 3.4). It is also worth noting that the unrestricted movement of livestock in Makhanda has led to the occurrence of faecal matter in the streets and other places in the community. Urban run-off carries animal faecal matter and solid waste into the streams that flow into the Bloukrans River. In both catchments, river water is used for livestock watering, and the presence of grazing livestock along the river banks leads to the introduction of animal excreta in the rivers.

Figure 3.4. Livestock grazing and drinking water at a site along the Bloukrans River in Belmont Valley, Makhanda, South Africa.

The other identified factors that can contribute to the contamination of the rivers with antibiotic residues, ARB, ARGs and other pollutants are urban run-off and storm water. Run-off from the community in the Bloukrans catchment often carries solid waste, animal excreta and other pollutants and discharges them into the Bloukrans River. This is worsened by indiscriminate dumping of solid waste into the drainage system and the tributaries of the Bloukrans River. In the Swartkops River catchment, storm water contributes to the microbial pollution of the river. The Kat, Markman and Motherwell storm water canals are heavily polluted with garbage and sewage and they discharge this polluted water into the Swartkops River. This may lead to the introduction of antibiotic-resistant bacteria, antibiotic resistant genes and antibiotic residues in the rivers.

Industrial effluent also contributes to the occurrence and emergence of ARB/ARGs in the catchments. The Bloukrans River catchment lies in a less industrialised area than the Swartkops River catchment, and therefore is less influenced by industrial effluent. However, both catchments receive effluent from institutions, health facilities and trading areas in addition to domestic effluent. In the Swartkops River catchment, effluent from different industries is channelled to WWTWs for treatment, and insufficient treatment of the wastewater may result in the discharge of effluent with a high content of biological and chemical pollutants in the river.

Lastly, agricultural run-off was also identified as a key driver of ARB/ARGs by the respondents of both the Bloukrans and Swartkops river catchments. Both catchments have been affected by low water levels due to climate change-induced drought. Despite this, these rivers support crop production. Farms are located along the river banks, and run-off from the farms may introduce ARB, ARGs, pesticides, biocides and other pollutants.

These findings indicate that anthropogenic activities in river catchments may be key drivers of the spread and emergence of ARB and ARGs. The anthropogenic drivers of microbial pollution and antibiotic resistance identified in these catchments are summarised in Table 3.3.

Table 3.3. Anthropogenic drivers of the occurrence of ARB and ARGs in the Bloukrans and Swartkops river catchments

Drivers	Effect on the occurrence of ARB and ARGs in the rivers	Mitigation
Poor solid waste management	Indiscriminate disposal of solid waste at illegal dumpsites, rivers, streams, drainage systems and storm water canals introduces ARB/ARGs in rivers.	Improving solid waste management Public awareness on indiscriminate disposal of waste
Discharge of poorly treated wastewater	Poorly treated wastewater introduces ARB/ARGs in the rivers.	Rehabilitation of wastewater treatment infrastructure, Application of new technologies for removing ARB/ARGs in wastewater
Poor animal husbandry	Living in close proximity with livestock and unrestricted movement of livestock in informal settlements increases the transmission of ARB/ARGs from animals to the environment and humans.	Proper management of animal excreta Improved animal husbandry practices
Urban run-off and storm water	Urban run-off and storm water carries solid waste, animal excreta and other pollutants from human settlement and discharges them into the river.	Public awareness on proper solid waste, improved management of storm water and solid waste in the community
Industrial effluent	May introduce bacteria, antibiotic residues, heavy metals, biocides, pesticides and some persistent organic compounds. These pollutants co-select for ARGs, promoting antibiotic resistance.	Monitoring effluent quality, pre-treatment of industrial effluent and use of nature-based solutions such as constructed wetlands
Agricultural run-off	The run-off from the farms along the river banks may potentially carry ARGs/ARB, pesticide residual, biocides and other pollutants.	Improving regulations on antibiotic, pesticide and biocide use in agriculture
Recreational activities	May introduce bacteria, ARB/ARGs in river through littering and human excreta. Open defecation near the river during recreation is a common practice.	Raising awareness on the public health effects of contamination of water sources

The severity of the impact of these key factors was ranked from the lowest (1) to the highest (6) as shown in Figure 3.5.

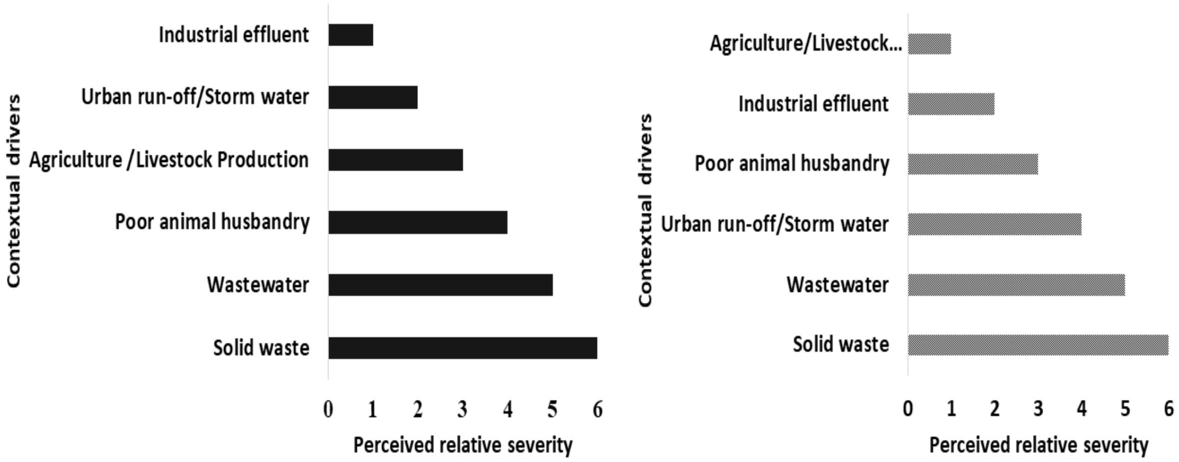

Figure 3.5. Perceived relative severity of the contextual drivers of the emergence and transmission of antibiotic resistance in the catchments (left Bloukrans River, right Swartkops River)

3.3.3 Potential contribution of recreational activities to the occurrence of ARB/ARGs in the river

The study assessed how recreation activities contribute to the occurrence of ARB/ARGs in rivers. The respondents expressed concerns about the poor state of the rivers in both catchments. They stated that rivers are polluted with litter at most sites and the water is often characterised by a blackish appearance and unpleasant odours and is not aesthetically pleasing. However, there are parts of the river where the river is clean and the water appears clear, and such sites are utilised for recreation and spiritual/cultural purposes. The recreation activities often involve contact with river water (Table 3.4). Although the respondents were aware of the risk of contracting waterborne diseases through recreation activities, the majority of the respondents did not believe they could play a role in preventing water pollution (66.4% Bloukrans River, 64.1% Swartkops River). The respondents stated that it is the responsibility of the Department of Water Affairs to ensure that water resources are protected from pollution, and only a small proportion of the community were involved in community activities that advocated for pollution prevention and proper management of water resources.

Table 3.4. Contribution of recreational activities to water pollution in the catchment

Variables	Bloukrans River catchment		Swartkops River catchment	
	Yes (%)	No (%)	Yes (%)	No (%)
Engages in recreational activities in or around the river	35	65	38.8	61.1
Comes into contact with river water during recreation	48.8	51.2	57.1	42.9
Observed some actions (e.g., littering, open defecation) by humans during recreation which could contaminate the river	77.6	22.4	72.9	27.1
Concerned about the quality of the river water	84.0	16.0	85.7	14.3
Think recreation activities contribute to contamination of river water	44.8	55.2	41.4	58.6
Is aware of the potential risk of contracting waterborne diseases during recreation activities	89.6	10.4	93.9	6.4
Is aware of the consequences of water pollutions on human health	47.6	52.4	54.1	45.9
Think they can play a role in preventing water pollution	33.6	66.4	35.9	64.1

3.3.4 Knowledge and practices regarding antibiotic resistance and antibiotic use at household level and antibiotics disposal

The majority (89.5%) of the respondents from the Bloukrans were not aware of the concept of antibiotic resistance, its causes and its effects, as shown in Table 3.5. A significant relationship between the respondent's education level and knowledge of antibiotic resistance ($\chi^2 = 8.17$, $p = 0.04$), sharing of antibiotics ($\chi^2 = 9.65$, $p = 0.0047$), consumption of leftover antibiotics ($\chi^2 = 11.25$, $p = 0.024$) and purchasing antibiotics without a prescription ($\chi^2 = 16.54$, $p = 0.002$) was noted. Gender had a significant relationship with adherence to antibiotic course ($\chi^2 = 5.75$, $p = 0.016$). Similarly, household income had a significant relationship with self-medication of antibiotics.

Table 3.5. Practices regarding antibiotic resistance and antibiotics among the respondents from the Bloukrans River catchment

Characteristics	**Self-medicates**		**Adherence to antibiotic course**		**Sharing of antibiotics**		**Consumption of antibiotic leftovers**		**Buys antibiotics without prescription**	
	χ2	**p -value**	**χ2**	**p- value**	**χ2**	**p-value**	**χ2**	**p-value**	**χ2**	**p-value**
Gender	0.17	0.679	5.75	0.016*	0.61	0.436	0.61	0.433	0.87	0.352
Age	3.16	0.367	2.23	0.527	1.78	0.619	2.6	0.458	7.7	0.053
Education	6.58	0.16	6.27	0.18	9.65	0.047*	11.25	0.024*	16.54	0.002*
Monthly household income	9.91	0.042*	6.98	0.137	5.23	0.264	4.85	0.303	8.89	0.064

The majority (70.4 %) of the respondents from the Swartkops catchment were not aware of antibiotic resistance, its causes and its effects. Household income had a significant effect on self-medication with antibiotics for both the Bloukrans and Swartkops rivers. There was no statistically significant relationship between gender and adherence to antibiotic treatment course (χ^2 = 0.21, p =0.646) (Table 3.6). Similarly, there was no statistically significant relationship between gender and purchasing antibiotics without a prescription (χ^2 = 0.42, p = 0.519), gender and sharing antibiotics with family and friends (χ^2 = 1.93, p = 0.164), gender and self-medication with antibiotics (χ^2 =1.31, p = 0.253), and, gender and consumption of leftover antibiotics (χ^2 = 0.03, p = 0.862). As shown in Table 3.6, neither the relationships between age and adherence to antibiotic treatment course (χ^2 = 0.2, p = 0.978), nor purchasing antibiotics without a prescription (χ^2 = 4.23, p = 0.238), sharing antibiotics (χ^2 = 1.42, p = 0.7), nor self-medication with antibiotics (χ^2 =1.04, p = 0.791) nor consumption of left-over antibiotics (χ^2 = 2.63, p = 0.452) were statistically significant. Similarly, level of education had no statistically significant relationship with adherence to antibiotic treatment course (χ^2 = 6.02, p = 0.111), purchasing antibiotics without prescription (χ^2 = 2.16, p = 0.54), sharing antibiotics (χ^2 = 3.7, p = 0.295), self-medication with antibiotics (χ^2 = 3.95, p = 0.267) and consumption of leftover antibiotics (χ^2 = 2.41, p = 0.492). However, household income had a statistically significant relationship on self-medication with antibiotics at household level (χ^2 = 9.56, p = 0.049). In contrast, household income had no statistically significant relationship with regard to adherence to antibiotic treatment course (χ^2 = 2.99, p = 0.56), purchasing antibiotics without a prescription (χ^2 = 3.68, p = 0.451), sharing antibiotics (χ^2 = 6.44, p = 0.169) and consumption of leftover antibiotics (χ^2 = 0.66).

Table 3.6. Practices regarding antibiotic resistance and antibiotics among the respondents from the Swartkops River catchment

Characteristics	**Self-medicates**		**Adherence to antibiotic course**		**Sharing of antibiotics**		**Consumption of antibiotic leftovers**		**Buys antibiotics without prescription**	
	χ2	**p -value**	**χ2**	**p- value**	**χ2**	**p-value**	**χ2**	**p-value**	**χ2**	**p-value**
Gender	1.31	0.253	0.21	0.646	1.93	0.164	0.03	0.862	0.42	0.519
Age	1.04	0.791	0.2	0.978	1.42	0.70	2.63	0.452	4.23	0.238
Education	3.95	0.267	6.02	0.111	3.7	0.295	2.41	0.492	2.16	0.54
Monthly household income	9.56	0.049*	2.99	0.56	6.44	0.169	0.66	0.956	3.68	0.451

In this study, poor handling and disposal of antibiotics was recorded in both catchments (Figure 3.6). The survey showed that 63.8% of the respondents from the Bloukrans catchment threw unconsumed or expired antibiotics in the garbage bin while 13.07% poured or flushed the unused or expired antibiotics in the kitchen sink or the toilet. Some of the respondents (20%) also admitted that they stock up the unused or unwanted antibiotics in the house while 3.07% stated that they returned the unused or expired medicines to a health facility or pharmaceutical outlet. Poor handling and disposal of antibiotics was also observed among the respondents from the Swartkops catchment. The majority (59.3%) of the respondents admitted that they keep unused and expired antibiotics at home while 14.8% dispose them in the bin as trash, 22.2% pour or flush the antibiotics in the kitchen sink or the toilet and 3.7% stated that they return the antibiotics to the health facility or a pharmaceutical outlet.

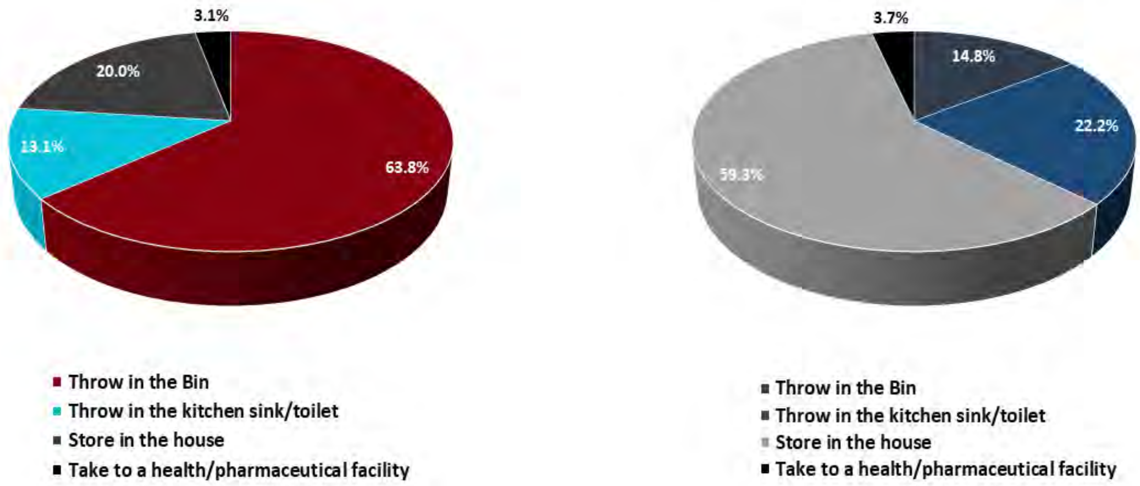

Figure 3.6. Handling and disposal of antibiotics among the respondents from the Bloukrans River catchment (left) and Swartkops River catchment (right)

3.4 Discussion

The study assessed the context-related sources and drivers of the occurrence and emergence of antibiotic resistant bacteria and genes in the Bloukrans and Swartkops rivers. Through a comprehensive analysis, insights into potential mitigation strategies were provided. One of the key findings from this investigation is that the sociodemographic characteristics of the study respondents in the catchments is a key driver of occurrence and emergence of antibiotic-resistant bacteria and genes in the Bloukrans and Swartkops rivers. This study shows that a high percentage of respondents from the Bloukrans River catchment did not attain education beyond high school. In contrast, a higher percentage of respondents from the Swartkops catchment had attained education beyond high school. It has been demonstrated that a lack of formal education may affect awareness and attitudes about infections, hand hygiene practices and poor disposal of waste among the public. These practices and behaviour may contribute to the occurrence and emergence of antibiotic resistance. Additionally, studies show that the level of education influences antibiotic use, antibiotic disposal and compliance with antibiotic treatment courses (Singh *et al.*, 2018; Gautron *et al.*, 2023). There was a high percentage of households with low household income, many living in informal settlements. Although these households had access to municipal services, they had poor access to service delivery in terms of social amenities such as water and sanitation. This places households from such communities at a higher risk of introducing ARB and ARGs into the environment through sewer leakages, poor solid waste disposal and poor sanitary conditions (Nomamiukor *et al.*, 2015).

In this study, noteworthy findings regarding anthropogenic drivers of the occurrence of ARB and ARGs in the Bloukrans and Swartkops rivers were revealed. The anthropogenic drivers identified include improper solid waste management, discharge of poorly treated wastewater, poor animal husbandry practices, urban run-off and storm water, industrial effluent and agricultural run-off. Anthropogenic activities such as indiscriminate dumping of solid waste in the rivers, along river banks, in the drainage system and storm water canals in the community may introduce unused/expired antibiotics, microbial and chemical pollutants (Ding *et al.*, 2022). In addition, illegal dumpsites in the Bloukrans and Swartkops River catchments are also grazing places for livestock. Livestock may pick up ARB and ARGs from the dumpsites and potentially transport them to rivers through excretion of faecal matter as they graze along the river banks. Disposal of inadequately treated wastewater can contribute to high concentrations of ARB and ARGs in rivers. Wastewater carries ARGs, antibiotic residuals and resistant and sensitive pathogenic organisms such as *Campylobacter spp, E. coli, Klebsiella spp., Shigella spp., Salmonella spp.* and *Vibrio spp.* (O'Flaherty and Cummins, 2017; Adams, Pretorius and Snow, 2019). Urban run-off from the catchments also contributes to the occurrence of ARB/ARG in the rivers. Urban run-off may transport ARGs and ARB from the community to the rivers. Antibiotic-resistant bacteria has been detected in urban run-off in China and in the Amazon watershed (Poma, Mamani and Iñiguez, 2016; Zhang *et al.*, 2016). Similarly, in South Africa, bacteria from sites impacted by urban run-off in rivers showed a higher prevalence of resistance to gentamicin and sulfamethoxazole (Tucker *et al.*, 2022). Therefore, heavily polluted storm water canals such as the Motherwell, Kat and Markman stormwater canals in the Swartkops River catchment may well introduce ARB/ARGs into the Swartkops River (Adams, Pretorius and Snow, 2019).

Run-off from livestock production farms along the Bloukrans and Swartkops rivers may also introduce ARB and ARGs in the river. Studies in South Africa indicate that antibiotic use in livestock production is high, which increases the possibility that run-off from livestock production farms along the rivers may introduce ARB and ARGs (Karama *et al.*, 2020; Pillay *et al.*, 2020; Sithole *et al.*, 2021). Additionally, poor animal husbandry practices such as keeping livestock in the backyards in informal settlements within the catchments, and allowing livestock to graze freely increases the potential for river pollution. This practice has been linked to high occurrences of ARGs and antibiotic zoonotic pathogens such as *Campylobacter*, *Salmonella*, *E. coli* and *Shigella* (Chala *et al.*, 2021). Animal excreta from livestock may therefore lead to a high concentration of ARB and ARGs being released into the Bloukrans and Swartkops rivers by livestock which graze by the river banks.

Discharge of industrial effluent is another factor that can contribute to the high prevalence of ARB/ARGs in rivers. Pollutants in industrial effluent can create stressful conditions for the bacteria community in rivers and therefore promote selective pressures to shift the resistome. The resistome refers to the collection of resistance traits in the microbiological community (Rodgers *et al.*, 2019). Under stressful conditions, the bacteria's survival mechanisms can develop resistance to antibiotics. This is mainly accomplished through co-selective pressures. Heavy metals, biocides, pesticides and some persistent organic compounds co-select for antibiotic resistance genes (Rodgers *et al.*, 2019; Hubeny *et al.*, 2021). When these pollutants are present in river water, selective pressure in the environment can increase the bacteria's resistance to antibiotics. Selective pressure enhances the exchange of ARGs through horizontal gene transfer (HGT) between pathogenic and non-pathogenic bacteria, including bacteria that may not be closely related (Hubeny *et al.*, 2021). Pollution from industrial effluents can be minimised through monitoring effluent quality and strict enforcement of laws on effluent quality. Proper management of industrial effluent through pre-treatment and use of nature-based solutions such as constructed wetlands may be effective (Bai *et al.*, 2022).

Additionally, the run-off from the farms along the river banks may potentially carry ARGs/ARB, pesticide residual, biocides and other pollutants. The ARB and ARGs in agriculture run off may originate from livestock on farms as well as soil treated with manure or sludge (Irfan and Almotiri, 2022). Additionally animal excreta and sludge may contain heavy metals and antibiotic residues such as nor-floxacin, ofloxacin, ciprofloxacin, trimethoprim, sulfamethoxazole and doxycycline. These pollutants may end up in rivers and this may lead to contamination of river water. Pesticides and biocides used for farm applications may also end up in rivers through agriculture run-off. The presence of all these pollutants in rivers may create sustained selection pressure that facilitate the emergence and prevalence of ARGs (Irfan and Almotiri, 2022; Reddy *et al.*, 2022).

All these factors regarding the identified anthropogenic drivers of ARB and ARGs occurrence and emergence in both rivers underscores the pressing need for robust measures to mitigate improper solid waste management, discharge of poorly treated wastewater, poor animal husbandry practices, urban run-off and storm water, industrial effluent and agricultural run-off in both catchments. These identified anthropogenic drivers of ARB and ARGs occurrence and emergence in the Swartkops and Bloukrans rivers align with those observed in rivers in certain previous studies (Table 3.1). This suggests that there are shared and consistent drivers and potential mitigation strategies across different contexts. However, importantly, the findings from this study reveal divergent anthropogenic drivers from some existing studies. The absence of certain anthropogenic drivers, including runoffs from dairy farms, horse breeding (Fuentes *et al.*, 2019) and the discharge of effluents from aquaculture (Tafoukt, Touati and Leangapichart, 2017) in Bloukrans and Swartkops rivers must be noted. This observation supports the argument that context-specific assessments are necessary for tailored interventions. The argument for localised strategies to address ARB and ARGs occurrence and emergence in river systems therefore stands.

As expected, a lack of knowledge regarding AMR and inappropriate disposal of antibiotics by the respondents were revealed as major drivers of potential ARB and ARGs occurrence and emergence in both rivers. The results also show that awareness and knowledge of antibiotic resistance is low in the community. The majority of the respondents (89.5%) were not aware of antibiotic resistance, its causes and linkage to river pollution, as well as its effects. Furthermore, this study shows that antibiotics are poorly handled and disposed into the environment at household level. Unconsumed antibiotics were reported to be mainly stocked up at home (20% and 59.3% for the Bloukrans and Swartkops rivers, respectively) or disposed of as solid waste (60.8% and 14.8% for Bloukrans and Swartkops rivers, respectively). Unconsumed antibiotics were also poured or flushed in the kitchen sink or the toilet (13.07% and 22.2% for the Bloukrans and Swartkops rivers, respectively). Poor disposal of antibiotics may lead to the emergence of antibiotic resistance in the environment through wastewater discharge and solid waste disposal (Ahmad, Malak and Abulreesh, 2021; Koch *et al.*, 2021). It has been demonstrated that a lack of knowledge about infections, hand hygiene practices, and poor disposal of waste among the general public contribute to the emergence and occurrence of ARB/ARGs in rivers (Singh *et al.*, 2018; Gautron *et al.*, 2023). It is evident that human behaviour and practices such as those described in this chapter play an important role in the occurrence and emergence of ARB and ARGs at catchment level. There is a need to incorporate good health seeking behaviours in antimicrobial awareness campaigns (Gautron *et al.*, 2023). The proactive inclusion of behavioural science in research, policy, and practice regarding antibiotic resistance is necessary (Chadborn *et al.*, 2023). At global level, behavioural insights and change is included in one of the five strategic areas of the quadripartite OH Priority Research Agenda for AMR (Chadborn *et al.*, 2023). However, incorporating behaviour change is still a challenge at national level, as antibiotic resistance is often regarded as a medical problem and is rarely addressed as a social problem (Minssen *et al.*, 2020).

3.5 Conclusion

The drivers of ARGs and ARB in the Bloukrans and Swartkops rivers were identified in this study. Socio-demographic characteristics of the catchment, anthropogenic activities such as, improper solid waste management, discharge of poorly treated wastewater, poor animal husbandry practices, urban run-off and storm water, industrial effluent, and agricultural run-off, and a lack of knowledge on AMR and inappropriate disposal of antibiotics are significant drivers of this concern. We consider these identified drivers as pivotal markers, and key factors to monitor and assess when evaluating and managing the risk of occurrence and emergence of antibiotic-resistant bacteria and genes in river systems in contexts similar to the Bloukrans and Swartkops rivers.

Notably, the absence of certain anthropogenic activities, such as effluent from dairy farms, horse breeding and aquaculture in this study, reinforces the importance of context-specific assessments. In addition, it is evident that tailored interventions are imperative for effective mitigation of antibiotic resistance in rivers. This study indicates that the occurrence and emergence of ARGs and ARB in rivers is inherent to anthropogenic-related sources, behavioural factors and water related activities in the catchments. Mitigating the occurrence and emergence of ARB and ARGs in rivers can be achieved through environmental education on the public health risks of indiscriminate solid waste disposal in the community, and improvements of solid waste management and wastewater treatment at community level. Increasing awareness of good practices with regard to antibiotic use and handling, and antibiotic resistance and its impact on health can also mitigate the emergence of antibiotic resistance in rivers. Adoption of good animal husbandry, and increased regulation of antibiotic use in livestock production may also play an important role in reducing contamination of rivers with ARB and ARGs. It may be difficult to combat antibiotic resistance without adopting effective interdisciplinary actions involving social science, environmental science, public health, agriculture, regulatory authorities and policy makers. There is need to address antibiotic resistance in river water using an integrated approach which will involve incorporation of preventive measures at household, community and local government levels.

CHAPTER 4: SPATIOTEMPORAL VARIATIONS IN THE OCCURRENCE OF *CAMPYLOBACTER* SPECIES IN THE BLOUKRANS AND SWARTKOPS RIVERS, EASTERN CAPE, SOUTH AFRICA

This chapter has been published in a peer-reviewed journal.
Chibwe, M., Odume, O. N. and Nnadozie, C. F. (2024) ‘Spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers, Eastern Cape, South Africa’, *Heliyon*, 10(7), p. e28774. https://doi.org/10.1016/j.heliyon.2024.e28774

4.1 Introduction

Campylobacter spp. are among the leading etiological agents for gastroenteritis in humans worldwide (Vereen *et al*., 2013). Although most *Campylobacter* infections are attributed to the consumption of contaminated food, infections can also occur through ingestion of contaminated water. Faecal contamination of surface water from different anthropogenic sources is a major contributor of *Campylobacter* in rivers. *Campylobacter* from water sources has been linked to waterborne outbreaks in many parts of the world (Kaakoush *et al*., 2015; Gilpin *et al*., 2020; Hyllestad *et al*., 2020; Mortensen *et al*., 2021). The occurrence of *Campylobacter spp.* in the environment therefore presents a public health risk for humans who may be exposed to this water.

Previous studies suggest that the occurrence of *Campylobacter* in water sources is influenced by seasons and human activities in the catchment. Studies have reported seasonal variation in levels of *Campylobacter* contamination in rivers in many parts of the world (Eyles *et al*., 2003; Sari Kovats *et al*., 2005; Fravalo *et al*., 2011; Mughini-Gras *et al*., 2016; Djennad *et al*., 2019). Seasonal variations can affect the presence, and recovery of *Campylobacter* from water, as well as the period of maximum risk of exposure to infection by water users. In addition to seasonal variations, the occurrence of *Campylobacters* species in rivers is also influenced by land use in the catchment (Eyles *et al*., 2003; Vereen *et al*., 2013). *Campylobacter* in river water mainly originates from anthropogenic activities, including faecal matter from animals, agricultural runoff and wastewater treatment plant effluent (Pitkänen, 2013; Mulder *et al*., 2021).

Bloukrans and Swartkops rivers lie near the east coast of South Africa in the Eastern Cape Province. Both rivers are important for recreational and agricultural purposes for local residents. However, these rivers are at risk of *Campylobacter* pollution. These rivers are affected by faecal pollution from livestock practices, dilapidated wastewater treatment plants, and agricultural practices. Furthermore, high population, unplanned settlements, poor management of water resources and infrastructure failure are leading to deteriorating water quality in these freshwater bodies. In addition to microbial pollutants from the community, antibiotic residues from healthcare facilities and wastewater also end up in the rivers (Adams, Pretorius and Snow, 2019; Mahlalela *et al.*, 2020; Vumazonke, Khamanga and Ngqwala, 2020).

The antibiotic residues that end up in rivers act as a selection pressure for the antibiotic-resistant microbial pathogens in the environment. Resistance of pathogens to antibiotics which are used for treatment is a major public health concern because the infections caused by these microorganisms become difficult to treat. This phenomenon leads to an increased duration of hospitalisation, high morbidity and mortality (Páll *et al.*, 2013). It is crucial to investigate the distribution of *Campylobacter* species across different locations and periods in these rivers to identify potential sources of contamination, estimate the risk of waterborne infections, and formulate targeted strategies to reduce the impact on human health. The paucity of comprehensive understanding of these variations currently hinders the implementation of effectual preventive measures and prompt responses to emerging public health threats associated with *Campylobacter* infections in the Bloukrans and Swartkops river systems. This study investigates the spatiotemporal patterns of *Campylobacter* species occurrence in the Bloukrans and Swartkops rivers, examining influences from seasonality and proximity to potential contamination sources.

. This involved analysing patterns of temporal-spatial occurrence, and considering factors such as meteorological conditions, physicochemical parameters, season of the year and sampling site. The study aimed to understand how these environmental variables influence the presence of *Campylobacter* species in the river systems. The innovation of this study lies in its pioneering nature, being the first to employ non-culture-based methods to investigate spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers of the Eastern Cape, South Africa. Furthermore, the study explored the complex interactions between meteorological conditions and physicochemical parameters and dynamics of waterborne pathogens in this specific geographical region for the first time.

4.2 Materials and methods

The study areas, sample collection, sample processing, DNA extraction, detection of *Campylobacter,* ARGs and virulence genes by conventional PCR are described in Chapter 2.

4.2.1 Statistical Analysis

To investigate the temporal dynamics, logistic regression was conducted to investigate the relationship between the meteorological conditions of the sampling days (independent variables) and the detection of *Campylobacter* (dependable variable). Additionally, logistic regression was conducted to determine the relationship between physicochemical parameters, the season of the year and the sampling site (independent variables) and *Campylobacter* detection (dependable variable). All statistical analyses were performed using R software version 4.2.0. A negative coefficient (b) indicated that an increase in the independent variable is associated with a decrease in the detection of *Campylobacter*. A positive "b" meant that an increase in the independent variable is associated with an increase in the detection of *Campylobacter*. The independent variable Odds Ratio (OR) represented the factor by which the odds changed for the dependent variable given a unit increase in the independent variable (values >1 indicated an increase in odds of *Campylobacter* occurrence while values < 1 indicated a decrease in the odds of *Campylobacter* detection). P-values ≤ 0.05 were considered significant.

4.3 Results

4.3.1 Physico-chemical characteristics of the river water

Results for the physico-chemical analysis of the river water showed that the highest river water temperature was 24.98ºC in summer while the lowest was 13.1ºC for the Bloukrans River (Figure 4.1). The mean water temperature across different sites along the river was 18ºC to 20.1ºC, while dissolved oxygen (DO) concentration ranged from 2.17 to 9.05 mg/l (Figure 4.1). Dissolved oxygen was highest (>8–9.6 mg/l) for the control site (BC) throughout all the seasons and lowest at site B1 (≤ 5.0 mg/l). The sites that recorded low dissolved oxygen concentration (B1, B2, and B3), also recorded high electrical conductivity (> 1000 µs/cm), and high turbidity. The mean pH ranged from 7.3 to 7.5, as shown in Figure 4.1.

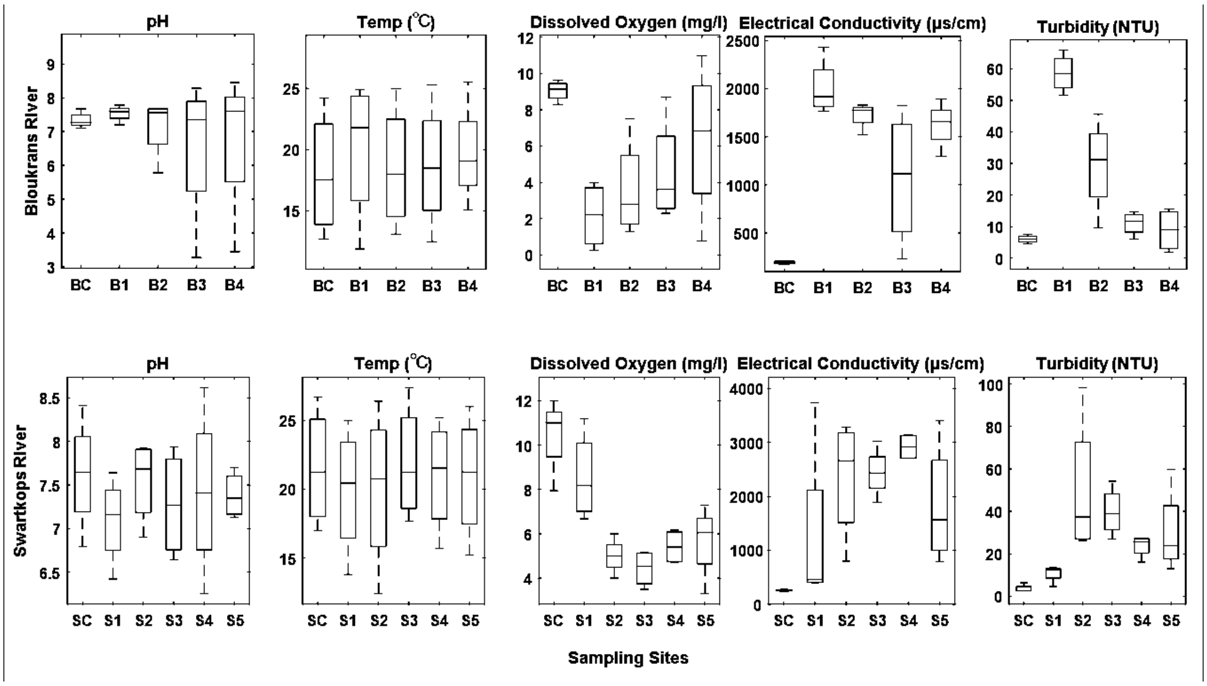

Figure 4.1. Spatial variations for physiochemical characteristics (pH, Temperature, Dissolved Oxygen, Electrical conductivity and Turbidity) of water samples collected from the Bloukrans and Swartkops rivers, Eastern Cape, South Africa

For the Swartkops River, the mean pH ranged from 7.1 to 7.62 (Figure 4.1). The lowest temperature was 15.3°C in winter while the highest was in summer (26.12°C). The mean temperatures across the different sites ranged from 19.9 to 21.6°C. Similar to the results obtained from the Bloukrans River, the control sites recorded high dissolved oxygen (> 8 mg/l) while human-impacted sites recorded lower dissolved oxygen (< 4 mg/l). The electrical conductivity and turbidity were high (> 1000 µs/cm and 40 NTU respectively) for the sites impacted by anthropogenic activities, similar to the figures recorded in the Bloukrans River. High conductivity for freshwater bodies is usually in the range of 0 to 1500 µs/cm. In this study conductivity above 1000 µs/cm was considered as high (Pitkänen, 2013). Figure 4.1 shows the mean physicochemical parameters across the sampling sites for the Swartkops River.

4.3.2 Detection of *Campylobacter* species, antibiotic resistance genes and virulence genes

Campylobacter was detected in 20 (60 %), and 21 (58%), of Bloukrans and Swartkops screened samples, respectively. Sequence analysis of the PCR products obtained was done by BLAST. All of the products corresponded to *C. jejuni*, and this was confirmed based on percentage identity (96%). Additionally, hippuricase-positive *Campylobacter* were detected in 46.7%

(14/30) of the Bloukrans River and 44.4% (16/36) of the Swartkops River samples. PCR products (816bp) obtained using the 16S rRNA primers specific for *Campylobacter* genus are shown in Figure 4.2.

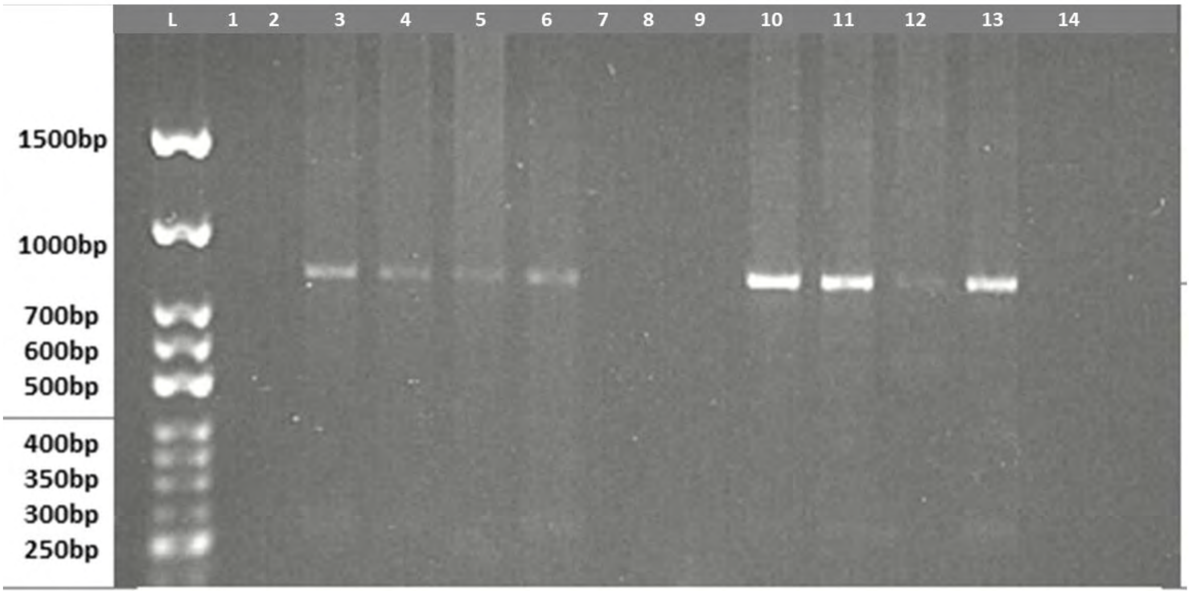

Figure 4.2. PCR products (816bp) using the 16S rRNA primers specific for *Campylobacter* genus. Lanes: L: Accuris SmartCheck 50bp DNA Ladder; 1: negative control; 2, 3, 4, 5, 6: bands from Bloukrans River water samples; 7: negative control; 8.9,10, 11, 12, 13 bands from Swartkops River water samples

Of the *Campylobacter*-positive water samples from the Bloukrans River, 4 (20%) were *cmeA* gene positive, 13 (65%) were *cmeB* gene positive and 2 (10%) were *cmeC* gene positive. In water samples from the Swartkops River, the genes *cmeA*, *cmeB* and *cmeC* were detected at 3 (14.3%), 11 (52%) and 2 (9.5%) respectively. Tetracycline resistance genes were detected in 14 (70%) and 16 (76%) of the water samples collected from Bloukrans and Swartkops rivers, respectively. The PCR products obtained using *tetO* primers specific for *Campylobacter* tetracycline resistant genes are shown in Figure 4.3. For both rivers, neither A2074G nor A2075G point mutations on 23S rRNA were detected. A2074G and A2075G point mutations of *Campylobacter* on the 23S rRNA gene are associated with erythromycin resistance.

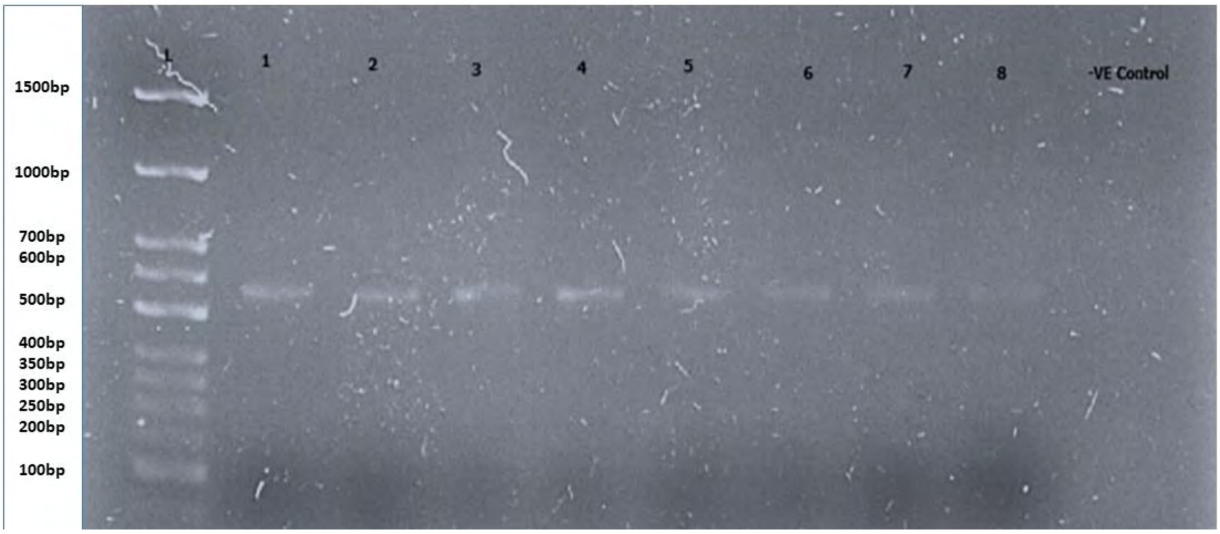

Figure 4.3. PCR products (559 bp) using *tetO* primers specific for *Campylobacter* tetracycline resistant genes Lanes: L: Accuris SmartCheck 50bp DNA Ladder; lanes 1,2,3,4 bands from Bloukrans River water samples; 5.6,7,8 bands from Swartkops River water samples –VE control: negative control

Virulence genes were also detected in the water samples, but the detection of virulence genes was low for both rivers. For the Bloukrans River, *cadF* was detected at 3 (15%) while *htrB* and *clpP* were detected at 2 (10%). The virulence genes *ciaB* and *ctdB* were detected at 1 (5%). Similarly, the percentage of virulence genes detected in water samples from the Swartkops River was also low. The gene *cadF* was detected at 1 (4.8%), while *htrB* and *ciaB* were detected at 0% and 9.5% respectively. In contrast, *clpP* and *ctdB* were detected at 3 (14.2%) and 1 (4.8%) respectively.

4.3.3 Influence of river water physico-chemical characteristics on *Campylobacter* detection

Logistic regression analysis shows that generally, physicochemical parameters did not have a significant relationship with *Campylobacter* occurrence ($\chi2 = 25.9$, $p = 0.001$, $n = 20$) ($\chi2 = 13.41$, $p = 0.02$, $n = 24$) for both the Bloukrans and Swartkops rivers, respectively. For the Bloukrans River, an increase in pH had a negative effect on the detection of *Campylobacter* ($b = -54.5$, OR = 0) but the relationship is not significant ($p = 0.999$). The temperature also had a negative effect on the detection of *Campylobacter* ($b = -15.87$, OR = 0) but the relationship is not statistically significant ($p = 0.998$). Similarly, dissolved oxygen had a negative effect on the detection of *Campylobacter* although the relationship is also not statistically significant ($p = 0.998$, OR = 0, $b = -22.78$). In contrast, electrical conductivity had a positive effect on the detection of *Campylobacter* but the relationship is not statistically significant ($p = 0.998$, OR

= 1.15, b = 0.14). The relationship between turbidity and the detection of *Campylobacter* was not statistically significant (p = 0.998, OR = 0.02, b = -3.94). For the Swartkops River, an increase in pH had a negative influence on the detection of *Campylobacter* (b = -0.09, OR = 0.92) but the relationship is not significant (p = 0.935). The results showed that temperature also had a positive influence on the detection of *Campylobacter* (b = 0.07, OR = 1.08) but with no statistically significant relationship (p = 0.699). Similarly, dissolved oxygen had a negative influence on the detection of *Campylobacter* with no significantly significant relationship (p = 0.183, OR = 0.37, b = -1). In contrast, electrical conductivity and turbidity both had a positive influence on the occurrence of *Campylobacter* (b = 0 for both) but this relationship was also not significant (p = 0.829, OR = 1 for electrical conductivity, and p = 0.973; OR = 1 for turbidity).

4.3.4 Spatial occurrence of *Campylobacter* species in river water

Overall, the sampling site had a significant effect on the detection of *Campylobacter* for both the Bloukrans ($\chi 2$ = 12.31, p =0.015) and Swartkops ($\chi 2$ = 19.89, p =0.001) rivers. Variation in *Campylobacter* detection rate was observed across the different sites for both the Bloukrans and Swartkops rivers. *Campylobacter* was detected at Sites B1, B2, B3, and B4, and Sites S2, S3, S4, and S5, of the Bloukrans and Swartkops rivers, respectively. In contrast, there was no detection of *Campylobacter* at the control sites for either the Bloukrans or Swartkops river sites BC and SC, respectively. *Campylobacter* was also not detected at the site (S1) immediately downstream of the control site for the Swartkops River.

4.3.5 Temporal occurrence of *Campylobacter* in the rivers

Meteorological conditions on the sampling days were recorded for both the Bloukrans and Swartkops rivers. The mean values for meteorological conditions on the sampling days for each season are presented in Table 4.1. Statistical analysis conducted to investigate the influence of meteorological conditions (air temperature, humidity, pressure, and precipitation) on the occurrence of *Campylobacter*. Results obtained show that these conditions had no significant effect on the occurrence of *Campylobacter* for either the Bloukrans ($\chi 2$ = 2.62, p = 0.624, n = 20) or Swartkops rivers ($\chi 2$ = 3.63, p = 0.458, n = 24).

In terms of temporal occurrence, *Campylobacter* was detected in water samples from both rivers across all the seasons of the year. Overall, the lowest percentage (%) of detection was recorded in spring while the highest was recorded in autumn (%). For the Bloukrans River samples, the highest prevalence (80%) was recorded in autumn, compared to summer (60 %), spring (60 %) and winter (60 %) samples (Figure 4.3). For the Swartkops River, *Campylobacter* was detected in 67% of the winter and autumn samples, and in 50% of samples in spring and summer (Figure 4.4). However, based on the outcome of the logistic regression analysis, the sampling season had no significant effect on the detection of *Campylobacter* for either the Bloukrans River ($\chi2 = 0.66$, $p = 0.883$) or the Swartkops River ($\chi2 = 0.69$, $p = 0.877$).

Table 4.1. Mean meteorological conditions (standard deviation) for the sampling days in each season

Season	Rainfall (mm)		Humidity (%)		Air temperature (°C)		Atmospheric pressure (kPa)	
	BR	SR	BR	SR	BR	SR	BR	SR
Winter	0 (0)	101.35 (0.35)	41.8 (9.31)	62.67 (9.81)	20.6 (3.29)	21 (1.55)	141.54 (31.55)	0.07 (0.1)
Spring	0 (0)	100.73 (0.19)	48.8 (23)	64 (2.19)	19.8 (4.38)	21 (1.1)	165.28 (77.89)	0.2 (0.31)
Summer	0.12 (0.27)	100.9 (0)	46.4 (13.15)	57 (6.2)	27.2 (1.1)	26.67 (1.03)	157.12 (44.48)	0.13 (0.21)
Autumn	0 (0)	101.07 (0.19)	36.6 (8.76)	60.5 (9.31)	26.8 (1.1)	23 (2.19)	123.92 (29.69)	0.55 (0.05)

BR= Bloukrans River, SR= Swartkops River

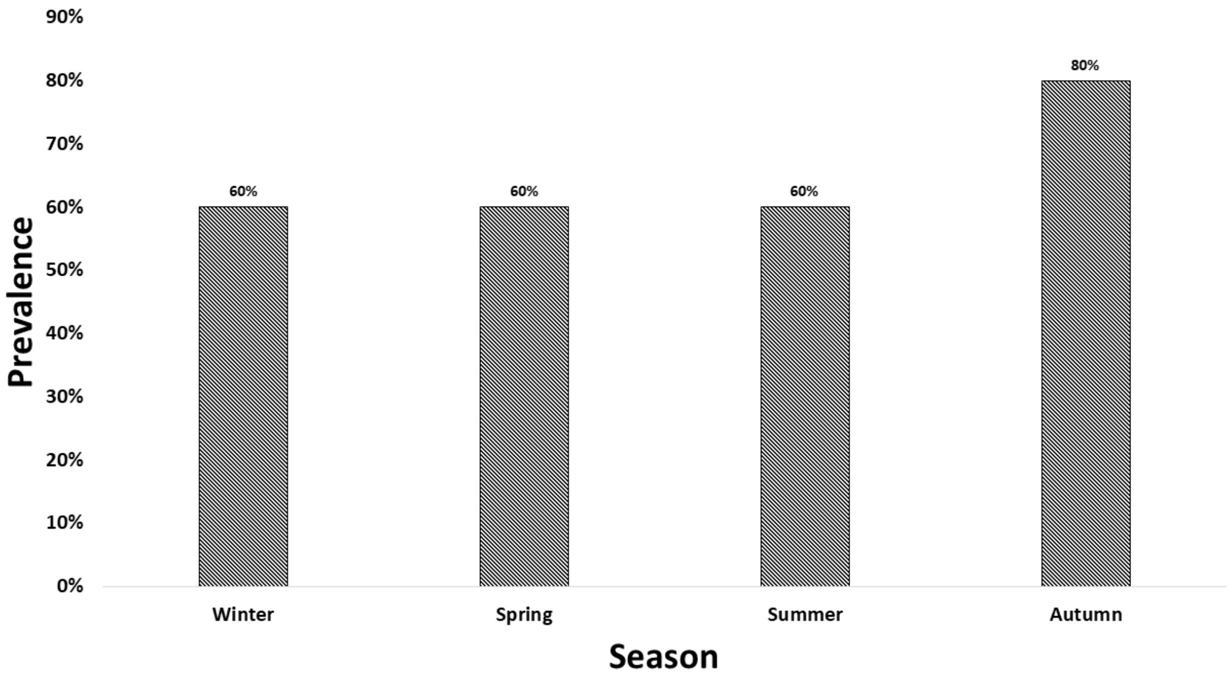

Figure 4.4. Seasonal occurrence of *Campylobacter* species for the Bloukrans River, Eastern Cape South Africa

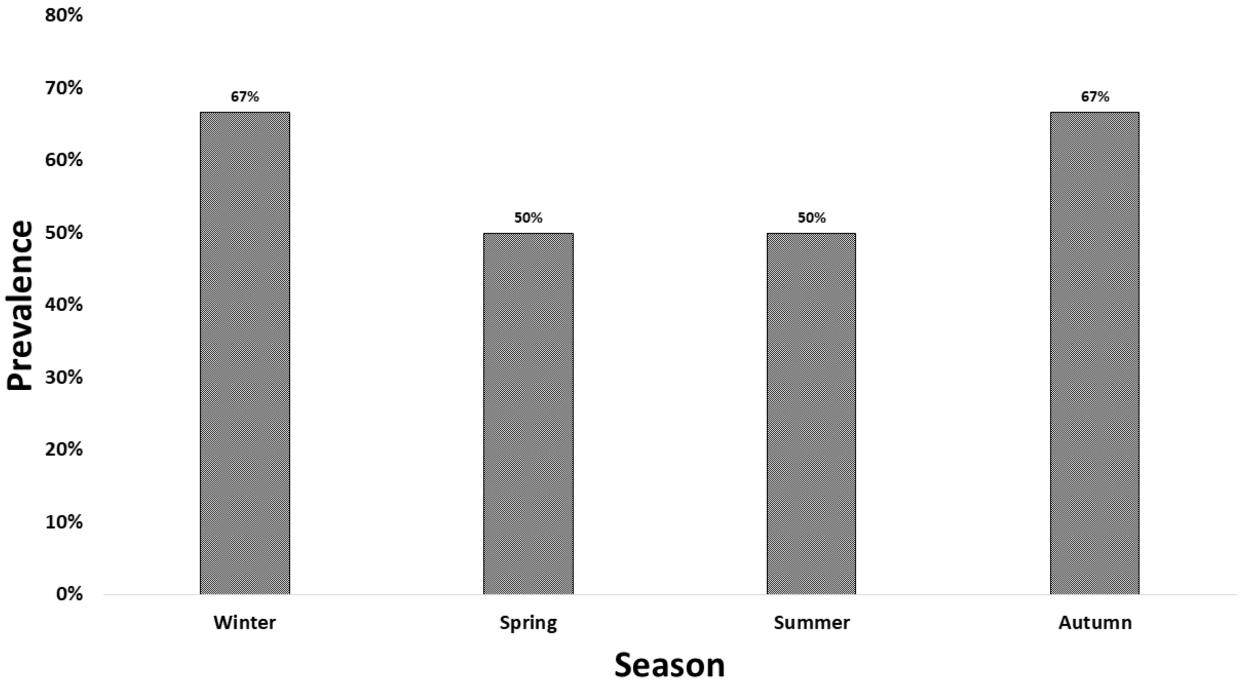

Figure 4.5. Seasonal occurrence of *Campylobacter* species for the Swartkops River, Eastern Cape South Africa

4.4 Discussion

This study investigated the spatial and temporal distribution of *Campylobacter spp.* and its antibiotic-resistant genes in the Bloukrans and the Swartkops Rivers, Eastern Cape, South Africa. The influence of meteorological conditions, physicochemical parameters, season of the year, and sampling site on the detection of *Campylobacter* (dependable variable) were assessed. This study ascertained whether these rivers are potential sources of *Campylobacter* and the periods of greatest *Campylobacter* exposure from the rivers to humans. The physicochemical quality of the river water is first considered, as these factors influence the occurrence of *Campylobacter* in the environment (Eyles *et al.*, 2003).

The results obtained suggest that the physiochemical properties (pH, dissolved oxygen level, turbidity, and electrical conductivity) of the water in the Bloukrans and Swartkops rivers are conducive to *Campylobacter* survival. It is notable that at sites where high turbidity, low dissolved oxygen, and high conductivity were recorded, the prevalence of *Campylobacter* was also high. The findings from this study correlate with those from previous studies. High turbidity, high electrical conductivity, and low dissolved oxygen favour the survival of *Campylobacter* in river water (Hilton *et al.*, 2001; Van Dyke *et al.*, 2010; Teh, Lee and Dykes, 2017). Turbidity may result from the high concentration of dissolved particles, and provide nutrients, which thereby favours the survival of bacteria present in the river, including *Campylobacter* (Cai *et al.*, 2021). In previous studies, a strong positive correlation has been found between turbidity and *Campylobacter* presence in environmental waters (Azhdarpoor *et al.*, 2019; Adewale *et al.*, 2020). Low dissolved oxygen at sites with a higher prevalence of *Campylobacter* was also expected because their survival is better at low dissolved oxygen concentrations (Smith *et al.*, 2016). However, the physicochemical characteristics of the river water did not show a significant influence on the occurrence of *Campylobacter* in either the Bloukrans or the Swartkops rivers.

It is noteworthy that the water temperatures (> 16°C), measured for the Bloukrans and Swartkops rivers in this study, may not be suitable for the growth of bacteria. It has been suggested that warm climates cause higher temperatures and longer UV exposure periods, which can lead to lower potential survival and consequent isolation rates (Diergaardt *et al.*, 2004). However, these bacteria have acquired the ability to thrive in harsh environmental conditions. Moreover, *Campylobacter* species exhibit different survival patterns, ascribed to their high genetic diversity and therefore interspecies differences in genes for stress response

(Elmonir *et al.*, 2022). For example, *Campylobacter jejuni* can survive longer in a viable but non-culturable (VBNC) form in freshwater, and in this form *Campylobacter jejuni* retains infectivity (Pitkänen, 2013). Furthermore, *Campylobacter jejuni* is also more tolerant to stress conditions than *Campylobacter coli* (Pitkänen, 2013; Karikari *et al.*, 2016). Therefore, this may render it more likely to be detected in rivers.

This study reports a prevalence of 60% *Campylobacter spp*. for the Bloukrans River and 58% for the Swartkops River, respectively. The prevalence of *Campylobacter spp*. in river systems can vary by region and country, as reported in previous studies. For example, *Campylobacter* occurrence has been reported to be 25% in Australia (Ahmed *et al.*, 2009), 26% in Canada (Jokinen *et al.*, 2011), 35.7% in Ghana (Karikari *et al.*, 2016), 41.5% in India (Baserisalehi, Al-Mahdi and Kapadnis, 2005) 46.6% to 53.3% in France (Denis, Tanguy, Chidaine, M.J. Laisney, *et al.*, 2011), 53.3% in Norway (Rosef, Rettedal and Lågeide, 2001) and 91% in the Yarra River estuary, Australia (Siddiqui *et al.*, 2019). *Campylobacter* occurrence (66.67 %) reported in the Bloukrans River in this study is similar to the 68.7% reported in Nigeria (Ugboma *et al.*, 2013) and 33 – 63% in Canada (Khan *et al.*, 2014). The differences observed in the prevalence from different studies could also be a result of different methods used for detection, local anthropogenic activities, and the physicochemical characteristics of the river water (Abulreesh, Paget and Goulder, 2006).

The sampling site had a significant effect on the detection of *Campylobacter* for both rivers. Variations in the occurrence of *Campylobacter* at different sites on both rivers reflects the various levels and types of anthropogenic activities that occur near the sites. The prevalence of *Campylobacter* was high for sites downstream of the WWTP discharge points, sites in areas where the predominant land use was agriculture, and sites where there were human settlements and poor solid waste management. The presence of *Campylobacter* in rivers impacted by human activities has been reported in previous studies (Strachan *et al.*, 2013; Otigbu *et al.*, 2018; Chukwu *et al.*, 2019; Mulder *et al.*, 2021). *Campylobacter* was detected at sites where animals accessed the river for drinking water, indicating that animals can act as the source of this pathogen (through faecal matter) as well as the transmission route (from river water to humans). The occurrence of *Campylobacter* in surface waters often suggests recent faecal contamination by livestock, runoffs from farm animal manure, birds, and insufficiently treated effluent or leakages from nearby septic tanks (Abulreesh, Paget and Goulder, 2006).

Meteorological conditions on the sampling day had no significant effect on the detection of *Campylobacter* for either river. There is limited information on the influence of meteorological conditions on the survival of *Campylobacter* in river water. The few studies available suggest that meteorological conditions on the sampling day may affect the detection of *Campylobacter* in rivers. Low air temperatures (14–15 °C or air temperature below 18 °C) support the occurrence of *Campylobacter spp.* in rivers (Wilkes *et al.*, 2011; Strakova *et al.*, 2022). In addition, *Campylobacter* detection is reported to be positively associated with an increase in rainfall (Vereen *et al.*, 2013).

This study did not find any significant effect of sampling season on the occurrence of *Campylobacter* for either river. However, the higher occurrence of *Campylobacter* in autumn for the Bloukrans River, and in summer for the Swartkops River is notable. Similarly, a high occurrence of *Campylobacter jejuni* and *Campylobacter coli* in river water was observed in autumn (Strakova *et al.*, 2022). Eyles *et al.* (2003) reported seasonal variations in levels of *Campylobacter* contamination in rivers, with high levels recorded in summer. Similar to the results obtained in this study, there was no significant seasonal effect on *Campylobacter* isolation in river water used for human consumption in Brittany, France (Denis, Tanguy, Chidaine, M.J. Laisney, *et al.*, 2011). However, season and temperature had significant effects on the occurrence of *Campylobacter* in the study by Wilkes *et al.* (2011). Rainfall experienced during autumn and summer may also lead to high occurrence of *Campylobacter* during these seasons. Rainfall may lead to an increase in the movement of *Campylobacter* from solid waste, sewer lines, wastewater treatment plants, livestock farms, and human settlements into rivers (Sterk et al. 2013).

The occurrence of virulence genes in *Campylobacter spp*. isolated from water samples from the Bloukrans and Swartkops rivers is reported in this study. The prevalence of virulence genes reported in this study ranged from 0% for *htrB* to 24% for *cadF*. These results correlate with a previous study which reported low percentage of detection of virulence genes in water samples in South Africa. The gene *cadF* was detected at 5.71% in the Eastern Cape (Igwaran and Anthony Ifeanyi Okoh, 2020). In contrast, a higher prevalence of virulence genes *cdtB* (63.2%), *cadF* (47.4%), *clpP* (31.6%) and *htrB* (0%) was reported in water samples from the Swartkops River in the Eastern Cape, and 90.9% of *Campylobacter jejuni* isolates from water samples from the North West province were reported to have expressed the *cadF* gene (Otigbu *et al.*, 2018; Chukwu *et al.*, 2019). It is worth noting that there is limited information on the virulent properties of *Campylobacter* species from water sources (Andrzejewska *et al.*, 2022). The virulence genes detected in this study are associated with adherence (*cadF*), environmental stress tolerance (*htrB*), invasion (*ciaB*), stress tolerance (*clpP*) and toxin production (*cdtB*). The presence of these virulence genes in the *Campylobacter* species from river water may increase the incidence of infection in humans who are exposed to river water (Andrzejewska *et al.*, 2022).

The *Campylobacter jejuni* detected in the river water in this study expressed antibiotic-resistant genes. The *Campylobacter tetO* gene detected in the positive water samples is plasmid-borne, and is acquired by *Campylobacter* through horizontal gene transfer (Connell *et al.*, 2003; Crespo *et al.*, 2016). The gene gives rise to tremendously high levels of resistance to tetracycline. The binding of the *tetO* genes to an open A site induces a conformational change leading to the release of the bound tetracycline molecule, so protein elongation is not interrupted (Luangtongkum *et al.*, 2009). The presence of *tetO* in the water samples was expected given the high rate of resistance against tetracycline observed among *Campylobacter* isolates from humans and the use of this antibiotic in veterinary medicine. The detection of multidrug efflux pump c*me*ABC genes in the rivers is a serious concern. The c*me*ABC genes encode proteins of different structures involved in extruding antimicrobials (Lin, Michel and Zhang, 2002). Another study in South Africa also reported the detection of *Campylobacter jejuni* isolates which exhibited multidrug resistance in estuarine water samples (Otigbu *et al.*, 2018).

The increase in antibiotic-resistant *Campylobacter* in these rivers is being driven by anthropogenic activities such as poor solid waste disposal, runoff from informal settlements, livestock grazing and discharge of wastewater. Additionally, the livestock industry in South Africa heavily relies on the use of antibiotics as prophylaxes or growth promoters. This has resulted in the misuse, overuse, and inappropriate use of antimicrobials, especially in intensive livestock production. This is worsened by weaknesses in the guidelines for veterinary antibiotic use in South Africa (Henton *et al.*, 2011; van den Honert, Gouws and Hoffman, 2018; Sithole *et al.*, 2021). The guidelines for veterinary antibiotic use permit the use of erythromycin, ampicillin, streptomycin and tetracycline in livestock (Henton *et al.*, 2011; van den Honert, Gouws and Hoffman, 2018; Sithole *et al.*, 2021). In South Africa, antibiotics for agricultural usage are less restricted than in most developed countries. The South African government, through the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act 36 of 1947, allows the non-prescription sale of antibiotics registered under stock feeds. This low restriction on antibiotic use leads to the high usage of antibiotics by farmers in South Africa (Otigbu *et al.*, 2018; Mupfunya, Qekwana and Naidoo, 2021).

A major limitation of this study is that the abundance and densities of *Campylobacter spp.* and their genes were not quantified in the samples, and this is a critical requirement to effectively determine the associated human health risk. More research is needed to quantify *Campylobacter* in rivers and link human *Campylobacter* infections to specific sources in rivers.

4.5 Conclusion

This study highlights the widespread and continual occurrence of potentially antibiotic-resistant *Campylobacter spp.* in the Bloukrans and Swartkops rivers. The occurrence of *Campylobacter* in water is linked to the physical condition of the rivers and anthropogenic activities prevailing in the catchment. *Campylobacter* infection may be endemic in South Africa, and the Bloukrans and Swartkops rivers are potential sources of *Campylobacter* exposure to humans. The evidence of the factors promoting the survival of *Campylobacter,* provided in this study is useful in monitoring *Campylobacter* in rivers. Water from these rivers is utilised for irrigation of crops (mainly vegetables), spiritual/cultural activities, recreation (mainly for children), drinking water for animals, and to a lesser extent, fishing. These rivers are an interface for human and animal activities and are a risk factor for the spread of *Campylobacter* species. It is therefore critical that the management of urban rivers is improved to prevent microbial contamination. Transmission of pathogens through contact with river water may play a role in the epidemiology of enteric diseases, including Campylobacteriosis, in this region. This study contributes valuable insights into the environmental factors influencing *Campylobacter* dynamics, which could help inform steered strategies for water quality management and public health interventions.

CHAPTER 5: SELECTIVE QUANTIFICATION OF VIABLE *CAMPYLOBACTER* AND ANTIBIOTIC RESISTANCE GENES IN RIVER WATER SAMPLES BY PROPIDIUM MONOAXIDE QUANTITATIVE POLYMERASE CHAIN REACTION

5.1 Introduction

Campylobacter species are among the leading etiological agents for bacterial gastroenteritis globally (Park *et al.*, 2011). Chapter 4 highlighted the widespread and continual occurrence of potentially antibiotic-resistant *Campylobacter spp.* in the Bloukrans and Swartkops rivers. The detection of antibiotic resistance genes (ARGs) of *Campylobacter* bacterial origin in these water sources indicates a potential hazard, and suggests that these water sources may act as significant points of risk for the presence of *Campylobacter* strains that are resistant to antibiotics. Resistance of pathogens to antibiotics used for treatment is a major public health concern because the infections caused by these microorganisms become difficult to treat (Páll *et al.*, 2013). With water sources being a potential transmission route, the occurrence of *Campylobacter spp.* in rivers presents a public health risk for humans exposed to this water.

, Quantitative microbial risk assessment (QMRA) is used to estimate the risk for an adverse health outcome resulting from exposure to pathogens. Traditionally the exposure assessment stage of QMRA relies on culture-based enumeration data or quantification using quantitative PCR. Culture-based methods involve directly measuring viable organisms through multiplication of host cells (for parasites and viruses parasites) or in a medium (for bacteria) (Haas, 2020). However, *Campylobacter* can switch into a viable but non-culturable (VBNC) state after protracted exposure to water due to environmental stress, and culture-based methods are unable to detect viable but non-culturable (VBNC) forms of *Campylobacter* (Bronowski, James and Winstanley, 2014). Therefore, *Campylobacter spp.* may not be detectable even if the water is contaminated and infection outbreak is possible (Hu and Kuo, 2011; Pitkänen, 2013). Molecular methods such as Polymerase chain reaction (PCR) enable rapid, specific, and highly sensitive detection of the presence of *Campylobacter* in water without prior cultivation (Gu *et al.*, 2013; Canciu *et al.*, 2021). Quantitative polymerase chain reaction (qPCR) is utilised to detect and measure the quantity of target genomic material in a sample without the need for culturing (Gu *et al.*, 2013; Cangelosi and Meschke, 2014; Canciu *et al.*, 2021; Stingl *et al.*, 2021). Additionally, for the purposes of risk assessment, a viability PCR assay can be employed to distinguish between live and dead cells (Cangelosi and Meschke, 2014). This involves incubating samples with a DNA binding dye such as propidium monoazide (PMA). Propidium monoazide (PMA) penetrates the cell membranes of dead cells because the integrity of their cell membrane is compromised. In the cells, the azide groups of PMA covalently bind to cellular DNA to form irreversible nitrogen-carbon bonds under photolysis. This subsequently inhibits the amplification of DNA from dead cells during PCR, while only the DNA from viable cells is amplified (Gensberger *et al.*, 2014; Li, Xin and Li, 2015; Yuan *et al.*, 2018). Viability PCR presents a distinct advantage in that it specifically excludes the detection of DNA of dead *Campylobacter* bacteria, but allows for the detection of viable but non-culturable (VBNC) cells, and therefore takes into account the infectious potential of the viable but non-culturable (VBNC) state (Gensberger *et al.*, 2014; Li, Xin and Li, 2015; Yuan *et al.*, 2018). Consequently, the data obtained through this method prove valuable for assessing the risk of infection.

This chapter documents the estimation of the quantity of viable *Campylobacter* cells and selected antibiotic resistance genes in the river water samples using PMA-qPCR. The estimated concentration of viable *Campylobacter* and ARGs is important to determine human exposure risk.

The effect of PMA treatment in selectively quantifying viable *Campylobacter* cells and their antibiotic resistance genes (ARGs) recovered from river water samples was also investigated. The *Campylobacter* cells, along with their ARGs, were recovered from the water samples using a combination of centrifugation and membrane filtration methods. Specifically, the chapter assessed whether the concentration of *Campylobacter* and their ARGs, detectable by qPCR, differed between the PMA-treated and untreated portions, emphasizing the capacity of PMA treatment to selectively detect only viable cells in a sample. The hypothesis was that PMA treatment would significantly reduce the concentration of *Campylobacter* and their ARGs compared to untreated portions, highlighting the ability of PMA treatment in selectively detecting viable cells in a sample.

5.2 Materials and methods

The methods employed in this chapter are described in detail in Chapter 2. Briefly, the methods employed involved water sampling, sample processing, and treatment of portions of the samples with PMA, DNA extraction for both PMA and non-PMA samples and finally quantification of the concentration using qPCR. The obtained C_T values were converted to concentrations as described in Chapter 2.

5.2.1 Data analysis

The estimated concentration of *Campylobacter* 16S rRNA, *tetO* and *cmeB* genes in PMA and non-PMA treated samples were entered in a Microsoft Excel spreadsheet and analysed using R version 4.2.

The post ANOVA Tukey test was used to determine whether there was a significant difference between the means of *Campylobacter* 16S rRNA, ARGs (*cmeB* and *tetO*) genes in PMA treated and non-PMA treated samples. The value of $p < 0.05$ was considered statistically significant.

5.3 Results

5.3.1 Validation of the qPCR protocol

According to the standard curve for the 16S rRNA, the C_T value at which no amplification in at least one replicate was observed was 30. This C_T value was regarded as the C_T cut-off value. Any samples with a C_T value higher than or equal to the negative control or C_T cut off value of the specific target gene were considered below the limit of detection. The limit of detection (LOD) is the lowest concentration that can be detected with reasonable certainty (95% CI) (Bustin *et al.*, 2009; Kralik and Ricchi, 2017; Al Salah, Laffite and Poté, 2019). The results obtained for *tetO* and *cmeB* assays showed that the C_T value cut-off were 27.9 for *tetO* and 26.04 for *cmeB* (Figure 5.1) according to the first standard dilution with no amplification in at least one replicate for each respective standard curve. Any samples with C_T value higher than or equal to negative control or 27.9 and 26.04 for *tetO* and *cmeB* gene were considered below the limit of detection.

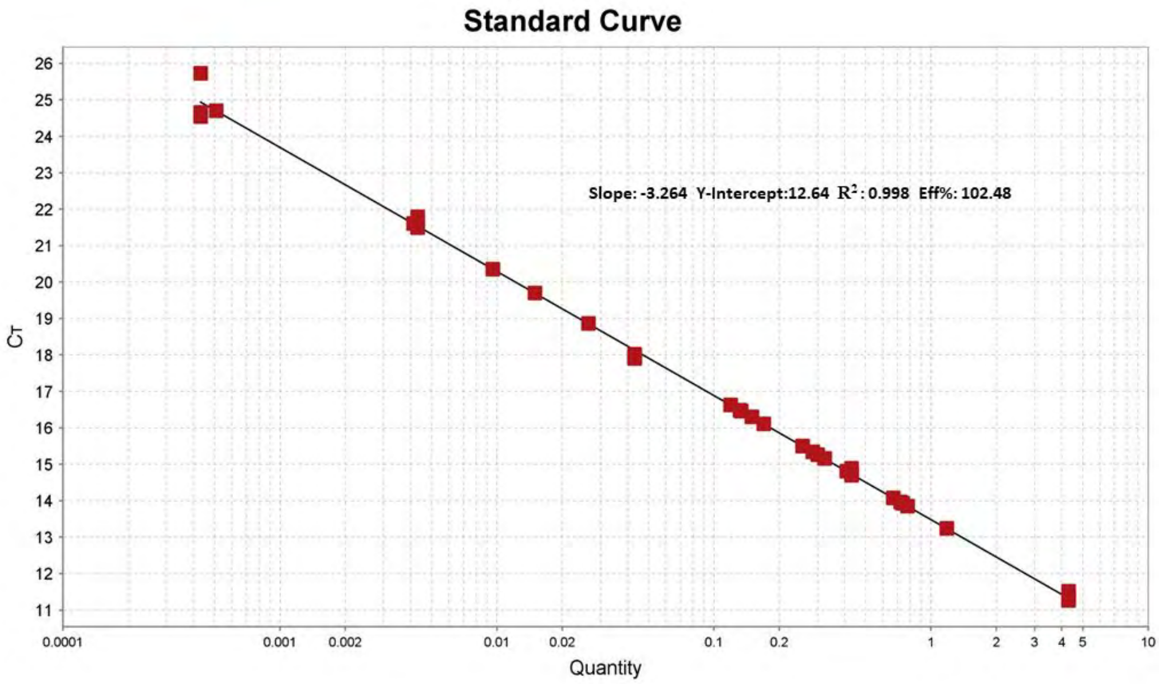

Figure 5.1. Quantitative Polymerase Chain Reaction data for the purified DNA (*cmeB*) used in producing a standard curve exhibiting linearity to more than six orders of magnitude

The analytical specificity of the qPCR assay was confirmed by melt-curve analysis and gel electrophoresis. The qPCR assay results considered in this chapter are only those which generated melt-curve single peaks. Additionally, the amplification efficiency, slope of the standard curve and correlation coefficient (R^2) (Table 5.1) fell within the specifications described in Section 2.6.2.

Table 5.1. Summary of qPCR amplification efficiency, slope and R^2 values for all qPCR assays in this study

Target gene	Efficiency (%)	Slope	R^2
16S rRNA	98.7–102.1	-3.132–-3.342	0.912–0.997
cmeB	92.0–103.1	-3.281–-3.532	0.923–0.998
tetO	94. –105.6	-3.191–-3.265	0.934–0.981

Quantitative PCR (qPCR) was employed to assess whether the concentration of *Campylobacter* 16S rRNA, *cmeB* and *tetO* genes detectable by qPCR differed between the PMA-treated and untreated portions, highlighting the ability of PMA treatment to selectively detect only viable cells in a sample. The qPCR efficiencies of the tested primers ranged from 92% to 105.6%, and the standard curves exhibited linearity to more than six orders of magnitude ($R^2 > 0.9$) (Figure 5.1 and Table 5.1).

5.3.2 Estimated concentrations of *Campylobacter* cells (*Campylobacter* 16S rRNA) in non-PMA and PMA treated river water samples

The mean concentrations of the *Campylobacter* 16S rRNA gene (copies/ml) at different sites of the Bloukrans and Swartkops rivers are shown in Figure 5.2. For the Bloukrans River, the mean concentration of the *Campylobacter* 16S rRNA gene ranged from 3.91×10^4 to 3.73×10^5 copies/ml for non-PMA treated samples and 2.1×10^4 to 1.75×10^5 copies/ml for PMA treated samples (Figure 5.2). For Swartkops River, the mean concentration of the *Campylobacter* 16S rRNA gene ranged from 2.21×10^4 to 1.45×10^5 copies/ml for non-PMA treated samples and 0 (no detection) at site S2 to 1.11×10^4 copies/ml for PMA treated samples (Figure 5.2). There was a statistically significant difference between the concentration of *Campylobacter* in PMA treated samples, and non-PMA treated samples ($p = 0.018$).

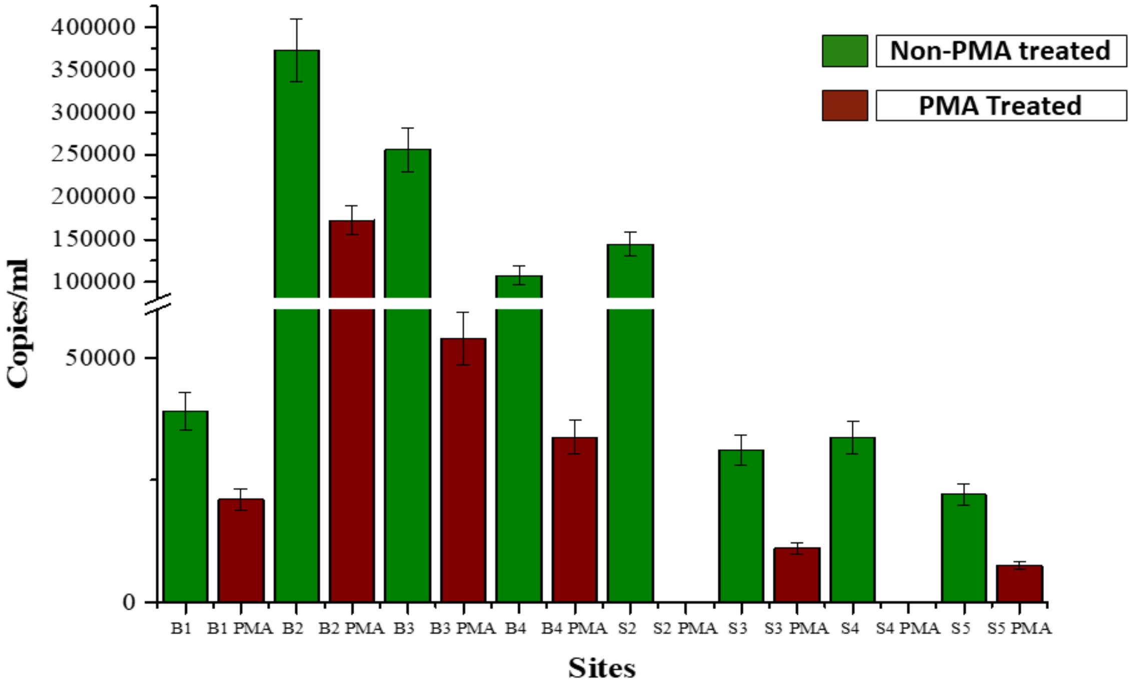

Figure 5.2. Estimated concentrations of *Campylobacter* 16S rRNA genes in water samples collected from different sites along the Bloukrans River (B1 to B4) and Swartkops River (S2 to S5). *Campylobacter* was not detected in all samples from site BC (on Bloukrans River) and sites SC and S1.

5.3.3 Estimated concentrations of tetracycline resistant gene (*tetO*) in non-treated and PMA treated river water samples

The concentration of tetracycline-resistant genes (*tetO*) in the water samples was estimated. For the Bloukrans River, the mean concentration of tetracycline-resistant genes (*tetO*) ranged from 5.52 × 10^1 to 1.58 × 10^4 copies/ml for non-PMA treated samples and 4.75 × 10^1 to 2.71 × 10^3 copies/ml for PMA treated samples (Figure 5.3). There was no detection of *tetO* in PMA treated samples from site B1. The site immediately after the wastewater discharge point (B2) recorded the highest concentration of *tetO*. For the Swartkops River, the mean concentration of tetracycline-resistant genes (*tetO*) ranged from 1.3 × 10^2 to 3.65 × 10^2 for non-PMA treated samples and 115.96 to 325.12 copies/ml for PMA treated samples (Figure 5.3). Similar to what was observed for the Bloukrans River, the site impacted by wastewater (Site S3) recorded the highest concentration for both *tetO*. There was a statistically significant difference between the concentration of tetracycline resistant genes (*tetO*) in PMA treated and non-PMA samples ($p = 0.032$).

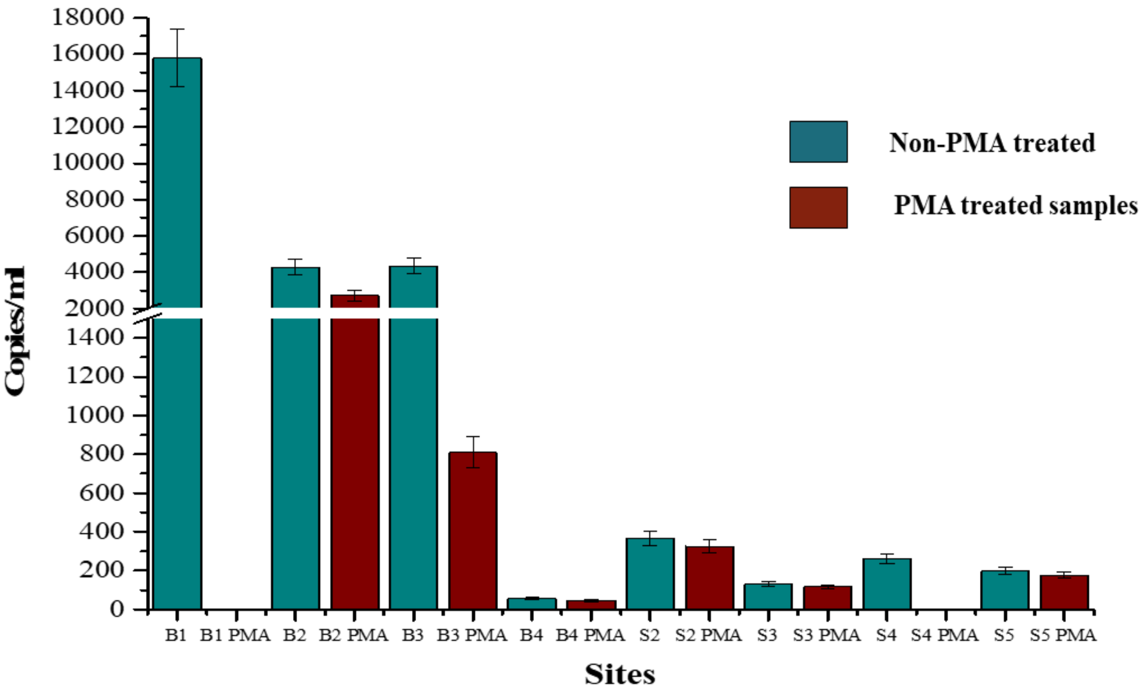

Figure 5.3. Estimated concentrations of *tetO* genes in water samples collected from different sites along the Bloukrans River (B1 to B4) and Swartkops River (S2 to S5). There was no detection of *tetO* genes in any samples from site BC (on Bloukrans River) and sites SC and S1.

5.3.4 Estimated concentrations of *Campylobacter* multi-drug resistance gene B (*cmeB*) in non-treated and PMA treated river water samples

The concentration of *Campylobacter* multi-drug resistance gene B (*cmeB*) ranged from 3.64 × 10^2 to 7.30 × 10^5 for non-PMA treated samples 7.8 × 10^1 to 2.76 × 10^4 copies/ml for the PMA treated samples from the Bloukrans River (Figure 5.4). There was no detection of *cmeB* at site B1, and site B3 recorded the highest concentration of *cmeB*. For the Swartkops River, the concentration of *cmeB* ranged from 2.53 × 10^1 to 8.03 × 10^3 for non-PMA treated samples and 1.71 × 10^1 to 7.39 × 10^3 copies/ml for the PMA treated samples (Figure 5.4). Similar to what was observed for the Bloukrans River, the site immediately downstream of the wastewater discharge point (Site S3) recorded the highest concentration for *cmeB* gene. A statistically significant difference between the concentration of *cmeB* in PMA treated and non-PMA was observed ($p = 0.017$).

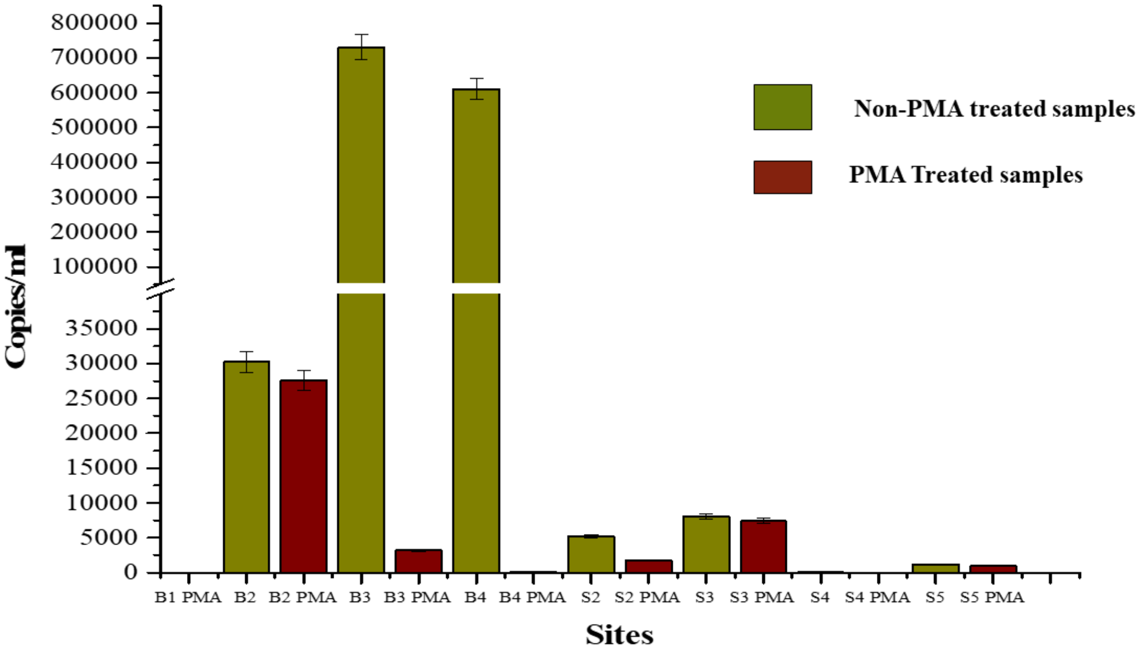

Figure 5.4. Estimated concentrations of *cmeB* genes in water samples collected from different sites along the Bloukrans River (B1 to B4) and Swartkops River (S2 to S5). There was no detection of *cmeB* genes in any samples from site BC (on Bloukrans River) and sites SC and S1.

5.4 Discussion

The analysis of the concentration of *Campylobacter* and its ARGs in river water through qPCR of PMA-pre-treated *Campylobacter* cells recovered from water samples was undertaken to specifically determine the concentration of viable *Campylobacter* and its ARGs, to avoid overestimating DNA quantifications. This distinction is important for the accurate calculation of exposure doses for studies assessing the risk of human exposure to antibiotic resistant *Campylobacter* in rivers. Previous studies have mostly reported the presence or absence of *Campylobacter spp.* and ARGs in rivers based on results obtained using conventional culture-based methods and PCR (Jokinen *et al.*, 2012; Szczepanska, Spica and Klawe, 2017; Otigbu *et al.*, 2018; Chukwu *et al.*, 2019; Strakova *et al.*, 2021). Quantitative PCR has also been used to report the concentration of *Campylobacter* and ARGs, but this has often been done without distinguishing the concentrations of viable *Campylobacter* in water samples (Hokajärvi *et al.*, 2013; Banting *et al.*, 2016). This study provides the first opportunity to seek an understanding of the actual concentration of viable *Campylobacter* and its ARGs in river water using PMA-qPCR. The use of PMA treatment prior to DNA extraction followed by conducting qPCR prevents amplification of DNA from dead cells, allowing only the DNA from viable cells to be amplified during PCR. This provides a more accurate estimate of the actual viable microbial load in samples. This information on the concentration of viable *Campylobacter* and its ARGs in river is critical for human health risk assessment.

As expected, PMA treatment prior to DNA extraction clearly reduced the concentrations of *Campylobacter* 16S rRNA, *cmeB* and *tetO* genes in the water samples. There was a significant reduction in concentration of the 16S rRNA gene and the antibiotic-resistant genes in PMA treated samples ($p < 0.05$). These results agree with those reported in previous studies (Gensberger *et al.*, 2014; Li *et al.*, 2014; Golpayegani *et al.*, 2019). PMA treatment is reported to have led to the reduction in the concentration of *E. coli* and *Enterococci* in wastewater (Li *et al.*, 2014) and *Escherichia coli*, *Enterococcus spp.* and *Pseudomonas aeruginosa* in drinking water and processed water (Gensberger *et al.*, 2014). Similarly, PMA treatment of water samples from a swimming pool prior to qPCR assays resulted in the reduction in the concentration of *Pseudomonas aeruginosa* in swimming pools (Golpayegani *et al.*, 2019). The reduction in concentration indicates that PMA-qPCR is able to selectively detect only viable cells in a sample. Therefore PMA-qPCR may be considered effective in selectively detecting the concentration of viable bacteria in water. This suggests that estimation of the concentration of antibiotic resistance *Campylobacter* recovered from water samples without PMA treatment may lead to over estimation of the risk of exposure or infection.

5.5 Conclusion

The motivation of this study was to determine whether PMA pre-treatment before PCR affects the abundance of detected *Campylobacter* cells and their antibiotic resistance genes (ARGs) recovered from river water samples, and to estimate the concentrations of viable antibiotic resistant *Campylobacter* in river water samples. For the Bloukrans River, the concentration of viable *Campylobacter* 16S rRNA gene ranged from 2.1×10^4 to 1.75×10^5 copies/ml, while the concentration for the Swartkops River ranged from 0.472×10^1 to 1.11×10^4 copies/ml. Although PCR-based methods are not able to distinguish between live and dead cells, culture-based methods enable identification of viable cells but exclude those that cannot be cultivated. *Campylobacter* can enter into a VNBC state, which may lead to underestimation of its concentration, particularly in environmental samples (Hazeleger, Jacobs-reitsma and Besten, 2016; Lanzl *et al.*, 2020). PMA treatment combined with PCR proves a valuable method of detection, highlighting the fact that viable *Campylobacter* cells were present in the river water samples investigated in this study. Based on the results obtained in this study and literature studies, PMA-treatment showed a significant reduction in the abundance of bacteria and their antibiotic resistance genes (ARGs) recovered from river water samples. The concentration of viable antibiotic-resistant *Campylobacter* measured in river the water samples can be used for exposure dose estimation for risk assessment studies without exaggerating abundance of viable microorganisms. PMA treatment prior to DNA extraction is therefore a critical procedure in estimating the concentration of viable bacteria and their antibiotic resistance genes (ARGs) in river water samples for the assessment of human exposure/infection risk to ARB and ARGs in rivers.

CHAPTER 6: ASSESSMENT OF RISK OF EXPOSURE TO CAMPYLOBACTER SPECIES AND THEIR ANTIBIOTIC-RESISTANT GENES FROM SELECTED RIVERS IN THE EASTERN CAPE, SOUTH AFRICA

This chapter has been published in a peer reviewed journal
Chibwe, M., Odume, O. N. and Nnadozie, C. F. (2023) 'Assessment of risk of exposure to *Campylobacter* species and their antibiotic-resistant genes from selected rivers in the Eastern Cape, South Africa', *Environmental Pollution*, 338(June), p. 122625. https://doi.org/10.1016/j.envpol.2023.122625.

6.1 Introduction

Globally, rivers provide several benefits, including clean water, wildlife habitat, recreation, and spiritual and cultural activities. However, they are also a source of pathogenic microbes, due to the discharge of pollutants from anthropogenic sources (Khatri and Tyagi, 2015). Contamination of rivers by microbial pathogens ultimately leads to the emergence of waterborne diseases (Nwabor *et al.*, 2016). Contaminated rivers play a critical role in the transmission of pathogens, including antibiotic-resistant ones. South Africa is a water-scarce country, and communities rely on their freshwater bodies such as rivers for irrigation, domestic water, recreation, spiritual, and cultural activities (Molobela and Pramod, 2011; Njage and Buys, 2015; Mboweni and De Crom, 2016; Sekwadi *et al.*, 2018). Rivers are increasingly being recognised as reservoirs of clinically important antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs), and they have the potential to transmit these to humans (Abia *et al.*, 2018). Furthermore, ARGs can be exchanged between environmental bacteria, human/animal commensals, and pathogens. The increase in pathogen resistance to clinically relevant antibiotics is a global concern, as this can lead to increased mortality associated with infectious diseases, including waterborne infections (O'Flaherty and Cummins, 2017).

Campylobacter species are one of the major causes of waterborne infections, and are one of the key pathogens responsible for bacterial gastroenteritis. *Campylobacter* infections are characterised by watery, non-bloody, non-inflammatory diarrhoea, abdominal pain and fever (Shobo *et al.*, 2016). The infections are usually self-limiting, but more severe cases can result in Guillain-Barre syndrome, reactive arthritis and abortion (Aksomaitiene *et al.*, 2019). Despite *Campylobacter* infections being self-limiting, patients are treated with antibiotics when infections prolong. The antibiotics used in the treatment of infections due to *Campylobacter* species are macrolides (erythromycin, azithromycin, and clarithromycin), fluoroquinolones (ciprofloxacin) and tetracycline (Chukwu *et al.*, 2020). Globally, the prevalence of *Campylobacter* infections is on the rise, and this phenomenon, coupled with the tendency of these bacterial species to resist the actions of antibiotics, renders them a serious public health threat (Aksomaitiene *et al.*, 2019; Igwaran and Okoh, 2019).

Antibiotic-resistant *Campylobacter* and ARGs have been detected in river water (Moore, Caldwell and Millar, 2001; Van Dyke *et al.*, 2010; Andrzejewska *et al.*, 2022) and this can pose health risks to humans exposed to infected water bodies. The common potential routes of human exposure to ARB and ARGs in environmental water are ingestion, dermal contact and inhalation (Manaia, 2017; Shuai *et al.*, 2021).

Existing regulations for water quality are based on routine monitoring of indicator organisms and physicochemical parameters. For many years water quality management and research have been based on these parameters (Okeyo *et al.*, 2018). Despite evidence that recreational water is a known reservoir and transmission path for ARGs and ARB, current regulations for recreational water quality do not consider antibiotic-resistant *Campylobacter* or any other antibiotic-resistant pathogens. The regulations only consider indicator organisms. For example, the WHO guidelines for recreational water stipulate the minimum concentrations for intestinal *Enterococci* (≤ 40/100 ml), while the European Union (EU) bathing waters directive 2006 (2006/7/EC) guideline values for excellent water quality specify less than 100 CFU/100 ml for *Enterococci*, and less than 250 CFU/100 ml for *Escherichia coli* (Efstratiou and Tsirtsis, 2009). Similarly, the guidelines for Canadian recreational water quality only consider *Escherichia coli* (≤ 235 CFU/100 ml) and *Enterococci* (≤ 70 CFU/100 ml) (Saleem *et al.*, 2023). The Australian guidelines for recreational water quality and aesthetics also do not consider antibiotic-resistant bacteria, but do stipulate the concentrations for *Enterococci* organisms (≤ 35/100 ml) and faecal coliform organisms (≤ 150/100 ml) (NRMMC-EPHC-AHMC, 2006). The South African water quality guidelines for recreation state that faecal coliform counts, or *E. coli*, should not exceed 130 CFU/ 100 mL (DWAF, 1996). Currently, no guideline considers antibiotic-resistant bacteria and their antibiotic resistance genes (ARGs), and therefore there are no set limits. However, WHO guidelines have recognised antibiotic resistance as an emerging pollutant in freshwater, including in recreational waters, rivers and lakes (WHO, 2021).

Antibiotic-resistant strains of various bacteria, including *Campylobacter*, have been detected in recreational waters in many parts of the world (O'Flaherty *et al.*, 2019; Tyagi and Kumar, 2021). Studying the exposure dose of ARGs through different routes (ingestion, inhalation and skin contact) can contribute to a better understanding of the potential health risks of antibiotic microbial resistance (AMR) in human-related environments. Currently, there is no standard method for exposure risk assessment of pathogenic bacteria and ARGs from environmental waters. However, a few studies have provided a convenient and reliable way of assessing the exposure dose to human populations (L. Li *et al.*, 2020; Shuai *et al.*, 2021). In these studies, the intake burden/dose of targeted ARGs or bacterial genus was estimated from the product of the target concentration in water and the ingestion rate/ inhalation rate/ skin surface area for dermal contact. Ingestion rate refers to the estimated volume of water which water users typically ingest during an average session of their chosen activity in water (Leonard *et al.*, 2018). Inhalation rate (20 m^3/day per person) is an approximated average volume of air inhaled per person per day (USEPA, 2006), while skin surface area is an approximated value of 16,000 cm^2, based on literature data (Li et al. 2020). The exposure data (dose) estimated through this method can be used to estimate the human health risks. The estimated exposure dose of *Campylobacter* and associated ARGs through accidental ingestion of recreational water can provide an evaluation of the potential health risk of antibiotic-resistant *Campylobacter spp.* in selected rivers in the Eastern Cape, South Africa. Accidental ingestion is emphasised here because the water from the rivers is not expected to be intentionally ingested (used as drinking water) without some level of treatment. The extent to which recreational water contributes to human exposure to Campylobacteriosis remains uncertain. To our knowledge, this is the first study designed to estimate potential human exposure (dose) to *Campylobacter* and associated ARGs through accidental ingestion of water from rivers used for recreation. This study aimed to assess the potential human exposure to *Campylobacter* and its ARGs from selected rivers through accidental ingestion during swimming, by calculating human hourly intake of ARGs and the average hourly ingested dose rates of *Campylobacter spp*. The estimated exposure dose of *Campylobacter* and associated ARGs through accidental ingestion of recreational water can contribute to the evaluation of the potential health risk of antibiotic-resistant *Campylobacter spp.* in aquatic environments.

6.2 Materials and Methods

The materials and methods are described in detail in Chapter 2. Briefly, the study involved determination of physico-chemical parameters, quantification of *Campylobacter* 16S rRNA, *tetO* and *cmeB* in PMA treated samples only. Only the concentration obtained from PMA samples was used to assess the risk of human exposure.

6.2.1 Assessment of human exposure risk

To assess the human exposure risk to *Campylobacter spp.* and ARGs, the major exposure pathways were first identified. The author acknowledges that the major exposure pathways for the two rivers are ingestion, inhalation and skin contact. However, in this study, ARGs on skin and aerosol samples were not determined, and therefore the risk of exposure through these two pathways was not assessed. Exposure to river water via ingestion during swimming is of short duration compared to the daily intake of ARGs via ingestion from drinking the water. Therefore, the event intake of the ARGs during swimming was evaluated for an hour instead of daily duration, which is a method determined by Shuai *et al.* (2021). The human intake of *Campylobacter* ARGs from a river per hour (copy/ h) during recreational swimming was calculated by multiplying the concentration of ARGs (copy/L) by the ingestion rate (21 ml/h on average) following the method by Shuai *et al.* (2021). The ingestion rate of (21 ml/h) for recreational swimming was calculated based on data from a previous study (Dorevitch *et al.*, 2011). The event intake of the pathogens at the genus level was calculated by multiplying the 16S rRNA gene concentration (copy/L) with the ingestion rate (21 ml/h on average). Human intake of *Campylobacter* ARGs (copy/hr) was calculated by multiplying the concentration (copy/L) by the ingestion rate (21 ml/h on average) (Shuai *et al.*, 2021).

6.3 Results

6.3.1 Detection of *Campylobacter* and ARGs

This study analysed river water samples from the Bloukrans respectively and Swartkops rivers in the Eastern Cape, South Africa. Water samples were collected from control sites and sites impacted by anthropogenic activities. Water quality parameters were measured on-site before collecting water samples, given that these parameters are critical to the survival of *Campylobacter* species in the aquatic environment. The physicochemical parameters recorded are presented in Table 6.1. Additionally, we measured average *Escherichia coli* concentrations of 144 – 362 CFU/ml and 21 – 196 CFU/ml for the Bloukrans and Swartkops rivers, which indicates faecal contamination.

Table 6.1. Physico-chemical characteristics of the water samples from the Bloukrans River (BC to B4) and Swartkops River (SC to S5)

Site	pH	Temperature (°C)	Dissolved Oxygen (mg/L)	Electrical Conductivity (us/cm)	Turbidity (NTU)
BC	7.2 (0.1)	22.1 (3.0)	8.7 (0.5)	187.5 (17.7)	6.0 (0.7)
B1	7.6 (0.0)	22.4 (3.6)	2.5 (2.1)	2151.5 (393.9)	54.0 (3.4)
B2	7.7 (0.0)	22.5 (3.5)	2.8 (1.0)	1647.0 (181.0)	39.5 (9.0)
B3	7.4 (0.2)	22.4 (4.2)	3.4 (1.5)	1116.5 (447.6)	11.6 (1.6)
B4	7.6 (0.0)	22.3 (4.5)	6.8 (1.2)	1472.0 (251.7)	8.0 (8.5)
SC	7.2 (0.1)	22.9 (5.4)	11.5 (0.7)	247.5 (9.2)	2.6 (0.0)
S1	7.2 (0.1)	22.1 (4.2)	10.1 (1.6)	408.0 (11.3)	12.5 (0.0)
S2	7.4 (0.7)	22.9 (5.0)	5.5 (0.7)	1515.0 (1011.2)	36.7 (14.7)
S3	7.4 (0.8)	23.5 (5.6)	4.6 (0.8)	2457.5 (804.0)	44.9 (13.2)
S4	7.9 (1.0)	22.6 (3.7)	5.4 (0.9)	3131.0 (15.6)	21.7 (8.0)
S5	7.4 (0.4)	22.9 (4.4)	6.7 (0.9)	994.5 (290.6)	17.6 (6.4)

Mean (standard deviation)

For the Bloukrans River, twenty (20) river water samples from five (5) sites influenced by different anthropogenic activities were analysed. *Campylobacter* was detected in 13 samples (60%). Of the *Campylobacter*-positive samples, 22.72% were positive for *cmeA*, while 52.27% were positive for *cmeB,* 15% were positive for *cmeC,* and 61% were positive for *tetO*. All the samples positive for *Campylobacter* were also positive for at least one *Campylobacter* antibiotic-resistant gene.

For the Swartkops River, twenty-four (24) river water samples from six (6) sites influenced by different anthropogenic activities were analysed. *Campylobacte*r was detected in 14 samples (58%). Of the *Campylobacter*-positive samples found, *cmeA*, *cmeB*, *cmeC*, and *tetO* genes were detected at concentrations of 14.29%, 50%, 7.14%, and 50%, respectively. None of the river samples from the control sites (BC and SC) showed positive results for *Campylobacter* or any of the antibiotic-resistance genes being tested. The same observations were also made for site S1 on the Swartkops River. This site lies downstream of the control site and is one of the least impacted by human activities.

6.3.2 Quantification of *Campylobacter* 16S rRNA genes

In Figure 6.1, Sites B1, B2, B3 and B4 are sampling sites for the Bloukrans River, and S3, S4, S5 are sites on the Swartkops River. For the Bloukrans River, the mean concentration of the *Campylobacter* 16S rRNA gene ranged from 3.38×10^4 to 1.75×10^5 copies/ml (Figure 6.1). The highest concentration was detected at Site B2, which lies downstream of a wastewater discharge point. *Campylobacter* 16S rRNA gene was detected at higher concentrations in the water samples from sites along the Bloukrans River while sites on the Swartkops River recorded lower concentrations. For the Swartkops River, the mean concentration of the *Campylobacter* 16S rRNA gene ranged from 0.472×10^1 to 1.11×10^4 copies/ml (Figure 6.1). The highest concentration was detected at Site S2, which lies downstream of a wastewater discharge point.

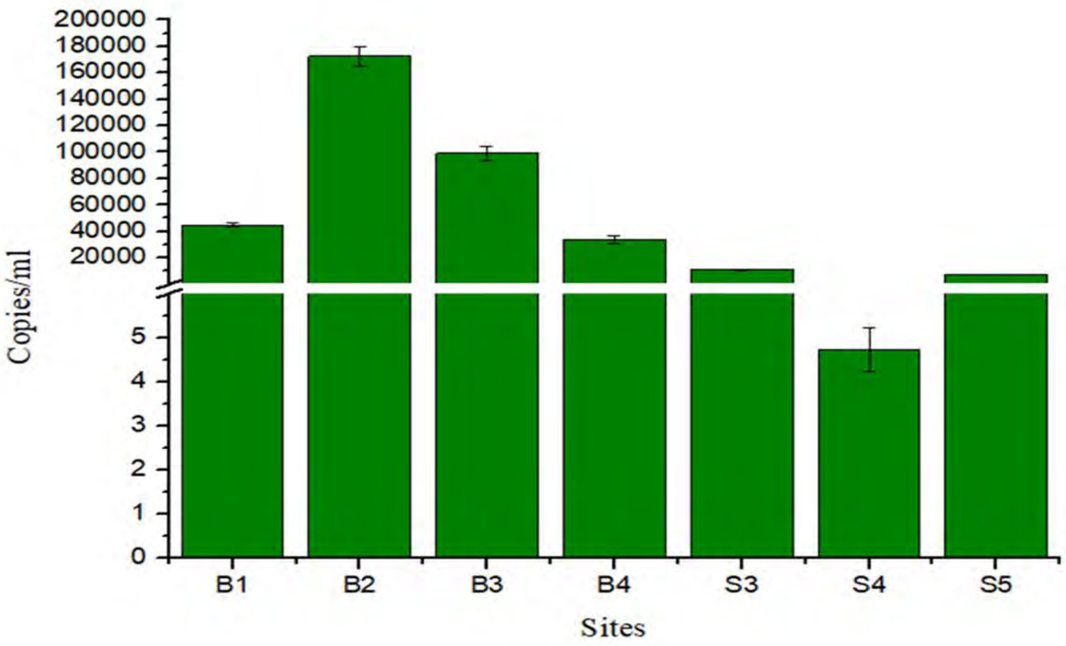

Figure 6.1. Quantification of the concentration of the *Campylobacter* 16S rRNA gene (in gene copies/ml) for the water samples collected from sites along the Bloukrans (Sites B1–B4) and Swartkops (Sites S3–S5) rivers, respectively.

6.3.3 Quantification of *Campylobacter* antibiotic resistance genes

Figure 6.2 shows the concentration of the *cmeB* and *tetO* (in gene copies/ml) for the water samples collected from sites along the Bloukrans and Swartkops rivers. Sites B2, B3, and B4 are sampling sites from the Bloukrans River, and S2, S3, S4, and S5 are from the Swartkops River. Although *cmeA* and *cmeC* were detected during standard PCR, their concentration was not determined during qPCR. Therefore, only the concentrations of *cmeB* and *tetO* are reported.

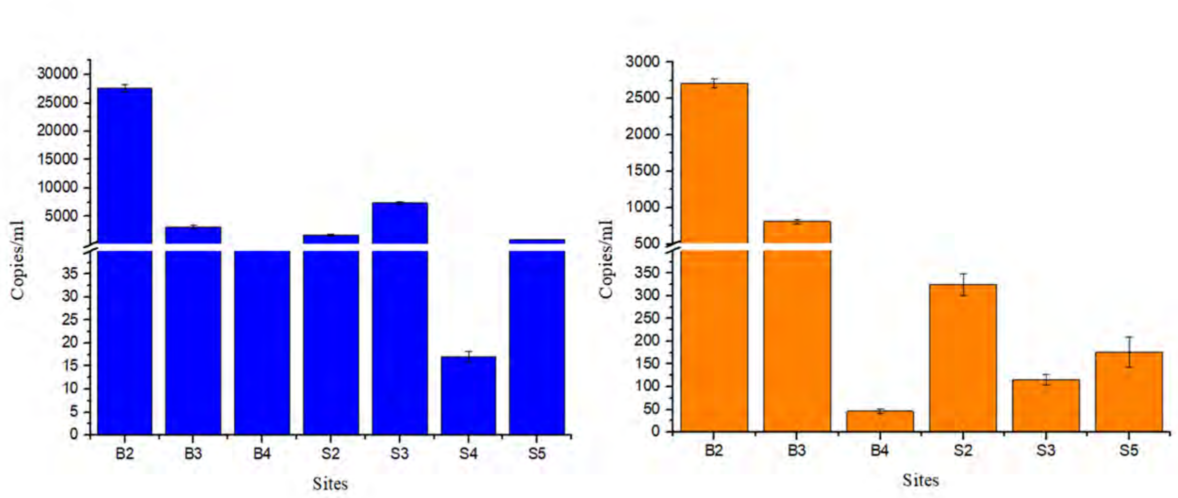

Figure 6.2. Quantification of the concentration of *cmeB* (left) and *tetO* (right) genes (in copies/ml) for the water samples collected from sites B2–B4 (Bloukrans) and S3–S5 (Swartkops) rivers, respectively.

For the Bloukrans River, the mean concentration of tetracycline-resistant gene (*tetO*) ranged from 4.75 × 10^1 to 2.71 × 10^3 copies/ml, while *Campylobacter* multi-drug resistance gene B (*cmeB*) ranged from 7.8 × 10^1 to 2.71 × 10^4 copies/ml. Site B2 downstream of the Wastewater Treatment Works (WWTW) recorded the highest concentration of both *tetO* and *cmeB*. For the Swartkops River, the mean concentration of *tetO* ranged from 115.962 to 325.123 copies/ml while *Campylobacter* multi-drug resistance gene B, (*cmeB*) ranged from 1.72 × 10^1 to 2.71 × 10^3 copies/ml (Figure 6.2). Similar to the results observed for the Bloukrans River, the site immediately downstream of the Swartkops WWTW (Site S3) had the highest concentration for both *cmeB* and *tetO*.

6.3.4 Assessment of human exposure to *Campylobacter* and antibiotic-resistant genes

The intake burden for *Campylobacter* 16S rRNA gene ranged from 7.1 × 10^5 – 3.7 × 10^6 copies/h for the Bloukrans River while that of the Swartkops River ranged from 9.9 × 10^1 – 2.3 × 10^5 copies/h (Figure 6.3).

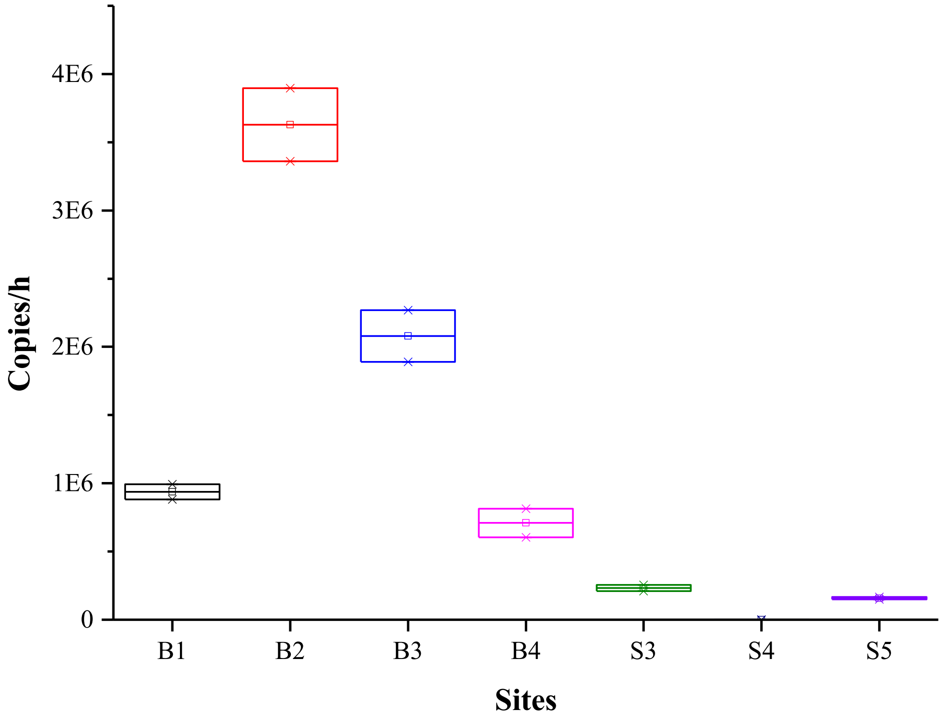

Figure 6.3. Estimated intake burden (copies/h) of *Campylobacter* 16S rRNA gene for one hour of swimming in the river at sites along the Bloukrans and Swartkops rivers, respectively. Sites B1, B2, B3 and B4 are sampling sites for the Bloukrans River, and S3, S4, S5 are for the Swartkops River.

The intake burden (copies/h) of *Campylobacter* ARGs for the Bloukrans River ranged from $1.64 \times 10^4 - 5.8 \times 10^5$ copies/h for *cmeB* (Figure 6.4) and $1.0 \times 10^3 - 5.7 \times 10^4$ copies/h for *tetO*, while the intake burden for Swartkops River ranged from $3.6 \times 10^2 - 1.551 \times 10^5$ copies/h for *cmeB* and $9.98 \times 10^2 - 5.7 \times 10^4$ copies/h for *tetO* (Figure 6.5).

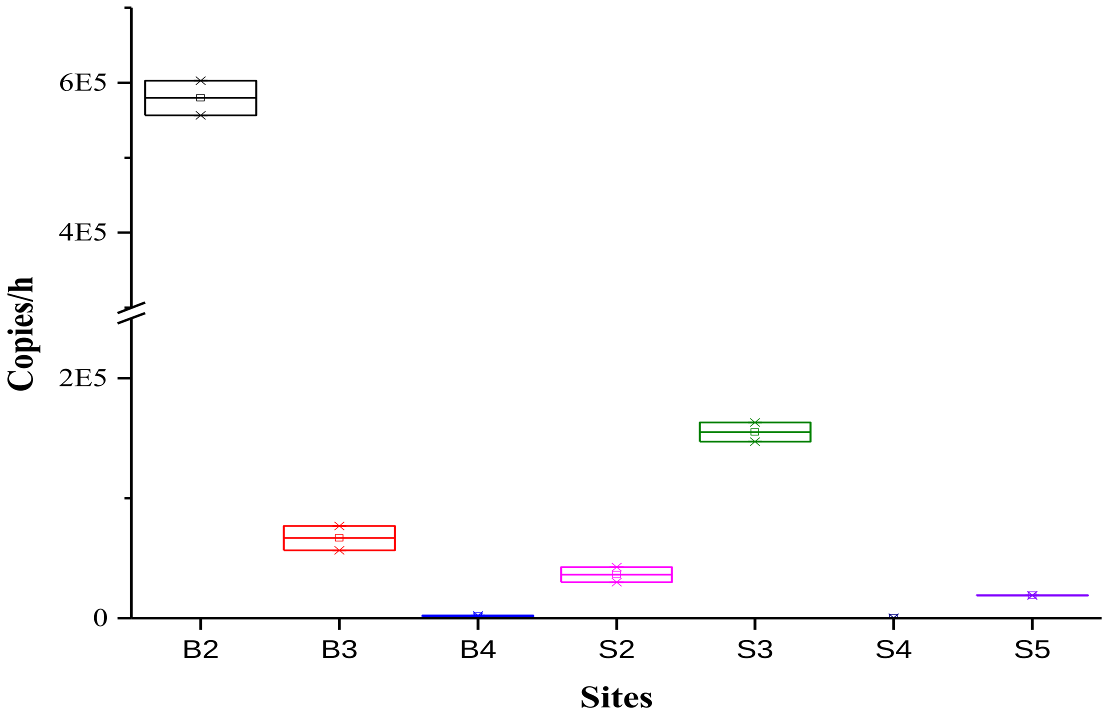

Figure 6.4. Estimated intake burden (copies/h) of *cmeB* for one hour of swimming in the water at sites along the Bloukrans and Swartkops rivers in the Eastern Cape, South Africa

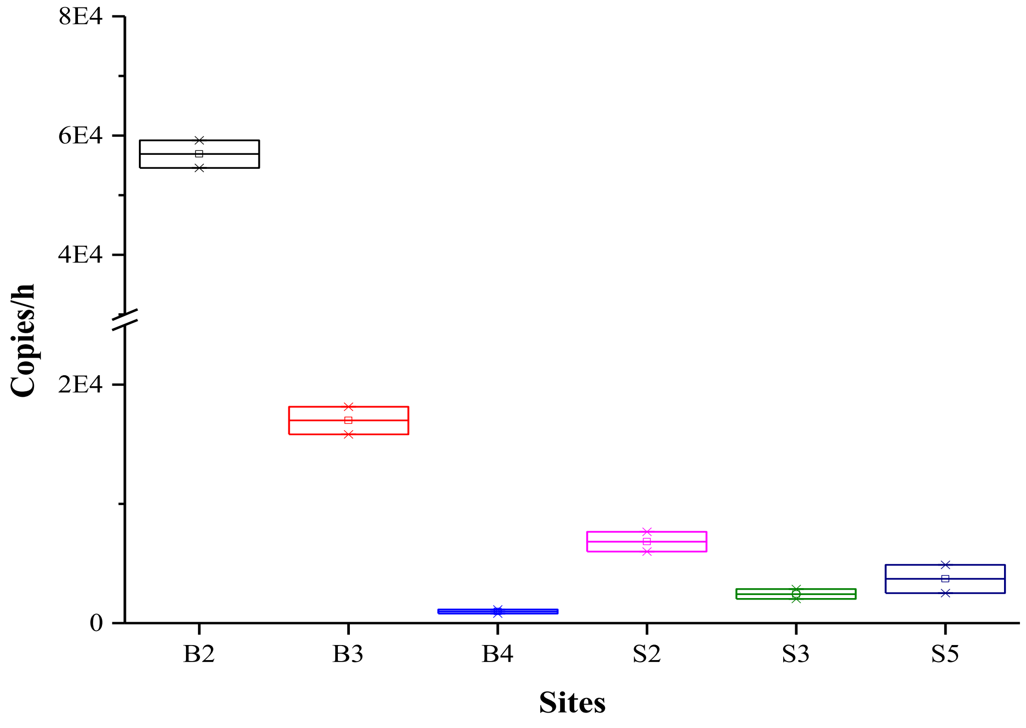

Figure 6.5. Estimated intake burden (copies/h) of *tetO* genes for one hour of swimming in the water at sites along the and Bloukrans and Swartkops rivers in the Eastern Cape, South Africa

6.4 Discussion

Campylobacter infections may result in severe symptoms in children, immunocompromised individuals, and the elderly (Barker *et al.*, 2020). Exposure to *Campylobacter* antibiotic-resistant genes may result in even more severe effects (Otigbu *et al.*, 2018). Exposure to infected water in the environment has been identified as a risk factor for *Campylobacter* infection, and has been linked to outbreaks of waterborne diseases (Pitkänen, 2013; Gorham and Lee, 2016). This study assessed potential human exposure to *Campylobacter* and its ARGs from river water samples from the Bloukrans and Swartkops rivers, Eastern Cape, South Africa.

Results of the prevalence of *Campylobacter* (60% and 58%) in water samples from the Bloukrans and Swartkops rivers, respectively, are comparable with results from other rivers around the world. In Bangladesh, *Campylobacter spp*. was detected in 60% of untreated river water used as drinking water for ducks at a farm (Uddin *et al.*, 2021). Similarly, in the

Netherlands, *Campylobacter* was detected in 66% of surface water samples (Mulder *et al.*, 2021). In some instances, the prevalence of *Campylobacter* infection has been relatively higher or lower than that recorded in this study on the Bloukrans and Swartkops rivers. For example, in Mozambique, *Campylobacter spp.* was detected in 95% of Inkomati river water samples. This higher prevalence could be because the Inkomati River receives run-off from large-scale poultry production in the catchment (Taviani *et al.*, 2022). In contrast, a lower prevalence of 8.1% for *Campylobacter spp.* in river water from Poland was reported (Andrzejewska *et al.*, 2022). The concentration of ARGs in this study is lower than that reported by Zhang *et al.* (2022) where the absolute abundance of total ARGs in river water ranged from 2.71×10^6 to 8.35×10^8 copies/L. Similarly, a mean concentration of the ARGs *intI1, suls, tets, erms and qnrs* was reported in the Yangtze River Delta in China at 1.23×10^9, $1.86{\times}10^8$, 1.99×10^7, 6.83×10^6, and 4.23×10^5 copies/L (Yang *et al.*, 2020). These concentrations were higher than those recorded in this study because they did not specifically target *Campylobacter* ARGs. Only limited studies have specifically quantified *Campylobacter* ARGs in river water. The lower concentrations found in this study may also be attributed to the treatment with propidium monoazide, which led to the detection of only viable DNA.

For the Bloukrans River, the mean concentration of the *Campylobacter* 16S rRNA gene ranged from 3.38×10^4 to 1.75×10^5 copies/ml (Figure 6.1). The highest concentration was detected at site B2, a site that lies downstream of a wastewater discharge point. For the Swartkops River, the mean concentration of the *Campylobacter* 16S rRNA gene ranged from 0.472×10^1 to 1.11×10^4 copies/ml (Figure 6.1). The highest concentration was detected at site S2, which lies downstream of a wastewater discharge point. The level of *Campylobacter* in the Bloukrans River is high than that reported in Brisbane, Australia by Ahmed *et al.* (2009). Considering how low an infectious dose is (500 CFU can cause illness), the current concentrations of *Campylobacter* in Bloukrans River could pose significant health risks.

Campylobacter and ARG prevalence and their concentrations were higher in the Bloukrans River samples than in the Swartkops River samples. The prevalence of ARGs recorded for the Bloukrans River was higher than that for the Swartkops River. The high values observed in the Bloukrans River can be attributed to the differences in width and flow rate. The Swartkops River has a higher depth, width, and flow rate than the Bloukrans River, and therefore the quantity of pathogens is reduced due to dilution (O'Flaherty *et al.*, 2019). In addition, the Bloukrans River receives a large volume of insufficiently treated effluent from the nearby dysfunctional and dilapidated Wastewater Treatment Works (WWTW), and this can contribute to high concentrations of *Campylobacter* (Vereen *et al.*, 2013).

The prevalence of *Campylobacter* is influenced by the water quality of the rivers. The physicochemical properties of the water samples from the Bloukrans and Swartkops rivers indicate that *Campylobacter* can thrive in both rivers (Hilton *et al.*, 2001; Van Dyke *et al.*, 2010; Culotti and Packman, 2015; Teh, Lee and Dykes, 2017). Based on the *Escherichia coli* concentrations of the water samples (144–362 CFU/ml and 21–196 CFU/ml) from Bloukrans and Swartkops rivers, respectively, the presence of *Campylobacter* could be inferred, as this pathogen is associated with faecal-contaminated water. In addition, the high turbidity (44.9–54 NTU), low dissolved oxygen (2.5–4.6 mg/L), and high conductivity (2151.5–3131 µs/cm) recorded in these rivers, which are impacted by wastewater discharge, agricultural activities, livestock grazing, and run-off from nearby informal settlements, all promote the proliferation of *Campylobacter* species (Pitkänen, 2013; Gorham and Lee, 2016). This correlates with the results from this study were *Campylobacter* and its ARGs concentrations were higher for sites which recorded high turbidity, low dissolved oxygen and high electrical conductivity. These observations also correlate with previous studies which have reported the presence of *Campylobacter* in rivers highly impacted by human activities (Strachan *et al.*, 2013; Otigbu *et al.*, 2018; Chukwu *et al.*, 2019; Mulder *et al.*, 2021).

For both rivers, the prevalence of *Campylobacter* was high for sites downstream of the WWTP effluent discharge points, sites in areas where the predominant land use was agriculture, where animals accessed the river for drinking water, and sites with nearby informal settlements lacking solid waste management systems. The presence of *Campylobacter* in rivers impacted by human activities has been reported in previous studies (Strachan *et al.*, 2013; Otigbu *et al.*, 2018; Chukwu *et al.*, 2019; Mulder *et al.*, 2021). Frequent faecal contamination of the water by livestock, farm animal manure runoffs, solid waste, discharge of poorly treated wastewater, and urban runoff from the community into these rivers may lead to high concentrations of viable *Campylobacter* (Abulreesh, Paget and Goulder, 2006; Karikari *et al.*, 2016). Runoff from livestock production farms along the Bloukrans and Swartkops rivers has been implicated as a source of ARGs as well as ARB in the river. Local studies in South Africa indicate that antibiotic use in livestock production is high (Pillay *et al.*, 2020; Sithole *et al.*, 2021). This practice has been linked to the high spread and transmission of antibiotic-resistant zoonotic pathogens such as *Campylobacter*, *Salmonella*, *E. coli* and *Shigella* (Chala *et al.*, 2021). Studies suggest that animals poorly absorb antibiotics in their guts and may excrete high concentrations of antibiotic residues in their faecal matter and urine. Animal excreta from livestock may therefore lead to a high concentration of ARB and ARGs being released into the Bloukrans and Swartkops rivers by livestock which graze the river banks.

The Bloukrans and Swartkops rivers are both seriously impacted by pollution inputs from insufficiently treated effluent from several dysfunctional wastewater treatment works (WWTW). Antibiotic-resistant pathogens, including *Campylobacter* and their genes, are introduced into the rivers through the discharge of wastewater into the rivers. Wastewater carries both resistant and sensitive pathogen organisms such as *Campylobacter spp, E. coli, Klebsiella spp., Shigella spp., Salmonella spp.* and *Vibrio spp.* (Fouz *et al.*, 2020). In addition, the wastewater may also contain ARG and antibiotic residuals which are then discharged into the rivers, making the rivers hot spots for the transmission and development of antimicrobial resistance (Berendonk *et al.*, 2015; Adams, Pretorius and Snow, 2019; Phungela *et al.*, 2022).

The fate of the ARGs is multi-faceted; they can be diluted, adsorbed onto particulate matter, degrade through biological and non-biological processes, or be taken up by aquatic microorganisms through horizontal gene transfer (HGT) (Chen and Dubnau, 2004; Lapara *et al.*, 2015). Dilution of ARGs does occur in high-flowing rivers (Lapara *et al.*, 2015; Jerde *et al.*, 2016). However, in the case of the Bloukrans and Swartkops rivers, which are located in the drought-prone Eastern Cape in South Africa, dilution may not necessarily reduce the concentration of ARGs. The ARGs can interact with minerals and humic substances, and are immobilised in sediments (Nnadozie and Odume, 2019). ARGs will persist for longer in rivers owing to immobilization, and extended persistence of ARGs provides an opportunity for resuspension during high-flow events or shear stress (Turner, Uy and Everhart, 2015). It can be argued that degradation, a significant process which influences the fate of ARGs in the freshwater environment, may occur either through natural sunlight exposure or biotic processes involving DNases. The DNases are secreted by the organotrophic microbiome while metabolizing large organic molecules, before they are assimilated into cells. ARGs adsorb to sediments and their components, which protects them from biological degradation (Turner, Uy and Everhart, 2015). Data regarding the capacity of sunlight to degrade ARGS are still scarce, however, generally, sunlight-mediated degradation does not eliminate ARGs in the freshwater environment. Therefore, ARGs can be assimilated by competent bacteria within the environment and incorporated into the bacterial genome (Yoon, Doddb and Lee, 2018; Nnadozie and Odume, 2019). Based on this discussion, it can be deduced that ARGs may persist in rivers. It is therefore feasible that humans could be exposed to ARGs via drinking water, consumption of crops irrigated with contaminated water, and through recreational activities (Kaakoush *et al.*, 2015). Microbial pollution of these rivers can be mitigated through adequate treatment of wastewater, controlled grazing and proper solid waste management in the community.

With regard to assessment of the risk of exposure, the exposure dose of *Campylobacter* via accidental ingestion to humans, that is, $7.1 \times 10^5 - 3.7 \times 10^6$ copies/h, which was recorded for the Bloukrans River in this study, is comparable to the $2.67 \times 10^3 - 4.18 \times 10^6$ copies/h calculated by Shuai *et al.* (2021) for the dissemination of total antibiotic-resistant opportunistic pathogens via swimming. Accidental ingestion remains the main exposure pathway to humans in recreational water and is the focus of this study. The study by Shuai *et al.* (2021) investigated the exposure dose of ARGs and antibiotic-resistant opportunistic pathogens during swimming in a pool. The exposure dose/ intake burden /targeted ARG or bacterial genus was estimated from the product of the target concentration in water and ingestion rate. Ingestion rate is an approximation of the volume of water that water users typically ingest in an average session of their chosen activity (Leonard *et al.*, 2018). The exposure dose of *Campylobacter* via accidental ingestion of Bloukrans river water is higher than that calculated by Shuai *et al.* (2021) for antibiotic-resistant opportunistic *Mycobacterium* ($7.47 \times 10^2 - 1.41 \times 10^7$ copies/h) via swimming in pools. This difference was expected because swimming pool water is treated, and would be of better microbiological and physico-chemical quality than river water.

The exposure dose of ARGs for one hour through accidental ingestion of water from the Bloukrans and Swartkops rivers is comparable to the reported exposure dose via ingestion of drinking water (10^4–10^5 copies/day) (L. Li *et al.*, 2020). The intake burden (copies/hr) of *Campylobacter* ARGs for the Bloukrans River ranged from $1.64 \times 10^4 - 5.8 \times 10^5$ copies/hr for *cmeB* and $1.0 \times 10^3 - 5.7 \times 10^4$ copies/h for *tetO*. In contrast, the intake burden for Swartkops River ranged from $3.6 \times 10^2 - 1.551 \times 10^5$ copies/h for *cmeB* and $9.98 \times 10^2 - 5.7 \times 10^4$ copies/h for *tetO*. Therefore, just by accidentally ingesting water from the Bloukrans River, one can be exposed to the same dose of ARGs as one would be by drinking the water intentionally. This indicates an increased risk to human health through accidental ingestion. These results also suggest that the route of waterborne transmission of ARGs from these rivers poses a high risk of ARG exposure via accidental ingestion to local residents who use the rivers for recreation or other activities, such as baptism/cultural rituals.

Exposure to natural waters has been identified as a risk factor for *Campylobacter* infection (Pitkänen, 2013; Gorham and Lee, 2016). Furthermore, exposure to natural waters used for recreation has been linked to outbreaks of waterborne diseases. A previous study demonstrated that ingestion of contaminated recreational water has been linked to outbreaks of waterborne diseases in the US (Gorham and Lee, 2016). Ingestion of contaminated water is just one of the major routes of human exposure. The Swartkops and Bloukrans rivers also support other anthropogenic activities such as fishing and crop irrigation, and both are water sources for livestock such as goats and cattle. Therefore, other routes of human exposure to *Campylobacter* and its ARGs in these catchments include consumption of vegetables irrigated with river water, consumption of fish, and indirect exposure through consumption of meat and other products of animals which may have consumed this water. These potential routes of exposure need to be further investigated. In addition, the health risks caused by the potential intake of *Campylobacter* ARGs should be further explored. Most importantly, measures to reduce the health risks associated with exposure to *Campylobacter* and related antibiotic-resistance genes in polluted river environments are critical for the benefit of people who rely on the rivers for their livelihoods.

6.5 Conclusions, practical applications of this work, and future research perspectives

This study demonstrates that the Swartkops and Bloukrans rivers may represent a source of contamination by *Campylobacter* and its antibiotic-resistant genes. The pathogen and ARGs can be transmitted to humans who ingest the water during recreation, which can result in infection or illness. These rivers are also utilised for fishing and irrigation, and are a source of water for livestock. Further investigation of other potential routes of exposure needs to be conducted. It is important to evaluate and monitor the quality of river water, and to improve surveillance of waterborne diseases caused by pathogens such as *Campylobacter*. The propagation of antibiotic-resistance genes in aquatic environments, and subsequent human exposure to these genes in these environments, may pose public health risks. Quantitative microbial risk assessment (QMRA) is a generally suitable method to evaluate and quantify this health risk. QMRA approach assesses the potential health risks posed by a particular pathogen by combining the information on occurrence, exposure, and dose-response. From this study, the exposure doses of *Campylobacter* ARGs of $71.64 \times 10^4 – 5.8 \times 10^5$ copies/h for *cmeB* and $1.0 \times 10^3 – 5.7 \times 10^4$ copies/h for *tetO* for a one-hour swimming event at the Bloukrans River, and $3.6 \times 10^2 – 1.551 \times 10^5$ copies/h for *cmeB* and $9.98 \times 10^2 – 5.7 \times 10^4$ copies/h for *tetO* at the Swartkops Rivers have been estimated. This work contributes towards the body of knowledge in closing the current research gap on possible human exposure to antibiotic-resistant *Campylobacter*, and in general, exposure to ARB through freshwater sources. However, this study has only considered the ingestion pathway; other pathways for the exposure assessment step of the QMRA process during recreational activities in freshwater systems must be considered in future studies.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 General Introduction

*Campylobacte*r species are increasingly associated with human diarrhoea infections. This phenomenon constitutes a significant health concern in South Africa, where diarrhoea is a primary cause of morbidity and mortality in children. Compounding this issue is the growing resistance of *Campylobacter* species to antibiotics. Bacteria are becoming resistant to clinically relevant antibiotics, which leads to treatment failure, long duration of illnesses, high morbidity, longer periods of hospitalisation and in severe cases, death. (Gothwal and Shashidhar, 2015). Other indirect effects include economic costs, loss of productivity, and reduction in the quality of life at an individual level (Bueno *et al.*, 2018; Iwu and Patrick, 2021). Although the etiological agent has been known for a considerable period, its recent recognition as a major contributor to waterborne diseases adds to the existing concerns. In South Africa the increasing prevalence of antibiotic-resistant bacteria and their genes in freshwater systems, including rivers, further magnifies the potential threat to the exposed population. A high concentration of pollutants such as antibiotic-resistant bacteria (ARB) and antibiotic resistant genes (ARGs) have been reported in local rivers (Abraham, 2011; Molobela and Pramod, 2011). This study investigated the context-related drivers, occurrence and human exposure to antibiotic resistant *Campylobacter* in selected river systems in the Eastern Cape, South Africa. This thesis addresses certain knowledge gaps identified in the introduction and literature review (Chapter1), which include (i) the context-related drivers of antibiotic-resistant bacteria and genes in the Bloukrans and Swartkops rivers in the Eastern Cape, South Africa (Chapter 3), ii) spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers, Eastern Cape, South Africa (Chapter 4), iii) risk of exposure to *Campylobacter* species and their antibiotic-resistant genes from selected rivers in the Eastern Cape, South Africa (Chapter 6). And also, by examining context-related drivers of occurrence, spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers, this study contributed to a holistic framework for assessing the risk of the emergence and transmission of antibiotic-resistant *Campylobacter* in these rivers. This study was conducted in two river systems, the Bloukrans and Swartkops rivers in the Eastern Cape, South Africa. Both rivers are important for recreational and agricultural purposes for local residents. However, these rivers are at risk of *Campylobacter* pollution. These rivers are affected by faecal pollution from livestock practices, dilapidated wastewater treatment plants and agricultural practices. This chapter provides a summary of the major findings in line with the objectives and the results which have discussed in the previous chapters. Additionally, the implications of the results regarding our understanding of the emergence and spread of *Campylobacter* and antibiotic-resistant genes on river water management, implications for management and policy, and the study limitations/recommendations are presented in this chapter. It is worth acknowledging that the insights from this study extend to other ARB, beyond *Campylobacter*.

7.1.1 Assessment of the context-related drivers of antibiotic resistant bacteria and genes in the Bloukrans and Swartkops river catchments in the Eastern Cape, South Africa

This study aimed to identify context-related drivers contributing to the heightened occurrence/ emergence of antibiotic-resistant bacteria and genes in the Bloukrans and Swartkops rivers in the Eastern Cape, South Africa. While this study did not include a formal correlational analysis, it successfully highlighted specific anthropogenic pressures (improper solid waste management, discharge of poorly treated wastewater, poor animal husbandry practices, urban run-off and storm water, industrial effluent, and agricultural run-off), socio-demographic characteristics of the catchment, and a lack of knowledge regarding AMR and inappropriate disposal of antibiotics as significant contributors. Poor wastewater treatment and solid waste disposal in the community are increasing the concentration of ARB and ARGs in the rivers. Unequal access to health care and municipal services, as well as extreme wealth inequalities are evidence of social inequalities (Truter, 2015; Schellack *et al.*, 2017; Weaver *et al.*, 2017; Manderson, 2020). Consequently, erratic water supply, poor water quality, broken sewer pipes and uncollected solid waste is commonly experienced, especially in historically black-dominated townships. This may contribute to the outbreak of diseases such as diarrhoea in these communities and subsequently increase the spread of ARB and ARGs. Poor animal husbandry, agricultural run-off, urban run-off, storm water and industrial effluent are other factors that contribute to the spread of ARB/ARGs at catchment level. This chapter also highlighted how the spread and emergence of ARB/ARGs resistance is largely driven by poor handling/disposal of antibiotics. Significantly, the findings from this study revealed divergent anthropogenic drivers compared to other studies. The absence of certain anthropogenic drivers such as runoffs from dairy farms, horse breeding and discharge effluents from aquaculture in the Bloukrans and Swartkops rivers is noted. This observation supports the argument that context-specific assessments are necessary for tailored interventions. This observation underlines the necessity for targeted interventions to mitigate the impact of these context drivers on water quality.

7.1.2 Spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers, Eastern Cape, South Africa

Chapter 4 investigated the spatiotemporal patterns of *Campylobacter* species' occurrence in the Bloukrans and Swartkops rivers, examining influences of meteorological conditions, physicochemical parameters, season and sampling site proximity to potential contamination sources. Significant insights into the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers were revealed. Initially, it was anticipated that there would be a connection between seasonality and *Campylobacter* occurrence, given common assumptions about its influence. Seasonality in *Campylobacter* occurrence in surface waters has been reported in previous studies. A higher occurrence of *Campylobacter* in autumn and summer has been reported in previous studies (Eyles *et al.*, 2003; Strakova *et al.*, 2022). However, the findings from this study challenge these assumptions, the sampling season did not significantly impact *Campylobacter* prevalence in this study. The results from this study suggest that risk of infection on exposure to *Campylobacter* in both rivers investigated persists across all seasons. This observation suggests a reconsideration of temporal dynamics traditionally associated with the safety of rivers used for recreation and irrigation purposes. In correlation, a previous study also reported no significant seasonal effect on *Campylobacter* isolation in river water used for human consumption in Brittany, France (Denis, Tanguy, Chidaine, M. J. Laisney, *et al.*, 2011). However, season and temperature had significant effects on the occurrence of *Campylobacter* in a study by Wilkes *et al.* (2011). The occurrence of *Campylobacter* and its ARGs in rivers throughout all the seasons suggests that *Campylobacter* infections may be endemic in the study area. It may also suggest that the rivers are constantly being contaminated with *Campylobacter* and its ARGs, and that the rivers serve as potential sources of exposure to humans.

Although *Campylobacter* and its ARGs were detected throughout the year, there were variations in the occurrence between different sites along the rivers. The prevalence of *Campylobacter* was higher for sites impacted by wastewater, agriculture, urban run-off, solid waste and grazing livestock. Similar to what has been reported in previous studies, this study demonstrated the presence of *Campylobacter* in rivers impacted by human activities (Strachan *et al.*, 2013; Otigbu *et al.*, 2018; Chukwu *et al.*, 2019; Mulder *et al.*, 2021). The occurrence of

Campylobacter in surface waters indicates recent faecal contamination (Abulreesh, Paget, and Goulder, 2006). Chapter 4 reported the detection of *Campylobacter* at various sites throughout the year, which suggests that the Bloukrans and Swartkops rivers may be constantly re-contaminated by faecal matter. The occurrence of *Campylobacter* is also influenced by meteorological conditions such as rainfall and temperature.

Based on this study, it is evident that physicochemical characteristics influence the occurrence of ARB/ARGs in the rivers. For example, the high turbidity, high electrical conductivity and low dissolved oxygen observed in the rivers favours the occurrence of *Campylobacter*. The findings from this study correlate with those from previous studies (Hilton *et al.*, 2001; Van Dyke *et al.*, 2010; Culotti and Packman, 2015; Teh, Lee and Dykes, 2017).

7.1.3 Selective quantification of viable *campylobacter* cells and selected antibiotic resistance genes by PMA-qPCR

The detection of *Campylobacter* and ARGs in river water indicates a potential hazard, and suggests that rivers may act as significant points of exposure to *Campylobacter* and ARGs. To estimate the risk of exposure to *Campylobacter*, it is important to quantify the concentration of viable *Campylobacter* in river water. Chapter 5 investigated the efficacy of PMA treatment in selectively quantifying the viable *Campylobacter* cells and their ARGs recovered from river water samples. The *Campylobacter* cells, along with their ARGs, were retrieved from the water samples using a combination of centrifugation and membrane filtration methods. Chapter 5 assessed whether PMA-qPCR is able to selectively detect the concentration of viable *Campylobacter* cells and their ARGs in river water samples. The results obtained in this study show that PMA treatment prior to DNA extraction clearly reduced the concentrations of *Campylobacter* 16S rRNA, *cmeB* and *tetO* genes in the water samples. This correlates with the findings of previous studies where PMA treatment has reduced the concentration of bacteria cells in water samples (Gensberger *et al.*, 2014; Li *et al.*, 2014; Golpayegani *et al.*, 2019). The reduction in the concentration as reported in Chapter 5 indicates that PMA-qPCR was able to selectively quantify the concentrations of *Campylobacter* and ARGs from viable cells. Therefore PMA-qPCR is effective in detecting the concentration of viable bacteria in water (Chapter 5). Estimation of the concentration of antibiotic resistance *Campylobacter* species in water without PMA treatment may lead to over estimation of the risk of exposure or infection. In risk assessment, PMA treatment prior to DNA extraction is critical in estimating the risk of human exposure/infection to ARB and ARGs in rivers. Furthermore, PMA-qPCR is a better option for detecting fastidious pathogens such as *Campylobacter*, which are difficult to detect using culture-based methods (Golpayegani *et al.*, 2019).

7.1.4 Assessment of risk of exposure to *Campylobacter* species and their antibiotic-resistant genes from selected rivers in the Eastern Cape, South Africa

Contaminated rivers play a critical role in the transmission of *Campylobacter* and antibiotic-resistant genes (ARGs). Assessment of the risk of human exposure to antibiotic-resistant bacteria can contribute to a better understanding of the potential health risks of human exposure to ARB/ARGs from river water. Chapter 6 assessed the potential human exposure to *Campylobacter* and its ARGs through unintentional ingestion during swimming in the Bloukrans and Swartkops Rivers, to determine the number of gene copies a person could ingest after swimming in the contaminated water for one hour (human intake burden). The human intake burden of *Campylobacter* 16S rRNA copies per one-hour swimming event ranged from $9.9 \times 10^1 – 3.7 \times 10^6$ copies/h. The human intake burden of *Campylobacter* ARGs ranged from $3.6 \times 10^2 – 5.8 \times 10^5$ copies/h for *cmeB* and $9.98 \times 10^2 – 5.7 \times 10^4$ copies/h for *tetO*. These quantitative data contribute valuable information for risk assessment models and public health planning. Unintentional ingestion of contaminated river water during recreation, cultural, or religious activities may lead to human exposure to ARGs, posing a health risk. As described in Chapter 3, the Bloukrans and Swartkops rivers play an important role in the social and cultural lives of people in the community, hence human exposure to water in these rivers may pose health risks to the community. The information presented in Chapter 6 shows the level of human exposure to ARB/ARGs contributable through recreational activities in the Swartkops and Bloukrans rivers. The assessment of actual exposure provided important understanding, confirming that individuals who engage in recreational water activities in both rivers face a substantial risk of exposure to *Campylobacter*.

7.1.5 A holistic framework for assessing the risk of occurrence, emergence and transmission of antibiotic-resistant bacteria and genes in rivers

Lastly, the outcome of this study is a holistic framework (Figure 7.1) which integrates physicochemical conditions and seasons with social dimensions to assess the risk of emergence and transmission of ARB and ARGs in rivers like the Bloukrans and Swartkops used for recreation, irrigation, and domestic purposes. By carefully evaluating anthropogenic pressures, considering physicochemical conditions and seasonality and exploring meteorological influences, this framework highlights the complex factors which contribute to the emergence of pathogens in recreational water. The evaluation of the actual exposure further highlights the intricate nature of these health risks that encompass the potential for infections associated with recreational water. Significantly, this framework could be valuable to water regulatory bodies, water resource managers and public health agencies. Stakeholders can benefit from the framework to enhance their risk assessment practices, implement targeted preventive measures, and develop guidelines that ensure the safety of individuals engaging in recreational activities in the rivers.

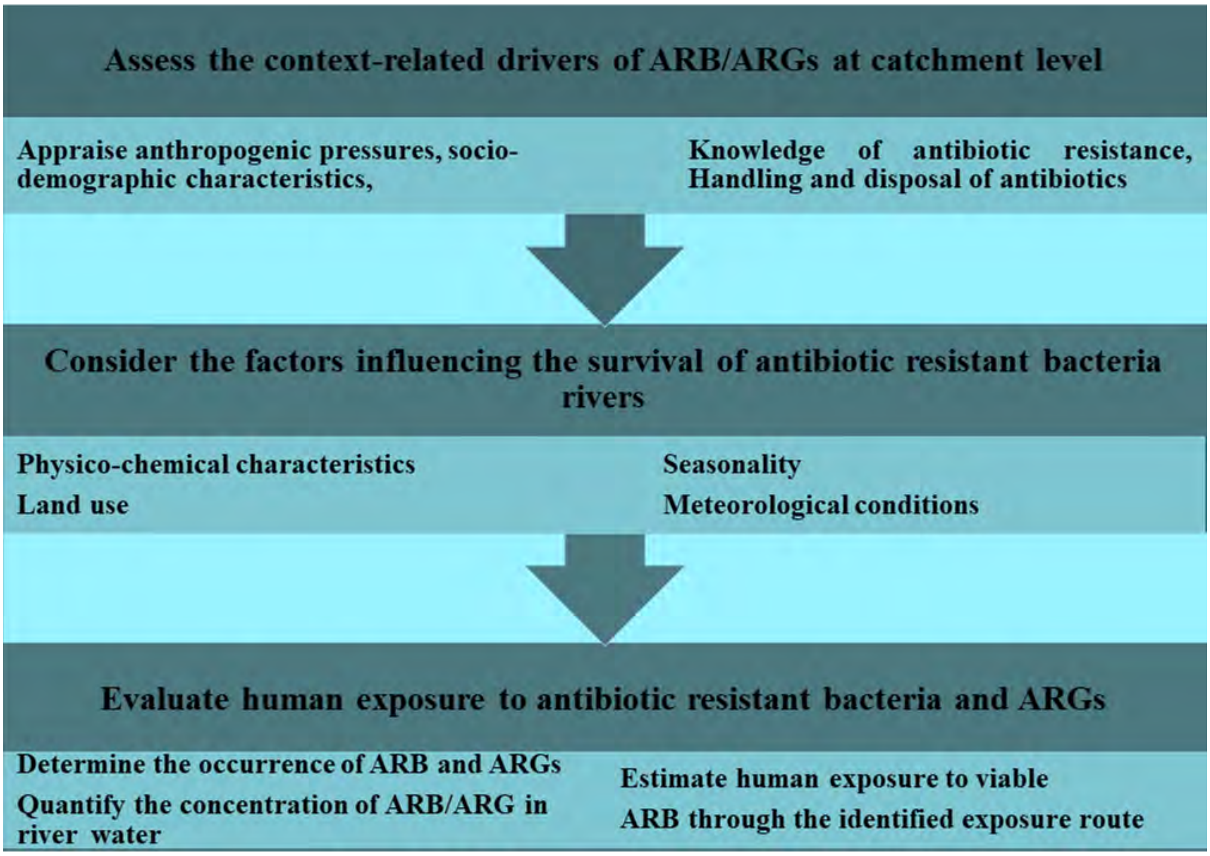

Figure 7.1. A framework for assessing the risk of occurrence, emergence and transmission of antibiotic-resistant bacteria and genes in recreational rivers

7.2 Implications of the study for the understanding of the emergence and transmission of *Campylobacter* and antibiotic-resistant genes in river water

This study highlights the widespread and continual occurrence of *Campylobacter spp.* in the Bloukrans and Swartkops rivers, which may be potentially antibiotic-resistant. Furthermore, the detection of high concentrations of viable *Campylobacter* and ARGs *Campylobacter* in these rivers shows that there is a high risk of transmission of viable antibiotic-resistant *Campylobacter* from rivers to humans. The occurrence of *Campylobacter* in river water has been linked to the physical condition of the rivers, anthropogenic activities prevailing in the catchment, socio demographic characteristics of the community and a lack of knowledge regarding AMR and inappropriate use and disposal of antibiotics. The concurrence between specific anthropogenic pressures, favourable environmental conditions and *Campylobacter* occurrence in the selected rivers emphasises the urgency of implementing targeted interventions in the Bloukrans and Swartkops rivers.

7.3 Implications for management and policy

This study shows that the spread and emergence of antibiotic-resistant *Campylobacter* is a multifaceted problem. Therefore, it may be difficult to combat the spread of antibiotic resistance in rivers without adopting effective interdisciplinary actions involving social science, environmental science, public health, agriculture, regulatory authorities and policy makers. There is a need to prevent the spread and transmission of antibiotic-resistant *Campylobacter* in river water using an integrated approach which will involve incorporation of preventive measures at a household, community and local government level. First of all, it is important to recognise antibiotic resistance in general as a sociodemographic and behavioural problem that is driven by complex factors. Mitigation and preventative actions on reducing the spread of antibiotic resistance in rivers can be achieved through public health and environmental awareness campaigns at community level. Improvements in provision of municipal services and awareness regarding the public health risks of indiscriminate solid waste disposal in the community, and improvements in solid waste management and wastewater treatment at a community level can reduce the spread of ARB/ARGs. Additionally, promoting good health-seeking behaviours and increasing awareness regarding antibiotic use and handling can also help in preventing the spread of ARB/ARGs in rivers. Adoption of good animal husbandry and increased regulation of antibiotic use in livestock production may also play an important role in reducing contamination of rivers with ARB and ARGs.

At a policy level, the South African National AMR strategic framework needs to incorporate the socio-economic dimension of antibiotic resistance, and pay more attention to addressing antibiotic resistance in environmental sources such as rivers. Furthermore, regulation and monitoring of hospital effluent, pharmaceutical effluent, wastewater, and industrial effluent quality is essential to prevent the spread of ARB and ARGs in rivers. Additionally, water quality guidelines need to incorporate monitoring of antibiotic-resistant bacteria and antibiotic residues in addition to the variables currently under consideration.

With respect to practical implications, the ability of the framework developed in this study to capture these interactions within the local context positions it as a valuable tool for regulatory bodies, water resource managers and public health agencies.

7.4 Study limitations and recommendations for future studies

This study attempted to fill the knowledge gaps outlined in Chapter 1. However, the findings of this study have also highlighted several research gaps that need to be investigated in future studies. The first limitation is that this study did not consider the risk of human infection due to ingestion of antibiotic-resistant *Campylobacter* but only considered the risk of exposure. This was due to the lack of a dose-response model for antibiotic resistant *Campylobacter*. Currently, there is no existing dose response (D-R) model for any antibiotic-resistant bacteria. The second limitation is that this study only considered human exposure through accidental ingestion of *Campylobacter* and its ARGs antibiotic resistant genes during swimming. However, human exposure to ARB/ARGs in river water can occur through other exposure routes. Other potential exposure routes that should be considered in future studies include dermal exposure and inhalation during recreation, exposure through the consumption of crops irrigated with contaminated river water or zoonotic exposure through consumption of meat/milk from animals that drink contaminated river water. Further research should be done on attributing the human disease burden of waterborne campylobacteriosis to specific sources at river catchment level.

7.5 Conclusion

In conclusion, socio-demographic characteristics, anthropogenic activities, a lack of awareness about antimicrobial resistance (AMR), and improper antibiotic disposal are the key drivers of the occurrence of antibiotic-resistant genes (ARGs) and antibiotic-resistant bacteria (ARB) in Bloukrans and Swartkops rivers. The identified drivers constitute pivotal markers for assessing and managing the risk of antibiotic-resistant bacteria and genes in river systems. The need for education on antimicrobial resistance and its link to river pollution, improved waste management, and wastewater treatment at different catchments is emphasized. The absence of certain anthropogenic activities in this study, that are found in other river catchments, reinforces the need for context-specific assessments, and the importance of tailored interventions for effective antibiotic resistance mitigation in rivers. The study's findings highlight the widespread occurrence of potentially antibiotic-resistant *Campylobacter spp*. in the Bloukrans and Swartkops rivers, presenting concerns about human exposure during various activities. Implementing measures for waste disposal, adequate treatment of wastewater from wastewater treatment plants, and pollution control is essential to prevent microbial contamination, safeguard water quality, and mitigate the risk of waterborne diseases associated with antibiotic-resistant *Campylobacter* in the Bloukrans and Swartkops rivers. The quantitative microbial risk assessment (QMRA) approach employed in this study offers valued insights into estimating exposure doses, contributing to our knowledge of human exposure to antibiotic-resistant *Campylobacter* through freshwater sources. However, the study acknowledges the importance of future research to explore other routes of human exposure to *Campylobacter* and its ARGs in these rivers, such as inhalation and dermal contact during recreational activities, consumption of vegetables irrigated with river water, consumption of fish, and indirect exposure through consumption of meat and meat other products of animals which may have consumed this water.

REFERENCES

Abia, A. L. K. *et al.* (2018) ‘Metagenomic analysis of the bacterial communities and their functional profiles in water and sediments of the Apies River, South Africa, as a function of land use’, *Science of The Total Environment*, 616–617, pp. 326–334. doi: 10.1016/J.SCITOTENV.2017.10.322.

Abraham, W. (2011) ‘Megacities as sources for pathogenic bacteria in rivers and their fate downstream’, *International Journal of Microbiology*, pp. 1–13. doi: 10.1155/2011/798292.

Abulreesh, H. H., Paget, T. A. and Goulder, R. (2006a) ‘*Campylobacter* in waterfowl and aquatic environments: Incidence and methods of detection’, *Environmental Science and Technology*, 40(23), pp. 7122–7131. doi: 10.1021/ES060327L.

Adams, J. B., Pretorius, L. and Snow, G. C. (2019) ‘Deterioration in the water quality of an urbanised estuary with recommendations for improvement’, *Water SA*, 45(1), pp. 86–96. doi: 10.4314/wsa.v45i1.10.

Adefisoye, M. A. and Okoh, A. I. (2017) ‘Ecological and public health implications of the discharge of multidrug-resistant bacteria and physicochemical contaminants from treated wastewater effluents in the Eastern Cape, South Africa’, *Water (Switzerland)*, 9(8), pp. 1–18. doi: 10.3390/w9080562.

Adewale, O. *et al.* (2020) ‘Risk Assessment of traditional faecal pollution markers in three streams in a suburb of Akure , Nigeria’, *Jordan Journal of Earth and Environmental Sciences*, 11(2), pp. 93–97. ISSN 1995-6681

Adewumi, J. R., Ilemobade, A. A. and Zyl, J. E. Van (2010) ‘Treated wastewater reuse in South Africa : Overview , potential and challenges’, *Resources, Conservation & Recycling*, 55(2), pp. 221–231. doi: 10.1016/j.resconrec.2010.09.012.

Ahmad, I., Malak, H. A. and Abulreesh, H. H. (2021) ‘Environmental antimicrobial resistance and its drivers : a potential threat to public health’, *Journal of Global Antimicrobial Resistance*, 27, pp. 101–111. doi: 10.1016/j.jgar.2021.08.001.

Ahmed, W. *et al.* (2009) ‘Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR’, *Water Research*, 43(19), pp. 4918–4928. doi: 10.1016/j.watres.2009.03.041.

Ahsan, A. *et al.* (2022) ‘Antibiotic resistance pattern and molecular detection of ESBL-associated genes in *E. coli* from surface and wastewater of Islamabad capital territory , Pakistan’, *Journal of Water and Health*, 20(4), pp. 601–609. doi: 10.2166/wh.2022.216.

Aksomaitiene, J. *et al.* (2019) ‘Overlap of antibiotic resistant *Campylobacter jejuni* MLST genotypes isolated from humans, broiler products, dairy cattle and wild birds in Lithuania’, *Frontiers in Microbiology*, 10, pp. 1–8. doi: 10.3389/fmicb.2019.01377.

Allel, K. *et al.* (2020) ‘Socioeconomic factors associated with antimicrobial resistance of *Pseudomonas aeruginosa* , *Staphylococcus aureus* , and *Escherichia coli* in Chilean hospitals (2008 – 2017)’, pp. 1–11. https://doi.org/10.26633/RPSP.2020.30

Alonso, R. *et al.* (2005) ‘MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli* strains’, *Journal of Microbiological Methods*, 63, pp. 99–103. doi: 10.1016/j.mimet.2005.03.013.

Andrzejewska, M. *et al.* (2022) ‘Genetic relatedness, virulence, and drug susceptibility of *Campylobacter* isolated from water and wild birds’, *Frontiers in Cellular and Infection Microbiology*, 12, pp. 1–10. doi: 10.3389/fcimb.2022.1005085.

Assawatheptawee, K. *et al.* (2017) ‘Occurrence of Extended-Spectrum and AmpC-Type β-Lactamase Genes in *Escherichia coli* isolated from water environments in Northern Thailand’, *Microbes and Environments*, 32(3), pp. 293–296. doi: 10.1264/jsme2.ME17050.

Asuming-Bediako, N. *et al.* (2019) ‘*Campylobacter* at the human – food interface : The African perspective’, *Pathogens*, 8(87), pp. 1–30. doi:10.3390/pathogens8020087

Ateba, C. N. *et al.* (2020) ‘Occurrence of antibiotic-resistant bacteria and genes in two drinking water treatment and distribution systems in the North-West Province of South Africa’, *Antibiotics*, 9(745), pp. 1–17. doi:10.3390/antibiotics9110745

Atta, H. I. *et al.* (2022) ‘Detection of extended spectrum beta-lactamase genes in strains of *Escherichia coli* and *Klebsiella pneumoniae* isolated from recreational water and tertiary hospital waste water in Zaria , Nigeria’, *International Journal of Environmental Health Research*, 32(9), pp. 2074–2082. doi: 10.1080/09603123.2021.1940884.

Azhdarpoor, A. *et al.* (2019) ‘Relationship between turbidity and microbial load of water in Salman Farsi Dam Reservoir’, *Journal of Environmental Pollution and Management*, 2(2), pp. 2–5. ISSN: 2639-7269

Bai, S. *et al.* (2022) ‘Constructed wetlands as nature-based solutions for the removal of antibiotics: performance, microbial response, and emergence of antimicrobial resistance (AMR)’, *Sustainability*, 14(14989), pp. 1–12. doi.org/10.3390/su142214989

Balta, I. *et al.* (2023) ‘Overview of Virulence and Antibiotic Resistance in *Campylobacter spp.* livestock isolates’, *Antibiotics*, 12(402), pp. 1–19. doi: org/10.3390/antibiotics12020402.

Baniga, Z. and Hounmanou, Y. M. G. (2020) ‘Genome-Based analysis of β -Lactamase-

Producing *Escherichia coli* in the aquatic environment and Nile Perch (*Lates niloticus*) of Lake', *Frontiers in Microbiology*, 11(108), pp. 1–11. doi: 10.3389/fmicb.2020.00108.

Banihashemi, A., Van Dyke, M. I. and Huck, P. M. (2012) 'Long-amplicon propidium monoazide-PCR enumeration assay to detect viable *Campylobacter* and *Salmonella*', *Journal of Applied Microbiology*, 113(4), pp. 863–873. doi: 10.1111/j.1365-2672.2012.05382.x.

Bankier, C. *et al.* (2018) 'A comparison of methods to assess the antimicrobial activity of nanoparticle combinations on bacterial cells', *PLoS ONE*, 13(2). doi: 10.1371/journal.pone.0192093.

Banting, G. S. *et al.* (2016) 'Evaluation of various *Campylobacter*-specific quantitative PCR (qPCR) assays for detection and enumeration of campylobacteraceae in irrigation water and wastewater via a miniaturized most-probable-number-qPCR assay', *Applied and Environmental Microbiology*, 82(15), pp. 4743–4756. doi: 10.1128/AEM.00077-16.

Banu, R. A. *et al.* (2021) 'Extended Spectrum Beta-Lactamase *Escherichia coli* in river waters collected from two cities in Ghana , 2018 – 2020', *Tropical Medicine and Infectious Disease*, 6(105), pp. 1–19.

Barker, C. R. *et al.* (2020) 'Microevolution of *Campylobacter jejuni* during long-term infection in an immunocompromised host', *Scientific Reports*, 10(1), pp. 1–11. doi: 10.1038/s41598-020-66771-7.

Bartley, P. S. *et al.* (2019) 'Antibiotic resistance in Enterobacteriaceae from surface waters in urban Brazil highlights the risks of poor sanitation', *American Journal of Tropical Medicine and Hygiene*, 100(6), pp. 1369–1377. doi: 10.4269/ajtmh.18-0726.

Baserisalehi, M., Al-Mahdi, A. Y. and Kapadnis, B. P. (2005) 'Antimicrobial susceptibility of thermophilic *Campylobacter spp.* isolated from environmental samples', *Indian journal of medical microbiology*, 23(1), pp. 48–51. doi: 10.4103/0255-0857.13874.

Bate, G., Smailes, P. and Adams, J. (2004) 'A water quality index for use with diatoms in the assessment of rivers', *Water SA*, 30(4), pp. 493–498. ISSN 0378-4738

Beceiro, A., Tomás, M. and Bou, G. (2013) 'Antimicrobial resistance and virulence : a successful or deleterious association in the bacterial world ?', 26(2), pp. 185–230. doi: 10.1128/CMR.00059-12.

Bengtsson-Palme, J. (2017) 'Antibiotic resistance in the food supply chain: where can sequencing and metagenomics aid risk assessment?', *Current Opinion in Food Science*, 14, pp. 66–71. doi: 10.1016/j.cofs.2017.01.010.

Bengtsson-Palme, J. *et al.* (2019) 'Industrial wastewater treatment plant enriches antibiotic resistance genes and alters the structure of microbial communities a', *Water Research*, 162(162), pp. 437–445. doi: 10.1016/j.watres.2019.06.073.

Benites, C. *et al.* (2022) ‘Prevalence, tetracycline resistance and *tet (O)* gene identification in pathogenic *Campylobacter* strains isolated from chickens in retail markets of Lima,Peru’, *Antibiotics*, 11(1580), pp. 1–12.

Berendonk, T. U. *et al.* (2015) ‘Tackling antibiotic resistance: The environmental framework’, *Nature Reviews Microbiology*, 13(5), pp. 310–317. doi: 10.1038/nrmicro3439.

Bessong, P. O. *et al.* (2009) ‘Spatial distribution of diarrhoea and microbial quality of domestic water during an outbreak of diarrhoea in the Tshikuwi community in Venda, South Africa’, *Journal of Health, Population and Nutrition*, 27(5), pp. 652–659. doi: 10.3329/jhpn.v27i5.3642.

Bester, L. A. and Essack, S. Y. (2012) ‘Observational study of the prevalence and antibiotic resistance of *Campylobacter spp.* from different poultry production systems in KwaZulu-Natal, South Africa’, *Journal of Food Protection*, 75(1), pp. 154–159. doi: 10.4315/0362-028X.JFP-11-237.

Bolinger, H. and Kathariou, S. (2017) ‘The current state of macrolide resistance in *Campylobacter spp.*: Trends and impacts of resistance mechanisms’, *Applied and Environmental Microbiology*, 83(12). doi: 10.1128/AEM.00416-17.

Bronowski, C., James, C. E. and Winstanley, C. (2014) ‘Role of environmental survival in transmission of *Campylobacter jejuni*’, *FEMS Microbiology Letters*, 356(1), pp. 8–19. doi: 10.1111/1574-6968.12488.

Bueno, I. *et al.* (2018) ‘Systematic review: Impact of point sources on antibiotic-resistant bacteria in the natural environment’, *Zoonoses and Public Health*, 65(1), pp. e162–e184. doi: 10.1111/zph.12426.

Bustin, S. A. *et al.* (2009) ‘The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments’, *Clinical Chemistry*, 55(4), pp. 611–622. doi: 10.1373/clinchem.2008.112797.

Cai, G. *et al.* (2021) ‘Spatiotemporal variation of bacterial communities in three cascade reservoirs in a southern city of China’, *Water Supply*, 21, pp. 2532–2542. doi: 10.2166/ws.2021.072.

Canciu, A. *et al.* (2021) ‘Modern Analytical Techniques for Detection of Bacteria in Surface and Wastewaters’, *Sustainability*, 13(7229), pp. 1–25. doi:i.org/10.3390/su13137229.

Cangelosi, G. A. and Meschke, J. S. (2014) ‘Dead or alive : Molecular assessment of microbial viability’, *Applied and Environmental Microbiology* 80(19), pp. 5884–5891. doi: 10.1128/AEM.01763-14.

Carter, P. E. *et al.* (2009) 'Novel clonal complexes with an unknown animal reservoir dominate *Campylobacter jejuni* isolates from river water in New Zealand', *Applied and Environmental Microbiology*, 75(19), pp. 6038–6046. doi: 10.1128/AEM.01039-09.

Chadborn, T. *et al.* (2023) 'An approach for embedding behavioural science in antimicrobial resistance One Health research', *Journal of Infection and Public Health*, 16, pp. 134–140. doi: 10.1016/j.jiph.2023.11.001.

Chala, G. *et al.* (2021) 'Identification and characterization of *Campylobacter* species in livestock , humans , and water in livestock owning households of peri-urban Addis Ababa , Ethiopia : A One Health approach', *Frontiers in Public Health*, 9, pp. 1–11. doi: 10.3389/fpubh.2021.750551.

Chaturvedi, P. *et al.* (2021) 'Dissemination of antibiotic resistance genes , mobile genetic elements , and ef fl ux genes in anthropogenically impacted riverine environments', *Chemosphere*, 273, p. 129693. doi: 10.1016/j.chemosphere.2021.129693.

Chen, I. and Dubnau, D. (2004) 'DNA uptake during bacterial transformation', *Nature Reviews Microbiology*, 2(242), pp. 1–9. doi: 10.1038/nrmicro844.

Chen, P. *et al.* (2014) '*Escherichia coli* strains isolated from multiple rivers in Southern', *Applied and Environmental Microbiology*, 82(6), pp. 1889–1897. doi: 10.1128/AEM.03222-15.

Cho, S. *et al.* (2019) 'Genetic characterization of antimicrobial-resistant *Escherichia coli* isolated from a mixed-use watershed in Northeast Georgia, USA', *International Journal of Environmental Research and Public Health*, 16(3761), pp. 1–14.

Cho, S., Jackson, C. R. and Frye, J. G. (2023) 'Freshwater environment as a reservoir of extended-spectrum β -lactamase-producing Enterobacteriaceae', *Journal of Applied Microbiology*, 134 , pp. 1–22.

Chukwu, M. O. *et al.* (2019) 'Characterization and phylogenetic analysis of *Campylobacter* species isolated from paediatric stool and water samples in the northwest province, south africa', *International Journal of Environmental Research and Public Health*, 16(12), pp. 1–22. doi: 10.3390/ijerph16122205.

Chukwu, M. O. *et al.* (2020) 'Mixed aetiology of diarrhoea in infants attending clinics in the North-West province of South Africa: potential for sub-optimal treatment', *Pathogens*, 9(3), pp. 1–18. doi: 10.3390/pathogens9030198.

CODEX (2006) 'CODEX alimentarius commission procedural manual Sixteenth edition', *Joint FAO/WHO Food Standards Programme*, pp. 1–167.

Antimicrobial Resistance Collaborators (2022) 'Global burden of bacterial antimicrobial resistance in 2019 : a systematic analysis', *Lancet*, 399, pp. 629–655. doi: 10.1016/S0140-6736(21)02724-0.

Connell, S. R. *et al.* (2003) 'Ribosomal protection proteins and their mechanism of tetracycline resistance.', *Antimicrobial agents and chemotherapy*, 47(12), pp. 3675–81. doi: 10.1128/aac.47.12.3675-3681.2003.

Conte, D. *et al.* (2017) 'Characterization of CTX-M enzymes, quinolone resistance determinants, and antimicrobial residues from hospital sewage, wastewater treatment plant, and river water', *Ecotoxicology and Environmental Safety*, 136, pp. 62–69. doi: 10.1016/j.ecoenv.2016.10.031.

Crespo, M. D. *et al.* (2016) 'Novel plasmid conferring kanamycin and tetracycline resistance in the turkey-derived *Campylobacter jejuni* strain 11601MD', *Plasmid*, 86, pp. 32–37. doi: 10.1016/j.plasmid.2016.06.001.

Culotti, A. and Packman, A. I. (2015) '*Pseudomonas aeruginosa* facilitates *Campylobacter jejuni* growth in biofilms under oxic flow conditions', *FEMS Microbiology Ecology*, 91(12). doi: 10.1093/femsec/fiv136.

Dabbousi, A. A. *et al.* (2022) 'High rates of macrolide and fluoroquinolone resistance in human campylobacteriosis in the Middle East and North Africa', *Future Microbiology*, (July). doi: 10.2217/fmb-2021-0302.

Dale, K. *et al.* (2010) 'Reported waterborne outbreaks of gastrointestinal disease in Australia are predominantly associated with recreational exposure', *Australian and New Zealand Journal of Public Health*, 34(5), pp. 527–530. doi: 10.1111/j.1753-6405.2010.00602.x.

Dalu, T. *et al.* (2018) 'River sediment metal and nutrient variations along an urban – agriculture gradient in an arid austral landscape : implications for environmental health', *Environmental Science and Pollution Research*, 25, pp. 2842–2852.

Denis, M., Tanguy, M., Chidaine, B., Laisney, M. J., *et al.* (2011) 'Description and sources of contamination by *Campylobacter spp.* of river water destined for human consumption in Brittany, France', *Pathologie Biologie*, 59(5), pp. 256–263. doi: 10.1016/j.patbio.2009.10.007.

Derakhshan, S., Navidinia, M. and Haghi, F. (2021) 'Antibiotic susceptibility of human-associated *Staphylococcus aureus* and its relation to agr typing , virulence genes , and biofilm formation', *BMC Infectious Diseases*, 21(627), pp. 1–10. doi: doi.org/10.1186/s12879-021-06307-0.

Department of Water Affairs and Forestry (DWAF). Resource directed management of water quality: Introduction, 2nd ed.; Water resource planning systems series, sub-series No. WQP 1.7.6. DWAF: Pretoria, South Africa, 2006; ISBN 0-621-36786-9.

Díaz-Gavidia, C. *et al.* (2021) 'Isolation of ciprofloxacin and Enterobacterales from vegetables and river water is strongly associated with the season and the sample type', *Frontiers in Microbiology*, 12, pp. 1–14. doi: 10.3389/fmicb.2021.604567.

Diergaardt, S. M. *et al.* (2004) 'The occurrence of *Campylobacter* in water sources in South Africa', *Water Research*, 38, pp. 2589–2595. doi: 10.1016/j.watres.2004.03.004.

Ding, D. *et al.* (2022) 'Effect of cattle farm exposure on oropharyngeal and gut microbial communities and antibiotic resistance genes in workers', *Science of the Total Environment*, 806(100), p. 150685. doi: 10.1016/j.scitotenv.2021.150685.

Djennad, A. *et al.* (2019) 'Seasonality and the effects of weather on *Campylobacter* infections', *BMC Infectious Diseases*, 19(1), pp. 1–10. doi: 10.1186/s12879-019-3840-7.

Dorevitch, S. *et al.* (2011) 'Water ingestion during water recreation', *Water Research*, 45(5), pp. 2020–2028. doi: 10.1016/J.WATRES.2010.12.006.

Dufour, A. P. *et al.* (2017) 'Ingestion of swimming pool water by recreational swimmers', *Journal of Water and Health*, 15(3), pp. 429–437. doi: 10.2166/WH.2017.255.

DWAF (1996) *Water Quality Guidelines Volume 8 Field Guide South African Water Quality*.

DWAF (2006) *Resource Directed Management of Water Quality: Introduction*.

Van Dyke, M. I. *et al.* (2010) 'The occurrence of *Campylobacter* in river water and waterfowl within a watershed in southern Ontario, Canada', *Journal of Applied Microbiology*, 109(3), pp. 1053–1066. doi: 10.1111/j.1365-2672.2010.04730.x.

Efstratiou, M. A. and Tsirtsis, G. (2009) 'Do 2006 / 7 / EC European Union bathing water standards exclude the risk of contact with *Salmonella* or *Candida albicans* ?', *Marine Pollution Bulletin*, 58(7), pp. 1039–1044. doi: 10.1016/j.marpolbul.2009.02.007.

Ekdahl, K. and Andersson, Y. (2004) 'Regional risks and seasonality in travel-associated campylobacteriosis.', *BMC infectious diseases*, 4(1), p. 54. doi: 10.1186/1471-2334-4-54.

Elmonir, W. *et al.* (2022) 'Survival capability of *Campylobacter upsaliensis* under environmental stresses', *BMC Research Notes*, 15(47). doi: 10.1186/s13104-022-05919-2.

Evers, E. G. *et al.* (2008) '*Campylobacter* source attribution by exposure assessment', *International Journal of Risk Assessment and Management*, 8. doi: 10.1504/IJRAM.2008.016151.

Eyles, R. *et al.* (2003) 'Spatial and temporal patterns of *Campylobacter* contamination underlying public health risk in the Taieri River, New Zealand', *Journal of Environmental Quality*, 32(5), pp. 1820–1828. doi: 10.2134/JEQ2003.1820.

Fernando, D. M. *et al.* (2016) 'Detection of antibiotic resistance genes in source and drinking water samples from a first nations community in Canada', *Applied and Environmental Microbiology*, 82(15), pp. 4767–4775. doi: 10.1128/AEM.00798-16.

Ficetola, G. F. and Miaud, C. (2008) 'Species detection using environmental DNA from water samples', *Biology letters*, 4, pp. 423–425. doi: 10.1098/rsbl.2008.0118.

Fouz, N. *et al.* (2020) 'The contribution of wastewater to the transmission of antimicrobial resistance in the environment : Implications of mass gathering settings', *Tropical Medicine and Infectious Disease*, 5(33). doi: 10.3390/tropicalmed5010033.

Fravalo, P. *et al.* (2011) 'Description and sources of contamination by *Campylobacter spp.* of river water destined for human consumption in Brittany , France ` res destine ´ e Description et origines de contamination par *Campylobacter spp*. d ' eau de rivie', 59, pp. 256–263. doi: 10.1016/j.patbio.2009.10.007.

Freitas, D. Y. *et al.* (2019) 'Extended spectrum gram-negative bacteria recovered from an Amazonian lake near the city of Belém , Brazil', *Frontiers in Microbiology*, 10. pp. 1–13. doi: 10.3389/fmicb.2019.00364.

Fuentes, M. D. *et al.* (2019) 'Assessment of antibiotic levels, multi-drug resistant bacteria and genetic biomarkers in the waters of the Rio Grande River between the United States-Mexico Border', 9(23), pp. 1–13.

Gaardbo Kuhn, K. *et al.* (2018) 'Determinants of sporadic *Campylobacter* infections in Denmark: a nationwide case-control study among children and young adults', *Clinical Epidemiology*, Volume 10, pp. 1695–1707. doi: 10.2147/CLEP.S177141.

Gahamanyi, N. *et al.* (2020) 'Prevalence, risk factors, and antimicrobial resistance profiles of thermophilic *Campylobacter* species in humans and animals in Sub-Saharan Africa: A systematic review', *International Journal of Microbiology*, 2020. doi: 10.1155/2020/2092478.

García, A., Fox, J. G. and Besser, T. E. (2010) 'Zoonotic Enterohemorrhagic Escherichia coli: A One Health Perspective', *ILAR Journal*, 51(3)

Gautron, J. M. C. *et al.* (2023) 'Using intersectionality to study gender and antimicrobial resistance in low- and middle-income countries', *Health Policy and Planning*, pp. 1–16. https://doi.org/https://doi.org/10.1093/heapol/czad054.

Gensberger, E. T. *et al.* (2014) 'Evaluation of quantitative PCR combined with PMA treatment for molecular assessment of microbial water quality', *Water Research*, 67(0), pp. 367–376. doi: 10.1016/j.watres.2014.09.022.

Gharbi, M. *et al.* (2022) 'Distribution of virulence and antibiotic resistance genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler chickens in Tunisia', *Journal of Microbiological methods*, 55, pp. 1273–1282. doi: 10.1016/j.jmii.2021.07.001.

Ghunaim, H. *et al.* (2015) ‘Analysis of resistance to antimicrobials and presence of virulence/stress response genes in *Campylobacter* isolates from patients with severe diarrhoea’, *PLoS ONE*, 10(3), pp. 1–16. doi: 10.1371/journal.pone.0119268.

Gibreel, A. *et al.* (2004) ‘Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002 , with special reference to *tet* (*O*) -Mediated tetracycline resistance’, *Antimicrobial Agents and Chemotherapy*, 48(9), pp. 3442–3450. doi: 10.1128/AAC.48.9.3442.

Gilpin, B. J. *et al.* (2020) ‘A large scale waterborne Campylobacteriosis outbreak, Havelock North, New Zealand’, *Journal of Infection*, 81(3), pp. 390–395. doi: 10.1016/j.jinf.2020.06.065

Golpayegani, A. *et al.* (2019) ‘Propidium monoazide-quantitative polymerase chain reaction (PMA-qPCR) assay for rapid detection of viable and viable but non-culturable (VBNC) *Pseudomonas aeruginosa* in swimming pools’, *Journal of Environmental Health Science and Engineering*, 17(1), pp. 407–416. doi: 10.1007/s40201-019-00359-w.

Gorham, T. J. and Lee, J. (2016) ‘Pathogen loading from Canada geese faeces in freshwater: potential risks to human health through recreational water exposure’, *Zoonoses and Public Health*, 63(3), pp. 177–190. doi: 10.1111/zph.12227.

Gothwal, R. and Shashidhar, T. (2015) ‘Antibiotic pollution in the environment: A review’, *Clean Soil, Air,Water*, 43(4). doi: 10.1002/clen.201300989.

Goulart, D. B. *et al.* (2022) ‘Effect of danofloxacin treatment on the development of fluoroquinolone Resistance in *Campylobacter jejuni* in calves’, *Antibiotics*, 11.

Gqomfa, B., Maphanga, T. and Shale, K. (2022) ‘The impact of informal settlement on water quality of Diep River in Dunoon’, *Sustainable Water Resources Management*, 8(1), pp. 1–18. doi: 10.1007/s40899-022-00629-w.

Gu, G. *et al.* (2013) ‘Occurrence and population density of *Campylobacter jejuni* in irrigation ponds on produce farms in the Suwannee River watershed’, *Canadian Journal of Microbiology*, 59(5), pp. 339–346. doi: 10.1139/cjm-2013-0027.

Guo, X. *et al.* (2022) ‘Multi-media occurrence of antibiotics and antibiotic resistance genes in East Dongting Lake’, 10, pp. 1–12. doi: 10.3389/fenvs.2022.866332.

Guzman-otazo, J. *et al.* (2019) ‘Diarrheal bacterial pathogens and multi- resistant enterobacteria in the Choqueyapu River in La Paz , Bolivia’, *PLoS ONE*, 14(1), pp. 1–20.

Haas, C. N. (2020) ‘Quantitative microbial risk sssessment and molecular biology: Paths to integration’, *Environmental Science and Technology*, 54(14), pp. 8539–8546. doi: 10.1021/acs.est.0c00664.

Haberecht, H. B. *et al.* (2019) 'Antimicrobial-resistant *Escherichia coli* from environmental waters in Northern Colorado', *Journal of Environmental and Public Health*, pp. 1–13.

Hamandawana, H., Atyosi, Y. and Bornman, T. G. (2020) 'Multi-temporal reconstruction of long-term changes in land cover in and around the Swartkops River Estuary , Eastern', *Environmental Monitoring and Assessment*, 192(173), pp. 1–14.

Hamiwe, T. *et al.* (2019) 'Occurrence of *Enterococci* harbouring clinically important antibiotic resistance genes in the aquatic environment in Gauteng, South Africa', *Environmental Pollution*, 245, pp. 1041–1049. doi: 10.1016/j.envpol.2018.11.040.

Haque, A. *et al.* (2014) 'ESBL-producing Enterobacteriaceae in environmental water in Dhaka ,Bangladesh', *Journal of Infection and Chemotherapy*, 20(11), pp. 735–737. doi: 10.1016/j.jiac.2014.07.003.

Harder-Lauridsen, N. M. *et al.* (2013) 'Gastrointestinal illness among triathletes swimming in non-polluted versus polluted seawater affected by heavy rainfall, Denmark, 2010-2011', *PLoS ONE*. 8(11), p. e78371. doi: 10.1371/journal.pone.0078371.

Hassen, B. *et al.* (2020) 'Genetic characterization of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from wastewater and river water in Tunisia : predominance of CTX-M-15 and high genetic diversity', pp. 44368–44377.

Hazeleger, W. C., Jacobs-reitsma, W. F. and Besten, H. M. W. Den (2016) 'Quantification of growth of *Campylobacter* and Extended Spectrum β -Lactamase producing bacteria sheds light on black box of enrichment procedures', *Frontiers in Microbiology*, 7, pp. 1–9. doi: 10.3389/fmicb.2016.01430.

Haziq, M. A. and Panezai, S. (2017) 'An empirical analysis of domestic water sources, consumption and associated factors in Kandahar City, Afghanistan', *Resources and Environment*, 7(2), pp. 49–61. doi: 10.5923/j.re.20170702.03.

Henton, M. M. *et al.* (2011) 'Part VI. Antibiotic management and resistance in livestock production', *South African Medical Journal*, 101(8), pp. 583–586.

Hilton, C. L. *et al.* (2001) 'The recovery of *Arcobacter butzleri* NCTC 12481 from various temperature treatments', *Journal of Applied Microbiology*, 91(5), pp. 929–932. doi: 10.1046/j.1365-2672.2001.01457.x.

Hoefnagels, N., Margaret, P. and Sinenhlanhla, I. (2022) 'Makhanda : Exploring the mise-en-scène of a city under threat', *Urban Forum*,. doi: 10.1007/s12132-022-09467-7.

Hokajärvi, A. *et al.* (2013) 'Occurrence of thermotolerant *Campylobacter spp.* and adenoviruses in Finnish bathing waters and purified sewage effluents', pp. 120–134. doi: 10.2166/wh.2012.192.

Van den Honert, M. S., Gouws, P. A. and Hoffman, L. C. (2018) ‘Importance and implications of antibiotic resistance development in livestock and wildlife farming in South Africa: A Review’, *South African Journal of Animal Sciences*, 48(3), pp. 401–412. doi: 10.4314/sajas.v48i3.1.

Hoven, C. Van Der *et al.* (2017) ‘The impact of various land uses on the microbial and physicochemical quality of surface water bodies in developing countries : Prioritisation of water resources management areas’, *Environmental Nanotechnology, Monitoring & Management*, 8, pp. 280–289. doi: 10.1016/j.enmm.2017.10.006.

Hu, T. and Kuo, P. (2011) ‘Isolation of *Campylobacter spp* in surface waters of Taiwan’, *Journal of Microbiology, Immunology and Infection*, 44(1), pp. 15–20. doi: 10.1016/j.jmii.2011.01.004.

Hu, Y. *et al.* (2013) ‘Molecular typing of CTX-M-producing *Escherichia coli* isolates from environmental water , swine feces , specimens from healthy humans , and human patients’, *Applied and Environmental Microbiology*, 79(20), pp. 5988–5996. doi: 10.1128/AEM.01740-13.

Hubeny, J. *et al.* (2021) ‘Industrialization as a source of heavy metals and antibiotics which can enhance the antibiotic resistance in wastewater, sewage sludge and river water’, *PLoS ONE*, pp. 1–24. doi: 10.1371/journal.pone.0252691.

Hull, D. M. *et al.* (2021) ‘Antimicrobial resistance and interspecies gene transfer in *Campylobacter coli* and *Campylobacter jejuni* isolated from food animals, poultry processing, and retail meat in North Carolina, 2018–2019’, *PLoS ONE*, 16, pp. 1–23. doi: 10.1371/journal.pone.0246571.

Hung, P. Van *et al.* (2011) ‘Genetic relatedness and identification of clinical strains of genus Campylobacter based on dnaJ , 16S rRNA , groEL , and rpoB gene sequences’, *Microbiology Culture Collection*, 27(1), pp. 1–12.

Hyllestad, S. *et al.* (2020) ‘Large waterborne *Campylobacter* outbreak: Use of multiple approaches to investigate contamination of the drinking water supply system, Norway, June 2019’, *Eurosurveillance*, 25(35), pp. 1–10. doi: 10.2807/1560-7917.ES.2020.25.35.2000011.

Igwaran, A. and Okoh, A. I. (2019) ‘Human campylobacteriosis: A public health concern of global importance’, *Heliyon*, 5(11), p. e02814. doi: 10.1016/j.heliyon.2019.e02814.

Igwaran, A. and Okoh, Anthony I. (2020) ‘Campylobacteriosis agents in meat carcasses collected from two district municipalities in the Eastern Cape province, South Africa’, *Foods*, 9(2), pp. 1–18. doi: 10.3390/foods9020203.

Igwaran, A. and Okoh, Anthony Ifeanyi (2020) ‘Occurrence, virulence and antimicrobial resistance-associated markers in *Campylobacter* species isolated from retail fresh milk and water samples in two district municipalities in the Eastern Cape Province, South Africa', *Antibiotics*, 9(7), pp. 1–18. doi: 10.3390/antibiotics9070426.

Iovine, N. M. (2013) 'Resistance mechanisms in *Campylobacter jejuni*', *Virulence*, 4(3), pp. 230–240. doi: 10.4161/viru.23753.

Irfan, M. and Almotiri, A. (2022) 'Antimicrobial resistance and its drivers — A review', *Antibiotics*, 11(1362). doi: 10.3390/ antibiotics11101362.

Iwu, C. D., Korsten, L. and Okoh, A. I. (2020) 'The incidence of antibiotic resistance within and beyond the agricultural ecosystem: A concern for public health', *Microbiology Open*, 9(9), pp. 1–28. doi: 10.1002/mbo3.1035.

Iwu, D. C. and Patrick, M. S. (2021) 'An insight into the implementation of the global action plan on antimicrobial resistance in the WHO African region : A roadmap for action', *International Journal of Antimicrobial Agents*, 58(4), p. 106411. doi: 10.1016/j.ijantimicag.2021.106411.

Jang, J. *et al.* (2013) 'Pathogenic *Escherichia coli* Strains Producing Extended-Spectrum β - Lactamases in the Yeongsan River Basin of South Korea', *Environmental Science and Technology*, 47, pp. 1128–1136. .doi.org/10.1021/es303577u

Janssen, R. *et al.* (2008) 'Host-pathogen interactions in *Campylobacter* infections: The host perspective', *Clinical Microbiology Reviews*, 21(3), pp. 505–518. doi: 10.1128/CMR.00055-07.

Jerde, C. L. *et al.* (2016) 'Influence of stream bottom substrate on retention and transport of vertebrate environmental DNA', *Environmental Science and Technology*, 50, pp. 8770–8779. doi: 10.1021/acs.est.6b01761.

Johnson, A. *et al.* (2020) 'Extended-spectrum beta-lactamase (ESBL)-positive *Escherichia coli* presence in urban aquatic environments in Kanpur, India', *Journal of Water and Health*, 18(5), pp. 849–854. doi: 10.2166/wh.2020.065.

Jokinen, C. *et al.* (2011) 'Molecular subtypes of *Campylobacter spp.*, *Salmonella enterica*, and *Escherichia coli* O157:H7 isolated from faecal and surface water samples in the Oldman River watershed, Alberta, Canada', *Water research*, 45(3), pp. 1247–1257. doi: 10.1016/J.WATRES.2010.10.001.

Jokinen, C. C. *et al.* (2012) 'An enhanced technique combining pre-enrichment and passive filtration increases the isolation efficiency of *Campylobacter jejuni* and *Campylobacter coli* from water and animal fecal samples', *Journal of Microbiological Methods*, 91(3), pp. 506–513. doi: 10.1016/j.mimet.2012.09.005.

Kaakoush, N. O. *et al.* (2015) ‘Global epidemiology of *Campylobacter* infection’, *Clinical Microbiology Reviews*, 28(3), pp. 687–720. doi: 10.1128/CMR.00006-15.

Karama, M. *et al.* (2020) ‘Occurrence and antimicrobial resistance profiles of *Campylobacter jejuni, Campylobacter coli*, and *Campylobacter upsaliensis* in beef cattle on cow-calf operations in South Africa’, *Foodborne Pathogens and Disease*, 17(7), pp. 440–446. doi: 10.1089/fpd.2019.2703.

Karikari, A. B. *et al.* (2016) ‘Occurrence and susceptibility patterns of *Campylobacter* isolated from environmental water sources’, *African Journal of Microbiology Research*, 10(37), pp. 1576–1580. doi: 10.5897/AJMR2016.8296.

Kariuki, S. *et al.* (2022) ‘Antimicrobial Resistance Rates and Surveillance in Sub-Saharan Africa : Where Are We Now ?’, *Infection and Drug Resistance*, 15, pp. 3589–3609. doi: doi.org/10.2147/IDR.S342753.

Kashappanavar, S. R. *et al.* (2018) ‘Comparision of five different enrichment broth-agar combinations for the isolation of *Campylobacter jejuni*’, *International Journal of Livestock Research*, 8(6), pp. 102–112. doi: 10.5455/ijlr.20171123092351.

Kashoma, I. P. *et al.* (2016) ‘Prevalence and antimicrobial resistance of *Campylobacter* isolated from dressed beef carcasses and raw milk in Tanzania’, *Microbial Drug Resistance*, 22(1), pp. 40–52. doi: 10.1089/mdr.2015.0079.

Kayman, T. *et al.* (2019) ‘Antibiotic resistance of *Campylobacter jejuni* isolates recovered from humans with diarrhoea in Turkey’, *Journal of Medical Microbiology*, 68, pp. 136–142. doi: 10.1099/jmm.0.000890.

Khan, I. U. H. *et al.* (2014) ‘A national investigation of the prevalence and diversity of thermophilic *Campylobacter* species in agricultural watersheds in Canada’, *Water Research*, 61, pp. 243–252. doi: 10.1016/j.watres.2014.05.027.

Khatri, N. and Tyagi, S. (2015) ‘Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas’, *Frontiers in Life Science*, 8(1), pp. 23–39. doi: 10.1080/21553769.2014.933716.

Kim, J. *et al.* (2016) ‘An improved culture method for selective isolation of *Campylobacter jejuni* from wastewater’, *Frontiers in Life Science*, 7, pp. 1–8. doi: 10.3389/fmicb.2016.01345.

Kim, Jong-chul *et al.* (2015) ‘Regulation of oxidative stress resistance in *Campylobacter jejuni* ,a microaerophilic foodborne pathogen’, *Frontiers in Microbiology*, 6, pp. 1–12. doi: 10.3389/fmicb.2015.00751.

Kimera, Z. I. *et al.* (2020) ‘Assessment of drivers of antimicrobial use and resistance in poultry and domestic pig farming in the Msimbazi river basin in Tanzania’, *Antibiotics*, 9(12), pp. 1–

20. doi: 10.3390/antibiotics9120838.

Koch, N. *et al.* (2021) 'Environmental antibiotics and resistance genes as emerging contaminants : Methods of detection and bioremediation', *Current Research in Microbial Sciences*, 2, p. 100027. doi: 10.1016/j.crmicr.2021.100027.

Konkel, M. E. *et al.* (1999) 'Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product', 37(3), pp. 510–517.

Kovač, J. *et al.* (2014) 'The evidence for clonal spreading of quinolone resistance with a particular clonal complex of *Campylobacter jejuni*', *Epidemiology and Infection*, 142(12), pp. 2595–2603. doi: 10.1017/S0950268813003245.

Kovanen, S. *et al.* (2016) 'Tracing isolates from domestic human *Campylobacter jejuni* infections to chicken slaughter batches and swimming water using whole-genome multilocus sequence typing', *International Journal of Food Microbiology*, 226, pp. 53–60. doi: 10.1016/j.ijfoodmicro.2016.03.009.

Kralik, P. and Ricchi, M. (2017) 'A basic guide to real time PCR in microbial diagnostics: Definitions, parameters, and everything', *Frontiers in Microbiology*, 8, pp. 1–9. doi: 10.3389/fmicb.2017.00108.

Kruger, A. C. and Nxumalo, M. (2017) 'Surface temperature trends from homogenized time series in South Africa : 1931 – 2015', *International Journal of Climatology*, 37, pp. 2364–2377. doi: 10.1002/joc.4851.

Kurincic, M., Klancnik, A. and Mozina, S. S. (2012) 'Effects of Efflux Pump Inhibitors on', *Microbial Drug Resistance*, 18(5), pp. 492–501. doi: 10.1089/mdr.2012.0017.

Kusi, J., Ojewole, C. O. and Ojewole, A. E. (2022) 'Antimicrobial resistance development pathways in surface waters and public health implications', *Antibiotics*, 11(821), pp. 1–22.

Lanzl, M. I. *et al.* (2020) 'Variability in lag-duration of *Campylobacter spp.* during enrichment after cold and oxidative stress and its impact on growth kinetics and reliable detection', *Food Research International*, 134, p. 109253. doi: 10.1016/j.foodres.2020.109253.

Lapara, T. M. *et al.* (2015) 'Multiple discharges of treated municipal wastewater have a small effect on the quantities of numerous antibiotic resistance determinants in the Upper Mississippi River', *Environmental Science and Technology*, 49, pp. 11509–11515. doi: 10.1021/acs.est.5b02803.

Larsson, D. G. J. and Flach, C. F. (2022) 'Antibiotic resistance in the environment', *Nature Reviews Microbiology*, 20(5), pp. 257–269. doi: 10.1038/s41579-021-00649-x.

Leila, S. Ben *et al.* (2016) 'Characteristics of extended-spectrum β -lactamase (ESBL) - and pAmpC beta-lactamase-producing Enterobacteriaceae of water samples in Tunisia', *Science of* *the Total Environment,* 550, pp. 1103–1109. doi: 10.1016/j.scitotenv.2016.01.042.

Leonard, A. F. C. *et al.* (2018) ‘A coliform-targeted metagenomic method facilitating human exposure estimates to *Escherichia coli*-borne antibiotic resistance genes’, *FEMS Microbiology Ecology*, 94(3). doi: 10.1093/femsec/fiy024.

Lévesque, S. *et al.* (2011) ‘Determination of the optimal culture conditions for detecting thermophilic *Campylobacters* in environmental water’, *Journal of Microbiological Methods*, 86(1), pp. 82–88. doi: 10.1016/j.mimet.2011.04.001.

Li, D. *et al.* (2014) ‘Quantification of viable bacteria in wastewater treatment plants by using propidium monoazide combined with quantitative PCR (PMA-qPCR)’, *Journal of Environmental Sciences (China)*, 26(2), pp. 299–306. doi: 10.1016/S1001-0742(13)60425-8.

Li, H., Xin, H. and Li, S. F. Y. (2015) ‘Multiplex PMA-qPCR assay with internal amplification control for simultaneous detection of viable *Legionella pneumophila*, *Salmonella typhimurium*, and *Staphylococcus aureus* in environmental waters’, *Environmental Science and Technology*, 49(24), pp. 14249–14256. doi: 10.1021/acs.est.5b03583.

Li, L. *et al.* (2020) ‘Municipal solid waste treatment system increases ambient airborne bacteria and antibiotic resistance genes’, *Environmental Science and Technology*, 54(7), pp. 3900–3908. doi: 10.1021/ACS.EST.9B07641.

Li, N. *et al.* (2020) ‘Airborne antibiotic resistance genes in Hong Kong kindergartens’, *Environmental Pollution*, 260, p. 114009. doi: 10.1016/J.ENVPOL.2020.114009.

Li, X. *et al.* (2020) ‘Antimicrobial resistance risk assessment models and database system for animal-derived pathogens’, *Antibiotics*, 9829, pp. 1–16. doi: 10.3390/antibiotics9110829.

Lin, J., Michel, L. O. and Zhang, Q. (2002) ‘CmeABC Functions as a multidrug efflux system in *Campylobacter jejuni*’, *Antimicrobial Agents and Chemotherapy*, 46(7), pp. 2124–2131. doi: 10.1128/AAC.46.7.2124.

Linton, D., Owen, R. J. and Stanley, J. (1996) ‘Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals’, *Research in Microbiology*, 147(9), pp. 707–718. doi: 10.1016/s0923-2508(97)85118-2.

Liu, F. *et al.* (2022) ‘Global epidemiology of campylobacteriosis and the impact of COVID-19’, *Frontiers in Cellular and Infection Microbiology*, 12(979055).
doi: 10.3389/fcimb.2022.979055.

Lluque, A. *et al.* (2017) ‘Virulence and antimicrobial resistance in *Campylobacter spp.* from a Peruvian Pediatric Cohort’, *Scientifica*, pp. 1–8. doi: doi.org/10.1155/2017/7848926.

Louis, V. R. *et al.* (2005) ‘Temperature-driven *Campylobacter* seasonality in England and ales.’, *Applied and environmental microbiology*, 71(1), pp. 85–92. doi: 10.1128/AEM.71.1.85-

92.2005

Luangtongkum, T. *et al.* (2009) ‘Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence’, *Future Microbiology*, 4(2), pp. 189–200. doi: 10.2217/17460913.4.2.189.

Luvhimbi, N. *et al.* (2022) ‘Water quality assessment and evaluation of human health risk of drinking water from source to point of use at Thulamela municipality, Limpopo Province’, *Scientific Reports*, , pp. 1–17. doi: 10.1038/s41598-022-10092-4.

MacKellar, N., New, M. and Jack, C. (2014) ‘Climate trends in South Africa observed and modelled trends in rainfall and temperature for South Africa : 1960 – 2010’, *South African Journal of Science*, 110 (7), pp. 1–13.

Magana-Arachchi, D. N. and Wanigatunge, R. P. (2020) "Ubiquitous waterborne pathogens" *Waterborne Pathogens*. Elsevier. doi: 10.1016/b978-0-12-818783-8.00002-5.

Mahlalela, P. T. *et al.* (2020) ‘Drought in the Eastern Cape region of South Africa and trends in rainfall characteristics’, *Climate Dynamics*, 55(9–10), pp. 2743–2759. doi: 10.1007/s00382-020-05413-0.

Makowska, N., Koczura, R. and Mokracka, J. (2016) ‘Class 1 integrase, sulfonamide and tetracycline resistance genes in wastewater treatment plant and surface water’, *Chemosphere*, 144, pp. 1665–1673. doi: 10.1016/j.chemosphere.2015.10.044.

Manaia, C. M. (2017) ‘Assessing the risk of antibiotic resistance transmission from the environment to humans: Non-direct proportionality between abundance and risk’, *Trends in Microbiology*, 25(3), pp. 173–181. doi: 10.1016/j.tim.2016.11.014.

Manderson, L. (2020) ‘Prescribing, care and resistance: antibiotic use in urban South Africa’, *Humanities and Social Sciences Communications*, 7(1), pp. 1–10. doi: 10.1057/s41599-020-00564-1.

Mangadze, T. *et al.* (2019) ‘Water quality assessment in a small austral temperate river system (Bloukrans River system, South Africa): Application of multivariate analysis and diatom indices’, *South African Journal of Botany*, 125, pp. 353–359. doi: 10.1016/j.sajb.2019.08.008.

Masiya, T. *et al.* (2019) ‘Theoretical and empirical researches in urban managementt’, *Theoretical and Empirical Researches in Urban Management*, 14(2), pp. 20–40.

Maugeri, A. *et al.* (2023) ‘Socio-economic, governance and health indicators shaping antimicrobial resistance: an ecological analysis of 30 European countries’, *Globalization and Health*, pp. 1–12. doi: 10.1186/s12992-023-00913-0.

Mboweni, T. J. and De Crom, E. P. (2016) ‘A narrative interpretation of the cultural impressions on water of the communities along the Vaal River, Parys, Free State', *The Journal for Transdisciplinary Research in Southern Africa*, 12(1), pp. 1–7. doi: 10.4102/td.v12i1.345.

Mendonça, E. P. *et al.* (2015) 'Campylobacteriosis: an emerging zoonosis, underdiagnosed and underreported by public health agencies in Brazil', *Bioscience Journal*, 31(5), pp. 1458–1474. doi: 10.14393/bj-v31n1a2015-27524.

Meurer, L., Payne, W. and Guffey, J. S. (2020) 'Visible light as an inhibitor of *Campylobacterjejuni*', *International Journal of Antimicrobial Agents*, 55(1), p. 105818. doi:10.1016/j.ijantimicag.2019.09.022

Mileng, K. *et al.* (2021) 'Isolation and antibiotic sensitivity of *Campylobacter* species from fecal samples of broiler chickens in North West Province, South Africa', *Veterinary World*, 14(11), pp. 2929–2935. doi: 10.14202/vetworld.2021.2929-2935.

Minssen, T. *et al.* (2020) 'Social, cultural and economic aspects of antimicrobial resistance', *Bulletin of the World Health Organization*, 98(12), pp. 823–82A.doi: 10.2471/BLT.20.275875.

Molobela, P. I. and Pramod, S. (2011) 'Management of water resources in South Africa: A review', *African Journal of Environmental Science and Technology*, 5(12), pp. 993–1002. doi: 10.5897/ajest11.136.

Moore, J., Caldwell, P. and Millar, B. (2001) 'Molecular detection of *Campylobacter spp*. in drinking, recreational and environmental water supplies', *International Journal of Hygiene and Environmental Health*, 204(2–3), pp. 185–189. doi: 10.1078/1438-4639-00096.

Moore, J. E. *et al.* (2001) 'Occurrence of *Campylobacter spp*. in water in Northern Ireland : implications for public health', *The Ulster Medical Journal*, 70(2), pp. 102–107.

Moore, J. E. *et al.* (2006) 'The epidemiology of antibiotic resistance in *Campylobacter*', *Microbes and Infection*, 8, pp. 1955–1966. doi: 10.1016/j.micinf.2005.12.030.

Morpeth, S. C. *et al.* (2014) 'Making standards for quantitative real-time pneumococcal PCR', *Biomolecular Detection and Quantification*, 2, pp. 1–3. doi: 10.1016/j.bdq.2014.11.003.

Mortensen, N. *et al.* (2021) 'Characteristics of hospitalized patients during a large waterborne outbreak of *Campylobacter jejuni* in Norway', *PLoS ONE*, 16, pp. 1–11. doi: 10.1371/journal.pone.0248464

Moyo, P. *et al.* (2023) 'Prevention of antimicrobial resistance in sub-Saharan Africa: What has worked? What still needs to be done?', *Journal of Infection and Public Health*, 16(4), pp. 632–639. doi: 10.1016/j.jiph.2023.02.020.

Mpondo, L., Ebomah, K. E. and Okoh, A. I. (2021) 'Multidrug-resistant *Listeria* species shows abundance in environmental waters of a key district municipality in South Africa', *International Journal of Environmental Research and Public Health*, 18(2), pp. 1–12. doi:

10.3390/ijerph18020481.

Mughini-Gras, L. *et al.* (2016) ‘Quantifying potential sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*’, *Water Research*, 101, pp. 36–45. doi: 10.1016/j.watres.2016.05.069.

Mughini Gras, L. *et al.* (2012) ‘Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: A combined case-control and source attribution analysis’, *PLoS ONE*, 7(8). doi: 10.1371/journal.pone.0042599.

Mulder, A. C. *et al.* (2021) ‘Tracing the animal sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*’, *Water Research*, 187(2020), p. 116421. doi: 10.1016/j.watres.2020.116421.

Mupfunya, C. R., Qekwana, D. N. and Naidoo, V. (2021) ‘Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga , South Africa’, *Veterinary Medicine Science*, 7, pp. 112–121. doi: 10.1002/vms3.334

Muraleedharan, C. *et al.* (2019) ‘Occurrence of extended-spectrum β -lactamase-producing bacteria in urban Clinton River habitat’, *Journal of Global Antimicrobial Resistance*, 16, pp. 225–235. doi: 10.1016/j.jgar.2018.10.007.

Musa, K. *et al.* (2023) ‘Laboratory surveillance, quality management, and its role in addressing antimicrobial resistance in Africa : A narrative review’, *Annals of the New York Academy of Sciences*, 12(1313), pp. 1–12.

Nakayama, T. *et al.* (2017) ‘Water metagenomic analysis reveals low bacterial diversity and the presence of antimicrobial residues and resistance genes in a river containing wastewater from backyard aquacultures in the Mekong’, *Environmental Pollution*, 222, pp. 294–306. doi: 10.1016/j.envpol.2016.12.041.

Navarro-Gonzalez, N. *et al.* (2014) ‘*Campylobacter* shared between free-ranging cattle and sympatric wild ungulates in a natural environment (NE Spain)’, *EcoHealth*, 11, pp. 333–342. doi: 10.1007/s10393-014-0921-3.

Nelson, J. M. *et al.* (2007) ‘Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry : A public health success story’, *Clinical Infectious Diseases*, 44, pp. 977–980. doi: 10.1086/512369.

Ngqwala, N. P. and Muchesa, P. (2020) ‘Occurrence of pharmaceuticals in aquatic environments : A review and potential impacts in South Africa’, *South African Journal of Science*, 116(7), pp. 1–7. doi.org/10.17159/ sajs.2020/5730

Njage, P. M. K. and Buys, E. M. (2015) ‘Pathogenic and commensal *Escherichia coli* from irrigation water show potential in transmission of extended spectrum and AmpC β-lactamases determinants to isolates from lettuce', *Microbial Biotechnology*, 8(3), pp. 462–473. doi: 10.1111/1751-7915.12234.

Nkosi, M., Mathivha, F. I. and Odiyo, J. O. (2021) 'Impact of Land Management on Water Resources , a South African Context', *Sustainability*, 13(701). doi: doi.org/ 10.3390/su13020701.

Nnadozie, C. F. and Ngoni, N. (2023) 'A method of recovering the very low concentration of pathogens in river water by combining centrifugation and membrane filtration', *MethodsX*, 11, p. 102291. doi: 10.1016/j.mex.2023.102291.

Nnadozie, C. F. and Odume, O. N. (2019) 'Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes', *Environmental Pollution*, 254, p. 113067. doi: 10.1016/j.envpol.2019.113067.

Nocker, A. *et al.* (2007) 'Use of propidium monoazide for live/dead distinction in microbial ecology', *Applied and Environmental Microbiology*, 73(16), pp. 5111–5117. doi: 10.1128/AEM.02987-06.

Nocker, A., Cheung, C. and Camper, A. K. (2006) 'Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs . dead bacteria by selective removal of DNA from dead cells', *Journal of Microbiological methods*, 67, pp. 310–320. doi: 10.1016/j.mimet.2006.04.015.

Nolan, T. M. *et al.* (2023) 'Land use as a critical determinant of faecal and antimicrobial resistance gene pollution in riverine systems', *Science of the Total Environment*, 871, p. 162052. doi: 10.1016/j.scitotenv.2023.162052.

Nomamiukor, B. O. *et al.* (2015) 'Living conditions are associated with increased antibiotic resistance in community isolates of *Escherichia coli*', *Journal of Antimicrobial Chemotherapy*, 70, pp. 3154–3158. doi: 10.1093/jac/dkv229.

NRMMC-EPHC-AHMC (2006) 'Australia guidelines for water recycling: Managing health and environmental risks (Phase 1)', *National Water Quality Management Strategy*, p. 415.

Nwabor, O. *et al.* (2016) 'Water and waterborne diseases : A review', *International Journal of Tropical Disease & Health*, 12(4), pp. 1–14. doi: 10.9734/IJTDH/2016/21895.

Nylen, G. *et al.* (2002) 'The seasonal distribution of *Campylobacter* infection in nine European countries and New Zealand.', *Epidemiology and infection*, 128(3), pp. 383–90. doi: 10.1017/s0950268802006830.

Nzima, B. *et al.* (2020) 'Resistotyping and extended-spectrum beta-lactamase genes among *Escherichia coli* from wastewater treatment plants and recipient surface water for reuse in South Africa', *New Microbes and New Infections*, 38, p. 100803.

doi:10.1016/j.nmni.2020.100803

O’Flaherty, E. *et al.* (2018) ‘Human exposure assessment to antibiotic-resistant *Escherichia coli* through drinking water’, *Science of the Total Environment*, 616–617, pp. 1356–1364. doi: 10.1016/j.scitotenv.2017.10.180.

O’Flaherty, E. *et al.* (2019) ‘The potential human exposure to antibiotic resistant-*Escherichia coli* through recreational water’, *Science of the Total Environment*, 650, pp. 786–795. doi: 10.1016/j.scitotenv.2018.09.018.

O’Flaherty, E. and Cummins, E. (2017) ‘Antibiotic resistance in surface water ecosystems: Presence in the aquatic environment, prevention strategies, and risk assessment’, *Human and Ecological Risk Assessment*, 23(2), pp. 299–322. doi: 10.1080/10807039.2016.1247254.

Obi, C. L. *et al.* (2004) ‘Profiles of antibiotic susceptibilities of bacterial isolates and physico-chemical quality of water supply in rural Venda communities , South Africa’, *Water SA*, 30(4), pp. 515–520.

Odiyo, J. O. and Makungo, R. (2012) ‘Water quality problems and management in rural areas of Limpopo Province, South Africa’, *Water Pollution*, 164, pp. 135–146. doi:10.2495/WP120121

Odjadjare, E. E. *et al.* (2012) ‘Prevalence of multiple antibiotics resistant (MAR) *Pseudomonas* species in the final effluents of three municipal wastewater treatment facilities in South Africa’, pp. 2092–2107. doi: 10.3390/ijerph9062092.

Odume, O. N. *et al.* (2012) ‘The impact of water quality deterioration on macroinvertebrate communities in the Swartkops River , South Africa : a multimetric approach ’, *African Journal of Aquatic Science*, 37(2), pp. 191–200. doi: 10.2989/16085914.2012.670613.

Odume, O. N. *et al.* (2022) ‘Governance and institutional drivers of ecological degradation in urban river ecosystems : Insights from case studies in African cities’, *Sustainability*, 14(14147). https://doi.org/10.3390/ su142114147

Ogunlaja, A. *et al.* (2022) ‘Antibiotic resistomes and their chemical residues in aquatic environments in Africa’, *Environmental Pollution*, 312, p. 119783. doi: 10.1016/j.envpol.2022.119783.

Okeyo, A. N. *et al.* (2018) ‘Vibrio species in wastewater final effluents and receiving watershed in South Africa: Implications for public health’, *International Journal of Environmental Research and Public Health*, 15(6). doi: 10.3390/IJERPH15061266.

On, S. L. W. *et al.* (2017) ‘Minimal standards for describing new species belonging to the families campylobacteraceae and helicobacteraceae ’:, pp. 5296–5311. doi:10.1099/ijsem.0.002255

Ortega-Paredes, D. *et al.* (2020) ‘High quantities of multidrug-resistant *Escherichia coli* are present in the Machángara urban river in Quito, Ecuador’, *Journal of Water and Health*, 18.1, pp. 67–76. doi: 10.2166/wh.2019.195.

Osiemo, M. M., Ogendi, G. M. and Erimba, C. M. (2019) ‘Microbial quality of drinking water and prevalence of water-related diseases in Marigat urban centre, Kenya’. doi: 10.1177/1178630219836988.

Otigbu, A. C. *et al.* (2018) ‘Antibiotic sensitivity profiling and virulence potential of *Campylobacter Jejuni* isolates from estuarine water in the Eastern Cape Province, South Africa’, *International Journal of Environmental Research and Public Health*, 15(5). doi: 10.3390/ijerph15050925.

Ouko, T. T. *et al.* (2021) ‘Prevalence and risk factors associated with *Campylobacter* infection in diarrheal patients in Busia County, Kenya’, pp. 657–680. doi: 10.4236/aim.2021.1111048.

Páll, E. *et al.* (2013) ‘Human impact on the microbiological water quality of the rivers’, *Journal of Medical Microbiology*, 62, pp. 1635–1640. doi: 10.1099/jmm.0.055749-0.

Park, S. H. *et al.* (2011) ‘Multiplex PCR assay for the detection and quantification of *Campylobacter spp*., *Escherichia coli* O157:H7, and *Salmonella* serotypes in water samples’, *FEMS Microbiology Letters*, 316(1), pp. 7–15. doi: 10.1111/j.1574-6968.2010.02188.x.

Paruch, L., Paruch, A. M. and Sørheim, R. (2020) ‘DNA-based faecal source tracking of contaminated drinking water causing a large *Campylobacter* outbreak in Norway 2019’, *International Journal of Hygiene and Environmental Health*, 224, p. 113420. doi: 10.1016/j.ijheh.2019.113420.

Phungela, T. T. *et al.* (2022) ‘The impact of wastewater treatment effluent on Crocodile River quality in Ehlanzeni District, Mpumalanga Province, South Africa’, *South African Journal of Science*, 118(7/8), pp. 1–8. doi: 10.17159/sajs.2022/12575.

Pillay, S. *et al.* (2020) ‘Characterisation of *Campylobacter spp*. isolated from poultry in Kwazulu-Natal, South Africa’, *Antibiotics*, 9(2). doi: 10.3390/antibiotics9020042.

Pitkänen, T. (2013) ‘Review of *Campylobacter spp.* in drinking and environmental waters’, *Journal of Microbiological Methods*, 95(1), pp. 39–47. doi: 10.1016/j.mimet.2013.06.008.

Pitkanen, T. and Hanninen, M.-L. (2017) ‘Members of the family campylobacteraceae : *Campylobacter*’, *Global Water Pathogens Project*.

Poma, V., Mamani, N. and Iñiguez, V. (2016) ‘Impact of urban contamination of the La Paz River basin on thermotolerant coliform density and occurrence of multiple antibiotic resistant enteric pathogens in river water, irrigated soil and fresh vegetables’, *SpringerPlus*. doi: 10.1186/s40064-016-2132-6.

Porte, L. *et al.* (2016) ‘Impact of changing from staining to culture techniques on detection rates of *Campylobacter spp*. in routine stool samples in Chile’, *BMC Infectious Diseases*, 16(1), pp. 1–6. doi: 10.1186/s12879-016-1546-7.

Potgieter, N. *et al.* (2020) ‘Human enteric pathogens in eight rivers used as rural household drinking water sources in the northern region of South Africa’, *International Journal of Environmental Research and Public Health*, 17(6). doi: 10.3390/ijerph17062079.

Pratt, A. and Korolik, V. (2005) ‘Tetracycline resistance of Australian *Campylobacter jejuni* and *Campylobacter coli* isolates’, *Journal of Antimicrobial Chemotherapy*, 55(4), pp. 452–460. doi: 10.1093/jac/dki040.

Rajasekar, A. *et al.* (2023) ‘Occurrence of antibiotic resistance genes and potentially pathogenic bacteria in the Yangtze River tributary (Nanjing section) and their correlation with environmental factors’, *Environmental Research Communications*, 5(035001). https://doi.org/10.1088/2515-7620/acbd8c

Ramaite, K., Ekwanzala, D. M. and Momba, N. B. M. (2022) ‘Prevalence and molecular characterisation of aquatic environments’, *Pathogens*, 11(674).
https://doi.org/10.3390/pathogens11060674

Ravel, A. *et al.* (2016) ‘Non food-related risk factors of campylobacteriosis in Canada: a matched case-control study’, *BMC Public Health*, pp. 1–12. doi: 10.1186/s12889-016-3679-4.

Reddy, S. *et al.* (2022) ‘Antimicrobial resistance in urban river ecosystems’, *Microbiological Research*, 263(April), p. 127135. doi: 10.1016/j.micres.2022.127135.

Reddy, S. and Zishiri, O. T. (2017) ‘Detection and prevalence of antimicrobial resistance genes in *Campylobacter spp*. isolated from chickens and humans’, *Onderstepoort Journal of Veterinary Research*, 84(1), pp. 1–6. doi: 10.4102/ojvr.v84i1.1411.

Reddy, S. and Zishiri, O. T. (2018) ‘Genetic characterisation of virulence genes associated with adherence, invasion and cytotoxicity in *Campylobacter spp.* isolated from commercial chickens and human clinical cases’, *Onderstepoort Journal of Veterinary Research*, 85(1), pp. 1–9. doi: 10.4102/ojvr.v85i1.1507.

Ravel, A. *et al.* (2016) ‘Non food-related risk factors of campylobacteriosis in Canada : a matched case-control study’, *BMC Public Health*, pp. 1–12. doi: 10.1186/s12889-016-3679-4

Robinson, T. P. *et al.* (2016) ‘Antibiotic resistance is the quintessential One Health issue’, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 110(7), pp. 377–380. doi: 10.1093/trstmh/trw048.

Rodgers, K. *et al.* (2019) ‘Can the legacy of industrial pollution influence antimicrobial resistance in estuarine sediments?', *Environmental Chemistry Letters*, 17(2), pp. 595–607. doi: 10.1007/s10311-018-0791-y.

Rosef, O., Rettedal, G. and Lågeide, L. (2001) 'Thermophilic *Campylobacter* in surface water: a potential risk of campylobacteriosis', *International journal of environmental health research*, 11(4), pp. 321–327. doi: 10.1080/09603120120081791.

Runcharoen, C. *et al.* (2017) 'Whole genome sequencing of ESBL- producing *Escherichia coli* isolated from patients, farm waste and canals in Thailand', pp. 1–11. doi: 10.1186/s13073-017-0471-8.

Sahin, O. *et al.* (2012) 'Molecular evidence for zoonotic transmission of an emergent, highly pathogenic *Campylobacter jejuni* clone in the United States', *Journal of Clinical Microbiology*, 50(3), pp. 680–687. doi: 10.1128/JCM.06167-11.

Al Salah, D. M. M., Laffite, A. and Poté, J. (2019) 'Occurrence of bacterial markers and antibiotic resistance genes in Sub-Saharan Rivers receiving animal farm wastewaters', *Scientific Reports*, 9(1), pp. 1–10. doi: 10.1038/s41598-019-51421-4.

Saleem, F. *et al.* (2023) 'Same - day *Enterococcus* qPCR results of recreational water quality at two Toronto beaches provide added public health protection and reduced beach days lost', *Canadian Journal of Public Health*, 114, pp. 676–687. doi: 10.17269/s41997-023-00763-8.

Sari Kovats, R. *et al.* (2005) 'Climate variability and *Campylobacter* infection: An international study', *International Journal of Biometeorology*, 49(4), pp. 207–214. doi: 10.1007/s00484-004-0241-3.

Schang, C. *et al.* (2016) 'Presence and survival of culturable *Campylobacter spp*. and *Escherichia coli* in a temperate urban estuary', *Science of the Total Environment,* 569–570, pp. 1201–1211. doi: 10.1016/j.scitotenv.2016.06.195.

Schellack, N. *et al.* (2017) 'A situational analysis of current antimicrobial governance, regulation, and utilization in South Africa', *International Journal of Infectious Diseases*, 64, pp. 100–106. doi: 10.1016/j.ijid.2017.09.002.

Schielke, A., Rosner, B. M. and Stark, K. (2014) 'Epidemiology of campylobacteriosis in Germany - insights from 10 years of surveillance.', *BMC infectious diseases*, 14, p. 30. doi: 10.1186/1471-2334-14-30.

Schönberg-Norio, D. *et al.* (2004) 'Swimming and *Campylobacter* infections.', *Emerging infectious diseases*, 10(8), pp. 1474–7. doi: 10.3201/eid1008.030924.

Schreiber, C. *et al.* (2015) 'The impact of land use on microbial surface water pollution', *International Journal of Hygiene and Environmental Health*, 218(2), pp. 181–187. doi: 10.1016/j.ijheh.2014.09.006.

Sekwadi, P. G. *et al.* (2018) ‘Waterborne outbreak of gastroenteritis on the KwaZulu-Natal Coast, South Africa, December 2016 / January 2017’, *Epidemiology and Infection*, 146, pp. 1318–1325.

Sekyere, J. O. (2016) ‘Current state of resistance to antibiotics of last-resort in South Africa : A review from a public health perspective’, *Frontiers in Public Health*, 4(209), pp. 1–11. doi: 10.3389/fpubh.2016.00209.

Shagieva, E., Demnerova, K. and Michova, H. (2021) ‘Waterborne isolates of *Campylobacter jejuni* are able to develop aerotolerance, survive exposure to low temperature, and interact with *Acanthamoeba polyphaga*’, 12, pp. 1–10. doi: 10.3389/fmicb.2021.730858.

Shobo, C. O. *et al.* (2016) ‘Antibiotic resistance profiles of *Campylobacter* species in the South Africa private health care sector’, *Journal of Infection in Developing Countries*, 10(11), pp. 1214–1221. doi: 10.3855/jidc.8165.

Shuai, X. *et al.* (2021) ‘Dissemination of antibiotic resistance genes in swimming pools and implication for human skin’, *Science of the Total Environment*, 794, p. 148693. doi: 10.1016/j.scitotenv.2021.148693.

Siddiqui, M. T. *et al.* (2019) ‘Co - occurrence of ESBLs and silver resistance determinants among bacterial isolates inhabiting polluted stretch of river Yamuna, India’, *International Journal of Environmental Science and Technology*, 16(10), pp. 5611–5622. doi: 10.1007/s13762-018-1939-9.

Sierra-arguello, Y. M. *et al.* (2018) ‘Fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli* from poultry and human samples assessed by PCR-restriction fragment length polymorphism assay’, *PLoS ONE*, 13(7), pp. 1–9.
https://doi.org/10.1371/journal.pone.0199974

Silva, J. *et al.* (2011) ‘*Campylobacter spp.* As a foodborne pathogen: A review’, *Frontiers in Microbiology*, 2, pp. 1–12. doi: 10.3389/fmicb.2011.00200.

Singh, A. K. *et al.* (2018) ‘Prevalence of antibiotic resistance in commensal *Escherichia coli* among the children in rural hill communities of Northeast India’, *PLoS ONE*, 13(6), pp. 1–15. doi.org/10.1371/journal.pone.0199179:

Sippy, R. *et al.* (2012) ‘Occurrence and molecular analysis of *Campylobacter* in wildlife on livestock farms’, *Veterinary Microbiology*, 157(3–4), pp. 369–375.
doi:10.1016/j.vetmic.2011.12.026

Sithole, V. *et al.* (2021) ‘Occurrence, antimicrobial resistance, and molecular characterization of *Campylobacter spp.* in intensive pig production in South Africa’, *Pathogens*, 10(4). doi: 10.3390/pathogens10040439.

Smith, S. *et al.* (2016) ‘The impact of environmental conditions on *Campylobacter jejuni* survival in broiler faeces and litter’, *Infection Ecology & Epidemiology*, 8686. doi: 10.3402/iee.v6.31685.

Snyman, Y. *et al.* (2021) ‘Characterisation of mobile colistin resistance genes (mcr - 3 and mcr - 5) in river and storm water in regions of the Western Cape of South Africa’, *Antimicrobial Resistance & Infection Control*, 2, pp. 1–9. doi: 10.1186/s13756-021-00963-2.

Sproston, E. L., Wimalarathna, H. M. L. and Sheppard, S. K. (2018) ‘Trends in fluoroquinolone resistance in *Campylobacter*’, *Microbial genomics*. doi: 10.1099/mgen.0.000198.

Stingl, K. *et al.* (2021) ‘Challenging the “gold standard” of colony-forming units - Validation of a multiplex real-time PCR for quantification of viable *Campylobacter spp.* in meat rinses’, *International Journal of Food Microbiology*, 359. doi: 10.1016/j.ijfoodmicro.2021.109417.

Strachan, N. J. C. *et al.* (2013) ‘Identifying the seasonal origins of human campylobacteriosis’, *Epidemiology and Infection*, 141(6), pp. 1267–1275. doi: 10.1017/S0950268812002063.

Strakova, N. *et al.* (2021) ‘A rapid culture method for the detection of *Campylobacter* from water environments’, *International Journal of Environmental Research and Public Health*, 18(6098). doi: 10.3390/ ijerph18116098.

Strakova, N. *et al.* (2022) ‘The effect of environmental conditions on the occurrence of *Campylobacter jejuni* and *Campylobacter coli* in wastewater and surface waters’, *Journal of Applied Microbiology*, 132, pp. 725–735. doi: 10.1111/jam.15197.

Szczepanska, B., Spica, D. and Klawe, J. J. (2017) ‘Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas’, *BMC Microbiology*, 17(80), pp. 1–9. doi: 10.1186/s12866-017-0991-9.

Tafoukt, R., Touati, A. and Leangapichart, T. (2017) ‘Characterization of OXA-48-like-producing Enterobacteriaceae isolated from river water in Algeria’, 120, pp. 185–189. doi: 10.1016/j.watres.2017.04.073.

Taljaard, S. *et al.* (2023) ‘Advancing ecosystem accounting in estuaries: Swartkops Estuary case study’, *South African Journal of Science*, 119(3), pp. 1–11. doi.org/10.17159/sajs.2023/14303

Tapela, K. and Rahube, T. (2019) ‘Isolation and antibiotic resistance profiles of bacteria from influent, effluent and downstream : A study in Botswana’, 13(15), pp. 279–289. doi: 10.5897/AJMR2019.9065.

Taviani, E. *et al.* (2022) ‘Occurrence of waterborne pathogens and antibiotic resistance in water supply systems in a small town in Mozambique’, *BMC Microbiology*, 22(243), pp. 1–11.

Teh, A. H. T., Lee, S. M. and Dykes, G. A. (2017) 'The influence of dissolved oxygen level and medium on biofilm formation by *Campylobacter jejuni*', *Food Microbiology*, 61, pp. 120–125. doi: 10.1016/j.fm.2016.09.008.

Thobela, M. S. (2017) Characterization of *Campylobacter* isolates from a South African population. Doctoral dissertation, University of the Witwatersrand, Johannesburg, South Africa.

Thornber, K. *et al.* (2022) 'Antimicrobial resistance in aquaculture environments: Unravelling the complexity and connectivity of the underlying societal drivers', *Environmental Science and Technology*, 56, pp. 14891–14903. doi: 10.1021/acs.est.2c00799.

Tissera, S. and Lee, S. M. (2013) 'Isolation of Extended Spectrum ß-lactamase (ESBL) producing bacteria from urban surface waters in Malaysia', *Malaysian Journal of Medicine and Science*, 20(3), pp. 14–22.

Traoré, A. N. *et al.* (2016) 'The Impact of human activities on microbial quality of rivers in the Vhembe District , South Africa', *International Journal of Environmental Research and Public Health*, 13(817). doi: 10.3390/ijerph13080817.

Truter, I. (2015) 'Antimicrobial prescribing in South Africa using a large pharmacy database: A drug utilisation study', *Southern African Journal of Infectious Diseases*, 30(2), pp. 52–56. doi: 10.1080/23120053.2015.1054181.

Tucker, K. *et al.* (2022) 'Spatiotemporal investigation of antibiotic resistance in the urban water cycle influenced by environmental and anthropogenic activity', *Microbiology Spectrum*, 10(5). doi: 10.1128/spectrum.02473-22

Turner, C. R., Uy, K. L. and Everhart, R. C. (2015) 'Fish environmental DNA is more concentrated in aquatic sediments than surface water', *Biological Conservation*, 183, pp. 93–102. doi: 10.1016/j.biocon.2014.11.017.

Tyagi, N. and Kumar, A. (2021) 'Evaluation of recreational risks due to exposure of antibiotic-resistance bacteria from environmental water: A proposed framework', *Journal of Environmental Management*, 279, p. 111626. doi: 10.1016/j.jenvman.2020.111626.

Uddin, N. *et al.* (2021) 'Occurrence and multidrug resistance of *Campylobacter spp.* at duck farms and associated environmental and anthropogenic risk factors in Bangladesh', *BMC Infectious Diseases*, 21(1139), pp. 1–17. doi: 10.1186/s12879-021-06834-w.

Ugboma, A. N. *et al.* (2013) 'Prevalence of *Campylobacter* species in ground water in Sokoto ', *Veterinary World*, 6(6), pp. 285–287. doi: 10.5455/vetworld.2013.285-287.

USEPA (2006) 'National primary drinking water regulations: Long term 2 enhanced surface water treatment rule (40 CFR Parts 9, 141, and 142)', *Federal Register*, 71(3), pp. 654–786.

Veltcheva, D. *et al.* (2022) ‘Emerging patterns of fluoroquinolone resistance in *Campylobacter jejuni* in the UK [1998 – 2018]’, *Microbial genomics*, 8, pp. 1–9. doi: 10.1099/mgen.0.000875.

Vereen, E. *et al.* (2013) ‘Landscape and seasonal factors influence *Salmonella* and *Campylobacter* prevalence in a rural mixed use watershed’, *Water Research*, 47(16), pp. 6075–6085. doi: 10.1016/j.watres.2013.07.028.

Vivas, M. C., Caicedo, L. D. and Castillo, J. E. (2019) ‘Occurrence of β -Lactamase-Producing gram-negative bacterial isolates in water sources in Cali city, Colombia’, *International Journal of Microbiology*, 2019, pp. 1–8. doi.org/10.1155/2019/1375060

De Vries, S. P. *et al.* (2015) ‘Motility defects in *Campylobacter jejuni* defined gene deletion mutants caused by second-site mutations’, *Microbiology*, 161, pp. 2316–2327. doi: 10.1099/mic.0.000184.

Vumazonke, S., Khamanga, S. M. and Ngqwala, N. P. (2020) ‘Detection of pharmaceutical residues in surface waters of the Eastern Cape Province’, *International Journal of Environmental Research and Public Health*, 17(11), pp. 1–13. doi: 10.3390/ijerph17114067.

Van der Walt, A. and Fitchett, J. (2020) ‘Statistical classification of South African seasonal divisions on the basis of daily temperature data’, *South African Journal of Science,* 116(9), pp. 1–15. doi.org/10.17159/ sajs.2020/7614

Wang, Y. *et al.* (2022) ‘Antibiotic resistance genes in land fill leachates from seven municipal solid waste land fills : Seasonal variations, hosts, and risk assessment’, *Science of the Total Environment*, 853(266), p. 158677. doi: 10.1016/j.scitotenv.2022.158677.

Weaver, M. J. T. *et al.* (2017) ‘Water service delivery challenges in a small South African municipality : Identifying and exploring key elements and relationships in a complex social-ecological system’, *Water SA*, 43(3), pp. 398–408. doi:10.4314/wsa.v43i3.04.

Wegener, H. C., Aarestrup, F. M. and McDermott, P. F. (2008) ‘Transmission of antibiotic resistance from food animals to humans’, in *Campylobacter,. American Society of Microbiology*, pp. 645–665. doi: 10.1128/9781555815554.ch36.

Weis, A. M. *et al.* (2016) ‘Genomic comparison of *Campylobacter spp.* and their potential for zoonotic transmission between birds, primates, and Livestock’, 82(24). doi:10.1128/AEM.01746-16.

WHO (2021) 'Guidelines on recreational water quality' Volume 1 Coastal and Fresh Waters.

Wieczorek, K. and Osek, J. (2013) ‘Antimicrobial Resistance Mechanisms among *Campylobacter*’, *BioMed Research International*, pp. 1–12.
doi: doi.org/10.1155/2013/340605.

Wilbers, G. *et al.* (2014) ‘Spatial and temporal variability of surface water pollution in the

Mekong', *Science of the Total Environment*, 485–486, pp. 653–665. doi:10.1016/j.scitotenv.2014.03.049.

Wilkes, G. *et al.* (2011) 'Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds', *Water Research*, 45(18), pp. 5807–5825. doi: 10.1016/j.watres.2011.06.021.

Williams, D. *et al.* (2009) 'Dynamic Existence of Waterborne Pathogens within River Sediment Compartments . Implications for Water Quality Regulatory Affairs', *Environmental Science & Technology*, 43(6), pp. 1737–1743. doi: doi:10.1021/es802321w.

Wilson, D. J. *et al.* (2008) 'Tracing the source of campylobacteriosis', *PLoS Genetics*, 4(9). doi: 10.1371/journal.pgen.1000203.

Xiong, W. *et al.* (2015) 'Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China', *Microbial Ecology*, 70(2), pp. 425–432. doi: 10.1007/s00248-015-0583-x.

Yamashita, N., Katakawa, Y. and Tanaka, H. (2017) 'Occurrence of antimicrobial resistance bacteria in the Yodo River basin, Japan and determination of beta-lactamases producing bacteria', *Ecotoxicology and Environmental Safety*, 143, pp. 38–45. doi: 10.1016/j.ecoenv.2017.04.053.

Yang, J. *et al.* (2020) 'Persistence of antibiotic resistance genes from river water to tap water in the Yangtze River Delta', *Science of the Total Environment*, 742, p. 140592. doi: 10.1016/j.scitotenv.2020.140592.

Yang, J. *et al.* (2022) 'Dynamic distribution and driving mechanisms of antibiotic resistance genes in a human-intensive watershed', *Water Research*, 222(July), p. 118841. doi: 10.1016/j.watres.2022.118841.

Yoon, Y., Doddb, M. C. and Lee, Y. (2018) 'Elimination of transforming activity and gene degradation during UV and UV/H2O2 treatment of plasmid-encoded antibiotic resistance genes', *Environmental Science water Research & Technology*, 4(9). doi: 10.1039/c8ew00200b.

Yuan, Y. *et al.* (2018) 'Detection of viable *Escherichia coli* in environmental water using combined propidium monoazide staining and quantitative PCR', *Water Research*, 145, pp. 398–407. doi: 10.1016/j.watres.2018.08.044.

Zhang, H. *et al.* (2022) 'Seasonal distribution of antibiotic resistance genes in the Yellow River water and tap water , and their potential transmission from water', *Environmental Pollution*, 292(PA), p. 118304. doi: 10.1016/j.envpol.2021.118304.

Zhang, S. *et al.* (2016) 'Antibiotic concentration and antibiotic-resistant bacteria in two shallow urban lakes after stormwater event', *Environmental Science pollution Research*, 23, pp. 9984–

9992. doi: 10.1007/s11356-016-6237-9.

Zheng, J. I. E. *et al.* (2006) ‘Adherence to and invasion of human intestinal epithelial cells by *Campylobacter jejuni* and *Campylobacter coli* isolates from retail meat products’, *Journal of Food Production*, 69(4), pp. 768–774. doi: 10.4315/0362-028X-69.4.768.

Zhong, Y. *et al.* (2021) ‘Characterization of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* isolates from from Jurong Lake, Singapore with whole-genome-sequencing’, *International Journal of Environmental Research and Public Health*, 18(937), pp. 1–12. doi.org/10.3390/ijerph18030937

Zingela, Z. *et al.* (2022) ‘A prospective descriptive study on prevalence of catatonia and correlates in an acute mental health unit in Nelson Mandela Bay, South Africa’, *PLoS ONE*, 17(3), pp. 1–12. doi: 10.1371/journal.pone.0264944.

Zirnstein, G. *et al.* (1999) ‘Ciprofloxacin resistance in *Campylobacter jejuni* isolates: Detection of *gyrA* resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis’, *Journal of Clinical Microbiology*, 37(10), pp. 3276–3280. doi: 10.1128/jcm.37.10.3276-3280.1999.

Zou, H. *et al.* (2019) ‘of Antibiotic-resistant bacteria in rural environments in China by using CTX-M-Producing *Escherichia coli* as an Indicator’, *Microbial Drug Resistance*, 25(7). doi: 10.1089/mdr.2018.0431.

APPENDIX A: Questionnaire survey

Survey on microbial pollution and context-related drivers of the occurrence and emergence of antibiotic resistant bacteria in the Bloukrans and Swartkops river catchments

You are invited to participate in a survey on microbial pollution and assessing the context-related drivers of the occurrence and emergence of antibiotic resistant bacteria in river water and river governance. The survey also seeks to identify local solutions to urban river management challenges such as water pollution and antibiotic resistance at catchment level.

Your participation in this study is completely voluntary. If you agree to participate you are free to withdraw from the survey at any point. Your survey responses will be kept strictly confidential. Your information will be coded, and your identity will not be made known to others through the research and writing.

As part of the project, I am asking permission to take a picture, audio and video record you while participate in the research. Should you not wish to be recorded; only notes will be taken. This research is conducted in accordance with Rhodes University`s policy on Research Ethics.

If you have questions about the survey, please contact the project leaders at mariechibwe@gmail.com or c.nnadozie@ru.ac.za or n.odume@ru.ac.za.

A. Demographic characteristics

1. What is your gender?
  - ○ Male
  - ○ Female

2. What is your age?
  - ○ Less than 25
  - ○ 25 – 34
  - ○ 35 – 44
  - ○ Above 45

3. What is your highest level of education?
  - ○ Primary school
  - ○ Secondary school
  - ○ Diploma
  - ○ Bachelor’s degree/Masters/PhD

    - Other

4. Please indicate your monthly household income
    - Less than R2000
    - R2000 – R5000
    - R5000 – R 10 000
    - R10 000 – R20 000
    - Greater than R20 000
5. Please indicate the type of dwelling you reside in
    - Formal settlement with municipal services
    - Informal settlement with municipal services
    - Informal settlement without municipal services
6. What are some of the major reasons you think are contributing to pollution of the river?

1____

2____

3____

4____

B. Significance of the Bloukrans/Swartkops River to the community

7. What benefits does the river provide to the community?

i. ____

ii. ____

iii. ____

iv. ____

v. ____

vi. ____

8. Rank the identified benefits according to their importance to the community (least important (1) to very important (6)).

1____

2____

3____

4____

5____

6____

9. Have you visited the River in the last 1year?

- ○ Yes

- ○ No

10. When you are at the River, what activities do you usually do?

1.____
2.____
3.____
4.____

11. Think about the water quality of Bloukrans Rivers. Which box do you think best describes the condition of the river? (Tick)

- ○ Very bad
- ○ Quite bad
- ○ Quite good
- ○ Very good

12. Please explain the reasons for your answer

____

____

____

____

C. Microbial pollution of the river

13. List the major activities within the catchment contribute to microbial pollution of river water?

i. ____
ii. ____
iii. ____
iv. ____
v. ____
vi. ____

14. Please rank the identified activities in terms of the activity with the greatest negative effect on water quality in the river?

i. ______________________________

ii. ______________________________

iii. ______________________________

iv. ______________________________

v. ______________________________

vi. ______________________________

15. Are there specific sites along the Bloukrans/Swartkops River and within the catchment where you are concerned about the water quality?

Please name the sites

1.______________________________

2.______________________________

3______________________________

4.______________________________

16. Where does your drinking water come from?

- Municipal tap
- Borehole
- Surface water (river, spring, stream)
- Tap from community water scheme
- Dug well
- Other (please specify)

17. Do you use river water for domestic purposes?

- Yes
- No

18. Do you believe exposure to the river negatively impacts on human health?

- Yes
- No

D. Recreational activities along the river

19. Do you engage in recreational activities in or around the river?

- Yes
- No

20. Do you come in contact with river water during recreation?

- Yes
- No

21. Have you observed some actions (e.g. littering, open defecation) that humans do during recreation that can introduce contaminate the river?

- Yes
- No

22. Are you concerned about the quality of the river water?

- Yes
- No

23. Do you think recreation activities contribute to contamination of river water?

- Yes
- No

24. Are you aware of the potential risk of contracting waterborne diseases during recreation activities?

- Yes
- No

25. Are you aware of the health consequences of water pollutions on human who get exposed to river water?

- Yes
- No

26. Do you think you can play a role in preventing water pollution?

- Yes
- No

27. Are there any cultural or religious practices that take place at the river that you know? Please share below.

____

____

____

E. Health-seeking behaviour, antibiotic use and handling at household level

28. Have you or anyone in your household suffered from diarrhoea the past 6 months?

- Yes
- No

29. Which class of people mainly suffers from diarrhoea?

    - Children
    - Women
    - Men
    - Older people
    - others (please specify) ____

30. Did the person seek healthcare?

- Yes
- No

31. Did the person submit stool sample?

- Yes
- No

32. Was the person admitted to the hospital for this illness?

- Yes
- No

33. Did the person self- medicate with antibiotics?

- Yes
- No

34. Do you or any family member borrow or share antibiotics / medications with others?

- Yes
- No

35. Do you or any family member stop antibiotic /medications intake once the person starts to feel better?

- Yes
- No

36. Does your family consume the leftover antibiotics/ medications?

- Yes

- No

37. Do you or your family follow the same prescription for taking medicine after facing a recurrence of symptoms?

- Yes
- No

38. How do you dispose-off the leftover antibiotics/medications?

- Throw in the bin
- Pour in the sink or toilet
- Other

F. Animal husbandry

39. Do you keep livestock at home?
- Yes
- No

40. What animals do you keep? (tick)
- Cattle
- Goats
- Sheep
- Pigs
- Poultry
- Donkeys

Others: specify________________

41. What animal rearing method do you use?
- Free grazing
- Zero grazing
- Mixed

42. Where do you get drinking water for animals?
- Piped water

- Borehole
- River water

Others; specify____________________________

43. Are your animals confined or they freely move in the community?

- Confined
- Not Confined

44. What do you use to treat your sick animals?

- Nothing
- Veterinary drugs only
- Herbal remedies only
- A mixture of both vet drugs and herbal remedies

45. How do you dispose your animal wastes?

- bury
- spray on farmlands
- other ___________________

G. Accountability

46. In your opinion who should be most responsible for ensuring water quality and quantity in the Bloukrans/Swartkops River

1.____
2.____
3____

47. Have you participated in any of the following activities concerning the river?

- Bloukrans/Swartkops River basin group meetings/activities
- Volunteer water quality monitoring
- Awareness, training and education programmes on rivers
- Bloukrans/Swartkops Catchment Management Forum

End

Thank you for your participation!

APPENDIX B: Stakeholder's workshop

Community stakeholder's workshop

25th January, 2022 – Dolphins Leap Conference & Events Centre, Humewood, Port Elizabeth.

Programme:

Time	Step and detail	Facilitator & materials
09.00	**Coffee and Tea on arrival**	
Welcome and Introduction		
09.30 *15 mins*	**Welcome and introductions** Each person introduces themselves and answers ice-breaker question, e.g. What motivated you to come here today?	Facilitator 1
10 mins	**Introduction to the workshop and key concepts:** • Microbial pollution of urban rivers • We all depend on rivers and our actions in the community may affect river water quality	Facilitator 1 & 2
5 mins	**Purpose and structure of the day:** • What we want to understand • Structure of the day	Facilitator 1 & 2
STEP 1 – SIGNIFICANCE OF RIVERS		
10.00 *30 mins*	**Introduction:** • Rivers play an important role in our daily lives. • Understanding the significance of rivers helps in proper management of rivers • If we can identify and appreciate the significant roles that rivers play in our lives we can contribute to better management of rivers. • Today in this workshop, and as we bring together different groups of people in the catchment to discuss the significance of rivers in the community. **Activity:** In your group, discuss the significance of the river to the community List at least 6 most important uses of river water in the catchment. Rank these uses in order of importance, from the least important to the most important. Share the main points with the plenary and cluster.	Facilitator 3 & 4 Flip-chart paper Markers
STEP 2 – DRIVERS OF MICROBIAL POLLUTION OF RIVERS		
10.30 *30 mins*	**Introduction** • Importance of understanding our context - We can never know everything about a situation – but if we share our knowledge we know a lot more.	All facilitators Map

	• We are all teachers and learners • Overview of how anthropogenic activities within the catchment are currently affecting river water **Activity – Help us to understand your context better** In small groups (10 mins): • Using the map for the catchment, illustrate how anthropogenic activities in the catchment may introduce microbial pollutants in rivers • Ask each group to identify their top 6 anthropogenic activities • Ask each group to rank the 6 key anthropogenic activities in increasing order of severity In plenary (15 mins): • Each group to feedback concerns and present their map to all participants.	Post-its Markers
STEP 2 – RECREATION ACTIVITIES IN THE CATCHMENT		
11.00 *30 mins*	**Introduction** Recreation activities may contribute to microbial contamination of river water. Humans may also be exposed to microbial pollutants such as pathogenic bacteria, antibiotic resistant bacteria and antibiotic resistant genes. **Activity** In small groups • List the types of recreation activities that are conducted in the river. • On the map, identify specific sites on the river that are used for recreation activities. • What activities conducted during recreation activities can can contribute to microbial contamination of the river. In plenary: Come together, share what was discussed and present the map	All facilitators Map Post-its Markers
Step 3 – MITIGATION MEASURES		
11.30 *60 mins*	**Introduction:** We have identified the causes and sources of microbial contamination of river water. Based on this information, policymakers can develop evidence-based policies that are targeted and effective in preventing and reducing microbial pollution of rivers This may contribute to the broader national and global efforts to mitigating microbial contamination of rivers. **Activity:** In your groups discuss the mitigation measures that should be employed to prevent microbial contamination of the Bloukrans/Swartkops rivers.	All facilitators Post-its Markers

	For each identified driver of microbial contamination, state how contamination can be mitigated	
12.30 *15 mins*	**Closing: Thanks and Next steps** Round of closing reflections - one word or phrase? Flag follow up joint workshop to feedback and elicit further input	Facilitator 1 & 2
12.45	***LUNCH***	

RHODES UNIVERSITY
Where leaders learn

Human Ethics Committee
Rhodes University Ethical Standards Committee
PO Box 94, Makhanda, 6149 South Africa
Email: s.manqele@ru.ac.za

www.ru.ac.za/research/research/ethics
NHREC Registration No. REC-241114-045

07/09/2021
Dr. Chika Nnadozie
Email: C.Nnadozie@ru.ac.za
Review Reference: 2021-4864-6101

Dear Dr Chika Nnadozie

Title: Investigating the multiple risk dimensions associated with Campylobacteriosis - a key poverty-related disease of South African urban source water environments

Principal Investigator: Dr Chika Nnadozie
Collaborators: Prof Nelson Odume, Dr. Dennis Junior Choruma, Ms. Mary Chibwe, Ms. Nnadipha Ngoni, Mr. Bashar Haruna Gulumbe, Mr. Ali Andrew

This letter confirms that the above research proposal has been reviewed and **APPROVED** by the Rhodes University Human Ethics Committee (RU-HEC). Your Approval number is: 2021-4864-6101

Approval has been granted for 1 year. An annual progress report will be required in order to renew approval for an additional period. You will receive an email notifying when the annual report is due.

Please ensure that the ethical standards committee is notified should any substantive change(s) be made, for whatever reason, during the research process. This includes changes in investigators. Please also ensure that a brief report is submitted to the ethics committee on the completion of the research. The purpose of this report is to indicate whether the research was conducted successfully, if any aspects could not be completed, or if any problems arose that the ethical standards committee should be aware of. If a thesis or dissertation arising from this research is submitted to the library's electronic theses and dissertations (ETD) repository, please notify the committee of the date of submission and/or any reference or cataloging number allocated.
Sincerely,
Sincerely,

RHODES UNIVERSITY
Where leaders learn

Arthur Webb

Prof Arthur Webb
Chair: Human Ethics Committee
Cc: Siyanda Manqele – Ethics Coordinator