Antimicrobial resistance screening and profiles: a glimpse from the South African perspective

B. Genthe, L. Ndlela and T. Madlala

ABSTRACT

According to the Centre for Disease Dynamics Economics and Policy, South Africa represents a paradox of antibiotic management similar to other developing countries, with both overuse and underuse (resulting from lack of access) of antibiotics. In addition, wastewater reuse may contribute towards antibiotic resistance through selective pressure that increases resistance in native bacteria and on clinically relevant bacteria, increasing resistance profiles of the common pathogens. Sediments of surface water bodies and wastewater sludge provide a place where antibiotic resistance genes are transferred to other bacteria. Crop irrigation is thought to be a potential source of exposure to antibiotic-resistant bacteria through the transfer from the water or sludge into crops. The objectives of this study were to examine the antibiotic-resistance profiles of *Escherishia coli* from three agricultural locations in the Western Cape, South Africa. Using a classical microbiology culture approach, the resistance profiles of *E. coli* species isolated from river water and sediments, farm dams and their sediments and a passive algal wastewater treatment ponds and sediment used for crop irrigation were assessed for resistance to 13 commonly used antibiotics. Randomly selected *E. coli* isolates from the sediment and water were tested for resistance. 100% of *E. coli* isolates were resistant to sulphamethoxazole, highlighting its relevance in the South African context. In river water and farm dam samples, only the *E. coli* isolated from sediment were found to be resistant to fluoroquinolone or fluorifenicol. In the wastewater treatment ponds, the resistance profiles of *E. coli* isolated from sediments differed from those isolated from effluent, with 90% of the effluent isolates being resistant to ampicillin. Isolates from the sediment were less resistant (40%) to ampicillin, whereas all the isolates from the pond water and sediment samples were resistant to sulphamethoxazole. These results illustrate the importance of developing a better understanding of antibiotic resistance in agriculture and wastewater scenarios to ensure remedial measures take place where the greatest benefit can be realised especially in countries with limited financial and infrastructural resources. Moreover, the potential for passive algal treatment as an effective, feasible alternative for wastewater treatment is highlighted, with comparable resistance profiles and a reducing overall resistance in the sediment samples.

Key words | agriculture irrigation water, antibiotic resistant bacteria, *E. coli*, river water, sediment, wastewater

B. Genthe (corresponding author)
L. Ndlela
T. Madlala
Water Centre, Smart Places, CSIR,
P.O. Box 320, 11 Jan Celliers Road, Stellenbosch 7599,
South Africa
E-mail: *bgenthe@csir.co.za*

T. Madlala
Department of Earth Science, University of Western Cape,
Private Bag X17, Bellville 7535,
South Africa

HIGHLIGHTS

- The study provides an overview of antibiotic-resistant *E. coli* isolates in surface waters and sediments and wastewater and its sediments in a rural setting in South Africa.
- The study highlights the potential for exposure to antibiotic-resistant bacteria through crop irrigation in agriculture.

doi: 10.2166/wh.2020.034

- All *E. coli* isolates from water and sediment samples used for irrigation were resistant to sulphamethoxazole.

INTRODUCTION

The WHO predicts that globally deaths from antibiotic-resistant infections could increase to 10 million deaths annually by 2050 and that antimicrobial resistance (AMR) could force up to 24 million people into extreme poverty due to the combined effects of AMR on human health as well as food systems by 2030 (WHO 2019). The development of resistance in microorganisms against commonly used antibiotics can be exacerbated through the use of antibiotics in livestock farming, unregulated administration of antibiotics and patient default of antibiotic treatment. South Africa represents a paradox of antibiotic management similar to other developing countries, with both overuse and underuse (resulting from lack of access) of antibiotics (Duse 2011; Laxminarayan *et al.* 2013). A further concern is that wastewater reuse must be encouraged as a measure to provide alternative water sources to provide resilience to climate change (Adewumi *et al.* 2010) and can contribute towards antibiotic resistance through selective pressure that increases antibiotic resistance in native bacteria (Kraemer *et al.* 2019). There is also growing concern that environmental concentrations of antibiotics exert selective pressure on clinically relevant bacteria. In some African countries, inadequate wastewater treatment facilities may also contribute to the increased resistance profiles of the common pathogens (Momba *et al.* 2006). Irrigation with water containing antibiotic-resistant bacteria may lead to the uptake of these bacteria into the crops, leading to an additional source of exposure to antibiotic-resistant pathogens (Ruimy *et al.* 2010; Hirneisen *et al.* 2012; Drissner & Zürcher 2014; Nüesch-Inderbinen *et al.* 2015; Thanner *et al.* 2016). It is estimated that between 2000 and 2010, the antibiotic consumption in the world increased 35%, with Brazil, Russia, India, China and South Africa accounting for 76% of this increase, although their contribution in population increase was only 25% (Van Boeckel *et al.* 2014). This increase in global consumption was attributed both to more affordable antibiotics in low-income countries, as well as inadequate prescription or over-the-counter purchases (Van Boeckel *et al.* 2014). Amoxicillin, sulphamethoxazole/trimethoprim and ciprofloxacin contributed to 75% of the total oral antibiotic consumption in the African region, whilst beta-lactams were 70% of the consumed antibiotics in the Western Pacific region; amoxicillin, macrolides and quinolones were also the majorly consumed antibiotics in the Eastern Mediterranean as well as the European region. The trend observed was similar for the most consumed classes of antibiotics across the global region, with beta-lactams, macrolides and fluoroquinolones reflected, in the African region; however, sulphonamides were consumed more than macrolides (WHO 2018).

Escherishia coli is sometimes used as a sentinel for monitoring antimicrobial drug resistance in faecal bacteria because it is found more frequently in a wide range of hosts, acquires resistance easily and is a reliable indicator of resistance in other pathogenic bacteria (Tadesse *et al.* 2012). Resistance to one of the most widely used antibacterial drugs for the oral treatment of urinary tract infections caused by *E. coli,* fluoroquinolones, is very widespread (WHO 2014). The incorrect use of antimicrobial drugs, for instance in animal husbandry, supports the development and selection of resistant bacteria. Antibiotics in sewage, treated effluent, sewage sludge and water sediments allow for the selection of antibiotic resistance (Fouz *et al.* 2020). In addition, exposure to antibiotic-resistant bacteria may be through the irrigation of crops. Selection for antibiotic resistance is not confined to the human body or to hospitals, clinics and farms. Selection takes place anywhere an antibiotic is present, especially in natural environments, most notably sewage and surface water sediments, where antibiotics are likely to be coupled with high densities of various microorganisms. Large amounts of antibiotics and biocides end up in sewage sludge, making it a source for the development of antibiotic resistance (Fouz *et al.* 2009). When dewatered sludge is applied as fertiliser to agricultural land, there is a renewed risk of introducing both antibiotics and resistant strains into the food supply.

E. coli is normally effectively treated by the antibiotics ampicillin, cloxacillin, colistin sulphate, doxycycline, enrofloxacin, florfenicol, fosfomycin, gentamicin, kanamycin, nalidixic acid, penicillin, streptomycin, sulphamethoxazole, trimethoprim, sulphonamides and tetracycline (Hertz *et al.* 2014; Tekin *et al.* 2018; Madappa 2019).

Antibiotics are not only given to humans and animals for the treatment of infections. Certain antibiotics, when given in low, sub-therapeutic doses, are known to improve feed conversion efficiency (more output, such as muscle or milk, for a given amount of feed) and/or may promote greater growth, most likely by affecting gut flora.

Antibiotic resistance has shown up soon after their development resistance for some antibiotics such as linizolid and methicillin developed only 1–2 years after its development. An extensive evaluation of antibiotic resistance on a global scale was conducted by Laxminarayan *et al.* (2013). The complexities in low- to middle-income countries stem from a combination of overuse and underuse as previously mentioned of certain antibiotics. In addition, the unregulated and/or misinformed use of antibiotics results in continued mortalities in adolescents from treatable infections and increased resistance to certain classes of antibiotics that are more easily accessible. This, coupled with inadequate wastewater treatment and poor data collation contribute to greater challenge of tackling antibiotic resistance. Within these developing countries, these are the combined considerations in understanding AMR. Compounding this are the limited comparable methods applicable in low and highly resource countries, with the disc diffusion method being the most commonly applied and standard method in research for screening (Syal *et al.* 2017). The AMR in different water sources in this study were assessed within this context.

Crop irrigation is thought to be a potential source of exposure to antibiotic-resistant bacteria through the transfer from the water or sludge into crops (Thanner *et al.* 2016). The objectives of this study were to examine the antibiotic-resistance profiles of *E. coli* from rural water supplies used for agriculture, including passive wastewater treatment systems, river waters and agricultural farm dams from three agricultural locations in the Western Cape, South Africa. Surface water sediments and wastewater sludge provide a place where antibiotic-resistance genes are transferred to other bacteria.

Using a classical microbiology approach, the resistance profiles of *E. coli* species detected from water and underlying sediments were assessed for resistance to 14 common antibiotics. These antibiotics included ampicillin, colistin sulphate, cloxacillin, doxycycline, enrofloxacin, florfenicol, fosfomycin, gentamicin, kanamycin, nalidixic acid, penicillin, streptomycin, tetracycline and trimethoprim–sulphamethoxazole. Randomly selected *E. coli* isolates from the sediment and surface water of wastewater and surface water were tested for resistance.

METHODOLOGY

Site description

The Western Cape has a warm temperate Mediterranean climate, with rainfall predominating during austral winter and early spring. There is a history of concern for water shortages and compromised water quality, and hence, the interest in reuse of wastewater with *E. coli* levels and nutrient concentrations being the major considerations. As part of independent research being carried out in projects looking at water quality issues in the Western Cape, an opportunity arose to investigate three agricultural sites in the Western Cape, South Africa to provide an indication of the occurrence of antibiotic-resistant bacteria in the water, used for the irrigation of crops. The sites included the Dwars river site in the upper reaches of the Berg River catchment, the Touws River site in the Gouritz catchment and the Brandwacht passive wastewater treatment works (WWTWs) also in the Gouritz Water Management Area (WMA) (Figure 1(1-1)–1(1-3)). The three sites offer a selection where agricultural activity takes place and could contribute to the exposure to antibiotic-resistant bacteria, through the irrigation of crops with water containing antibiotic-resistant bacteria.

Berg River – the Dwars River tributary

The Berg River has been a concern for agricultural use regarding the water quality over many years. The upper Berg River catchment is a mountainous sub-catchment of the Berg River catchment in the Western Cape and is

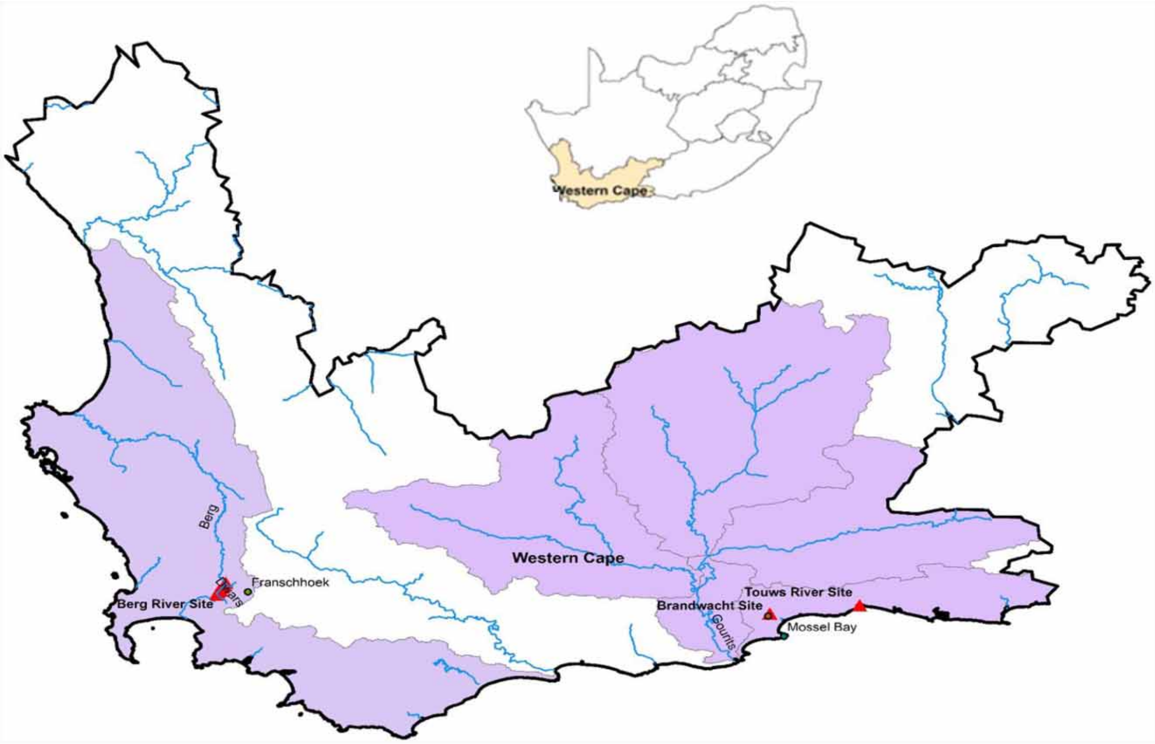

Figure 1 | Western Cape sampling sites. (1-1) Upper Berg River site in Berg River catchment. (1-2) Brandwacht wastewater treatment ponds. (1-3) Touws River site in Gouritz catchment. *(Continued.)*

bound by the Franschhoek and Drakenstein mountains to the south and south-west of the catchment. The main tributaries of the Berg River in this area are the Franschhoek, Wemmershhoek and Dwars Rivers and are used to irrigate crops. Samples were collected from the Dwaars tributary.

Touws River

The Touws River is part of the Coastal Belt in the Gouritz catchment which includes the Gouritz/Goukou/Duiwenhoks catchment. Land use is a mix of residential, natural vegetation and intense dairy farming with some fresh produce agriculture.

Brandwacht wastewater treatment works

The Brandwacht topography comprises land that drains into the Brandwacht WWTWs which serves a small population of 1,470 individuals or 398 households. The wastewater treatment system is a small one making use of seven earth ponds designed to allow gravity to flow from one pond to the next without electricity. Depending on the need, effluent may be made available for agricultural activities.

Sample collection

Surface water (1 l in sterile bottles) and sediment samples (100 g) were collected from different sites of the Touws river and farm dams, the Upper Berg river (surface water only), and the seven ponds of the Brandwacht WWTWs. Sediment samples from the centre of farm dams were collected in the benthic zone using a canoe or rowing boat (depending on the availability) and a Van Veen grab sampler. Samples were also collected from three sites in the littoral zone evenly distributed around the dam using a metal scoop, before combining the sediment samples into a composite sample in zip-lock plastic bags. In Brandwacht WWTW, 1 l grab samples were collected from the outlets of Pond 1–7 and from the sediment at these sites. Nitrile gloves were used for sample handling and to prevent contamination. All samples were collected using bailers, transferred to sterile water bottles and kept on ice, in the dark during transportation to the laboratory before analysis.

Figure 1 | Continued.

E. coli analysis and antibiotic-resistance assessment method

To determine the AMR profiles of *E. coli* isolates and to understand the role of WWTWs, surface water sources including farm dams and rivers in the development of AMR, samples from three different sites were collected in the Western Cape in South Africa. The antimicrobial susceptibility of *E. coli* isolates from surface waters, wastewater effluents and sediments were determined, using antibiotics used in the control of *E. coli* infections.

Detection of *E. coli* in samples

Using *E. coli* as an indicator organism, enumeration was conducted using the Colilert 18 method (IDEXX, South Africa) according to the manufacturer's specifications. Briefly, Colilert nutrient powder capsules were dissolved in 100 ml volumes of surface water samples. For sediment samples, 0.1 g samples of sediment were dissolved in volumes of 100 ml sterile tap water, sealed in 49 well Quanti-Trays and incubated at 35 °C, over an 18-h period. After incubation, samples tray wells were analysed based on colour changes. A change in medium from colourless to yellow indicated the presence of coliforms, whilst fluorescence of the yellow wells under UV light indicated the presence of *E. coli*.

Antimicrobial susceptibility testing

E. coli bacteria isolated from sediment and surface water samples were tested for resistance against various antibiotics. The *E. coli* originally cultured using the Colilert

Figure 1 | Continued.

18 method were extracted by removing 0.1 ml of medium from fluorescent wells (positive wells) using a needle and syringe. Spread plate antibiotic disc diffusion assays were conducted with the cell suspensions and monitored over a 48-h incubation period at 35 °C on nutrient agar (Merck, South Africa) plates. At least ten samples containing *E. coli* were tested in all three sites. Overall, 14 antibiotics were tested, namely, ampicillin, streptomycin, florfenicol, trimethoprim, colistin, enroflaxin, doxycycline, fosfomycin, nalidixic acid, tetramycin, sulphamethoxazole, gentamicin and kanamycin (Oxoid, South Africa). Zones of inhibition were recorded as either present (susceptible) or absent (resistant), according to the EUCAST method, with zones of inhibition typically indicated by no growth, when held up about 30 cm from the naked eye (Matuschek *et al.* 2014). The observed zones were generally greater than 5 mm, although only presence/absence criteria were used in this study.

Data analysis

Microsoft Excel was used to create figures and conduct trend analysis.

RESULTS AND DISCUSSION

The majority of *E. coli* isolates were resistant to ampicillin, penicillin, cloxacillin, sulphamethoxazole and trimethoprim (Table 1; Figure 2(a)–2(c)), whereas streptomycin, kanamycin, enrofloxacin, doxycycline and gentamycin remained effective against *E. coli* in the majority of isolates tested (Figure 2(a)–2(c)), ranging between 70 and 100% sensitivity.

The resistance profile of *E. coli* isolated from sediments differed from those isolated from surface water, with 90% of the surface water isolates being resistant to ampicillin. Isolates from the sediment were less resistant (40%) to ampicillin, whereas nearly all the isolates from the pond and

Figure 1 | Continued.

Table 1 | Antibiotic resistance of *E. coli* isolates (if over 50% of isolates were resistant then considered as resistant) and sensitivity (if less than 10% samples were resistant then considered as sensitive)

Upper Berg River water	Touws River water	Touws River sediment	Brandwacht WWTW water	Brandwacht WWTW sediment
Antibiotic resistance				
Ampicillin	Ampicillin	Ampicillin	Ampicillin	Sulphamethoxazole
Cloxacillin	Cloxacillin	Cloxacillin	Nalidixic acid	
Colistin	Colistin	Colistin	Sulphamethoxazole	
Pencillin	Penicillin	Fosphomycin		
Sulphamethoxazone		Penicillin		
Sulphonamides		Trimethoprime		
Antibiotic sensitivity				
Enrofloxacin	Doxycycline	Doxycycline	Doxycycline	Doxycycline
Florfenicol	Enrofloxacin	Enrofloxacin	Enrofloxacin	Enrofloxacin
Gentamycin	Gentamycin	Gentamycin	Gentamycin	Gentamycin
Kanamycin	Kanamycin	Kanamycin	Kanamycin	Kanamycin
Streptomycin	Nalidixic acid	Streptomycin	Tetracycline	

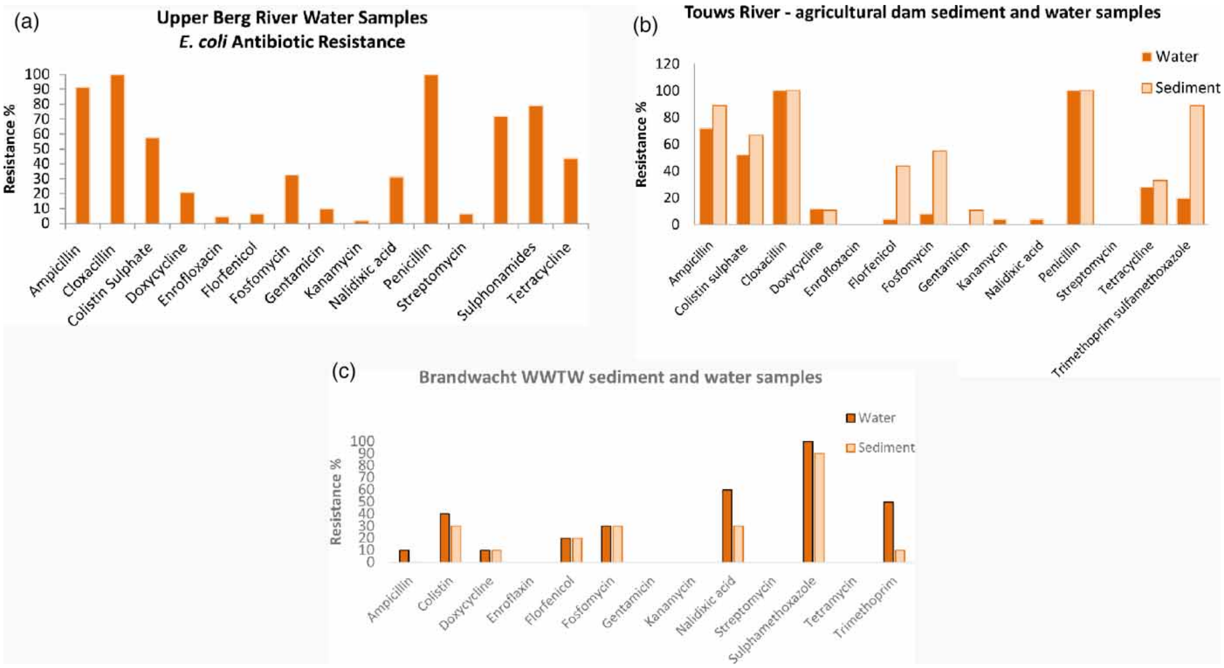

Figure 2 | (a) Percentage resistance to different antibiotics in *E. coli* isolates from Berg River water. (b) Percentage resistance to different antibiotics in *E. coli* isolates from Touws River water and sediment. (c) Percentage resistance to different antibiotics in *E. coli* from isolates from Brandwacht WWTW water and sediment.

sediment were resistant to sulphamethoxazole. In farm dams, only the *E. coli* isolates found in sediment were found to be resistant to fluorquinolone or fluorifenicol.

There is an indication of antibiotic resistance retained within the sediments of the Touws river, whilst this is only observed for sulphamethoxazole in the Brandwacht WWTWs.

In the wastewater ponds, the number of antibiotics that the *E. coli* were resistant to, increased in successive maturation ponds (Figure 3(b)), whereas in the *E. coli* isolated from sediments, the number of antibiotic they were resistant to started at 9 in pond 1 and only 5 in pond 7 (Figure 3(c)).

Penicillin and sulphonamides are examples of antibiotics which are known for overuse and persistence in the environment (Lobanovska & Pilla 2017). In the water and sediment samples, the greatest resistance across the sites is towards sulphamethoxazole, trimethoprim and penicillin. Ampicillin, which is a later generation of the penicillin classes and initially caused microbial sensitivity (Lobanovska & Pilla 2017), is now one of the antibiotics that *E. coli* isolates from the different sites were widely resistant to.

Sulphonamides such as sulphamethoxazole and/or the sulphamethoxazole–trimethoprim combination have been recorded as the more persistent antibiotics in the environment (Grenni *et al.* 2019), with an approximate 60-day degradation, with synergistic actions of the metabolite with other antibiotics resulting in a longer degradation time. This has led to the development of resistance genes against this antibiotic. In South Africa, HIV-positive patients are treated with low doses of this combination as preventative to opportunistic infections (Kaplan *et al.* 2009); however, 100% of *E. coli* isolates were resistant to sulphamethoxazole, highlighting its relevance in the South African context. This finding is supported by earlier work by Nyamukamba *et al.* (2019) in the Vaal triangle area, where sulphamethoxazole was detected in higher quantities than other antibiotics tested, which were interestingly below the limit of detection. This is interesting as this is one of the more prevalent antibiotics consumed in the African region in comparison to other global regions.

In surface water isolates of *E. coli* resistance to all of the antibiotics tested was detected, whereas in the maturation pond isolates, the *E. coli* isolates remained sensitive to streptomycin, enroflaxin and the majority of isolates remained sensitive to gentamycin and kanamycin (Figure 2(a)). In the agricultural dam water and sediment, more resistance was found in sediment isolates (Figure 2(b)).

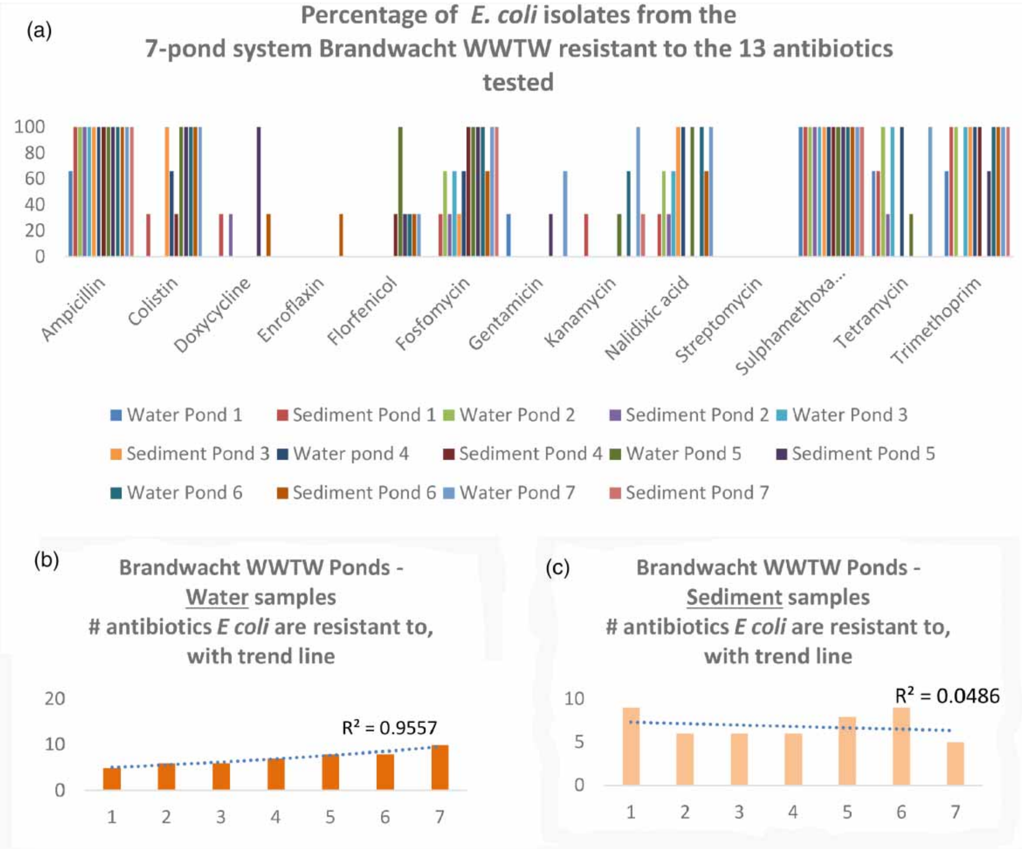

Figure 3 | (a) Percentage of *E. coli* isolates from both water and sediment samples in the seven pond Brandwacht WWTW that exhibit antibiotic resistance to the 13 antibiotics tested. (b) The number of antibiotics that *E. coli* isolates were resistant to in water samples of the seven maturation ponds. (c) The number of antibiotics that *E. coli* isolates were resistant to in sediment samples of the seven maturation ponds.

Passive wastewater treatment

The advent of passive wastewater treatment in developing countries offers a low cost alternative to the challenge of failing WWTWs system, which however consume large amounts of power, proving to be a challenge in developed countries as well (Hossain *et al.* 2010). Waste stabilisation ponds are a technology used prolifically by South African municipalities due to their simplicity, economy and reliability (Mambo *et al.* 2014a). In only very limited locations in South Africa are alternative wastewater treatment processes being trialled, such as constructed wetlands (Mthembu *et al.* 2013) and Algal Integrated Wastewater Pond Systems (AIWPS) or Integrated Algae Pond Systems (IAPS) as a municipal sewage treatment technology (Mambo *et al.* 2014b). When assessing compliance of this treatment, the effluent produced required additional tertiary treatment to meet the required standards in coliform and total suspended solids (Mambo *et al.* 2014a). In fact, it is recommended to decision-makers to engage in mitigating risks posed by poorly performing WWTWs by investing in in-stream biotechnologies concurrent to investing in the refurbishment of WWTWs (Mitchell *et al.* 2014).

The comparison of antibiotic-resistance profiles from samples of conventional and this treatment alternative provide useful information on the treatment efficiency and how AMR profiles may change in a passive treatment system. The use of algae to utilise pollutants for nutritional benefit in wastewater provides an environmentally friendly alternative to the conventional wastewater treatments, with higher retention times (Wang *et al.* 2010), which may explain the final reduced resistance profile in the sediment of pond 7 in Brandwacht WWTW, which uses gravitational energy for effluent flow into treatment ponds (Figure 2(c)).

In South Africa, a majority of the conventional WWTWs are dysfunctional and not treating water to the required standards (Momba *et al.* 2006). Coupled with the complexities of increased populations, climate change concerns, overuse and underuse of antibiotics; the challenge in screening and effective control of AMR in South Africa is critical.

The use of passive treatment in wastewater treatment, where residence times are significantly longer than in conventional wastewater treatment systems, may allow the reduction of antibiotic resistance, as shown by the lower numbers of antibiotics that the isolated *E. coli* were resistant to compared with surface water isolates (3 versus 6 antibiotics, respectively; Table 1). What is not known is whether the passive systems perform better than conventional WWTWs in reducing the presence of antibiotic-resistant bacteria. In the passive wastewater treatment system, the number of antibiotics that the *E. coli* isolated from the water samples were resistant to, increased in successive maturation ponds, whereas the reverse was seen in the *E. coli* isolated from sediment samples (Figure 3(b) and 3(c)).

These results illustrate the importance of developing a better understanding of antibiotic resistance in agriculture and wastewater scenarios to ensure remedial measures take place where the greatest benefit can be realised in countries with limited financial resources. Future research is needed to assess the contribution of conventional WWTWs to the growing antibiotic resistance of bacterial isolates and to establish the potential of passive treatment systems.

In South Africa (and most developing countries), pathology laboratories and water facility laboratories predominantly make use of the culture method to screen for AMR. This method, among many more sophisticated techniques, remains the gold standard method from a cost perspective. Among the methods to test for antibiotic resistance, there is still no standardised cohesive guide that is widely practised for reporting and comparable analysis in countries with limited resources, thereby making the classical microbiological culture technique the more feasible and standardised approach (Khan *et al.* 2019). The WHO has implemented an AMR surveillance project which recommends cultivation methods in support of the concept that a simplified, integrated, trans-sectoral surveillance system of bacterial resistance to antibiotics could be implemented on a global basis, the so-called Tricycle project (GLASS 2020).

The findings in this study indicate that the theory of wastewater treatment systems being a hub for horizontal antibiotic-resistance acquisition in pathogens is possible based on the increased resistance profiles in the sediment samples of conventional wastewater plants, where increased resistance has been linked to wastewater treatment (Manaia *et al.* 2018). This is observed in the surface water assessments of all the sites and in the sediment of the passive wastewater treatment plant. The use of passive wastewater treatment to manage persistence of sulphamethoxazole and other persistent antibiotics cannot be concluded within the limits of this research, especially considering that a 100% of isolates from each of the seven ponds were resistant to sulphamethoxazole. The sites indicated resistance to the largely used and persistent antibiotics, with increased resistance to fluoroquinolones in the animal influenced samples, this has been reported in other parts of the world where there is intense animal husbandry (Tang *et al.* 2017; Schulz *et al.* 2019), which may be due to the unaltered excretion of these antibiotics by the animals, into waters where inadequate treatment exists to effectively degrade or manage the impacts of genetic mutations in exposed bacteria. The comparison of the three sites indicates a global trend in resistance profile influence. Overall, the South African perspective is fairly bleak and complexed with less than 50% of the wastewater treatment systems in South Africa meeting national and international water quality standards for wastewater treatment (Mthembu *et al.* 2013; Mitchell *et al.* 2014). These findings are proof that South Africa's wastewater treatment systems are inadequate to meet the effluent required standards. This has resulted in the urgent need for the development and implementation of innovative systems to resolve the wastewater treatment constraints. This research, among the existing body of the literature, strengthens the call for a more stringent management approach of this challenge, with passive treatment showing a better sensitivity profile.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Adewumi, J. R., Ilemobade, A. A. & Van Zyl, J. E. 2010 Treated wastewater reuse in South Africa: overview, potential and challenges. *Resour. Conserv. Recycl.* **55**, 221–231. https://doi.org/10.1016/j.resconrec.2010.09.012.

Drissner, D. & Zürcher, U. 2014 Microbial safety of fresh fruits and vegetables. In: *Encyclopedia of Food Safety*, vol 3 (Y. Motarjemi, ed.). Elsevier, Oxford, UK.

Duse, A. G. 2011 The Global Antibiotic Resistance Partnership (GARP). *S. Afr. Med. J.* **101**, 8.

Elif Tekin, E., White, C., Manzhu Kang, T., Singh, N., Cruz-Loya, M., Damoiseaux, R., Van Savage, M. & Yeh, P. J. 2018 Prevalence and patterns of higher-order drug interactions in *Escherichia coli*. *Npj Syst. Biol. Appl.* **4**, 31.

Fouz, N., Pangesti, K. N. A., Yasir, M., Al-Malki, A. L., Azhar, E. L., Hill-Cawthorne, G. A. & Abd El Ghany, M. 2020 The contribution of wastewater to the transmission of antimicrobial resistance in the environment: implications of mass gathering settings. *Trop. Med. Infect. Dis.* **5** (1), 33. doi:10.3390/tropicalmed5010.

GLASS 2020 *Global Antimicrobial Resistance Surveillance System (GLASS) Report – Early Implementation 2020*. Available from: https://apps.who.int/iris/bitstream/handle/10665/332081/9789240005587-eng.pdf?ua=1

Grenni, P., Patrolecco, L., Rauseo, J., Spataro, F., Di Lenola, M., Aimola, G., Zacchini, M., Pietrini, F., Di Baccio, D., Stanton, I. C., Gaze, W. H. & Barra Caracciolo, A. 2019 Sulfamethoxazole persistence in a river water ecosystem and its effects on the natural microbial community and Lemna minor plant. *Microchem. J.* **149**, 103999. https://doi.org/https://doi.org/10.1016/j.microc.2019.103999.

Hertz, F. B., Løbner-Olesen, A. & Frimodt-Møller, N. 2014 Antibiotic selection of *Escherichia coli* sequence type 131 in a mouse intestinal colonization model. *Antimicrob. Agents Chemother.* **58** (10), 6139–6144. doi:10.1128/AAC.03021-14.

Hirneisen, K. A., Sharma, M. & Kniel, K. E. 2012 Human enteric pathogen internalization by root uptake into food crops. *Foodborne Pathog. Dis.* **9** (5), 396–405.

Hossain, F., Chang, N.-B., Wanielista, M., Xuan, Z. & Daranpob, A. 2010 Nitrification and denitrification in a passive on-site wastewater treatment system with a recirculation filtration tank. *Water Qual. Expo. Heal.* **2**, 31–46. https://doi.org/10.1007/s12403-010-0022-7.

Kaplan, J. E., Benson, C., Holmes, K. K., Brooks, J. T., Pau, A. & Masur, H. 2009 Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the national institutes of health, and the HIV medicine association of the infectious diseases society of America. *MMWR Recomm. Rep.* **58** (RR-4), 1–207. quiz CE1-4.

Khan, Z. A., Siddiqui, M. F. & Park, S. 2019 Current and emerging methods of antibiotic susceptibility testing. *Diagnostics* **9**, 49. https://doi.org/10.3390/diagnostics9020049.

Kraemer, S. A., Ramachandran, A. & Perron, G. C. 2019 Antibiotic pollution in the environment: from microbial ecology to public policy. *Microorganisms* **7** (6), 180.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, Z. A., Coates, A., Bergstrom, R., Wright, G. D., Brown, E. D. & Cars, O. 2013 Antibiotic resistance – the need for global solutions. *Lancet Infect. Dis.* **13**, 1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9.

Lobanovska, M. & Pilla, G. 2017 Penicillin's discovery and antibiotic resistance: lessons for the future? *Yale J. Biol. Med.* **90**, 135–145.

Madappa, T. 2019 *Escherichia coli (E. coli) infections. Medication*. https://emedicine.medscape.com/article/217485-medication#2

Mambo, P. M., Westensee, D. K., Render, D. S. & Cowan, A. K. 2014a Operation of an integrated algae pond system for the treatment of municipal sewage: a South African case study. *Water Sci. Technol. A J. Int. Assoc. Water Pollut. Res.* **69**, 2554–2561. https://doi.org/10.2166/wst.2014.187.

Mambo, P. M., Westensee, D. K., Zuma, B. M. & Cowan, A. K. 2014b The Belmont Valley integrated algae pond system in retrospect. *Water SA* **40**, 385–394.

Manaia, C. M., Rocha, J., Scaccia, N., Marano, R., Radu, E., Biancullo, F., Cerqueira, F., Fortunato, G., Iakovides, I. C., Zammit, I., Kampouris, I., Vaz-Moreira, I. & Nunes, O. C. 2018 Antibiotic resistance in wastewater treatment plants: tackling the black box. *Environ. Int.* **115**, 312–324. https://doi.org/10.1016/j.envint.2018.03.044.

Matuschek, E., Brown, D. F. J. & Kahlmeter, G. 2014 Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **20**, O255–O266. https://doi.org/10.1111/1469-0691.12373.

Mitchell, S. A., de Wit, M. O., Blignaut, J. N. & Crookes, D. 2014 *Waste Water Treatment Plants: The Financing Mechanisms Associated with Achieving Green Drop Rating*. WRC Report No. 2085/1/14.

Momba, M. N. B., Osode, A. N. & Sibewu, M. 2006 The impact of inadequate wastewater treatment on the receiving water bodies – case study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province. *Water SA* **32**, 687–692.

Mthembu, M. S., Odinga, C. A., Swalaha, F. M. & Bux, F. 2013 Constructed wetlands: a future alternative wastewater treatment technology. *Afr. J. Biotechnol.* **12** (29), 4542–4553.

Nüesch-Inderbinen, M., Zurfluh, K., Peterhans, S., Hächler, H. & Stephan, R. 2015 Assessment of the prevalence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in ready-to-eat salads, fresh-cut fruit, and sprouts from the Swiss market. *J. Food Prot.* **78**, 1178–1181. doi:10.4315/0362-028X. JFP-15-018.

Nyamukamba, P., Moloto, M. J., Tavengwa, N. & Ejidike, I. P. 2019 Evaluating physicochemical parameters, heavy metals, and antibiotics in the influents and final effluents of South African wastewater treatment plants. *Polish J. Environ. Stud.* **28**, 1305–1312. https://doi.org/10.15244/pjoes/85122.

Ruimy, R., Brisabois, A., Bernede, C., Skurnik, D., Barnat, S., Arlet, G., Momcilovic, S., Elbaz, S., Moury, F., Vibet, M. A., Courvalin, P., Guillemot, D. & Andremont, A. 2010 Organic and conventional fruits and vegetables contain equivalent counts of gram-negative bacteria expressing resistance to antibacterial agents. *Environ. Microbiol.* **12**, 608–615. doi:10.1111/j.1462-2920.2009.02100.

Schulz, J., Kemper, N., Hartung, J., Janusch, F., Mohring, S. A. I. & Hamscher, G. 2019 Analysis of fluoroquinolones in dusts from intensive livestock farming and the co-occurrence of fluoroquinolone-resistant *Escherichia coli*. *Sci. Rep.* **9**, 5117. https://doi.org/10.1038/s41598-019-41528-z.

Syal, K., Mo, M., Yu, H., Iriya, R., Jing, W., Guodong, S., Wang, S., Grys, T. E., Haydel, S. E. & Tao, N. 2017 Current and emerging techniques for antibiotic susceptibility tests. *Theranostics* **7**, 1795–1805. https://doi.org/10.7150/thno.19217.

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J. & McDermott, P. F. 2012 Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002. *Emerg. Infect. Dis.* **18** (5), 741–749. doi:10.3201/eid1805.111153.

Tang, Y., Sahin, O., Pavlovic, N., LeJeune, J., Carlson, J., Wu, Z., Dai, L. & Zhang, Q. 2017 Rising fluoroquinolone resistance in Campylobacter isolated from feedlot cattle in the United States. *Sci. Rep.* **7**, 494. https://doi.org/10.1038/s41598-017-00584-z.

Thanner, S., Drissner, D. & Walsh, F. 2016 Antimicrobial resistance in agriculture. *mBio* **7** (2), 02227-15. doi:10.1128/mBio.02227-15.

Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A. & Laxminarayan, R. 2014 Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *The Lancet Infect. Dis.* **14** (8), 742–750. https://doi.org/10.1016/S1473-3099(14)70780-7.

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y. & Ruan, R. 2010 Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. *Appl. Biochem. Biotechnol.* **162**, 1174–1186. https://doi.org1007/s12010-009-8866-7.

WHO 2014 *Antimicrobial Resistance: Global Report on Surveillance 2014.* WHO. Available from: https://www.who.int/drugresistance/documents/surveillancereport/en/

WHO 2018 *WHO Report on Surveillance of Antibiotic Consumption.* WHO. Available from: https://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf

WHO 2019 *No Time to Wait. Securing the Future From Drug-Resistant Infections*. Available from: https://www.who.int/news-room/detail/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis

First received 29 January 2020; accepted in revised form 22 September 2020. Available online 21 October 2020