

Full Length Research Paper

Molecular characterization of water-borne multi-drug resistant *Escherichia coli*

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Antibiotics are very important in the fight against infectious disease caused by bacteria and other microbes for decades. Today microbes have developed ways to resist antimicrobial agents targeted at them. We sought to characterize and simultaneously detect virulence genes associated water-borne antibiotic resistant *Escherichia coli*, obtained from water sampled from ground and surface water sources. The Analytical Profile Index (API) was used for the identifications of *E. coli* isolates. The Kirby-Bauer disk diffusion method was employed for susceptibility testing. A DNA-STRIP molecular assay technology designed for detection of shiga toxin genes was used for the molecular characterization. *E. coli* isolates showed a high (32.99%), resistance to penicillin, and was highly susceptible (93.8%) to nitrofurantoin. *E. coli* was confirmed Polymerase Chain Reaction (PCR). None of the confirmed multidrug resistant *E. coli* isolate had genes for *stx1* and *stx2*. However, the *eae* intimin virulence gene was found on 6% of the multidrug resistant *E. coli* isolates. It was concluded that *E. coli* has developed a very high resistance to the various antibiotics. Second, the genotype EHEC test based on the DNA-STRIP technology used in this study has proved to be efficient and reliable in the molecular characterization of the multi-drug resistant *E. coli* isolates. The DNA-STRIP Genotype EHEC technology test is therefore recommended for pathogenic *E. coli* detection and monitoring. There is also a need to revise strategies towards the multidrug resistance programme.

Key words: DNA-STRIP Technology, *Escherichia coli*, polymerase chain reaction, drug resistance, water borne.

INTRODUCTION

Antibiotic resistance is a major challenge worldwide. It is a threat towards the attainment of the Sustainable Development Goals (SDGs) on health as well as gains made in health and development (Assembly, 2015, 2011). Today antibiotic-resistant genes imparting resistance to various antibiotics have been recognized in

different water environments. This includes drinking water worldwide (Marathe et al., 2017; Odonkor and Addo, 2018). The major threat for health globally is the potential transmission of these genes from the environmental microbes unto human pathogens (Karkman et al., 2017). Prospect of drinking water passing on pathogens to

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people, thus causing disease, is well documented (Bengtsson-Palme and Larsson 2016; Pan and Chu, 2018).

The spread of multi-antibiotic resistant microbes in water environments is an important public health problem with stakeholders such as policy makers and physicians worried about their future capacity and ability to treat various infectious diseases (Fitzpatrick and Walsh, 2016; Da Silva et al., 2011; Schmidt, 2002). Ultimately, the life time of an antibiotic hinges largely on the rise and dissemination of resistant bacteria. However, early interest relating to antibiotic resistant bacteria has predominantly been focused on medical settings and nosocomial infections.

The persistence of antibiotics in waterways accounts for the increase in bacteria resistance to antibiotics as observed in water environments. This ensues through “selective pressure” and horizontal gene transfer (Alonso et al., 2001). The phenomenon of selective pressure normally occurs in the presence of antibiotics owing to the heightened persistence or development of microbial strains carrying resistance genes (Economou and Gousia, 2015; Holmes et al., 2016). These genes block the mechanism of cellular destruction caused by antibiotic compounds. Resistance genes are usually found on plasmids that are susceptible to horizontal gene transfer. This explains medium through which antibiotic resistant bacteria and associated genes are spread in the water environment (Cheng et al., 2016; Gothwal and Shashidhar, 2015; Schmidt, 2002). It is important to note that these plasmids are extrachromosomal gene structures, and have the ability to move thus, allowing for plasmid encoded genes to be transferred across cell membranes. This method of gene transfers permits profusion and allows variety of resistant microbes to increase quickly in water environments contaminated with antimicrobial compounds. These waterways may therefore become reservoirs and incubators for antibiotic resistant bacteria (Alonso et al., 2001; Odonkor and Addo, 2018).

Escherichia coli is commonly recognized as the principal channel for the spreading of antibiotic resistance genes and associated virulence vectors. This is due to their profusion within such environments (Cheng et al., 2016; Gothwal and Shashidhar, 2015; Schmidt, 2002; Tauxe et al., 1997). For example, β -Lactam antibiotics are known to account for about a half of the global antimicrobial utilization (Alpay-Karaoglu, 2007). Resistance to various β -lactam antibiotics such as penicillins and cephalosporins is usually facilitated by the β -lactamase enzymes. *E. coli* strains have also become resistant to ampicillin by plasmid-mediated class A β -lactamase enzyme such as TEM-1. They tend to mutate to extended spectrum activity (Odonkor and Addo 2011; Odonkor and Ampofo, 2013; Livermore, 1995). Epidemiological research findings show that TEM-1 is the most common plasmid mediated B -lactamase between

medical Gram-negative bacteria (Bush and Jacoby, 1997).

However, there are other genes that are of public importance and are specific to *E. coli*. These genes include *stx1* and *stx2*, the *eae* intimin gene, and the *ipaH* (invasion plasmid antigene H) gene (Odonkor and Ampofo, 2013). They do not only confer resistance to *E. coli* but they are also known to be toxin producing. Although there are several studies assessing multi drug resistance (MDR) in *E. coli*, not much work has been done on these genes specific to *E. coli*. The aim of this study was to therefore to characterize antibiotic-drug resistant water borne *E. coli* and simultaneously detect associated virulence genes.

MATERIALS AND METHODS

E. coli isolation and identification

E. coli isolates were obtained from drinking water samples collected from ground water and surface water sources. *E. coli* organisms were identified using conventional methods (such as catalase test, indole test, nitrate reduction test, etc). This was then confirmed with Analytical Profile Index (API 20E). The *E. coli* control strain ATCC 25922 was obtained and used as the positive control.

E. coli susceptibility testing

Antibiotic susceptibility testing was done using the Kirby-Bauer disk diffusion methods as described by the Clinical and Laboratory Standards Institute (CLSI) (Lacy et al., 2004). Confirmed *E. coli* isolates obtained from overnight cultures on nutrient agar brought was brought to an equivalent of 0.5 McFarland. This was done by the suspension of isolates in sterile saline until the desire turbidity (0.5 McFarland) was achieved. The suspension was then streaked on complete surface of dishes with Mueller Hinton agar. The *E. coli* isolates obtained were tested against 14 antibiotics that were in use at the time of this study. These antibiotics and their strength are shown (Table 1).

All plates were incubated at 37°C for 24 h. This was after the antibiotic disks were placed aseptically on the streaked Mueller-Hinton agar dishes. Clinical and Laboratory Standards Institute (CLSI) protocols were used to interpret the results (Lacy et al., 2004).

Molecular characterization

A DNA strip technology test (GenoType EHEC) was used for the molecular characterization. The GenoType EHEC allows for a combined characterization and identification of the following genes: *eae* intimin gene, *ipaH* (invasion plasmid antigene H) gene, *stx1* shiga toxin gene and *stx2* shiga toxin gene. Three steps were involved in this molecular characterization method as follows: DNA extraction from *E. coli* culture, a multiplex amplification with biotinylated primers and a reverse hybridization. A template ensuring interpretation of the banding pattern was obtained and used (Prère and Fayet, 2005)

RESULTS

Results in Table 2 show the number of *E. coli* isolates

Table 1. Antibiotics and their corresponding disc concentration used.

Antibiotic	Disc concentration
Amikacin (AMK)	30 µg
Ampicillin (AMP)	10 µg
Cefotaxime (CTX)	30 µg
Cefuroxime (CXM)	30 µg
Chloramphenicol (CHL)	30 µg
Ciprofloxacin (CIP)	5 µg
Co-trimoxazole (COT)	25 µg
Erythromycin (ERY)	15 µg
Gentamicin (GEN)	10µg
Nalidixic acid (NAL)	10 µg
Nitrofurantoin (NIT)	300 µg
Penicillin (PEN)	10 units
Pipemidic acid (PA)	20 µg
Tetracycline (TET)	30 µg

Table 2. Isolates of *E. coli* obtained from the water sources.

Water source	Number of <i>E. coli</i> isolates obtained	Total (%)
Surface water	62	63.90
Ground water	35	36.10
Total	97	100

Table 3. Antibiogram of water-borne *E. coli* isolates.

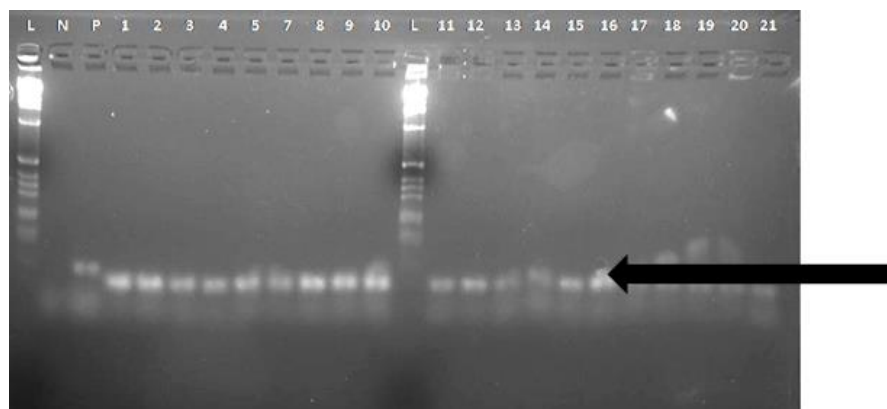
Antibiotic	Susceptibility			
	Disc concentration	Resistant number (%)	Intermediate number (%)	Sensitive number (%)
Amikacin (AMK)	30 µg	7 (7.22)	1 (1.03)	89 (91.75)
Ampicillin (AMP)	10 µg	11 (11.32)	41 (42.27)	45 (46.39)
Cefotaxime (CTX)	30 µg	4 (4.12)	4 (4.12)	89 (91.75)
Cefuroxime (CXM)	30 µg	28 (28.87)	18 (18.65)	51 (52.58)
Chloramphenicol (CHL)	30 µg	18 (18.56)	12 (12.37)	67 (69.07)
Ciprofloxacin (CIP)	5 µg	8 (8.25)	17 (17.53)	72 (74.22)
Co-trimoxazole (COT)	25 µg	10 (10.31)	6 (6.19)	81 (83.50)
Erythromycin (ERY)	15 µg	23 (23.71)	24 (24.74)	50 (51.55)
Gentamicin (GEN)	10µg	5 (5.15)	4 (4.12)	88 (90.72)
Nalidixic acid (NAL)	10 µg	4 (4.12)	6 (6.19)	87 (89.69)
Nitrofurantoin (NIT)	300 µg	4 (4.12)	2 (2.060)	91 (93.81)
Penicillin (PEN)	10 units	32 (32.99)	51 (52.58)	14 (14.43)
Pipemidic acid (PA)	20 µg	13 (13.40)	20 (20.62)	64 (65.98)
Tetracycline (TET)	30 µg	21 (21.45)	47 (48.45)	29 (29.90)

obtained from the various water sources. It can be seen from the table that 62 of the *E. coli* isolates accounting for 63.90% of the entire *E. coli* isolates obtained were from surface water sources and the rest (36.10%) were from ground water sources.

Results in Table 3 present the antibiogram patterns of the *E. coli* isolates tested against the 14 antibiotics used. Results show that the *E. coli* strains were most resistant to penicillin (32) representing 32.99%. On the other hand, the isolates were most susceptible to susceptible to

Table 4. Antibiotic resistance profile of *E. coli* isolates from the water sources.

No. of antibiotics	Isolates showing resistance	
	No.	(%)
One	10	17.24
Two	18	31.03
Three	13	22.41
Four	8	13.79
Five	3	5.17
Six	1	1.72
Seven	2	3.45
Eight	1	1.72
Nine	1	1.72
Ten	1	1.72

**Figure 1.** Diagram with arrow showing an amplicon on the gel.

nitrofurantoin (93.8%).

Results shown in Table 4 provide a summary of resistances. It can be observed from the table that 10 of the *E. coli* isolates were resistant to just one antibiotic, while one *E. coli* isolate was resistant to 10 out of the 14 antibiotics tested.

However, of great interest and importance as shown in Table 4 is that 48 out of the 97 (49.48%) *E. coli* isolates tested were resistant to 2 or more antibiotics. Thus, falls under the multi drug resistance classification (Hill et al., 2005).

Figure 1 shows a diagram of amplification of bands on the agarose gel. This is shown for the first few multi-drug resistant *E. coli* isolates. This is an important step, prior to hybridization. The success of DNA extraction and successful amplification is indicative of the presence of bands.

Results in Table 5 summarize the molecular characterization performed on the 48 multi drug resistant *E. coli* isolates. The PCR confirmed all isolates as *E. coli*. None of the confirmed multidrug resistant *E. coli* isolate had genes for *stx1* and *stx2*. However, the *eae* intimin

virulence gene was found on 6% of the multidrug resistant *E. coli* isolates.

DISCUSSION

Generally, a drug resistance refers to a phenomenon where there is a microbial resistance to at least two of the following classes of antibiotics: quinolones, aminoglycosides and lactams (Hill et al., 2005).

In this study, penicillin resistance was found to be very high. This has serious implications for public health, as the chances of these antibiotics curing infections caused by *E. coli* are significantly hampered. On the other hand, it is worth nothing that nearly all the 97 strains of *E. coli* (93.8%) found to be susceptible to nitrofurantoin, thus presenting a good choice for treatments. However, the rate at which these microbes are increasingly resistant to various antibiotics with an estimated over 10 million mortality attributable to antimicrobial-resistant infections annually by 2050 (O'Neill, 2018), the susceptibility of *E. coli* to just a single antibiotic as observed in this study

Table 5. Molecular characterization results.

Molecular parameter	Positive		Negative	
	Number	%	Number	%
<i>cc</i>	48	100	0	0
<i>uc</i>	48	100	0	0
<i>E. coli</i>	48	100	0	0
<i>ipaH</i>	0	0	48	100
<i>stx1</i>	0	0	48	100
<i>stx2</i>	0	0	48	100
<i>eae</i>	3	6	45	94

may not be considered a significant threat.

E. coli are generally apathogenic and belong to the enterobacteria group of microbes. They will not normally cause infections or disease. However, some strains such as Shiga toxin-producing *E. coli* (STEC), which are verotoxin-producing are known to possess pathogenic properties in humans as well as animals (Jaeger and Acheson, 2000).

Pathogenicity of a given bacteria strain such as *E. coli* is to a large extent influenced by virulence factors. In the case of *E. coli*, these factors include, capsule, adhesins, toxins and invasins, which are often present in a large genetic block on chromosome and may be horizontally transmitted between various strains. In this study, we found that 6% of the multiple resistant *E. coli* carried the *eae* virulence gene. Notwithstanding, a low percentage (6%) of virulence *eae* genes found on the stains of *E. coli*, the real danger is the potential to transfer these genes within the *E. coli* group and even with other members of the enterobacteria. Furthermore, presence of *eae* gene in water sources is of a grave public health concern, since spread of these virulence factors aside transmitting through food and water can also transfer from person to person (Karch et al., 2000).

Several disease outbreaks have been linked to the *eae* genes. The *E. coli* strains carrying *eae* gene have previously been isolated from adults who presented with the hemolytic-uremic syndrome. Similarly, it was also isolated in 101 children also presenting with hemolytic-uremic syndrome. However, in this case, the *E. coli* strains lacked the intestinal adherence factor intimin, which is known to be encoded by the *eae* gene (Bielaszewska et al., 2006). Gerber et al. (2002) reported that 97% of *E. coli* that were shiga toxin producing were isolated from children in Australia and Germany, were found to carry the *eae* gene. It worth noting that in this particular case the estimated mean incubation period was 8 days, longer than the 3 to 4-day incubation period previously reported for shiga-toxin-producing *E. coli* O157: H7.13 (Mead and Griffin, 1998).

Also, in this study, we found that all multiple resistant *E. coli* isolates were non *stx1* and *stx2* genes producing. *E. coli* carrying shiga toxin (*stx1* and *stx2*) genes are known

to possess pathogenic properties in humans as well as animals, thus they cause infections (Jaeger and Acheson, 2000).

E. coli are also known to be significant reservoirs of several genes that code for antibiotic resistance (Bucknell et al., 1997). Thus, major risk for public health lies in the potential for the transfer of resistant genes from environmental bacteria to human pathogens. The capacity of the resistant bacteria and associated genes to move across ecosystems is well documented (Von Wintersdorff et al., 2013).

Conclusion

This study found that all *E. coli* was highly resistant to penicillin and highly susceptible to nitrofurantoin. Furthermore, none of the isolates was gene producing for the virulence factor *Stx1* and *stx2*. However, 3 of the multidrug resistant *E. coli* isolates representing 6% were found to be *eae* virulence factor producing. Thus, the principal risk for public health is that resistance genes may be transmitted from environmental bacteria to human pathogens. We recommend the DNA•STRIP Genotype EHEC technology test is therefore recommended for pathogenic *E. coli* detection and monitoring. There is also a need to revise strategies towards the multidrug resistance programme.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Alonso A, Sanchez P, Martinez JL (2001). Environmental selection of antibiotic resistance genes: Minireview. *Environmental Microbiology* 3(1):1-9.
- Alpay-Karaoglu S, Ozgumus OB, Sevim E, Kolayli F, Sevim A, Yesilgil P (2007). Investigation of antibiotic resistance profile and TEM-type β -lactamase gene carriage of ampicillin-resistant *Escherichia coli* strains isolated from drinking water. *Annals of Microbiology* 57(2):281.
- Assembly G (2015). Sustainable development goals. SDGs Transform Our World, 2030.

- Bengtsson-Palme J, Larsson DJ (2016). Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environment International* 86:140-149.
- Bielaszewska M, Friedrich AW, Aldick T, Schürk-Bulgrin R, Karch H (2006). Shiga toxin activatable by intestinal mucus in *Escherichia coli* isolated from humans: Predictor for a severe clinical outcome. *Clinical Infectious Diseases* 43(9):1160-1167.
- Bucknell DG, Gasser RB, Irving A, Whithear K (1997). Antimicrobial resistance in Salmonella and *Escherichia coli* isolated from horses. *Australian Veterinary Journal* 75(5):355-356.
- Bush K, Jacoby G (1997). Nomenclature of TEM beta-lactamases. *The Journal of Antimicrobial Chemotherapy* 39(1):1-3.
- Cheng D, Xie Y, Yu Y, Liu X, Zhao S, Cui B, Bai J (2016). Occurrence and partitioning of antibiotics in the water column and bottom sediments from the intertidal zone in the Bohai Bay, China. *Wetlands* 36(1):167-179.
- Da Silva PEA, Von Groll A, Martin A, Palomino JC (2011). Efflux as a mechanism for drug resistance in Mycobacterium tuberculosis. *FEMS Immunology & Medical Microbiology* 63(1):1-9.
- Economou V, Gousia P (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance* 8:49.
- Fitzpatrick D, Walsh F (2016). Antibiotic resistance genes across a wide variety of metagenomes. *FEMS Microbiology Ecology* 92(2): fiv168.
- Fitzpatrick D, Walsh F (2016). Antibiotic resistance genes across a wide variety of metagenomes. *FEMS microbiology ecology* 92(2):fiv168.
- Gerber A, Karch H, Allerberger F, Verweyen HM, Zimmerhackl L B (2002). Clinical course and the role of Shiga toxin-producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: A prospective study. *The Journal of Infectious Diseases* 186(4):493-500.
- Gothwal R, Shashidhar T (2015). Antibiotic pollution in the environment: A review. *Clean–Soil, Air, Water* 43(4):479-489.
- Hill D, Rose B, Pajkos A, Robinson M, Bye P, Bell S, Elkins M, Thompson B, MacLeod C, Aaron SD, Harbour C (2005). Antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. *Journal of Clinical Microbiology* 43(10):5085-5090.
- Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJ (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet* 387(10014):176-187.
- Jaeger L, Acheson D (2000). Shiga Toxin-Producing *Escherichia coli* *Current Infectious Disease Reports* 2(1):61-67.
- Karch H, Bockemühl J, Huppertz HI (2000). Erkrankungen durch enterohämorrhagische *Escherichia coli* (EHEC). *Dtsch Arztebl* 97(36):36.
- Karkman A, Do TT, Walsh F, Virta MP (2017). Antibiotic-resistance genes in waste water. *Trends in Microbiology* 26(3):220-228.
- Lacy MK, Klutman NE, Horvat RT, Zapantis A (2004). Antibiograms: New NCCLS guidelines, development, and clinical application. *Hospital Pharmacy* 39(6):542-553.
- Livermore DM (1995). beta-Lactamases in laboratory and clinical resistance. *Clinical microbiology reviews* 8(4):557-584.
- Marathe NP, Pal C, Gaikwad SS, Jonsson V, Kristiansson E, Larsson DJ (2017). Untreated urban waste contaminates Indian River sediments with resistance genes to last resort antibiotics. *Water Research* 124:388-397.
- Mead PS, Griffin PM (1998). *Escherichia coli* O157: H7. *The Lancet* 352(9135):1207-1212.
- O'Neill J (2018). Tackling drug-resistant infections globally: Final report and recommendations. 2016. HM Government and Wellcome Trust: UK.
- Odonkor ST, Addo KK (2011). Bacteria resistance to antibiotics: recent trends and challenges. *International Journal of Biological and Medical Research* 2(4):1204-1210.
- Odonkor ST, Addo KK (2018). Prevalence of multidrug-resistant *Escherichia coli* isolated from drinking water sources. *International Journal of Microbiology*, 2018.
- Odonkor ST, Ampofo JK (2013). *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research* 4(1):2.
- Pan M, Chu LM (2018). Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China. *Science of the total environment* 624:145-152.
- Prère MF, Fayet O (2005). A new genetic test for the rapid identification of shiga-toxines producing (STEC), enteropathogenic (EPEC) *E. coli* isolates from children. *Pathologie Biologie* 53(8-9):466-469.
- Schmidt CW (2002). Antibiotic resistance in livestock: More at stake than steak. *Environmental Health Perspectives* 110(7):A396.
- Tauxe R, Kruse H, Hedberg C, Potter M, Madden J, Wachsmuth K (1997). Microbial hazards and emerging issues associated with produce a preliminary report to the national advisory committee on microbiologic criteria for foods. *Journal of Food Protection* 60(11):1400-1408.
- Von Wintersdorff CJ, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB, Savelkoul PH, Wolfs PF (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology* 7:173.