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Biofilms and Antimicrobial Susceptibility Profiles of *Escherichia coli* Recovered from Wastewater Treatment Plants in Kakamega Municipality, Kenya

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STUDIES have demonstrated high levels of ampicillin resistance among *Escherichia coli* occurring in wastewater and environmental systems in western Kenya. The current study investigated antimicrobial resistance profiles and biofilm formation abilities in presence and absence of sub-lethal ampicillin concentrations, of *E. coli* recovered from final sedimentation ponds of two wastewater treatment plants in Kakamega municipality. 34 non-duplicate *E. coli* isolates were recovered by direct plating of wastewater sample aliquots on MacConkey agar and their biofilm formation capabilities measured by crystal violet assay while disc diffusion and Polymerase Chain Reaction (PCR) techniques were used to determine their antibiotic resistance levels and ampicillin resistance genes respectively. The Spearman's Chi-square test at $P \leq 0.05$ was used to check for interdependence between antimicrobial resistance and biofilm formation capabilities. 58.8% of the isolates were multi-drug resistant (MDR) and 85.3% showed resistance to ampicillin, which was found to be encoded by bla_{TEM} in 65% and bla_{SHV} in 8.8%. The biofilm phenotype was exhibited by 61.8% of all the isolates, amongst which 23.6% showed a strong, 14.7% a moderate and 23.6% a weak propensity to form biofilms. This study revealed lack of association between antibiotic resistance and biofilm formation, but interestingly, ampicillin concentration of 8.0µg/ml triggered the highest biofilm biomass among the isolates. The findings drive at the conclusion that biofilm production among *E. coli* in wastewater treatment plants(WWTPs) does not correlate with antibiotic resistance, but may be an important protection mechanism against sub-lethal antimicrobial levels present in environmental milieu.

Keywords: Ampicillin resistance genes, Biofilms, *Escherichia coli*, Wastewater treatment plants.

Introduction

In many developing countries, waste management remains a challenge, especially in urban areas which are faced with a rapidly expanding population due to increasing rural-urban migration. In Kenya nearly all major towns are faced with the problem of expanding populations and settlements, that is not matched with wastewater management (KNBS, 2019). Recent studies have demonstrated the risks that these systems pose on communities, including acting as possible reservoirs of enteric pathogens and antimicrobial resistant strains (Malaho et al., 2018) besides being collecting vessels of residual antibiotics among other pollutants (Kimosop et al., 2016). Wastewater treatment in the region mainly rely on biological treatment processes whereby the wastewater slowly flow through filters into a series of four sedimentation ponds in which microbial biofilms digest organic matter and remove nutrients. The removal of nutrients in combination with prolonged exposure to sunlight is expected to eliminate parthogens and other host dependent microbes. However, biological treatment without chemical disinfection of effluents has been

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shown to only partially reduce the concentration of coliform bacteria in the order of 10^1-10^3 units and therefore such wastewater treatment plants (WWTPs) contribute to the contamination of surface water with antimicrobial-resistant bacteria (Blaak et al., 2015).

This study characterized *Escherichia coli* recovered from two WWTPs in Kakamega town in Western Kenya based on propensity to form biofilms, susceptibility to ten antibiotics and prevalence of two genes, $bla_{TEM,}$ and bla_{SHV}, associated with ampicillin resistance among Gram negative bacilli (Adegoke et al., 2016: Amira, 2013). This was premised on the fact that maintenance and spread of antimicrobial resistance (AMR) in hostile environments such as WWTPs could be linked to the capability of microbes to establish and coexist in surface-attached communities called biofilms (Anastasi et al., 2012). Antimicrobials in sub-lethal concentrations can induce biofilm formation apart from altering the microbial physiology and expression of some virulence genes (Mlynek et al., 2016; Silva et al., 2014). By forming biofilms, transient commensals and pathogens from antibiotics-fed livestock and humans are shielded from predation, UV-radiation, oxygen tension among other hazards and are able to acquire nutrients and exchange antimicrobial resistance genes that enhance their survival through the wastewater treatment processes (DePas et al., 2014; Macia et al., 2014; Rizzo et al., 2013). Such bacteria have the potential of becoming naturalized and thus transform these WWTPs into persistent point sources of surface and ground water contaminants (Jang et al., 2017).

Very few studies have investigated the biofilm formation capacities and the influence of residual antibiotics on survival of *E. coli* in environmental matrices (Ugwoke et al., 2019; Cornejova et al., 2015), yet high prevalence of antibiotic resistant *E. coli* in wastewater treatment systems in the region continue to be reported (Malaho et al., 2018; Wambugu et al., 2015; Atieno et al., 2013). This study therefore sought to investigate the survival mechanisms of *E. coli* in the WWTPs by determining their antimicrobial resistance profiles as well as their abilities to form biofilms under sub-lethal levels of ampicillin. Information generated herein emphasize the need to improve on wastewater treatment technologies employed in developing countries to enhance effective killing of bacteria and minimize contamination of water sources recieving effluents from the WWTPs, transmission of pathogens and AMR traits.

Materials and Methods

Study design and sample collection

A cross-sectional study approach was adopted and samples purposively collected from the final sedimentation ponds of the two wastewater treatment plants at Masinde Muliro University of Science and Technology (MMUST) (0031634N, 0696511E) and Shirere (0028313N, 0691850E). A total of 72 wastewater samples were asceptically collected in sterilized universal bottles, packed and transported in chiller boxes to the Microbiology laboratory at MMUST where they were processed within two hours.

Culture and isolation of Escherichia coli

E.coli was recovered from the samples by direct plating as described by Röderová et al. (2016).

An inoculum of each sample, after mixing thoroughly, was applied at a point on McConkey agar (HiMedia-India) using sterile swabs; and then streaked all over the medium surface by a sterilized wire loop. The culture plates were aerobically incubated at 37^{o}C for 24hrs. Presumptive *E. coli* (small pink colonies) were sub cultured on fresh McConkey agar to obtain pure cultures which were confirmed by standard biochemical tests (Cheesbrough, 2006).

Determination of levels of antimicrobial resistance

Resistance to ten antibiotics was determined by the Kirby Bauer disc diffusion technique following guidelines by the Clinical and Laboratory Standards Institute (CLSI, 2017). A colony from an 18 hrs old culture was picked on a flamed loop into sterile normal saline and its turbidity adjusted to 0.5 McFarland standards. A sterile cotton swab was dipped into each suspension then evenly swabbed on the surface of Mueller-Hinton agar (MH from HiMedia-India) three times while rotating through 60^o to obtain a uniform lawn of inoculum. Antibiotic discs impregnated with ampicillin (30µg), cefalexin (30µg), gentamicin (10µg), ceftriaxone (30µg), tetracycline (30µg), chloramphenicol (50µg), ciprofloxacin (30µg), cortrimoxazole (25µg), amoxyclav (30µg) and amikacin (30µg) (all from HIMedia-India) were placed on the inoculated media using a pair of flamed forceps (five per 100mm culture plate in

two sets per isolate). The cultures were incubated aerobically at 37°C for 18 hrs. *E. coli* ATCC 25922 was used as a control. The diameter of zone of inhibition (halo around and including the disc) for each antibiotic and every isolate was measured using a ruler and recorded in millimeters.

Detection of genes responsible for resistance to ampicillin

The protocol described by Kipkorir et al. (2016) was applied for DNA extraction and gene amplification with slight modifications. Purified Ampicillin-resistant *E. coli* cultures were pelleted, washed twice and thereafter suspended in 50 µl of DNase free water (molecular grade (MAGBIO-UK) in 2.0mL eppendorf tube (Thermofisher). The suspended cells were lysed by heating at 100°C for 10min in a heating block then centrifuged at 10,000rpm for 5min at 4°C and the supernatant was carefully aliquoted into a sterile eppendorf tube and stored at -20°C. Amplification of bla_{TEM} gene was done using the forward primer 5′-TCG GGG AAA TGT GCG-3′ and reverse primer 5′-TGC TTA ATC AGT GAG GCA CC-3′ while bla_{SHV} gene was amplified with 5′-GCC GGG TTA TTC TTA TTT GTC GC-3′ as forward primer and 5′-ATG CCG CCG CCA GTC A-3′ as reverse primer, described by Van et al. (2008) and made by Sigma-Aldrich United Kingdom). The PCR master mix was constituted with 13.3µL nuclease-free water, 2.0µL of 10X reaction buffer, 2.0µL of 10mM dNTP mixture, 0.25µL of each of 10µM forward and reverse primers, 2.0µL of the DNA template and finally 0.2µL of 5U/µL of Mag Taq Polymerase (MAGBIO- United Kingdom) to a final volume of 20.0µL in a 0.2mL thin walled PCR tube. The tubes were carefully loaded in a DNA thermal cycler pre-programmed to: Initial denaturation at 94°C for 5min; 35 cycles each consisting of 30sec denaturation at 94°C, 30sec annealing at the respective annealing temperatures in Table 1, and 1min extension at 72°C; followed by a final extension step for 10 min at 72°C. *E. coli* NCTC 13351 (a TEM-3 producer) and *Klebsiella pneumoniae* ATCC 700603 (SHV-18 producer) were positive controls while the negative control was an aliquot of the molecular grade water. Electrophoresis was done on 1.5% agarose gel under 1xTAE at 100 volts for 35min and the gene bands visualized through a UV illuminator (230nm).

Quantification of biofilm biomass

The biofilm assay was performed following the protocol described by Risal et al. (2018) with modifications. Each overnight culture in Tryptone Soy Broth (TSB (HiMedia-India) was diluted to OD_{600} = 0.2 (~ 10^8 CFU/mL), and three 180µL aliquots pippeted into wells of a 96-well microplate. The microplate was covered with a gas permeable membrane (ThermoFisher) and incubated in a gyrator (WiseCube Wis-10 WITEG Labortechnik-Germany) at 37°C with shaking at 100rpm for 24hrs. A medium without bacteria incubated under the same conditions was taken as a negative control while *E. coli* ATCC 25922 was the positive control. The spent culture media in each well was discarded and the wells carefully washed two times with phosphate buffered saline (PBS (Oxoid) water (pH=7.2) and placed without the lid in the incubator at 37°C for at least 30min to dry. 200µl of 0.3% Crystal violet solution (Oxoid) was added to each well and incubated for 5 minutes, in a well-ventilated area. The wells were washed twice with distilled water and excess water blotted out with a paper towel. 180µL of 33% glacial ethanoic acid solution was added and shaken at 100rpm for half a minute to dissolve the biofilm stain. Absorbance was read at 600nm (OD_{600}) in a Microplate Reader (AMP Platos R4PC) thrice in three independent experiments and the mean of the 9 measurements taken as the average OD_{600nm} for each isolate.

TABLE 1. Characteristics of Primers used in the study

Target gene	Primer name	Primer sequence (5′–3′)	Amplicon length, (bp)	Annealing Temp. (°C)	Source
bla_{TEM}	TEM	F: TCG GGG AAA TGT GCG R: TGC TTA ATC AGT GAG GCA CC	971	60	Van et al. (2008)
bla_{SHV}	SHV	F: GCC GGG TTA TTC TTA TTT GTC GC R: ATG CCG CCG CCA GTC A	1007	60	Van et al. (2008)

bla_{SHV} = Beta-lactam sulfurhydryl variable, bla_{TEM} = beta-lactam Temoneria

Effect of sub-MIC of ampicillin on biofilm formation

The effect of ampicillin (HiMedia-India) at different concentrations on abilities of the isolates to form biofilms were determined following the protocol described by Basar et al. (2016) with slight alterations. Various concentrations of ampicillin (256µg/mL to 0.5µg/mL) were prepared in 150µL of TSB and added to wells in columns 1-10, respectively on a sterile 96-well microtiter plate. 20µl of diluted (OD_{600nm} = 0.2) overnight culture was added to each well in columns 1-11. 20µL of TSB was added to each well of the 12th column to serve as the negative control. The 11th column contained inoculum in a drug-free medium to serve as the positive control. The microplates were sealed with a gas-permeable membrane and incubated at 37^{o}C while shaking at 100rpm for 24hrs. Quantification of biofilm biomass was done as described above.

Data analysis

The isolates were delineated as resistant or sensitive following the guidelines by CLSI (2017). Isolates that resisted at least three classes of drugs were categorised as multidrug resistant (MDR) and their Multiple Antibiotic Resistance index (MARI) was determined by the formula: MARI= a/(bxc); where a is the aggregate antibiotic resistant score, b is the number of antibiotics and c is the number of isolates (Adefisoye & Okoh, 2015). Differences in the diameters of zones of inhibition among the drugs were analyed by Kruskal Wallis test, and between the sources by Mann Whitney U test whereas relations among them were checked by the Spearman's correlation test at $P<0.05$.

The isolates were classified as non-biofilm producer ($OD \leq OD_c$); weak biofilm producers ($OD_c < OD \leq 2xOD_c$); moderate biofilm producers ($2xOD_c < OD \leq 4xOD_c$) and strong biofilm producers ($OD > 4xOD_c$). The optical density cut-off value (OD_c) was calculated as OD_c= Blank mean OD + 3 x standard deviation, following the criterion by Stepanović et al. (2007). The Chi square analysis (at $P<0.5$) was used to check for relationships in distribution of MDR Phenotypes between the sources, among the biofilm groups and the percentages of biofilm formation at various concentrations of ampicillin. The data was analyzed on IBM-SPSS version 20 software.

Results

Levels of antimicrobial resistance of the E. coli isolates

This study yielded 34 non-repetitive *E. coli* isolates of which 16 were from MMUST WWTP and 18 from Shirere WWTP. Ampicillin recorded the highest resistance levels 85.3%, whereas aminoglycosides (amikacin and gentamycin) showed 100% sensitivity as shown in Table 2. Kruskal Wallis test revealed significant differences across the ten antibiotics at P= 0.0001, whereas Mann Whitney U test showed lack of significant difference in their distribution between the two study sites at P= 0.848. The Spearman's correlation analysis revealed significant weak to moderate positive correlations between ampicillin and amoxiclav (r= 0.376, P= 0.028), cefalexin and ceftriaxone (r= 0.551, P= 0.001), cefalexin and ciprofloxacin (r= 0.594, P= 0.000), amoxiclav and tetracycline (r= 0.383, P= 0.025), amoxiclav and ciprofloxacin (r= 0.606, P= 0.0001) and between gentamicin and amikacin (r= 0.532, P= 0.001).

The MARI of the isolates from both plants was 0.322 and 0.312 for Shirere and MMUST, respectively. Spearman Chi-Square analysis showed lack of significant difference in proportions of MDR phenotypes among the isolates with respect to their sources P= 0.481. Overall, 58.8% of the isolates showed resistance to 3 or more classes of antibiotics with 38.2% showing resistance to 3 classes of antimicrobials, 17.6% to 4 classes in different combinations while 2.9% were resistant to 5 classes. The most prevalent Multiple Antimicrobial Resistance Phenotype (MARP) was AX-COT-TE as observed in 12.5% of isolates from MMUST and 11% of isolates from Shirere giving an overall prevalence of 11.8% (Table 3).

Ampicillin resistance genes

Among the ampicillin resistant isolates, 15% resistance could be associated to bla_{SHV} gene and 65% to the bla_{TEM} gene, although both genes were detected in one isolate recovered from MMUST WWTP as shown in Table 4 and Figs. 1, 2.

Biofilm formation capabilities of the isolates

In this study 43.8% of isolates recovered from the MMUST WWTP while 77.8% of the isolates from Shirere WWTP showed capacity to produce biofilms. Overall 61.8% of the isolates were biofilm producers, although only 23.6%

showed strong biofilm production capability, 14.7% moderate biofilm production capability and 23.6% weak biofilm production capability, whereas 38.2% were non-biofilm producers as presented in Table 5.

Correlation between biofilm formation and antimicrobial resistance

On testing for possible association between resistance to the antimicrobials and capacity to produce biofilms, Chi-square test revealed that the distribution of antimicrobial resistance was independent of the ability to produce biofilms (P> 0.05). Moreover, the distribution of ampicillin resistance genes was found to be uniform among biofilm producers and non- producers (P> 0.05).

Effect of sub minimum inhibitory concentrations of ampicillin on biofilm formation capabilities of the isolates

The MIC of ampicillin for all the isolates was above 256µg/mL. A general increase in the mean biofilm OD_{600nm} was observed from ampicillin concentration of 0.5 to 8.0µg/mL (Fig. 3) indicating that within this range, the isolates produced large biofilm masses. Chi-square test showed that increase in ampicillin concentration produced no significant change in numbers of biofilm producers (P= 0.951), indicating that sub-MIC ampicillin may increase biofilm formation in some *E. coli* strains without necessarily transforming non-producers into producers of biofilms.

TABLE 2. Antimicrobial resistance levels among isolates recovered from the two WWTPs

Antibiotic	Percentage resistance		
	MMUST (n= 16)	Shirere **(n= 18)**	**Total (n= 34)**
Ampicillin (AX)	87.5%	83.3%	85.3%
Ceftriaxone (CTR)	12.5%	0	5.9%
Chloramphenicol (C)	6.2%	22.2%	14.7%
Cefalexin (CN)	12.5%	5.6%	8.8%
Amoxiclav (AMC)	50%	55.6%	52.9%
Tetracycline (TE)	43.8%	38.9%	41.2%
Cotrimoxazole (COT)	75.0%	88.9%	82.4%
Ciprofloxacin (CIP)	25%	27.8%	26.5%
Amikacin (AK)	0	0	0
Gentamycin (GEN)	0	0	0

TABLE 3. Antibiogram patterns of the *E coli* isolates recovered from the two WWTPs

Pattern	Percentage of isolates		
	MMUST (n= 16)	Shirere **(n= 18)**	**Total (n= 34)**
AX-C-COT	6%	5.6%	5.9%
AX-COT-TE	12.5%	11%	11.8%
AX-COT- CIP	6%	0	2.9%
AX-AMC-TE-COT	12.5%	5.6%	8.8%
AX-AMC-COT-CIP	0	11%	5.9%
AX-CN-AMC-CTR-CIP	6%	0	2.9%
AX-CN-AMC-COT-CIP	0	5.6%	2.9%
AX-AMC-TE-COT-CIP	6%	5.6%	5.9%
AX-AMC-C-TE-COT	0	11%	5.9%
AX-AMC-CTR-TE-CN-CIP	6%	0	2.9%
AX-C-AMC-TE-COT-CIP	0	5.6%	2.9%

AX= Ampicillin, CTR= Ceftriaxone, CN= Cefalexin, AMC= Amoxiclav, C= Chloramphenicol, TE= Tetracycline, COT= Cotrimoxazole, CIP= Ciprofloxacin, AK= Amikacin and GEN= Gentamicin.

TABLE 4. Prevalence of ampicillin resistance genes among the *E coli* isolates

Gene	Amplicon size (bp)	Percentage of positive isolates		
		MMUST (n=9)	Shirere **(n=11)**	**Total (n=20)**
bla_{SHV}	1007	22.2%	9.1%	15%
bla_{TEM}	971	66.7%	63.6%	65%

bla_{SHV} = Beta-lactam sulfurhydryl variable, bla_{TEM} = Beta-lactam Temoneria

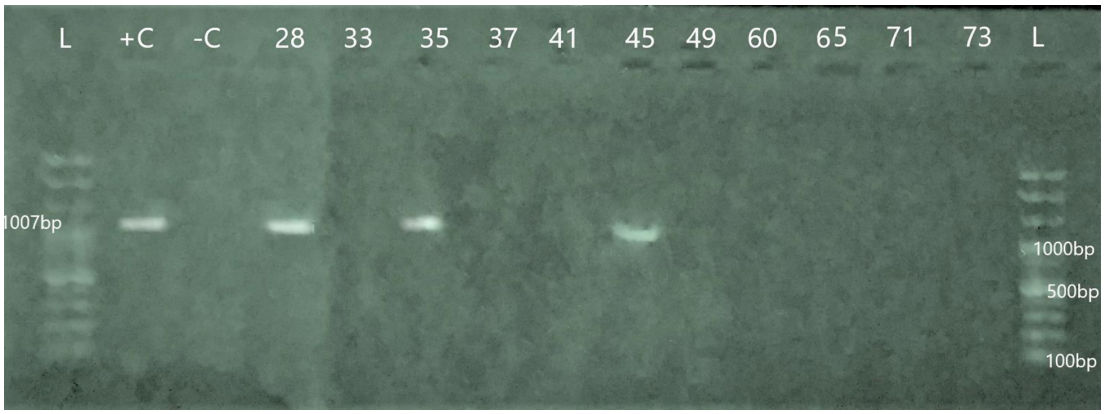

Fig. 1. Gel photo for bla_{SHV} gene in *E.coli* isolates from the two WWTPs [L = 100bp Ladder, +C = Positive control, -C = Negative control, 28 – 73 = Isolates with 28, 35 and 45 showing positive for bla_{SHV} gene]

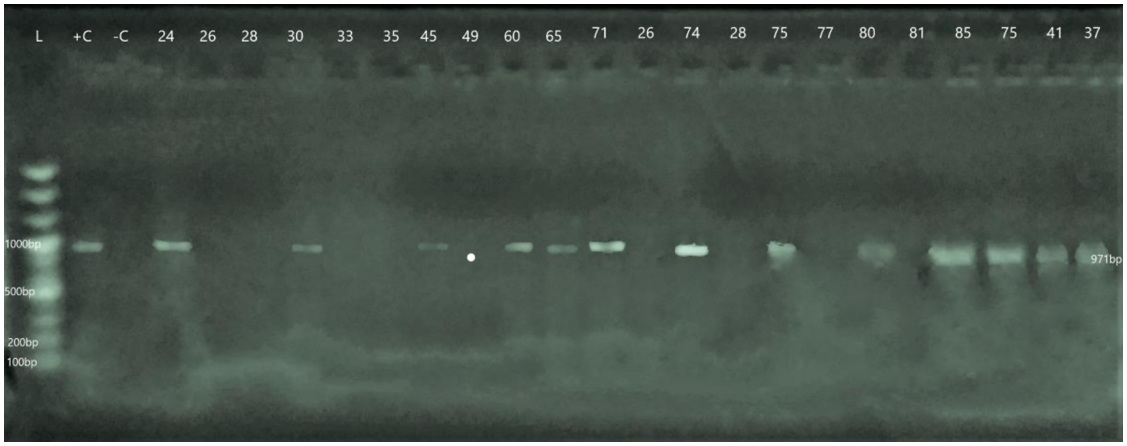

Fig. 2. Gel Photo for bla_{TEM} Gene in *E.coli* isolates from the two WWTPs [L = Ladder, +C = Positive control, -C = negative control, Isolates 24, 30, 45,60, 65, 71, 74, 75, 80, 81, 85, 73, 41 and 37 showing positive for blaTEM gene respectively]

TABLE 5. Categorization of *E. coli* isolates based on biofilm mass produced

Biofilm category	Percentage of isolates		
	MMUST (n= 16)	**Shirere (n= 18)**	**Total (n= 34)**
NB	56.3%	22.2%	38.2%
WB	12.5%	33.3%	23.6%
MB	12.5%	16.8%	14.7%
SB	18.8%	27.9%	23.6%

NB= Non-biofilm producer (OD<ODc), WB= Weak biofilm producer (ODc≤OD≤2ODc), MB= Moderate biofilm producer (2ODc<OD≤4ODc), SB= Strong biofilm producer (OD>4ODc).
ODc= Blank OD+3 Std deviation= 0.1264278.

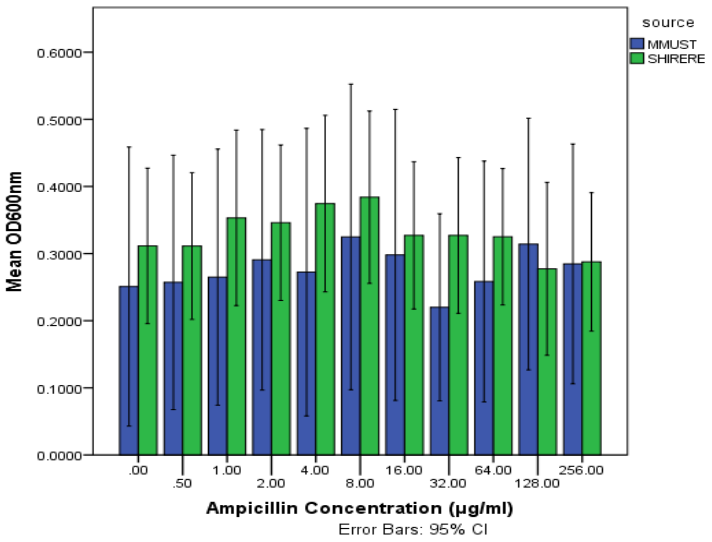

Fig. 3. Graph of mean OD against sub-MIC ampicillin for each site

Discussion

This study sought to determine the relationship between antibiotic resistance and biofilm formation capabilities of *E. coli* recorvered from two WWTPs in Western Kenya. Results herein showed a continued sustenance of high MDR phenotype in these environmental systems. The study ascertained the occurrence of bla_{TEM} and bla_{SHV} antimicrobial resistance genes as the major genes responsible for ampicillin resistance in *E. coli* from these environments. These genes can be plasmid mediated or expressed on chromosomes (Adegoke et al., 2016). They are responsible for the production of TEM and SHV extended spectrum β – lactamases (ESBL) apart from their narrow spectrum progenitors - TEM-1, TEM-2 and SHV-1 (Bush & Jacoby, 2010). These enzymes cleave the β – lactam ring in β – lactam antibiotics rendering them inactive and unable to bind the penicillin binding proteins (PBPs) and therefore building and maintenance of the peptidoglycan layer of the bacterial cell wall continues unaffected (Bush, 2013). The genes have been associated with resistance to penicillins, cephalosporins, and aztreonam (Gundran et al., 2019), with bla_{TEM} gene accounting for over 90% of ampicillin resistance in *E. coli* (Delmani et al., 2017). The current study recorded resistance to ampicillin, amoxyclav, ceftriaxone and cefalexin which could be closely associated to these resistance genes. Prevalence levels of 65% for bla_{TEM} genes in wastewaters that are not adequately treated is alarming as it exposes communities that use water sources receiving effluent discharges from these plants. This therefore calls for a risk assessment and management to minimize the occurrence and spread of these resistant bacteria in wastewater treatment plants and effluent receiving water bodies.

In this study, there was a mismatch between molecular (75%) and phenotypic resistance (85.3%), which perhaps could have been due to other resistance genes not tested for. Mechanisms such as alteration of outer membrane porins and expression of efflux pumps (Röderová et al., 2016) may also play an important role as determinants of ampicillin resistance. Nevertheless, the findings of the current study are in close agreement with other studies that have reported higher prevalence for bla_{TEM} compared to bla_{SHV} genes in both environmental and clinical situations. Amira (2013), reported 5% bla_{SHV} and 70.2 % bla_{TEM} genes in *E. coli* isolates that showed over 75% resistance to ampicillin, after recovery from wastewater in Alexandria- Egypt, whereas Röderová et al. (2016), reported that 57.1% and 19.0% of ampicillin resistant bacteria from hospital WWTPs outflow carried genes encoding TEM and SHV beta-lactamases respectively.

The current study findings further showed that *E. coli* recorvered from the two WWTPs significantly differed in their abilities to form biofilms, with 77.8% of isolates from Shirere WWTP being able to form biofilms as compared to 43.75% of those from MMUST WWTP. The study could not explain the difference in biofilm capabilities among *E. coli* within these two plants,

but considering the lack of significant differences in antibiograms and the close proximity of the two sites, most probabbly the difference in biofilm formation capabilities was indicative of other factors at play which therefore warrant further investigation. Being a stress-dependent response, biofilm formation could be greatly influenced by the prevailing physicochemical conditions in each WWTP apart from individual strain characteristics and their sources (Sharma et al., 2015; Naves et al., 2008).

Although both the phenotypic and genotypic resistance shown by the isolates in this study did not correlate with their abilities to produce biofilms, sub-MIC of ampicillin at 8.0µg/ml enhanced biofilm formation in some strains of *E. coli*. This observation therefore accentuated the fact that biofilm offers protection against exposure to antibiotics (Gupta, 2015; Naves et al., 2010). The extracellular polymeric matrix in biofilms shield microbes against stressors such as predators, chemical toxicants and fluctuations in temperature and osmotic pressure; allowing the microbe to persist at an interface by offering resistance to dehydration and access to nutrient-rich microenvironments apart from upregulation of enzymes that breakdown complex organic molecules into more easily metabolized substrates (Butler & Boltz, 2014; DePas, 2014). It can therefore be construed that the environment and its inherent challenges stimulate microbes to transform into biofilms, their genetic predisposition notwithstanding. In *Escherichia coli*, the sigma S factor which is encoded by the *rpoS* gene induces several stress resistance genes that codify biofilm formation in response to various environmental stresses (Sharma et al., 2015). Although the stresses may vary significantly within and between a given set of environmental matrices, different strains are able to utilize distinct pathways to initiate biofilm development (Chadha et al., 2014).

However, it has been shown that possesion of some resistance traits such as bla_{TEM} gene, may inhibit biofilm formation in some bacteria by disrupting type IV pili-mediated twitching motility and surface adherence (Naves et al., 2008). A study by Silva et al. (2014) on the other hand has shown that at half MIC of ampicillin biofilm formation increase among strong biofilm producer from bovine mastitis. Although the current study did not evaluate the levels of antibiotics in wastewater, a recent study by Kimosop et al. (2016), reported the occurrence of sub-lethal concentrations of ampicillin, amoxicillin, sulfamethoxazole, chloramphenicol, and ciprofloxacin in wastewater treatment systems in Western Kenya. This finding ascertains that *E. coli* may be stimulated to form biofilms within these WWTPs and thereby survive the treatment processes to contaminate subsequent aquatic milieu and other environmental matrices from which human exposure may occur.

Conclusion

Taken together, the findings of this study lead to the inference that residual ampicillin may promote biofilm production among *E. coli* in WWT systems and inadvertently enhance their environmental persistence. It is therefore imperative that developing countries evaluate the importance of biofilms and technologies employed in WWT systems, with the view of mitigating the risks of maintenance and transmission of pathogens and MDR traits.

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