Antibiotic Resistance Profile of Bacteria Isolated from Wastewater Systems in Eastern Ethiopia

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Abstract

Background : Antimicrobial resistance is one of the major public health challenges in the 21st century. In response, World Health Organizations launched a global action plan on antimicrobial resistance since 2015, which is, along with other objectives, aimed to strengthen knowledge of the spread of Antimicrobial resistance through surveillance and research. As wastewater systems are important reservoirs of communal human and animal bacteria, antibiotic residue and resistance genes they are considered as surveillance hotspots.

Objective : To assess the antibiotic resistance profile of proposed environmental resistance indicator bacteria in selected wastewater systems of Eastern Ethiopia from Feb. 2018 to Oct. 2019.

Method : Three wastewater management systems in Eastern Ethiopia, such as the activated sludge system of Dire Dawa University, waste stabilization pond of Haramaya University and a septic tank of Hiwot Fana Specialized University Hospital were monitored by sampled quarterly over 18 months. A total of 66 samples was collected from 11 sampling locations and a total of 722 Escherichia coli (151), Aeromonas spp. (142), P. aeruginosa (143), E. faecalis (144), and E. faecium (142) were isolated using selective culture media and biochemical tests. Their antimicrobial susceptibility was tested using standard Kirby-Bauer disk diffusion method on the surface of the Mueller-Hinton agar and the result was interpreted according to EUCAST guidelines. Multiple Antibiotic Resistance Index (MARI) was calculated for each isolate and its change in the course of treatment was evaluated.

Result : The highest percentage of resistance was seen for ampicillin among isolates of hospital wastewater effluent which is 36(94.7%), 33(91.7%) and 32(88.9%) for E. coli, E. faecalis, and E. faecium respectively. Lower rate of resistance was seen isolates from activated sludge system and gentamicin for isolates of activated sludge wastewater treatment system which is 10(16.4%), 8(13.3%), 11(18.9%), and 12(20.3%) for E. coli, E. faecalis, E. faecium , and P. aeruginosa respectively. MERI value was found to be variable across monitored sites and the course of the wastewater treatment process.

Conclusion : The study has found a high level of environmental antibiotic resistance indicator bacteria thrive in the wastewater systems most of which were multi-resistant. Hospital wastewater exhibits higher resistance than the other two wastewater systems. The multi-drug resistance index has significantly increased in the course of the wastewater treatment process, indicating the possible proliferation of resistance in the wastewater treatment system.

Introduction

Today antibiotic resistance is a growing public health concern around the world. Microorganisms resistant to commonly prescribed antibiotics are increasingly being found, and this lead World Health Organization (WHO) to declare antibiotic resistance a ‘major threat to public health’ (WHO, 2012). A return to the “pre-antibiotic era” would render many routine infections untreatable and would seriously affect healthcare practice (Gottlieb and Nimmo, 2011).

Wastewater systems are among the main anthropogenic sources of antibiotic resistance where antibiotic resistance develop, proliferate and discharged into the environment. Wastewater treatment plants (WWTP) are important reservoirs of commensal human and animal bacteria in which antibiotic resistant organisms, and/or, determinants persist in the final effluent and are released to the environment (Davison, 1999, Lorenz and Wackernagel, 1994, Thomas and Nielsen, 2005). However, data reported in previous publications are sometimes inconsistent and contradictory. For example, (Aminov et al., 2001) and (Auerbach et al., 2007) showed that due to the continuous exposure of bacteria to sub-inhibitory concentrations of antibiotics, wastewater treatment plants provide an environment that is potentially suitable for proliferation of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARBs). In contrary, (Suller and Russell, 2000) showed that continuous exposure of a triclosan-sensitive Staphylococcus aureus strain to sub-inhibitory concentrations of triclosan did not promote any changes in triclosan susceptibility or to other targeted antibiotics.

Despite the efforts to elucidate the role of wastewater treatment plans (WWTPs) in relation to antibiotic resistance, there is still no clear evidence that WWTPs, especially the biological treatment processes, are contributing to the proliferation of antibiotic resistance. Some studies suggest that WWTPs achieve a significant reduction in the number of ARBs ((Guo et al., 2015, Huang et al., 2012), while other research indicates that WWTPs serve as major contributors of ARBs and ARGs (Kim et al., 2010). These uncertainties may arise from research evaluating different treatment technologies, operational conditions, influent wastewater

quality or wastewater constituents, and different methodologies for the detection of ARBs and ARGs. Therefore, additional studies and analyses are needed to assess the role of wastewater treatment processes on proliferation and mitigation of antibiotic resistance. Hence, this study was conducted to assess resistance pattern of environmental resistance indicator and examine prepotency of wastewater systems to intensify antibiotic resistance of indicator organisms.

Methods

Study Setting and Sampling locations

Antibiotic resistance monitoring was conducted at selected wastewater systems (activated sludge system, waste stabilization pond, and septic tank system) in eastern Ethiopia from October 2018 to April 2019. Activated sludge system and waste stabilization pond were full scale plants receiving sewage from dormitories, cafeteria, animal farms and laboratories at Dire Dawa University and Haramaya University respectively (Figure 1). The third monitoring site is septic tank system receiving hospital wastewater at Hiwot Fana Specialized University Hospital (HFSUH). The Dire Dawa University wastewater treatment plant is activated sludge system (ASS) composed of preliminary waste treatment units (grit removal and stabilization basin), primary sedimentation tank (Dortmund tank), activated sludge system (aeration unit and secondary sedimentation) and waste oxidation pond. Haramaya University wastewater treatment plant is waste stabilization pond (WSP) composed of screening unit, two primary facultative pond and one maturation pond. Wastewater samples were collected at influent and effluent location at each unit operation/process in the course of wastewater treatment.

[Figure 1]

Sample collection

Wastewater samples were collected on quarterly basis in October 2018 - April 2019 from the specified sampling locations in the wastewater system. Samples were collected in two liters (2 L) plastic containers that were previously sterilized with 70% (v/v) alcohol and rinsed with deionized water prior to usage. During sampling, sample containers were rinsed three times with sample water before filling with the sample. To obtain flow representative sample, the actual samples were obtained by integrating grab samples collected in 30 minutes' interval in morning hours 8-11pm. After collection, the samples were protected from direct sunlight and transported in a cooler box containing ice packs to the laboratory for analyses. All samples were stored at 4°C and analyzed within 24 h of sample collection.

Wastewater Characteristics

Wastewater samples was analyzed for pH, at on-site using digital pH meter. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) and Total suspended solid were analyzed in laboratory according to standard methods (APHA, 2017)). Data on operational conditions such as flow rate, residence time and desludging rate of wastewater treatment plant were collected each time of the wastewater sampling.

Bacterial Enumeration, Isolation and Identification

Water samples were analyzed for the target bacterial using standard methods for the examination of water and wastewater (APHA, 2017). Samples were thoroughly mixed to distribute the bacteria uniformly prior to analysis. Serial dilution (10^{-2}-10^{-6}) of samples were prepared in sterile distilled water. Fifty milliliters (50 mL) from triplicates of dilution of each sample was filtered using a 0.45 µm, 47 mm, diameter, cellulosic white grid filter placed on filter holder. Approximately 25 ml of distilled water was first added to wet the filter paper. Selective medias were according to procedure recommended by manufacture and Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Membrane filters were aseptically transferred to 45mm petri dishes with the appropriate selective media.

R2A agar was used for enumeration of total heterotrophic bacteria after incubation at 37°C for 24 hours. mEndo-LES agar was used for total coliform and mFC agar for fecal coliform count after incubation at 37 and 44.5°C for 24 hours respectively. m-TEC agar for enumeration of Thermotolerant *E. coli 35*-37°C for 2 hours and at 44.5°±0.5°C for 22 hours. For isolation of *Enterococcus faecalis* and *Enterococcus faecium* m-Enterococcus agar was used. Plates were incubated at 37°C, and results were read after 24 and 48 h.

Maximum of five randomly selected presumptive Enterococcus colonies from mEnterococcus agar were subcultured on *Enterococcus* Differential Agar Base (TITG Agar Base) for the differentiation between *Enterococcus faecalis* and *Enterococcus faecium*. After an incubation at 35-37°C for 18-24 hours with 1% TTC solution, colonies with a deep red center and a narrow white periphery were identified as *Enterococcus faecalis*, whereas white or pale pink colored colonies were identified as *Enterococcus faecium*. Cetrimide Agar was used for isolation of *Pseudomonas aeruginosa* after incubation at 37°C for 48 h. We used *mADA*-V agar for isolation of *Aeromonas spp*. incubated in a temperature controlled incubator at incubation conditions showed in table 1. The plates were labeled with wastewater treatment plant, sampling location, date and sample number.

Table 1: Media and incubation conditions used for the enumeration, and primary isolation of the indicated bacteria from wastewater samples

Bacteria	Media	Incubation conditions
Total Heterotrophic count	R2A Agar	37°C; 24 h
Total coliforms	mEndo-LES agar	37°C; 24 h
Fecal coliforms	mFC agar	44.5°C; 24 h
Enterococcus spp. (E. faecalis and E. faecium)	mEnterococcus agar + TITG Agar Base	37°C; 48 h à 35-37°C for 18-24 hours
Eschrichia coli	m-TEC agar	35-37°C for 2 hours and at 44.5°±0.5°C for 22 hours
Aeromonas spp.	mADA-V agar	37°C; 24 h
Pseudomonas aeruginosa	Cetrimide agar	35◦C for 18 h

Antimicrobial susceptibility test

Two isolates per sample of each bacterial species were collected per sample to perform antimicrobial susceptibility testing (AST) except for hospital wastewater, for which three isolates were collected. The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates (Jorgensen J. and Turnidge J., 2015). Bacterial inoculum was prepared by suspending the freshly grown bacteria in 4–5 ml normal saline and the turbidity was adjusted to that of a 0.5 McFarland standard. Then this suspension was spread on over the entire surface of the Mueller-Hinton agar using cotton swab to produce confluent growth.

The susceptibility test was performed by placing paper disks impregnated with specific amounts of antibiotic on a lawn of bacteria grown on agar and aerobically incubated at 35 +_1°C for 18-24 hours. After an incubation period, the diameter for zone of inhibition, the area around the disk without bacterial growth, was measured.

Phenotypic resistance is often interpreted based on clinical standards and recommended breakpoints. A more reliable alternative for the interpretation of the antibiotic resistance of environmental bacteria may be the epidemiological cut-off (ECOFF) value developed by European Committee on Antimicrobial Susceptibility Testing (EUCAST), which, in a given taxonomic group, separates the populations with acquired resistance mechanisms (non-wild-type) from the wild-type populations that have no resistance. In contrast to clinical breakpoints, the ECOFF values are epidemiologically based, do not relate to the therapeutic efficiency and do not differ among different committees (Kahlmeter, 2014). The inhibition zone diameters for this study were interpreted according to EUCAST guidelines (EUCAST, 2018), except *E. coli* tested for tetracycline, *Enterococci* tested for which were evaluated by the Clinical Laboratory Standards Institute (CLSI, 2011) guidelines.

Table 2: Disk content and EUCAST breaking points of each antibiotic tested for specific indicator bacteria

Antibiotic class	Antibiotic	Code	Content (µg)	*E.coli*		*Enterococci spp.*		*P. aeruginosa*		*Aeromonas spp.*	
				<R	≥S	< R	≥S	<R	≥ S	R	S
β-Lactams	Ampicillin*	AMP	10 | 2	14	14	8	10	NR		NR	
	Amoxicillin/Clav	AMC30	20/10	19	19			NR		NR	
Cephalosporin	Ceftazidime	CAZ30	10	19	22	IR		17	17	21	24
	Cefepime	CFP	30	24	27	IR		21	21	24	27
Aminoglycosides	Gentamicin*	GEN10	10 | 30	14	17	8	8	15	15	NR	
	Amikacin	AMIK	30	15	18			15	18	NR	
fluoroquinolone	Levofloxacin	LVL5	5	23	19	15	15	22	22	24	27
	Ciprofloxacin	CIP5	5	24	26	15	15	26	26	24	27
carbapenem	Meropenem	MRP10	10	16	22	NR		18	24		
sulfonamides	Co-Trimoxazole	SxT25	1.25/23.75	11	14	23	23	NR		16	19

IR: intrinsically resistant, NR: not recommended, *we have used two type of ampicillin, and gentamicin disk for AST of *E. coli and* Enterococci *spp.*

Multiple Antibiotic Resistance Index (MARI): index was determined for each isolate by using the formula , where represents the number of antibiotics to which the test isolate depicted resistance and represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Krumperman, 1983). A MARI value of 0.2 indicates high-risk environment where antibiotics are often used (Osundiya et al., 2013, Christopher et al., 2013).

Analysis

Data analysis was done using descriptive and inferential statistical tools in R programing environment. A p-value of ≤0.05 was considered indicative of a statistically significant difference. Box plot graphs were chosen to illustrate the distribution of the MERI values using the mean values. In order to decide which statistical test should be used for determining the significance the data were first analyzed for their normal distribution using the Shapiro-Wilk test. The data were not normally distributed, and the Kruskal–Wallis test, a non-parametric version of the classical one-way analysis of variance (ANOVA), was used to determine variations in the level of antibiotic resistance (as measured by MERI) among studied bacterial groups. The result was used to assert weather antibiotic resistance level is significantly different among the three monitored systems and antibiotic resistance level varies in course of wastewater treatment progress.

Result

In the specified monitoring period, 66 samples were collected from 11 sampling locations in the three monitoring site in six monitoring rounds sampled quarterly Feb. 2018 to Oct. 2019 (Table 3). A total of 722 bacterial isolates proposed to indicate level of antibiotic resistance in the monitored wastewater systems were isolated and analyzed for their susceptibility to commonly prescribed antibiotics.

Table 3: Number of samples and bacterial isolates obtained per monitoring sites

	No. of sampling points	No. of sample	Number of isolate				
			E. coli	*E. faecalis*	*E. faecium*	*P. aeruginosa*	*Aeromonas spp.*
S	5	30	61	60	58	59	58
P	4	24	52	48	48	48	48
S	2	12	38	36	36	36	36
al	**11**	**66**	**151**	**144**	**142**	**143**	**142**

ASS: Activated Sludge System, WSP Waste Stabilization Pond, STS; Septic Tank System

Physicochemical and Bacteriologic characteristics of wastewater

Selected physicochemical and biological characteristics of wastewater analyzed were presented in Table 4. Wastewater characteristics (both physicochemical and microbial load) of the three systems was almost comparable. However animal farm waste entering Haramaya University waste stabilization pond was strongest waste both in organic and bacterial load with mean BOD and COD measure of 1108. 33 and 1275.33mg/L respectively and with $5.55*10^8$, $2.74*10^8$, and $1.13*10^8$cfu/100mL for *total coliform*, *fecal coliform*, *enterococci. Spp*. and *E. coli* respectively. pH of effluent from maturation pond in waste stabilization pond Haramaya University and oxidation pond of activated sludge system of Dire Dawa University were 9.25 and 9.45 respectively. Treatment efficiency of wastewater plants were presented in Table 4 as log reduction for specific physicochemical and bacterial contaminant. Efficiency of removal at log scale ranges from 0.83 for COD removal at WSP to 3.21 for total coliform at activated sludge system.

Antimicrobial susceptibility profile of bacterial isolates

This study evaluated ten commonly prescribed antibiotics against five group of bacteria proposed to indicate antibiotic resistance level in the environment. From the three monitored site and across the course of wastewater treatment 151 *E. coli* were isolated and tested for their resistance pattern against ten commonly prescribed antibiotics. Antibiotic resistance pattern of *E. coli* is presented in Figure 2. As shown, E. coli resistance is higher for β-Lactams and Cephalosporin groups such as ampicillin, amoxicillin/clavulanic acid while low resistant for Aminoglycosides (Gentamicin and Amikacin), and carbapenem (Meropenem) groups.

Table 4:Mean value of selected biological and physicochemical characteristics of row and effluent wastewater in the three monitoring sites and removal capacity of wastewater treatment facilities

Site	Characteristics	Total Coliform cfu/100ml	Fecal Coliform cfu/100ml	*Enterococci spp.* cfu/100ml	*E. coli* cfu/100ml	BOD mg/L	COD mg/L	TSS mg/L	pH
ASS	Row	$5.14*10^8$	$2.45*10^7$	$1.31*10^8$	$1.17*10^7$	737.67	931.33	707.67	7.23
	Effluent	$3.18*10^5$	$5.12*10^4$	$3.93*10^5$	$2.75*10^4$	73.17	119.33	63.50	9.45
	Log reduction	3.21	2.68	2.52	2.62	1.003	0.89	1.04	
WSP	Row	$5.55*10^8$	$2.74*10^8$	$1.13*10^8$	$9.87*10^6$	1108.33	1275.33	951.67	7.45
	Effluent	$9.9*10^6$	$7.28*10^5$	$3.33*10^5$	$5.36*10^4$	91.17	187.67	60.67	9.25
	Log reduction	1.74	2.57	2.53	2.26	1.08	0.83	1.19	
STS	Row	$1.39*10^8$	$7.93*10^7$	$9.3*10^7$	$1.53*10^7$				
	Effluent	$3.5*10^6$	$3.9*10^6$	$4.28*10^6$	$4.06*10^5$				
	Log reduction	1.59	1.3	1.33	1.57				

ASS: Activated Sludge System, WSP: Waste Stabilization Pond, STS: Septic Tank System, BOD; Biochemical Oxygen Demand, COD: Chemical Oxygen Demand, TSS: Total Suspended Solid

[Figure 2]
Isolates have shown reduced susceptibility for β-Lactams and Cephalosporin (Ampicillin, Amoxicillin/Clav and Ceftazidime) across all monitoring locations. Similarly, across all sites isolates have showed higher susceptibility to Aminoglycosides (Gentamicin and Amikacin), and carbapenem (Meropenem). The highest frequency of resistance was recorded against ampicillin 94.7% for *E. coli* isolates from Hospital wastewater, followed by ceftazidime with a frequency of 86.8% for *E. coli* isolates from hospital wastewater. Except ampicillin, amoxicillin/clav and ceftazidime, the resistance frequencies displayed by the isolates against other antibiotics were <50%, as shown in Figure 2.

From the three monitored site a total 286 enterococcus spp. (144 *E. faecalis* and 142 *E. faecium*) were isolated and their antibiotic resistance was tested against five commonly prescribed antibiotics such as ampicillin, gentamicin, levofloxacin, ciprofloxacin, and Co-Trimoxazole. The result of antibiotic susceptibility test was presented in Figure 3. Both isolates of Enterococcus spp. exhibit higher level of resistance for ampicillin and Co-Trimoxazole, while exhibiting higher susceptibility for gentamicin. Resistance of E. faecalis range from 13.3% for gentamicin in ASS waste water to 91.7% for ampicillin in STS of hospital wastewater. Similarly, antibiotic resistance of E. faecium is in range between 18.9% present for gentamicin in ASS waste water to 88.9% for ampicillin in STS of hospital wastewater.

[Figure 3]

A total of 143 pseudomonas aeruginosa were isolated from ASS (59), WSP (48) and STS (36). The isolates were tested for antibiotic resistance activity against seven antibiotics vis. ceftazidime, cefepime, gentamicin, amikacin, levofloxacin, ciprofloxacin, and meropenem and the result is presented in Figure 4. As showed, *pseudomonas aeruginosa* isolates expressed higher level of resistance for Cephalosporin such as ceftazidime and cefepime while showing higher susceptibility for gentamicin and meropenem. Resistance level is in range between 18% resistance to meropenem among isolates of ASS to 77.8% resistance to ceftazidime and cefepime among isolates of hospital STS.

Form 66 samples collected from the three sites 142 aeromonas spp. were isolated and their antibiotic resistance profile was tested against five comment antibiotics such as ceftazidime, cefepime, levofloxacin, ciprofloxacin, and Co-Trimoxazole. Isolates expressed higher level of antibiotic resistance Co-Trimoxazole, ceftazidime, and cefepime while expressing higher susceptibility for levofloxacin.

[Figure 4]

Susceptibility of bacterial isolates to different antimicrobials is shown Table 6. Isolates from hospital wastewater have showed elevated resistance characteristics for all isolates and drugs tested. Highest percentage resistance across all isolates was for AMP resistance for hospital wastewater which is 36(94.7%), 33(91.7%) and 32(88.9%) for *E. coli*, *E. faecalis,* and *E. faecium* respectively. Lower rate of resistance was seen GEN10 for activated sludge wastewater treatment system which is 10(16.4%), 8(13.3%), 11(18.9%), and 12(20.3%) *E. coli, E. faecalis, E. faecium, and P. aeruginosa* respectively. Except ampicillin, amoxicillin/clav and ceftazidime, the resistance frequencies displayed by the isolates against other antibiotics were <50%, as shown in Figure 5.

Accordingly, isolates from hospital wastewater have showed elevated resistance characteristics for all isolates and drugs tested while isolates of ASS expressed lower resistance for all isolates and tested drugs. Highest percentage resistance across all isolates was for ampicillin resistance for hospital wastewater which is 36(94.7), 33(91.7) and 32(88.9) for E. coli, E. faecalis, and E. faecium respectively. Lower rate of resistance was seen gentamicin for activated sludge wastewater treatment system which is 10(16.4), 8(13.3), 11(18.9), and 12(20.3) *E. coli, E. faecalis, E. faecium*, and *P. aeruginosa* respectively. Higher level of *E. coli* resistance was seen in hospital wastewater which is in range between 42.1% meropenem and 94.7% for ampicillin.

Table 5: Antibiotic resistance among isolates of environmental resistance indicator bacterial species by monitoring site

Site	Resistance phenotype	No. tested	Number and (%) resistant to antibiotic tested										MARI Mean (SD)
			β-Lactams		Cephalosporin		Aminoglycosides		fluoroquinolone		carbapenem	sulfonamides	
			AMP	AMC30	CAZ30	CFP	GEN10	AMIK	LVL5	CIP5	MRP10	SxT25	
ASS	E. coli	61	29(47.5)	28(45.9)	30(49.2)	19(31.2)	10(16.4)	13(21.3)	14(22.9)	17(27.87)	11(18)	15(24.6)	.30 (0.03)
	E. faecalis	60	26(43.3)	-	-	-	8(13.3)	-	13(21.6)	15(25)	-	16(26.7)	.26 (0.03)
	E. faecium	58	25(43.1)	-	-	-	11(18.9)	-	12(20.7)	15(25.9)	-	18(31)	.28 (0.04)
	P. aerugino	59	-	-	25(42.37)	23(39)	12(20.3)	12(20.3)	12(20.3)	16(27.1)	11(18.6)	-	.27 (0.03)
	Aero spp.	58	-	-	19(32.8)	21(36.2)	-	-	12(20.7)	17(29.31)	-	24(41.4)	.32 (0.04)
WSP	E. coli	52	28(53.8)	25(48.1)	27(51.9)	19(36.5)	13(25)	14(26.9)	17(32.7)	19(36.54)	15(28.9)	15(28.9)	.37 (0.03)
	E. faecalis	48	26(54.2)	-	-		11(22.9)	-	14(29.2)	17(35.4)	-	17(35.4)	.35 (0.03)
	E. faecium	48	23(47.9)	-	-		11(22.9)	-	13(27.1)	17(35.4)	-	21(43.7)	.35 (0.03)
	P. aeruginosa	48	-	-	24(50)	20(41.7)	9(18.75)	48(29.2)	14(29.2)	15(31.3)	13(27.1)	-	.32 (0.04)
	Aero spp.	48	-	-	16(33.3)	19(39.6)	-	-	13(27.1)	18(37.5)	-	29(60.4)	.40 (0.04)
STS	E. coli	38	36(94.7)	30(78.9)	33(86.8)	31(81.6)	15(39.5)	17(44.7)	21(55.26)	19(50)	16(42.1)	29(76.32)	.65 (0.03)
	E. faecalis	36	33(91.7)	-	-		13(36.1)	-	19(52.8)	21(58.3)	-	22(61.1)	.60 (0.04)
	E. faecium	36	32(88.9)	-	-		18(50)	-	23(63.9)	23(63.9)	-	27(75)	.68 (0.04)
	P. aerugino	36	-	-	28(77.8)	28(77.8)	16(44.4)	17(47.2)	17(47.22)	21(58.3)	17(47.2)	-	.57 (0.03)
	Aero spp.	36	-	-	26(72.2)	29(80.6)	-	-	19(52.8)	25(69.4)	-	25(69.4)	.69 (0..4)

ASS: Activated Sludge System, WSP: Waste Stabilization Pond, STS: Septic Tank System, AMP: Ampicillin, AMC30: Amoxicillin/Clav, CAZ30: Ceftazidime, CFP, Cefepime, GEN10: Gentamicin, AMIK: Amikacin, LVL5: Levofloxacin, CIP5: Ciprofloxacin, MRP10: Meropenem, SxT25: Co-Trimoxazole

Change in multidrug resistance level in course of wastewater treatment

Difference in antibiotic resistance level among the three monitored sites and its change in the course of wastewater treatment process was shown in box plots of Figure 6 and Figure 7 respectively. As shown in the boxplot 6, there is clear variation in MERI value at three monitored site with higher rate of being multidrug resistance in STS of the hospital wastewater.
[Figure 5]
Multidrug resistance level as measured by mean value of MERI, has also shown clear variation at each stage in the three monitored sites. Bar graph in Figure 6 below depicted change in mean MERI value in course of wastewater treatment process. For each of wastewater sites monitored effluent has higher level of MERI compared with row wastewater. Increase has also been shown at each stages for ASS and WSP.
[Figure 6]

Discussion

Antimicrobial Resistance is becoming the most significant public health problem of the 21st century and is linked to factors related to the overuse or misuse of antimicrobials in human and veterinary practice, and to environmental pollution (Gottlieb and Nimmo, 2011, Kahlmeter, 2014). Addressing the AMR phenomenon effectively requires close collaboration under a “one health” approach, taking into account the interconnections between human health, animal health and the environment (Sakkas et al., 2019). Hence in our study presented we examined the resistance profile proposed environmental resistance indicator bacteria from two University

wastewater treatment systems (ASS and WSP) and one hospital sewage septic tank system and change in intensity of resistance in course of wastewater treatment progress.

BOD to COD ratio is important aggregate measure of wastewater characteristics, indicating biodegradability of wastewater (Basri et al., 2019) and microbial community (Zhang et al., 2020, Osińska et al., 2019). Typical values for the ratio of BOD/COD for untreated domestic wastewater are in the range from 0.3 to 0.8 (Samudro and Mangkoedihardjo, 2010). BOD/COD ratio of the waste treated in ASS and WSP is in range between 0.79 of row to 0.61 of effluent and 0.87 of row to 0.46 at effluent respectively. This make it suitable for biological treatment, which make ASS and WSP the right choice for treatment of such waste. High level of efficiency of microbial removal was achieved in ranging 95% to 99% both at ASS and WSP. This may be related with interplay between sunlight, algal growth and elevated pH (Curtis et al., 1992).

The rate of isolation of resistant bacterial in the hospital wastewater was higher than the non-hospital environment for all indicator variables, this was statistically significant ($P < 0.001$). Similar observation was reported by Moges et al. (2014). The difference in environmental resistance between the three wastewater systems may be explained by different type of source wastewater. Influent wastewater to ASS is dominate by human waste which comprises both antibiotic resistant bacteria and antibiotic residues, a mixture that under favorable conditions, of high nutrient content and close contact between bacteria, may promote antibiotic resistance dissemination (Martinez, 2009). Whereas, influent wastewater to WSP, dominated by animal husbandry wastewater, which may comprise large amount of antibiotics residue, which in turn contribute for elevated rate isolation of antibiotic resistance bacteria (Martin et al., 2015). Factors other than the indiscriminate use of antibiotics in human medicine, animal husbandry, and agriculture may disrupt the microbial balance in favor of resistant bacteria. Hospitals are known to not only discharge pathogenic bacteria, most of which could be carrying resistance determinants into its wastewater, but also traces of antibiotics in urine, feces, as well as spilled and expired drugs that are improperly discarded into wash basins, are all channeled to the wastewater systems.

MARI has been used to estimate health risk associated with the spread of drug resistance in an environment. A MARI value of 0.2 (arbitrary) is used to differentiate between low- and high-health risk, and MARI greater than 0.2 suggests that a strain(s) of bacteria originate from an environment with high contamination or antibiotics usage (Christopher et al., 2013). The MARI estimates obtained for isolates from out study sites 0.287, 0.36, and 0.639, for ASS, WSP and STS respectively. These measures were all greater than 0.2, suggesting that the isolates originated from environments with high use or contamination of antibiotics. The high MARI values obtained in this study may suggest the exposure of the isolates to antibiotics pressure, which might have resulted from inappropriate use of antibiotic among the population in the study area, and may lead further to increase in the development of multidrug resistance overtime if appropriate measures are not put in place (Adefisoye and Okoh, 2016).

This study showed that intensity of resistance increase in course of wastewater treatment process. There is clear increase in measure of multidrug resistance profile of isolates in course of wastewater treatment process. This can be taken as indication of propensity of wastewater systems to intensify antibiotic resistance. Currently there is no clear evidence that whether resistance may develop in wastewater treatment plants (WWTPs) (Bouki et al., 2013). Couse effect relationship not yet well established between the presence of antibiotic resistance determinants in wastewater treatment plant and the favoring of resistant bacteria. However, there is established evidence that wastewater, or even treated wastewater, contain higher proportions of various resistant bacterial populations in relation to the respective proportions contained in other aquatic environment (Huang et al., 2012). As per former studies, the conditions in wastewater treatment plant are favorable for the proliferation of ARB, and non-resistant bacteria to acquire resistance genes (Davies, 2012). Goñi-Urriza et al. (2000) monitored the population of antibiotic resistant bacteria in effluent of wastewater treatment plant and receiving river, antibiotic susceptibility tests it was found that resistance against 21 out of the 22 antibiotics tested was significantly increased among the strains of *Enterobacteriaceae* and *Aeromonas spp.* collected downstream of the wastewater discharge point. Iwane et al. (2001) also reported that the ratio of tetracycline resistant coliforms increased by up to 6.8% downstream of a wastewater treatment plant.

Although this study addresses important Environmental health issues, it is not free from limitation. We are unable to identify the genes responsible for expressed resistance. We are also unable to determine level of antibiotic resistance determinants such as antibiotic residue, heavy metal concentration and antibiotic resistance genes in the wastewater systems. In addition, carbapenamase and extended spectrum betalactamase pattern of isolated bacterial species were not determined.

Conclusions

The study has found high level of environmental antibiotic resistance indicator bacteria thrive in the wastewater systems most of which were multi-resistant. Hospital wastewater exhibit higher resistance than the other two wastewater systems. Multi-drug resistance index has significantly increased in the advancement of wastewater treatment process for all wastewater treatment plants. This may indicate proliferation of resistance in the wastewater treatment system.

Abbreviations

AMR: antimicrobial resistance; ATCC: American Type Culture Collection; CLSI: Clinical Laboratory Standard Institute; DRERC: Departmental Research and Ethics Review Committee; EPHI: Ethiopian Public Health Institute; MDR: multi dregresistance; MIC: minimum inhibitory concentration; SOP: standard operatingprocedure; SPSS: Statistical Package for Social Science; WHO: World Health Organization.

Declarations

Authors' contributions

AT involved in raising initial idea, proposal development, the collection of samples, processing of samples in the laboratory, analysis and interpretation of data and in writing the manuscript. YA, TA, BS, DM, NB involved in the reviewing the proposal, commented in the method designing and reviewing drafts of the analysis. All authors read and approved the final manuscript

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

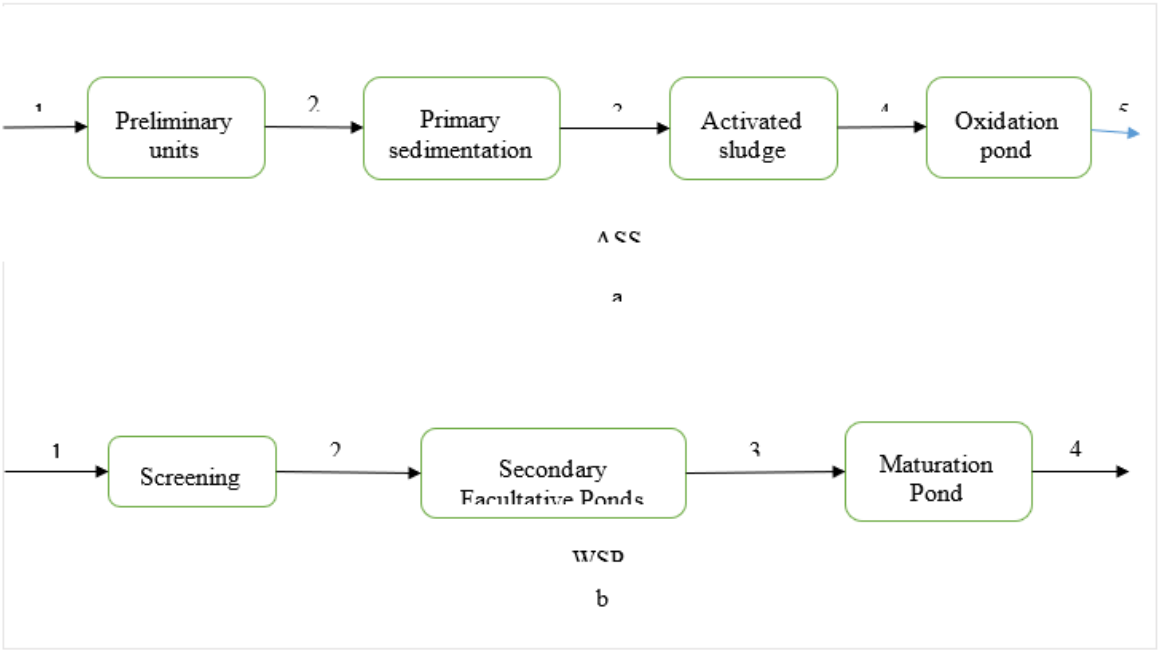

Figure 2

schematic diagram of unit operations and processes (a)activated sludge system at Dire Dawa University with the five sampling locations (b) waste stabilization pond at Haramaya university with the four sampling locations

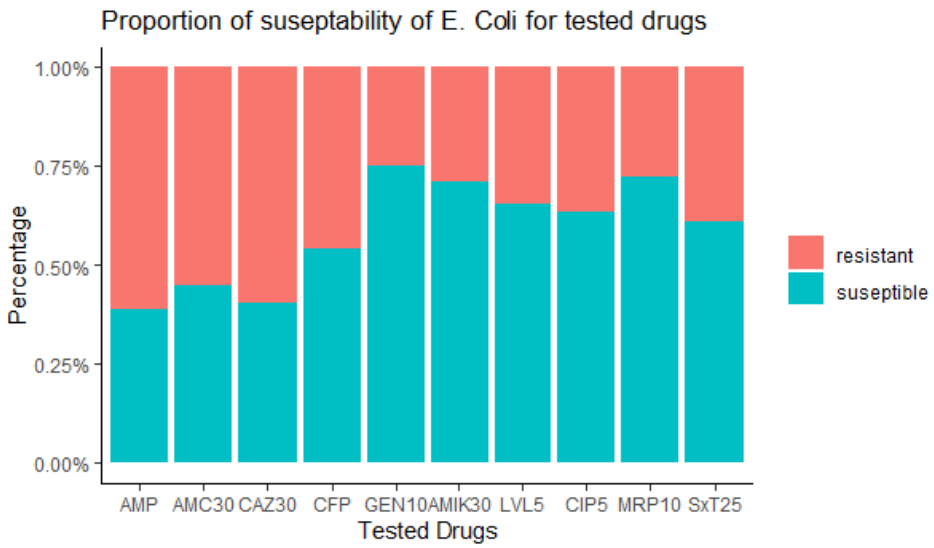

Figure 4

Level of antibiotic susceptibility among E. coli isolates from the three monitored sites

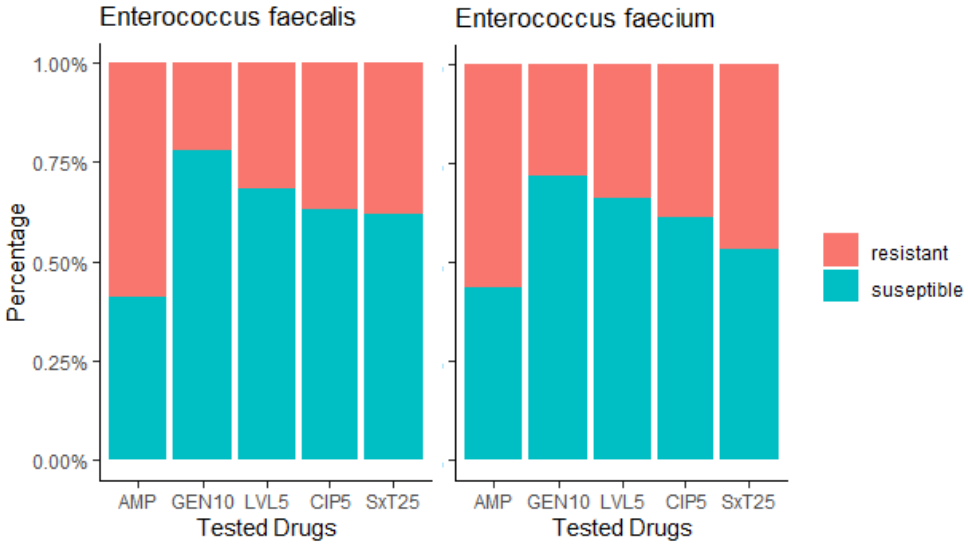

Figure 6

Level of antibiotic susceptibility among Enterococcus spp. isolated from the three monitored sites

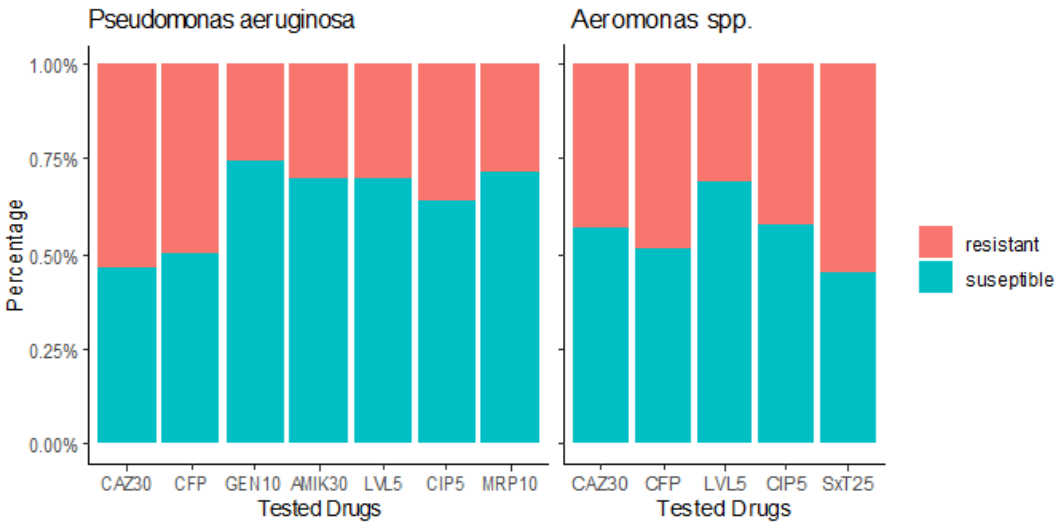

Figure 8

Level of antibiotic susceptibility among P.Aeruginosa and Aeromonas spp. isolated from the three monitored sites

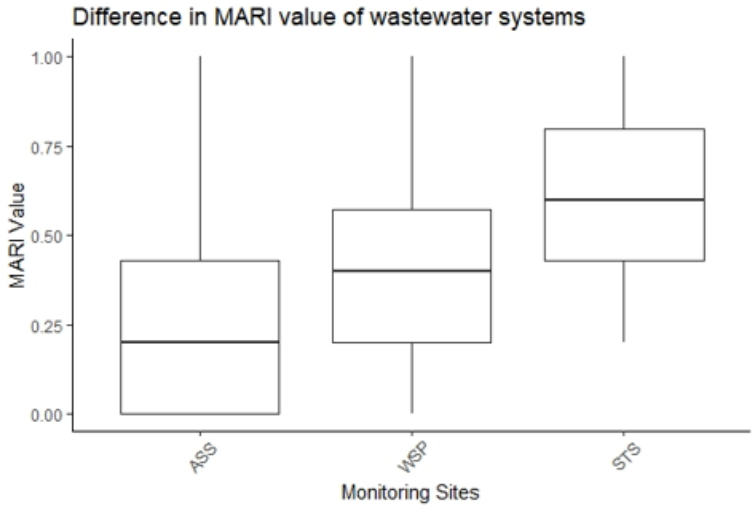

Figure 10

box plot of mean MARI value in the in the three wastewater systems

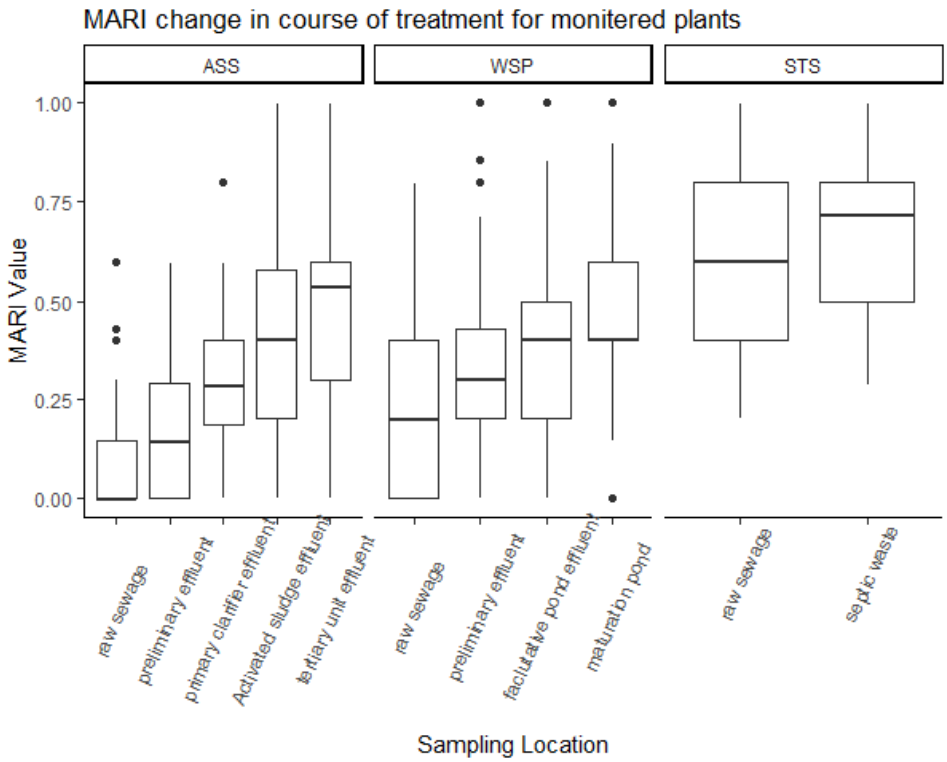

Figure 12

Change in MARI value in the course of waste water treatment in the three studied sites