Research Article

Prevalence of Multidrug-resistant Bacteria Isolates in Waste Water from Different Hospital Environment in Umuahia, Nigeria

[1]Uzoije U.N., *[2]Moses I.B., [2]Nwakaeze E.A., [3]Uzoeto H.O., [2]Otu J.O., [4]Egbuna N.R., [3]Ngwu J.N., [5]Chukwunwejim C. R., [6]Mohammed D.I., [7]Peter I.U., [2]Oke B., [2]Iroha I.R.

[1]Diagnostic Laboratory Unit, University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[2,*2]Department of Applied Microbiology, Faculty of Science Ebonyi State University, Abakaliki, Ebonyi State Nigeria.
[3]Department of Dental therapy, Faculty of Dental Health Federal College of Dental technology and Therapy, Trans-Ekulu, Enugu, Nigeria.
[4]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Science, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria.
[5]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.
[6]Dental Nursing Department, Federal College of Dental Technology and Therapy, Trans-Ekulu Enugu
[7] Department of Public Health, Faculty of Health Technology and Engineering Federal College of Dental technology and Therapy, Trans-Ekulu, Enugu, Nigeria.
***Corresponding author's E-mail:** ben_iyke70@yahoo.com

Received: 12-12-2020; **Revised:** 16-07-2021; **Accepted:** 24-07-2021; **Published on:** 15-08-2021.

ABSTRACT

The discharge of untreated hospital waste water into the environment is a major public health concern as this could result in the environmental spread of multidrug-resistant (MDR) bacteria. The dissemination of such MDR bacteria in waste water remains unexamined in most geographical area. This study assessed the prevalence of multidrug-resistant bacterial isolates in waste water from hospital environment in Umuahia, Nigeria. Exactly 200 waste water samples were collected from various diagnostic laboratory units of Michael Okpara University of Agriculture, Umudike (MOUAU) Health Centers and Federal Medical Center (FMC), Umuahia in 250 ml screw-capped, heat-sterilized bottles. Water samples were analyzed using standard microbiological techniques. Bacterial isolates from water samples were identified with API-20E test kit. Antibiotic susceptibility test was done using Kirby–Bauer disc diffusion method. Multiple antibiotic resistance index (MARI) of isolated bacteria was determined using standard formulae. A total of 147(73.5 %) bacterial species such as *S. aureus*, *Shigella* spp, *E. coli*, *Enterobacter* spp, *Proteus mirabilis*, and *Arizona* spp were identified from the waste water samples. Bacterial isolates exhibited resistance to tetracycline, trimethoprim-sulfamethoxazole, clindamycin, nalixidic acid, vancomycin, and chloramphenicol but very susceptible to imipenem. All isolates were multidrug-resistant with MARI values ranging from 0.5 - 0.8. This study revealed the presence of MDR bacteria in hospital waste water samples in Umuahia, Abia state, Nigeria. The threat and risk of exposure to such MDR bacteria is of public health significance and raises concern over poor management and disposal of hospital waste water or effluents.

Keywords: Multidrug-resistant bacteria, hospital environment, waste water, antibiotics

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2021.v69i02.003

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v69i02.003

INTRODUCTION

Wastewater has been observed as a niche for proliferation of bacteria due to vast present of optimum growth nutrient. Wastewater generated in most facilities are not usually recycled and may harbor multidrug resistant bacteria of public health significant. It is clear that the environment allows the proximity of transfer of resistant genes with multidrug determinant among bacterial isolates which can be disseminated further to sensitive bacteria.[1] Hospitals are known primary hotspots for selection of Multidrug resistant bacteria where several types of antibiotics and other pharmaceutical constituents at sub-therapeutic concentrations are discharged frequently inducing high selection pressure in the bacterial community.[2] In recent time, waste management in most hospital in developing country like Nigeria has been deem or consider unapt due to lack of proper waste management practice (as most hospital waste does not get appropriate treatment before being released to the nearby aquatic tributary or terrestrial environment) and inadequate advocacy on the impact of waste to human health. Wastewater generated from hospitals may include toilet flush, sinks, dish waters, bath tubs, washing machines and sewers released into the environment. They may carry both chemical and physical pollutants in the hospital environments, which may include hazardous substances, hormones and pharmaceuticals, toxins and poisonous organic materials both soluble and insoluble.[3] It is worth noting, that the discharge of hospital untreated wastewater to the environment greatly contributes to the environmental pool of diverse multidrug resistant bacteria and antimicrobial resistance genes.[4] The environmental pool of diverse multidrug resistant bacteria greatly influence and contribute to crossing resistance from one bacterial

species to another, also from one environment to another. This phenomenal trend could be possible via horizontal mobile genetic elements such as plasmids and transposons.[5,6] Bridget *et al.*[7] observed recurrent conjugative transfer of resistant genes in bacteria and found that more than 83 % of their environmental isolates had exchanged one or more resistant genes. Shakibaie *et al*.[8] reported horizontal transfer of resistant genes by conjugation from *Pseudomonas aeruginosa* to *E. coli*. As evidence in the study setting (Umuahia, Abia State) most hospital untreated waste samples are seen on terrestrial and water receiving bodies. This effluent from the hospital environment could contain multi-drug resistant (MDR) pathogenic bacteria capable of causing infection in humans and animals or commensal organisms capable of disseminating their resistance genetic markers to other bacterial species in the environment impacting natural ecosystem.[10] Several studies in many regions have evaluate the occurrence of multidrug-resistant isolate in hospital wastewater[1,9] and infer their findings to accumulation of wide spectrum of resistant genes in the environment. Importantly, the potential risk of dissemination of antimicrobial resistance agent to the ecology and its public health consequences is not underrated. Therefore, assessing the prevalence of multidrug resistant bacterial isolates from hospital environment in Umuahia, Abia State will be feasible and robust in decision making and contribute immensely to worldwide MDR Epidemiology surveillance.

MATERIALS AND METHODS

Study Areas

The Study areas were Michael Okpara University of Agriculture, Umudike (MOUAU) Medical and veterinary clinic and Federal Medical Center (FMC), Umuahia. It is situated within the Capital Territory, towards Ikwuano Local Government Area (5º 26'N 7º 34'E) South of Umuahia, Abia State Capital; about 10 kilometers from Umuahia town, along the Umuahia-Ikot Ekpene road, a direct route to the state capitals of Abia, Akwa-Ibom, and Cross River.

Ethical Approval

Ethical clearance was obtained from the Michael Okpara University Ethical Board, and the Ethical Committee of the Federal Medical Center, Umuahia with Ethical clearance number MOU/EB/000256 and FMC/UA/123C12

Sample collection and isolation of bacteria

Sample Collection

Two hundred (200) wastewater were collected from Michael Okpara University of Agriculture, Umudike (MOUAU) Health Center and Veterinary Clinic (Diagnostic Laboratory Units) and Federal Medical Center (FMC) Umuahia (from various sections) in 250 ml capacity screw-capped, heat-sterilized bottles according to Standard methods.[10]

Bacteriological analysis of water samples

Plate Count Method

A 1:10 dilution of wastewater was prepared by adding 1ml of wastewater to each of two petri dishes containing 9 ml of diluent (Ringer's solution of quarter strength); and with a fresh pipette, a 1:100 dilutions was prepared as stated above. One milliliter (1 ml) of undiluted wastewater was added to each of the two petri dishes, plus 20 ml of the required medium to each dish. This was mixed by rotating both clockwise and anticlockwise several times. It was incubated at 37 ºC for 18-24 hours. Bacterial colonies numbering between 30 and 300 was counted; and reported as number of colonies/ml of sample.[11,12]

Characterization of bacterial isolates

Bacterial isolates from the wastewater samples were identified and characterized by microscopic examination, standard conventional biochemical and physiological tests and with API 20E kits. Bacterial cultures were examined for colony morphology, cell morphology, haemolysis on blood agar, odour (or characteristic smell), motility, DNase test, Catalase, Coagulase, Citrate test, Triple Sugar Iron test, Gram stain reaction and sugar fermentation tests according to Microbiology Practical Handbook.[11] In addition, *Citrobacter*, *Klebsiella*, and *Enterobacter* species were confirmed using API 20E kit (Analytical Profile Index), a biochemical panel for identification and differentiation of members of the family Enterobacteriaceae and the software APIWEB (Biomérieux, France) was used to interpret results after incubation of the organism in each chamber according to the instruction provided by the manufacturer.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility of bacterial isolates to antimicrobials agent was determined using Kirby Bauer disc diffusion technique according to the Clinical and Laboratory Standards Institute guidelines (2016) on Mueller Hinton Agar (MHA) plate (Oxoid, Ltd). An overnight colony was transferred into test tube with 5 ml sterile water adjusted to obtain a turbidity matching of 0.5 McFarland turbidity standards. Standardized isolates were seeded on MHA plate and the following antibiotics were used: Penicillin (10 μg), ceftriaxone (30 μg), cefotaxime (30 μg), gentamicin (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), ciprofloxacin (5 μg), sulphonamide/trimethoprim (23.75/1.25 μg), chloramphenicol (30 μg), lincomycin (15 μg), erythromycin (15 μg), imipenem (10 μg), clindamycin (2 μg), vancomycin (30 μg), ceftazidime (30 μg) (Oxoid, Ltd). Antibiotic susceptible test results were interpreted as susceptible, intermediate or resistant according to the guidelines of Clinical and Laboratory Standards Institute.[13,14]

Determination of Multiple Antibiotic Resistance Index (MARI)

Isolates were reported as multidrug resistant (MDR) when they exhibit resistant to at least three or more antimicrobial classes. *E. coli* ATCC 25922 was used as a quality control organism. MARI value was determined using the formulae MARI = x/y, where "x" was the number of antibiotics to which test isolates displayed resistance while "y" is the total number of antibiotics to which the test organism has been evaluated for sensitivity.[14]

RESULTS

Distribution of Bacterial species isolated from Hospital waste water

A total of 147(73.5%) bacterial species were recovered from waste water emanating from hospitals in this study. Varying frequency of distribution of bacteria in different hospital waste water was seen with highest occurrence rate of 92 (46.0%) bacteria species isolated from College Veterinary Medical Clinic MOUAU (CVM) followed by 30 (15.0%) from University Health Centre Services MOUAU (UHS) and the least occurrence rate of 25 (12.5 %) was recovered from Federal Medical Centre MOUAU (FMC) (Table 1). Among all bacterial species, *S. aureus* was most predominant isolate recording 22.58 % and *Klebsiella pneumoniae* 16.67 % as the second most predominant isolate while *Shigella* species and *Citrobacter* species (3.33 %) were both the least predominant isolate from University Health Centre Services MOUAU (UHS) (Table 2). The frequency of bacteria isolated form wastewater samples from College Veterinary Medical, Clinic MOUAU (CVM) shows that *E. coli* was the most occurring bacteria recording 22.3%, *Enterobacter* species 11.9 % and *S. aureus* 10.8 % (Table 3). Other wastewater sample harbour high prevalence rate of 52.0 % of *S. aureus* and 12.0 % of both *Proteus mirabilis* and *Escherichia coli* while lower isolation rate of 2.0 % was documented against *Arizona* species, *Klebsiella pneumoniae* and *Enterobacter* species respectively (Table 4)

Antimicrobial resistant profile of bacterial species recovered from Hospital waste water

Majority of the bacterial species from UHS were phenotypically observed with resistant pattern of 50-100 % against most of the test antimicrobial agent in this current study (Table 5). *S. aureus* and *Enterobacter* species obtained from College Veterinary Medicine, Clinic MOUAU (CVM) wastewater exhibited high level of resistant to cephalosporin, glycopeptide, macrolide, lincosamide, Tetracycline, Trimethoprim-Sulfamethoxazole and Chloramphenicol (Table 6). *E. coli*, *S. aureus Enterobacter* species and *Arizona* species were all susceptible to ciprofloxacin and imipenem (Table 7). Majority of the isolate recovered from waste water demonstrate resistant to most of the antibiotics with MARI mean average value within the range of 0.5-0.8 (Table 8).

DISCUSSION

Diverse bacterial species: *Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Citrobacter* species, *Enterobacter* species, *Arizona* species and *Shigella* species of environmental and clinically significant was reported in hospital waste water in this study. The detection of these bacteria is due to the fact that, hospital wastewater contains a diverse group of pathogenic, commensal and environmental bacteria. This observation is in agreement with other studies.[9,15,16] *S. aureus* was the most predominant bacteria with isolation rate of 52.0%, 22.5%, 10.8% from the three studied locations. High-rate detection of *S. aureus* compared to other bacteria species is not surprising as it is one of the ubiquitous commensal bacteria commonly found in humans, animals, inanimate object etc. The characteristic composition of hospital wastewater and sewage make this reservoir a suitable ecological niche for the growth and spread of Multidrug resistance bacteria/genes due to selection pressure and horizontal gene transfer. High rate of MDR was detected in majority of the isolates in this study. Notably, *E. coli* was 50-100 % resistance to various antimicrobials, particularly to tetracycline, trimethoprim-sulfamethoxazole, gentamicin, ceftriaxone and ciprofloxacin that are commonly used in hospital environment. Similar high rate of multi-drug resistance among *E. coli* strains from clinical, environmental origin and food from hospital has been reported in previous studies in different countries.[9,17,18] Undoubtedly, the major mechanism of the observed multidrug resistance to antimicrobials is due to production of enzymes encoded for multidrug resistant genes carried on various plasmids, as such the presence of such gene in this *E. coli* isolates can contribute to horizontal transmission of multidrug resistance genes to other bacterial species in the wastewater and receiving downstream water bodies.[19]

Table 1: Hospital wastewater samples collected from different sources: (MOUAU UHS; MOUAU CVM and FMC UMUAHIA) and their bacterial total isolates

S/N	Wastewater Sources	Number of Samples	Number of Bacterial Isolated
1	University Health Centre Services, MOUAU (UHS)	60	30 (15.0 %)
2	College of Veterinary Medicine, Clinic MOUAU (CVM)	80	92 (46.0 %)
3	Federal Medical Centre, Umuahia (FMC)	60	25 (12.5 %)
	Total	**200**	**147(73.5 %)**

Table 2: Bacterial isolated from waste water samples from MOUAU (UHS)

Sources	No. collected	No. of Isolate	Bacteria							
			E. coli (%)	*S. aureus* (%)	*K. pneumoniae* (%)	*Arizona* spp (%)	*Enterobacter* spp (%)	*Shigella* spp (%)	*Citrobacter* spp (%)	*Proteus mirabilis* (%)
Ward	35	19	4 (21.05)	5 (26.32)	4 (21.05)	3 (15.78)	2 (10.53)	0 (0)	0 (0)	1 (5.26)
laboratory	15	7	2 (28.57)	0 (0)	1 (14.29)	1 (14.29)	2 (28.57)	0 (0)	1 (14.29)	0 (0)
Histopathology Lab	10	4	0 (0)	2 (50.0)	0 (0)	0 (0)	0 (0)	1 (25.0)	0 (0)	1 (25.0)
Total	**60**	**30**	**6 (20.0)**	**7 (22.58)**	**5 (16.67)**	**4 (13.33)**	**4 (13.33)**	**1 (3.33)**	**1 (3.33)**	**2 (6.67)**

Key: Histo Lab-Histopathology Laboratory, MOUAU-Michael Okpara University of Agriculture Umudike; UHS-University Health Services; n-number of bacterial isolates; (%)-percentage of number of bacteria

Table 3: Bacteria isolated from CVM, MOUAU

Sources	No. collected	No. Isolated	Bacteria							
			E. coli (%)	*S. aureus*(%)	*K. pneumoniae* (%)	*Arizona* spp (%)	*Enterobacter* spp (%)	*Shigella* spp (%)	*Citrobacter* spp (%)	*Proteus mirabilis* (%)
ML	35	16	7 (43.75)	5 (31.25)	1 (6.25)	1 (6.25)	0 (0)	0 (0)	0 (0)	2 (12.5)
PL	15	10	1 (10.0)	3 (30.0)	1 (10.0)	1 (10.0)	1 (10.0)	0 (0)	0 (0)	3 (30.0)
PP	15	47	10 (21.28)	2 (4.26)	7 (14.9)	8 (17.02)	8 (14.02)	5 (10.64)	4 (8.51)	3 (6.38)
PT	15	19	3 (15.79)	0 (0)	3 (15.79)	4 (21.05)	2 (10.53)	2 (10.53)	3 (15.79)	2 (10.5)
Total	**80**	**92**	**21 (22.83)**	**10 (10.87)**	**12 (13.04)**	**14 (15.23)**	**11 (11.96)**	**7 (7.61)**	**7 (7.61)**	**10 (10.8)**

Key: CVM = College Veterinary Medical, Clinic ML = Microbiology Laboratory, PL = Pathology Laboratory, PP = Pigpen, PT = Poultry farm

Table 4: Bacteria Isolates from FMC, Umuahia.

Sources	No. collected	No. Isolated	Bacteria					
			E. coli (%)	*S. aureus* (%)	*K. pneumoniae* (%)	*Arizona* spp (%)	*Enterobacter* spp (%)	*Proteus mirabilis* (%)
DL	30	20	3 (15)	10 (50)	2 (10)	1 (5)	2 (10)	2 (10)
HL	30	5	0 (0)	3 (60)	0 (0)	1 (20)	0 (0)	1 (20)
Total	**60**	**25**	**3 (12)**	**13 (52)**	**2 (8)**	**2 (8)**	**2 (8)**	**3 (12)**

Key: DL = Diagnostic Laboratory unit, HL = Histopathology unit

Table 5: Antibiotic resistance profile of bacteria isolated from wastewater samples from MOUAU (UHS)

Bacteria species	No. of isolated	Resistant rate to Antibiotic (%)												
		CRO	CIP	C	CN	E	DA	IPM	MY	NA	P	TE	SXT	VA
E. coli	6	1(16.7)	3(50)	6(100)	0(0.0)	ND	ND	0(0.0)	ND	3(50)	ND	6(100)	6(100)	ND
K. pneumoniae	5	3(60)	0(0.0)	1(20)	0(0.0)	ND	ND	0(0.0)	ND	3(60)	ND	5(100)	5(100)	ND
Arizona spp	4	4(100)	0(0.0)	0(0.0)	3(75)	ND	ND	0(0.0)	ND	1(25)	ND	4(100)	4(100)	ND
Enterobacter spp	4	0(0.0)	3(75)	2(50)	0(0.0)	ND	ND	0(0.0)	ND	2(50)	ND	3(75)	4(100)	ND
Shigella spp	1	0(0.0)	1(100)	1(100)	1(100)	ND	ND	0(0.0)	ND	1(100)	ND	1(100)	1(100)	ND
Citrobacter spp	1	1(100)	0(0.0)	1(100)	0(0.0)	ND	ND	0(0.0)	ND	1(100)	ND	1(100)	1(100)	ND
Proteus mirabilis	2	1(50)	1(100)	0(0.0)	1(100)	ND	ND	0(0.0)	ND	1(50)	ND	2(100)	2(100)	ND
S. aureus	7	ND	0(0.0)	7(100)	0(0.0)	5(71.4)	7(100)	0(0.0)	7(100)	7(100)	7(100)	7(100)	7(100)	6(85.7)

Key: MOUAU-Michael Okpara University of Agriculture Umudike, UHS= University Health Services, ND-Not Done, CRO-Ceftriaxone, CIP- Ciprofloxacin, C-Chloramphenicol CN-Gentamicin, E-Erythromycin, DA-Clindamycin, IPM- Imipenem, MY- Lincomycin, NA- Nalidixic acid, P- Penicillin G, TE- Tetracycline, SXT-Trimethoprim-Sulfamethoxazole, VA-Vancomycin.

Table 6: Antibiotics resistance profile of bacteria isolated from CVM, MOUAU

Bacteria species	No. of isolated	Resistant rate to Antibiotic (%)												
		CRO	CIP	C	CN	E	DA	IPM	MY	NA	P	TE	SXT	VA
E. coli	21	15(71.4)	0(0.0)	21(00)	3(14.3)	ND	ND	0(0.0)	ND	9(42.9)	ND	21(100)	21(100)	ND
K. pneumoniae	12	8(66.7)	3(25)	12(100)	0(0.0)	ND	ND	0(0.0)	ND	12(100)	ND	12(100)	12(100)	ND
Arizona spp	14	13(92.9)	7(46.7)	14(100)	0(0.0)	ND	ND	0(0.0)	ND	11(78.6)	ND	14(100)	7(50)	ND
Enterobacter spp	11	11(100)	0(0.0)	9(81.8)	0(0.0)	ND	ND	0(0.0)	ND	11(100)	ND	11(100)	11(100)	ND
Shigella spp	7	6(85.7)	7(100)	5(71.4)	0(0.0)	ND	ND	0(0.0)	ND	6(85.7)	ND	7(100)	7(100)	ND
Citrobacter spp	7	7(100)	0(0.0)	7(100)	5(71.4)	ND	ND	0(0.0)	ND	7(100)	ND	7(100)	7(100)	ND
Proteus mirabilis	10	5(50)	10(100)	9(90)	3(30)	ND	ND	0(0.0)	ND	6(60)	ND	10(100)	10(100)	ND
S. aureus	10	ND	5(100)	10(100)	0(0.0)	10(100)	10(100)	0(0.0)	8(80)	2(20)	6(60)	10(100)	10(100)	5(50)

Key: MOUAU-Michael Okpara University of Agriculture Umudike, CVM-college of Veterinary Medicine, ND-Not Done, CRO-Ceftriaxone, CIP- Ciprofloxacin, C-Chloramphenicol CN-Gentamicin, E-Erythromycin, DA-Clindamycin, IPM- Imipenem, MY- Lincomycin, NA- Nalidixic acid, P- Penicillin G, TE- Tetracycline, SXT-Trimethoprim-Sulfamethoxazole, VA-Vancomycin.

Table 7: Antibiotics resistance profile of bacteria Isolates from FMC, Umuahia

Bacteria species	No. of isolated	Resistant rate to Antibiotic (%)												
		CRO	CIP	C	CN	E	DA	IPM	MY	NA	P	TE	SXT	VA
E. coli	2	0(0.0)	0(0.0)	2(100)	0(0.0)	ND	ND	0(0.0)	ND	2(100)	ND	1(50)	2(100)	ND
K. pneumoniae	3	0(0.0)	2(66.7%)	3(100)	3(100)	ND	ND	0(0.0)	ND	3(100)	ND	3(100)	3(100)	ND
Arizona spp	2	1(50)	0(0.0)	0(0.0)	0(0.0)	ND	ND	0(0.0)	ND	2(100)	ND	2(100)	2(100)	ND
Enterobacter spp	2	0(0.0)	0(0.0)	1(50)	2(100)	ND	ND	0(0.0)	ND	2(100)	ND	2(100)	2(100)	ND
Proteus mirabilis	3	1(33.3)	2(66.7)	2(66.7)	3(100)	ND	ND	0(0.0)	ND	3(100)	ND	3(100)	3(100)	ND
S. aureus	13	ND	0(0.0)	11(84.6)	9(69.2)	13(100)	7(53.8)	0(0.0)	13(100)	9(69.2)	13(100)	13(100)	13(100)	13(100)

Key: FMC-Federal Medical Centre, ND-Not Done, CRO-Ceftriaxone, CIP- Ciprofloxacin, C-Chloramphenicol CN- Gentamicin, E-Erythromycin, DA-Clindamycin, IPM- Imipenem, MY-Lincomycin, NA- Nalidixic acid, P- Penicillin G, TE- Tetracycline, SXT-Trimethoprim-Sulfamethoxazole, VA- Vancomycin.

Table 8: Multidrug resistance patterns of bacteria species isolated from wastewater

Bacteria species	Resistant to Antibiotic	Number of test Antibiotic	Mean average MARI value
E. coli	CRO,CIP, C, CN, NA, TE,SXT	8	0.5
K. pneumoniae	CRO,CIP, C, CN, NA, TE,SXT	8	0.6
Arizona spp	CRO,CIP, C, NA, TE,SXT	8	0.5
Enterobacter spp	CRO,CIP, C, CN, NA, TE,SXT	8	0.6
Shigella spp	CRO,CIP, C, CN, NA, TE,SXT	8	0.6
Citrobacter spp	CRO,CIP, C, NA, TE,SXT	8	0.6
Proteus mirabilis	CRO,CIP, C, CN, NA, TE,SXT	8	0.7
S. aureus	CRO,CIP, C, CN, E, DA, MY, NA, P, TE,SXT, VA	12	0.8

Key: CRO-Ceftriaxone, CIP- Ciprofloxacin, C-Chloramphenicol CN- Gentamicin, E-Erythromycin, DA-Clindamycin, IPM- Imipenem, MY- Lincomycin, NA- Nalidixic acid, P- Penicillin G, TE- Tetracycline, SXT-Trimethoprim-Sulfamethoxazole, VA- Vancomycin

Multidrug resistance trend of majority of the waste water isolate recovered from CVM in this current study may be due to excessive use of antimicrobials particularly in veterinary medicine and thus mediate high selection pressure for resistant strains in the studied setting. The documented resistance to tetracycline in this study was unavoidable due to widespread use of oxytetracycline in community, hospital and animal husbandry in the country. MDR strains of *Citrobacter* species reported in this study is in support with high rate of antimicrobial resistance reported in Addis Ababa hospital[9] while previous findings also showed MDR *Citrobacter* species causing severe morbidity and mortality in hospitalized patients.[20] Multidrug resistant *Shigella* species, *Klebsiella pneumoniae, Enterobacter* species in this study is in agreement with other findings.[9,17] *Klebsiella pneumoniae* from hospital environment is widely known to carry genes coding for resistance to several antimicrobials including those producing ESBL and *Klebsiella pneumoniae* carbapenemase (KPC).[21]

A total of 17 isolate of *Enterobacter* species was recovered from WWS in this study and this isolate is also claimed to serve as the reservoir of antimicrobial resistance genes. They are known to acquire numerous mobile genetic elements which contribute to fitness of the organism to colonize several environments and hosts. Horizontal transfer of resistance genes from *Klebsiella pneumoniae* is implicated to be the main reason for wide occurrence of MDR *Enterobacter* species in hospital environment.[22] As such, detection of such MDR strains in hospital waste water sample in this study is an indication of risks associated with potential life-threatening infection as this isolates are causes of opportunistic nosocomial infections reported in most outbreaks[23] as well as possible dissemination of MDR isolates conferring genetic markers to other bacterial communities.[9] The observed 0.0% resistant to imipenem may result from low or in excessive use of carbapenem antibiotic in the studied setting. However, absent of carbapenem resistant in this study may not underscore its presence in waste water sample elsewhere but rather reflect the nature of the study design. *Proteus mirabilis* found in WWS from College of veterinary medicine/other sources were multidrug resistant as they resist majority of the test antibiotic. However, it is important to note that the *Proteus mirabilis* are opportunistic human and animal pathogens.[24] Other studies reported that *P. mirabilis* strain identified in wastewater samples from Casablanca City, Morocco also exhibiting resistance to naphthalene, anthracene and antibiotics.[25]

Arizona species exhibit Multidrug resistant in this study, although much is not known about this isolate but earlier study has reported the occurrence and distribution of serotypes of the *Arizona* group of Enterobacteriaceae among animals and man.[26] Since most of our WWS were from CVM the presence of *P. mirabilis* is certain as organisms of the *Arizona* group have been discovered in epizootic infections of animals in which the mortality was high.[26] In man they have appeared both in sporadic cases and in well-defined outbreaks of disease. The bacteria have been found in blood cultures and localized infections as well as in the stools of persons affected with gastro-enteritis.[26] The earlier findings show that *Arizona* species are primary excitants of disease while their multidrug resistant pattern is evidence in this study to support their pathogenicity. The observed MDR phenotype with MAR index within the range of 0.5-0.8 indicate high antibiotic contamination of hospital waste water due to excessive use of antibiotic and poor treatment of hospital waste in the studied area.

CONCLUSION

The findings of this study reported the presence of multidrug-resistant strains of *Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Citrobacter* species, *Enterobacter* species, *Arizona* species, and *Shigella* species in hospital waste water. The level of MDR in isolates obtained from CVM, UHS and FMC demonstrated similar antimicrobial resistant pattern of 50-100 % to most of the tested antibiotics with High MARI index of 0.5-0.8. Since most of this strain exist as allochthonous in waste water possible dissemination of such MDR strains carrying resistance genetic markers may impose high risk of spread of resistance genes to less sensitive autochthonous microbial communities in the environment. Therefore, waste water emanating from hospital environment should be treated before been released to the surrounding ecological niche. Concerted efforts in evaluating the resistant profile and virulence nature of waste water bacteria is necessary and is hereby recommended.

REFERENCES

1. Mustapha A and Imir T. Detection of Multidrug - Resistance Gram-Negative Bacteria from Hospital Sewage in North East, Nigeria. Frontiers in Environmental Microbiology, 2019; 5(1): 1-7.
2. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H. Antibiotic resistance-the need for global solutions. Lancet of Infectious Disease, 2013; 13(12): 1057–98.
3. Nick R. Wastewater. Wikipedia, 2018: https:// en.wikipedia.org /wiki/wastewater /p.1-8.
4. Mhongole OJ, Mdegela RH, Kusiluka LJ, Forslund A, Dalsgaard A. Characterization of *Salmonella* species from Wastewater used for Food Production in Morogoro, Tanzania. World Journal of Microbiology and Biotechnology, 2017; 33(3): 42.
5. Iroha IR, Amadi ES, Orji AE, Nwuzo AC, Ejikeugwu PC. Detection of Plasmid borne extended spectrum beta lactamase Enzymes from Blood and Urine Isolate of Gram Negative bacteria from a University Teaching Hospital in Nigeria. Current Research in Bacteriology, 2012; 2(3): 70-83.
6. Lazar V. Genome Wide Analysis Captures the Determinants of the Antibiotic Cross-Resistance Interaction network. Nature and Communication, 2014; 5: 4352-1038.

7. Bridgett P, Daniel C, Steven F. Antibiotic, Gene Transfer and Water Quality Patterns Observed in Waterways near Confined Animal Feeding Operations farms and wastewater treatment facilities. Water, air, soil, pollution, 2010; 11(270): 100-602.
8. Shakibaie MR, Jalilzadeh KA, Yamakanamardi SM. Horizontal Transfer of Antibiotic Resistance Genes Among Gram-negative bacteria in Sewage and Lake Water and Influence of some Physico-chemical Parameters of Water on Conjugation Process. Journal of Environmental Biology, 2009; 30: 45-49.
9. Tesfaye H, Alemayehu H, Desta AF, Eguale T. Antimicrobial Susceptibility Profile of Selected Enterobacteriaceae in Wastewater Samples from Health Facilities, Abattoir, Downstream Rivers and a WWTP in Addis Ababa, Ethiopia. Antimicrobial Resistance and Infection Control, 2019; 8: 134-136.
10. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part two, 2nd edn. Cambridge University Press, UK, 2010; pp. 143-180.
11. Iroha IR, Orji JO, Onwa NC, Nwuzo AC, Okonkwo EC, Ibiam EO, Nwachi AC, Afuikwa FN, Agah VM, Ejikeugwu EPC, Agumah NB, Moses IB, Ugbo EN, Ukpai EG, Nwakaeze EA, Oke B, Ogbu L, Nwunna E. Microbiology Practical Handbook. 1st Edition. Charlieteximage Africa (CiAfrica Press), 2019; pp. 344.
12. Ochei J and Kolhatkar A. Medical Laboratory Science: theory and practical. Sixth reprint. Tata McGraw-Hill publishing Coy. Ltd, New Delhi. 2007; pp 746-748.
13. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing M100S, 26th edition. 2016.
14. Moses IB, Esimone CO, Iroha IR, Ugbo EN, Orji JO, Nwuzo AC, Nwakaeze EA, Agumah NB, Emioye AA, Ukpai EG, Ogene LN. First report on the antibiotic resistance profiles and virulence genes of *Staphylococcus pseudintermedius* colonizing dogs and humans in Abakaliki, South-East Nigeria. Research Journal of Microbiology, 2020; 15: 22-34. DOI: 10.3923/jm.2020.22.34.
15. Asfaw T, Negash L, Kahsay A, Weldu Y. Antibiotic Resistant Bacteria from Treated and Untreated Hospital Wastewater at Ayder Referral Hospital, Mekelle, North Ethiopia. Advances in Microbiology, 2017; 7: 871-886.
16. Olutayo IF and Olalonpe SO. Extended Spectrum Beta Lactamase (ESBL) producing enteric bacteria from hospital wastewater, Ibadan, Nigeria. World News of Natural Sciences, 2019; 22: 62-74.
17. Adam M and Turgut I. Detection of Multidrug-resistant Gram-Negative Bacteria from Hospital Sewage in North East, Nigeria. Frontiers in Environmental Microbiology, 2019; 5: 1-7.
18. Lien TQ, Lan PT, Chuc NT, Hoa NQ, Nhung PH, Thoa NT, Diwan V, Tamhankar AJ, Stalsby LC. Antibiotic Resistance and Antibiotic Resistance Genes in *Escherichia coli* Isolates from Hospital Wastewater in Vietnam. International Journal of Environmental Research and Public Health, 2017; 14(7): 699-700.
19. Alekshun MN and Levy SB. Molecular Mechanisms of Antibacterial Multidrug Resistance. Journal of Cell, 2007; 128(6):1037–50.
20. Seboxa T, Amogne W, Abebe W, Tsegaye T, Azazh A, Hailu W, Fufa K, Grude N, Henriksen TH. High Mortality from Blood Stream Infection in Addis Ababa, Ethiopia, is due to Antimicrobial Resistance. Public Library of Science One, 2015; 10(12): 144-944.
21. Picao RC, Jones RN, Mendes RE, Castanheira M. *Klebsiella pneumonia* carbapenemase-producing Enterobacteriaceae testing susceptible to cefepime by reference methods. Journal of Clinical Microbiology, 2013; 51(7): 2388–90.
22. Davin-Regli A and Pages JM. *Enterobacter aerogenes* and *Enterobacter cloacae*; Versatile Bacterial Pathogens Confronting Antibiotic Treatment. Frontiers in Microbiology, 2015; 6: 392-401.
23. Pestourie N, Garnier F, Barraud O, Bedu A, Ploy MC, Mounier M. Outbreak of AmpC beta-lactamase-hyper-producing Enterobacter cloacae in a neonatal intensive care unit in a French teaching hospital. American Journal of Infection and Control, 2014; 42(4): 456–8.
24. Dominika D. Significance and Roles of *Proteus* species Bacteria in Natural Environments. Microbial Ecology, 2016; 72(4): 741–758.
25. Filali BK, Taoufik J, Zeroual Y, Dzairi FZ, Talbi M, Blaghen M. Waste water bacterial isolates resistant to heavy metals and antibiotics. Current Opinion in Microbiology, 2000; 41: 151–156.
26. Edwards PR, Alma CM, Mary AF. The Arizona group of Enterobacteriaceae in animals and man Occurrence and distribution. Bulletin of World Health Organization, 1956; 14(3): 511–528.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.