Trop J Nat Prod Res, May 2022; 6(5):826-830 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Occurrence of Extended Spectrum Beta Lactamase (ESBL) Producing Gram-Negative Bacteria in Wastewaters from Selected Hospitals in Ibadan, Oyo State, Nigeria

, Ajayi-Odoko O. Adenike* and Dayo A. Ayansina

Microbiology Programme, Bowen University, Iwo, Nigeria

ARTICLE INFO

Article history:
Received 08 May 2022
Revised 26 May 2022
Accepted 30 May 2022
Published online 04 June 2022

ABSTRACT

As an environmental antimicrobial resistance reservoir, untreated hospital wastewater plays a crucial role in the spread of antibiotic-resistant genes. The goal of this study was to look at the genetic drivers of ESBL resistance in Gram-negative bacteria isolates from hospital wastewater in Ibadan, Nigeria. A total of 408 bacterial isolates were obtained, with 54 being chosen for Polymerase Chain Reaction (PCR) testing due to resistance to more than three antibiotic classes. All the fifty-four ESBL producers were resistant to ceftazidime, while 40 (74.07%) showed resistance to cefotaxime. Percentage resistance to azithromycin, sulfamethoxazole trimethoprim, cefotaxime and streptomycin was 59.26 %, 64.81%, 74.07% and 75.93% respectively. Twenty-seven of the ESBL- producing isolates representing 50.00 % was resistant to oxacillin. Moreover, percentage resistance to ciprofloxacin and cefepime was 38.89% and 31.48% respectively. *Bla*$_{TEM-1}$ had the highest percentage frequency of occurrence of 85.19% while *bla*$_{CTX-M}$ had the least value of 7.41%. The percentage occurrence for resistant gene *bla*$_{SHV-2}$, and *bla*$_{SHV-1}$, was 20.37%, and 5.56% respectively. The presence of ESBL bacteria in hospital wastewater shows the role played by the discharge of untreated hospital wastewater in the horizontal spread of antibiotic resistance genes. As a result, Nigeria's tradition of discharging untreated hospital effluent into the environment poses a serious threat to public health.

Keywords: Antibiotic resistance, Extended Spectrum Beta-lactam, Hospital wastewater, Resistance Gene

Introduction

Antibiotic resistance (AR) has sparked widespread concern due to the decreased potency of conventional antibiotics in therapeutic applications.[1,2] Antibiotic-resistance in bacteria is such a global epidemic and a worldwide dilemma, and many studies have identified the clinical environment as a key player in antibiotic resistance challenges around the world.[3,4,5] Antimicrobial resistance (AMR) in bacteria expresses itself in a variety of ways. Bacteria that synthesize β-lactamase enzymes, which hydrolyze the β-lactam ring, are the main source of resistance to β-lactams. Different forms of β-lactamases inactivate various types of β-lactam antibiotics ESBLs are a type of enzyme that breaks down antibiotics like penicillin and cephalosporin. [6] In pathogenic bacteria under antibiotic selection pressure, gram-negative rod beta-lactamases are the most rapidly developing mechanisms of resistance. ESBLs have been the most source of bacterial resistance to all beta-lactam antibacterial agents, other than carbapenems and cephamycins, which are blocked by beta-lactamase inhibitors such clavulanic acid. Resistant genes can be passed down vertically (to bacterial offspring) or horizontally (to humans) (among bacteria of different taxonomic affiliations. According to Bush and Jacoby[7], there are approximately 300 ESBL subtypes, all of which are believed to originate from one of 3 main progenitor types: TEM-1, TEM-2, or SHV-1.

*Corresponding author. E mail: Omolola.ajayi@bowen.edu.ng
Tel: 08036321825

Citation: Ajayi-Odoko AO and Ayansina DA. Occurrence of Extended Spectrum Beta Lactamase (ESBL) producing Gram-Negative Bacteria in Wastewaters from Selected Hospitals in Ibadan, Oyo State, Nigeria. Trop J Nat Prod Res. 2022; 6(5):826-830.

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Genetic variations led to development of new *β*-lactamases having the capability of metabolizing third-generation cephalosporins and aztreonam.[8,9]

Hospital effluents, in particular, are a unique form of waste that is extremely dangerous due to its infectious and hazardous properties,[10] [11] and they are a major source of antibiotic-resistant bacteria[12] and antibiotics.[13] They are also regarded as hubs for antibiotic resistance, providing a breeding ground for antibiotic resistance genes to spread.[14-16]

The apparent significance of untreated wastewater as a transmission route for clinically important ARB and ARGs such as ESBL and associated genes at the Human-Environment Interaction has been highlighted. The study was motivated by the indiscriminate discharge of untreated hospital effluents directly into the environment in Nigeria as well as the lack of waste treatment systems in most hospitals.

Materials and Methods

Sample collection

Thirty-two effluents samples were obtained aseptically from six medical centres in Ibadan (three private and three public). Between the months of May and June 2019, and December 2019 and January 2020, samples were taken three times each. Prior to being released into the environment, wastewater from these health-care facilities was discharged directly into various drainage channels. Water samples were conveyed on ice to Bowen University's Department of Microbiology in Nigeria for analysis in less than six hours. After appropriate documentation was submitted, access to the selected hospitals for wastewater collection was authorized.

Bacteria isolation

Dilution of effluent samples was done aseptically up to 10^7 dilutions to yield countable bacterium colonies on agar plates On different agar media (eosin methylene blue (EMB), centrimide, MacConkey, salmonella-shigella, and nutritional agar), dilution 10^6 was plated for

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

enumeration and isolation of bacterium colonies. On the plates, colonies of varied morphologies were observed and streaked off onto new Nutrient agar plates for purification (colonies on nutrient agar were Gram-stained). Pure bacterium isolates were kept frozen in a broth containing 15% glycerol.

Antibiotic sensitivity testing

The test was performed using the Kirby-Bauer disc diffusion technique. Antibiotic sensitivity of isolates was tested using the following antibiotics discs CIP = Ciprofloxacin (5 µg), IMP = Imipenem (10 µg), AZM = Azithromycin (15 µg), SXT = Sulfamethoxazole (25µg), S = Streptomycim (10 µg), OX = Oxacillin (1 µg), CTX = Cefotaxime (30 µg), CAZ = Ceftazidime (30 µg). The zone of inhibition was evaluated after incubation and the results were compared to CLSI standards.[17]

DNA extraction

This was done using plasmid and chromosomal Presto™ Mini gDNA Bacteria Kit Protocol (Gram Negative Bacteria).

Genotyping of ESBL gene determinants

Using primers targeting the selected resistance genes, PCR was then used to check for the presence of ESBL resistance genes (Table 1). The PCR was performed with 1X Blend Master mix buffer (Solis Biodyne), 2.0 mM $MgCl_2$, 200 µM each deoxynucleoside triphosphate (dNTP) (Solis Biodyne), 20 µMol each primer, 2 units of Hot FIREPol DNA polymerase Proofreading Enzyme, 2 µl of extracted DNA, and sterile distilled water. A preliminary denaturation at 95°C for fifteen minutes was preceded by 35 amplification cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 60 seconds at 72°C in a Peltier PTC 200 Thermal Cycler. Then, at 72°C, a 10-minute extension procedure was done. On a 2% agarose gel, the amplified product was separated and electrophoresed at 80V for 1 hour and 30 minutes. Ethidium bromide staining was used to visualize DNA bands after electrophoresis. As a DNA molecular weight marker, a 100bp DNA ladder (Solis Biodyne) was used.

Results and Discussion

A total of 408 bacterial isolates were obtained, with 54 being chosen for PCR assay due to resistance to more than three antibiotic classes. Ceftazidime resistance was found in all fifty-four ESBL producers, while cefotaxime resistance was found in 40 (74.07%). Azithromycin, sulfamethoxazole-trimethoprim, cefotaxime, and streptomycin resistance percentages were 59.26 %, 64.81 %, 74.07 %, and 75.93 %, respectively. Oxacillin resistance was detected in 27 (50.00%) of the ESBL-producing isolates. Furthermore, ciprofloxacin and cefepime resistance percentages were 38.89% and 31.48%, respectively (Figure 1). Bacteria species isolated were identified using the ABIS online identification software based on the biochemical reaction results. Hospital wastewater is considered to be a major source for antibiotic resistance and other genetic factors which can disseminate AMR into the environment as it has high concentrations of antimicrobial drugs and human pathogens.[3,19] Antibiotic abuse or misuse in the environment and therapeutic settings could explain the exceptionally high level of antimicrobial resistance in bacteria found in this investigation.[20] In hospitals, antibiotics are frequently prescribed without absolute proof of infection or adequate medical rationale. As a suitable alternative for culture and drug susceptibility testing, toxic broad-spectrum antibiotics are routinely administered instead of narrow-spectrum antibiotics, resulting in considerable adverse reactions, systemic diseases, and the variety of drug-resistant mutants.[21] Since medications are readily available to the general public in underdeveloped nations, people may self-administer antibiotics, increasing the prevalence of drug-resistant strains. This study's high percentage of resistance to ceftazidime, ciprofloxacin, and sulphamethoxazole trimethoprim was similar to Adelowo *et al.* results.[22] Lower percentage resistance as compared to values obtained in this study to ciprofloxacin and ceftazidime have been reported by Zhang *et al.*[23] and Mustapha and Imir[24] respectively. Mutasim *et al.*[25] and Egbule[26] have also reported 90.5% and 93.5 % resistance to sulphamethoxazole trimethoprim. Zhang *et al.*,[23] reported 61.3% resistance to cefoxitin, though higher than 51.96 % resistance obtained in this study. Furthermore, 62.23% to streptomycin obtained in this study was similar to 63.0 % reported by Falodun and Oladimeji.[27] In 2017, Asfaw *et al.*[28] reported 92.9 % and 89.3 % resistance to cefotaxime and cefepime respectively, though higher than resistance percentages values obtained in this study (84.56 % and 69.6 % for cefotaxime and cefepime respectively). However, Mustapha and Imir [24] and Zaugi *et al.* [29] reported 44.0 % and 22.0 % resistance to cefotaxime and cefepime respectively (Figure 1).

The rate of antibiotic resistance found in this study (ceftazidime, ciprofloxacin, and oxacillin) could be due to the antibiotics employed in this study (ceftazidime, ciprofloxacin, and oxacillin) being widely available in drug shops. As a result, according to Calva *et al.*[30] Antibiotics most frequently reported by bacterial resistance in developing world are generally low-cost broad-spectrum antibiotics. As seen by Lau *et al.*,[31] pathogen resistance to these commonly accessible, low-cost, and extensively misused antibiotics will almost certainly translate into higher treatment costs, extended hospitalisation, and therapeutic failure, possibly leading to life-threatening infections and death.

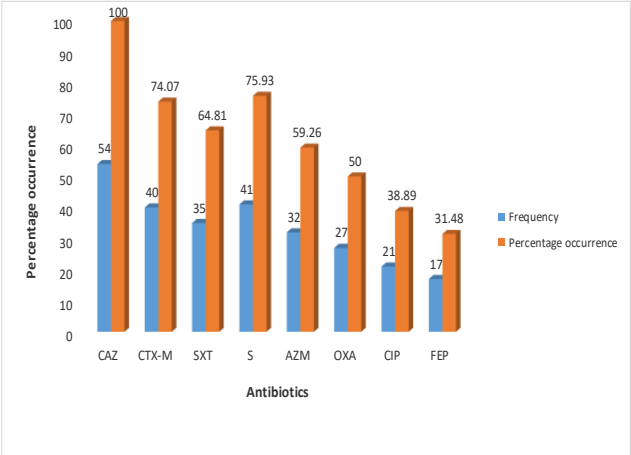

Figure 1: Resistance profile of ESBL-producing bacteria present in hospital effluent

KEY: CAZ=ceftazidime; CTX-M = cefotaxime; SXT = sulfamethoxazole-trimethoprim; S=streptomycim; AZM= azithromycin; OXA=oxacillin; CIP=ciprofloxacin, FEP=cefepime

Table 1: Primer sequences used to target ESBL resistance genes

Resistant genes	Primer sequence	Molecular weight (bp)	Ref
bla$_{SHV}$	F 5'- CGCCTGTGTATTATCTCCCT -3'	293	18
	R 5'- CGAGTAGTCCACCAGATCCT -3'		
bla$_{TEM}$	F 5'- TTTCGTGTCGCCCTTATTCC -3'	403	18
	R 5'- ATCGTTGTCAGAAGTAAGTTGG -3		
bla$_{CTX-M}$	F 5'- CGCTGTTGTTAGGAAGTGTG -3'	569	18
	R 5'- GGCTGGGTGAAGTAAGTGAC		

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Description of sampling sites

Site code	GPS coordinates	Type of health-care facility
AY	(7°21'49.7"N 3°51'52.7"E)	Public
AJ	(7°23'48.1"N 3°52'16.2"E)	Public
AR	(7°21'42.8"N 3°51'59.9"E)	Public
HL	(7°23'37.0"N 3°54'17.4"E)	Private
SL	(7°24'06.8"N 3°56'21.8"E)	Private
PC	(7°26'27.8"N 3°54'30.4"E)	Private

Footnote: Public hospitals provide secondary health care to Ibadan's lower and middle-classes population, whereas private hospitals serve the middle and upper classes

The isolates in this study showed various levels of multidrug resistance. Resistant gram-negative bacteria have been found in hospital effluents in similar numbers[32,33,34] *Bla*$_{TEM-1}$ had the highest percentage frequency of occurrence of 85.19% while *bla*$_{CTX-M}$ and had the least value of 7.41 %. The percentage occurrence for resistant gene *bla*$_{SHV-2}$, and *bla*$_{SHV-1}$, was 20.37%, and 5.56% (*bla*$_{SHV}$ = 25.93 %), respectively [Plates 1-3]. This is consistent with the study of Maha *et al*.[35] who found *bla*$_{TEM}$ to be the most dominant ESBL gene between Enterobacteriaceae isolates from patients in Khartoum, Sudan. This counters the findings of Fils *et al*.[36] who found *bla*$_{CTX-M}$ to be more prevalent than *bla*$_{TEM}$ and *bla*$_{SHV}$, and Ghafourian *et al.*[37]
When compared to other studied hospitals, *Klebsiella* spp. were the most abundant species, with the maximum incidence from both AR and SL, this is similar to Muller-Schulte *et al*.[38] and Fadare and Okoh [39] who found a significant prevalence of ESBL-producing bacteria among *Klebsiella* species in clinical samples and wastewater effluents, respectively.

Table 2: Occurrence of ESBL resistance genes from selected sampled hospitals

AY			AT			AJ			HL			SL			PC			Most probable organism
T	S	c	T	S	c	T	S	c	T	S	c	T	S	c	T	S	c	
1	-	-	2	3	-	1	-	-	2	-	-	2	-	-	-	1	-	*Klebsiella* spp.
4	1	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	*Salmonella* spp.
1	1	-	-	-	-	2	-	-	1	-	-	-	-	-	1	1	1	*Shigella* spp.
1	-	-	1	-	-	1	1	-	1	-	-	-	-	-	-	-	-	*Enterobacter* spp.
1	-	-	-	-	-	2	1	1	1	-	-	1	-	-	1	-	-	*Pseudomonas* spp.
2	2	-	-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	*Serratia* spp.
-	-	-	-	-	-	1	-	-	2	-	-	1	-	-	1	-	-	*Escherichia* spp.
1	-	-	-	-	-	-	-	-	1	-	-	2	1	-	1	-	-	*Proteus* spp.
2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Citrobacter* spp.

KEY: T = *bla*$_{TEM}$; S = *bla*$_{SHV}$; C = *bla*$_{CTX-M}$ Sampled sites code: AY, AR, AJ, HL, SL, PC"

This contradicts the findings of Eltai *et al.*[40] who found *E. coli* to be the most prevalent pathogen among enterobacteriaceae causing lower urinary tract infection among pediatric population. *Klebsiella* spp. incidence in both public and private hospital wastewaters at the same frequency could be useful indicators for monitoring AMR status in the environment, as they are regarded early markers of novel AMR emergence, as recommended by Berendonk *et al*.[3] and Navon-Venezia *et al*. [41] *Salmonella* spp from AY hospital had the highest percentage occurrence of *bla*$_{TEM}$ (20.37 %), with AJ hospital having the second highest percentage occurrence of *bla*$_{TEM}$. *Salmonella* and *Shigella* infections are among the most key public health critical problems. Every year, nontyphoidal *Salmonella* causes more than one billion cases of diarrhea worldwide, resulting in 3 million deaths.[42] The high AR incidence of *bla*$_{TEM}$ in *Salmonella* spp. could indicate that patients utilizing this public hospital are abusing medicines intended to treat salmonella-causing diseases. *Klebsiella* spp. harboring *bla*$_{SHV}$ were detected in AR and PC, with AR having the highest prevalence. In this investigation, the *bla*$_{SHV}$ gene was never found in any ESBL-positive *Escherichia, Salmonella, Serratia,* or *Citrobacter* species. Adekanbi *et al.* [5] found no blaSHV gene in *E. coli* isolated from effluents discharged by a sick bay in a university health care centre. Both the *bla*$_{TEM}$ and *bla*$_{SHV}$ genes were found in ESBL-producing E. coli isolated from individuals with urinary tract infections, according to Seyedjavadi *et al.*[43] Aleem *et al*. [44] reported resistance genes in *Pseudomonas* spp. in hospital water and surfaces, which is similar with the findings of this study. The presence of *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *bla*$_{TEM}$ in *Pseudomonas* spp. from AR was observed. *Pseudomonas aeruginosa* strains resistant to the *bla*$_{TEM}$ and *bla*$_{SHV}$ genes were also reported by Mohammad *et al*. [45.] *P. aeruginosa* strains produce a wide range of extended spectrum β-lactamases (ESBLs), which enable the bacteria to resist extended β–spectrum cephalosporins – for example cefotaxime, ceftriaxone, and ceftazidime, and they're becoming more prevalent.[46]

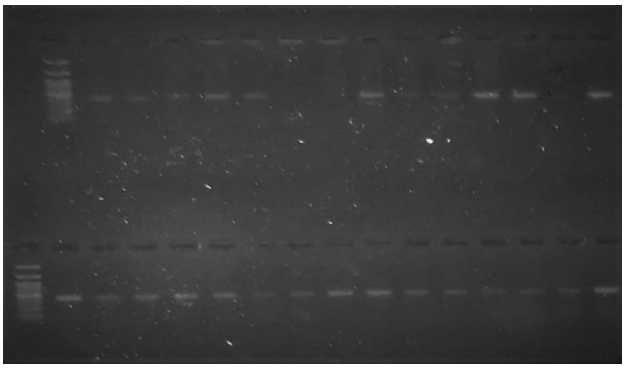

Plate 1: Gel image of *bla*$_{TEM-1}$ PCR amplification

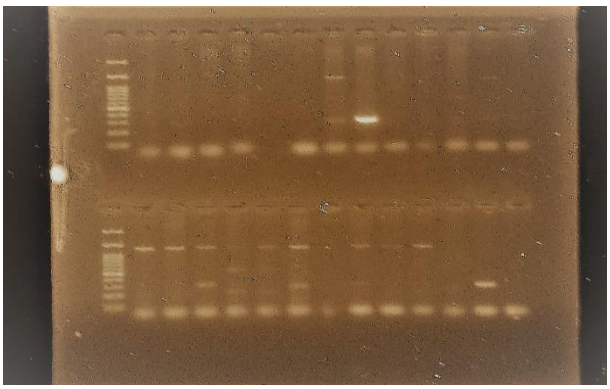

Plate 2: Gel image of *bla*$_{SHV-2}$ PCR amplification

Plate 3: Gel image of *bla*$_{CTX-M}$ PCR amplification

The evolution of ESBL-producing *P. aeruginosa* as a significant source of medical infections is increasing. Infections caused by resistant organisms are becoming extremely difficult to treat in hospitals due to rising resistance levels to the most frequently prescribed antibiotics.[47,48] *Bla*$_{CTX-M}$ resistance genes were detected in *Enterobacter, Citrobacter, Shigella*, and *Pseudomonas* species (with 50% percentage occurrence each from both private and public hospital) which is similar to Zeynudin *et al.*,[49] who reported blaCTX-M resistance genes in clinical strains of Gram-negative bacteria in Ethiopia.

Conclusion

The prevalence of ARB and ARGs in public hospital wastewaters is higher than in private hospitals, according to the findings of this study. There is a pressing need to raise public awareness about the dangers of antibiotic misuse, particularly among the city's lower-income residents. Furthermore, proper treatment of wastewater from care facility environments is suggested to avoid a public health crisis caused by the release of untreated sewage loaded down with antibiotic-resistant bacteria and their genes into the environment.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors would like to express their gratitude to the Medical Directors of all of the hospitals that were sampled for approving the collection of wastewater samples within their facilities.

References

1. Canton R and Morosini MI. Emergence and spread of antibiotic resistance following exposure to antibiotics. FEMS Microbiol Rev. 2011; 35:977-991
2. Rolain JM, Canton R, Cornaglia G. Emergence of antibiotic resistance: need for a new paradigm. Clin Microbiol Infect. 2012; 18:615-616.
3. Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Burgmann H, Sorum H, Norstrom M, Pons MN, Kreuzainger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez JL. Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol. 2015; 13:310-317
4. Adelowo OO, Caucci S, Banjo OA, Nnanna OC, Awotipe EO, Peters FB, Berendonk TU. Extended Spectrum Beta-Lactamase (ESBL)-producing bacteria isolated from hospital wastewaters, rivers and aquaculture sources in Nigeria. Environ Sci Poll Res. 2018; 25:2744–2755
5. Adekanmbi, AO, Akinpelu, MO, Olaposi, AV, Oyelade, AA. Diversity of Extended Spectrum Beta-lactamase (ESBL) genes in *Escherichia coli* isolated from wastewater generated by a Sick Bay located in a university health care facility. Gene Rep. 2020a; 20:100738.
6. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Biol Sci. 2015; 22(1):90-101.
7. Bush K and Jacoby GA. Updated functional classification of b-lactamases. Minireview. Antimicrob Agents Chemother. 2010; 54:969-976.
8. Slama TG. Gram-negative antibiotic resistance: there is a price to pay. Crit Care. 2008; 12(Suppl. 4):1-7
9. Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Catry B, Herman L, Haesebrouck F, Butaye P. Diversity of extended-spectrum b-lactamases and class C beta-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. Antimicrob. Agents Chemother. 2008; 52:1238-1243.
10. Verlicchi P, Galletti A, Petrovic M, Barceló D. Hospital effluents as a source of emerging pollutants: an overview of micro-pollutants and sustainable treatment options. J Hydrol. 2010; 389 (34):416-428.
11. Chagas TP, Seki LM, Cury JC, Oliveira JAL, Dávila AMR, Silva DM, Asensi MD. Multi-resistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro, *Brazil.* J Appl Microbiol. 2011; 111:572–581.
12. Huang JJ, Hu HY, Lu SQ, Li Y, Tang F, Lu Y, Wei B. Monitoring and evaluation of antibiotic resistant bacteria at a municipal wastewater treatment plant in China. Environ Int. 2012; 42:31–36.
13. Santos LM, Gros M, Rodriguez-Mozaz S, Delerue-Matos C, Pena A, Barceló D, Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters. The Sci Total Environ. 2013; 461-462:302-316.
14. Rozman U, Darja D, Mojca C and Sonja Šostar T. Hospital wastewater effluent: hot spot for antibiotic resistant bacteria. J Water Sanit Hygiene Dev. 2020; 10(2):171-172.
15. Zhang L, Ma X, Luo L, Hu N, Duan J, Tang Z, Zhong R, Li Y. The Prevalence and Characterization of Extended-Spectrum β-Lactamase- and Carbapenemase-Producing Bacteria from Hospital Sewage, Treated Effluents and Receiving Rivers. Int J Environ Res Pub Health. 2020: 17(4):1-13.
16. Soriano-Moreno DR, Yareta J, Rojas-Cosi AF, Fajardo-Loyola A, Leon-Luna D, Castillo-Quezada I. Hospital effluents as a reservoir of beta-lactamase- and carbapenemase-producing *enterobacteriaceae.* Rev Peru Med Exp Salud Publica. 2021; 3 8(2):302-307.
17. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. In: CLSI supplement M100, 27th ed. Clinical and Laboratory Standards Institute, Wayne 2018: 1-332.
18. Bali EB, Acik L, Sultan N. Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum-β-lactamase produced by *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella* isolates in a Turkish hospital. Afr J Microbiol Res. 2010; 4(8):650-654.
19. Hocquet D, Muller A, Bertrand X. What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. J Hosp Infect. 2016; 93:395-402
20. Silva AAL and Hoffer E. Resistance to antibiotics and heavy metals in *E. coli* from marine environment. Environ Toxicol Water Qual. 1993; 8:1–11.

21. Prescott LM, Harley JP, Klein DA. Microbiology, New York: Mc Graw Hill. 1999; 4:678-697.
22. Adelowo OO, Odion OI, Knecht C, Vollmers J, Bhatia M, Kaster AK, Müller JA. A survey of extended-spectrum beta-lactamase-producing Enterobacteriaceae in urban wetlands in southwestern Nigeria as a step towards generating prevalence maps of antimicrobial resistance. PLoS One. 2020; 15:0229451.
23. Zhang L, Ma X, Luo L, Hu N, Duan J, Tang Z, Rujie Zhong R, Li Y. The Prevalence and Characterization of Extended-Spectrum β-Lactamase- and Carbapenemase-Producing Bacteria from Hospital Sewage, Treated Effluents and Receiving Rivers. Int J Environ Res Pub Health. 2020; 17(4):1183.
24. Mustapha A and Imir T. Detection of Multidrug-Resistance Gram-Negative Bacteria from Hospital Sewage in North East, Nigeria. Front Environ Microbiol. 2019; 5(1):1-7.
25. Mutasim EI, Mohammed A, Abdullah MA, Bahaeldin KE. Phenotypic Characterization and Antibiotic Resistance Patterns of Extended-Spectrum β-Lactamase- and AmpC β-Lactamase-Producing Gram-Negative Bacteria in a Referral Hospital, Saudi Arabia. Canadian *2* Volume 2019, Article ID 6054694, 9 pages.
26. Egbule, OS. Detection and Transfer of Extended Spectrum Beta Lactamase Enzymes from Untreated Hospital Waste Water. Adv Microbiol. 2016; 6:512-520.
27. Falodun OI and Olademeji OS. Extended Spectrum Beta Lactamase (ESBL) producing enteric bacteria from hospital wastewater, Ibadan, Nigeria. World News of Nat Sci. 2019; 22:62-74.
28. Asfaw T, Negash L, Kahsay A, Weldu Y. Antibiotic Resistant Bacteria from Treated and Untreated Hospital Wastewater at Ayder Referral Hospital, Mekelle, North Ethiopia. Adv Microbiol. 2017; 7:871-886.
29. Zagui GS, de Andrade LN, Moreira NC, Silva TV, Machado GP, da Costa Darini AL, Segura-Muñoz SI. Gram-negative bacteria carrying *β*-lactamase encoding genes in hospital and urban wastewater in Brazil. Environ Monit Assess. 2020; 192(6):376.
30. Calva JJ, Ceron E, Bojalil R, Holbrook A. Antibiotics consumption in community of Mexico City. II Survery of purchases at pharmacies. Boletin Medico Hospital Infantil de Mexico. 1993; 50:145-150.
31. Lau SM, Peng MY, Chang Y. The microbiology of a pharmaceutical effluent and its public health implications. World J Microbiol Biotechnol. 2004; 20:167-171.
32. Moges F, Endris M, Belyhun, Y, Worku W. Isolation and characterization of multiple drug resistance bacterial pathogens from wastewater in hospital and nonhospital environments, Northwest Ethiopia. BMC Res Notes. 2014; 7:215.
33. Rabbani MAG, Howlader MZH, Kabir Y. Detection of multidrug resistant (MDR) bacteria in untreated wastewater disposals of hospitals in Dhaka City, Bangladesh. J Glob Antimicrob Resist. 2017; 10:120-125.
34. Wang Q, Wang P, Yang Q. Occurrence and diversity of antibiotic resistance in untreated hospital water. Sci Total Environ. 2018; 621:990-999.
35. Maha HD, Naser EB, Mutasim EI, Mohamed EH. Prevalence of extended-spectrum *β*-lactamase (ESBL) and molecular detection of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genotypes among Enterobacteriaceae isolates from patients in Khartoum, Sudan. PanAfr Med J. 2020; 37:213.
36. Fils PEL, Cholley P, Gbaguidi-Haore H, Hocquet D, Sauget M, Bertrand X. ESBLproducing *Klebsiella pneumoniae* in a University hospital: Molecular features, diffusion of epidemic clones and evaluation of cross-transmission. PLoSONE 2021; 16(3):e0247875.
37. Ghafourian S, Zamberi S, Vasanthakumari N, Afra K, Mohammad R Nourkhoda S. Incidence of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in patients with urinary tract infection. Sao Paulo Med J. 2012; 130(1):37-43.
38. Müller-Schulte E, Tuo MN, Akoua-Koffi C, Schaumburg F, Sören LB. High prevalence of ESBL-producing *Klebsiella pneumoniae* in clinical samples from central Côte d'Ivoire. Int J Infect Dis. 2020; 91:207-209.
39. Fadare FT and Okoh AI. Distribution and molecular characterization of ESBL, pAmpC. β-lactamases, and non-β-lactam encoding genes in *Enterobacteriaceae* isolated from hospital wastewater in Eastern Cape Province, South Africa. PLoS ONE 2021; 16(7):1-17.
40. Nahla OE, Asmaa AA, Khalid A, Anand SD, Eman W, Sara HA, Hadi MY. Molecular characterization of extended spectrum β-lactamases enterobacteriaceae causing lower urinary tract infection among pediatric population. Antimicrob Resist Infect Contr. 2018; 7:90-99.
41. Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol. Rev. 2017; 41:252–275.
42. Coburn B, Grassl GA, Finlay BB. "Salmonella, the host and disease: a brief review*,"* Immunol Cell Biol. 2007; 85(2):112–118.
43. Seyedjavadi SS, Goudarzi M, Sabzehali F Relation between *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes and acute urinary tract infections. J Acute Dis. 2016; 5:71–76.
44. Aleem M, Azeem AR, Rahmatullah S, Sidra Rahmatullah, Sufyan V, Shumyila N, Saadia A Prevalence of Bacteria and Antimicrobial Resistance Genes in HospitalWater and Surfaces. Cureus 2021; 13(10):18738.
45. Mohmmad B, Shahram SZ, Morteza SB, Alireza AM. Frequency of PER, VEB, SHV, TEM and CTX-M Genes in Resistant Strains of Pseudomonas aeruginosa Producing Extended Spectrum β-Lactamases. Jundishapur J Microbiol. 2015; 8(1):13783.
46. Shacheraghi F, Shakibaie MR, Noveiri H. Molecular Identification of ESBL genes *bla*$_{GES-1}$, *bla*$_{VEB-1}$, *bla*$_{CTX-M}$ *bla*$_{OXA-1}$, *bla*$_{OXA-4}$, *bla*$_{OXA-10}$ and *bla*$_{PER-1}$ in *Pseudomonas aeruginosa* strains isolated from burn patients by PCR, RFLP and sequencing techniques. Int J Biol life Sci. 2010; 3(6):138–142
47. Amirkamali S, Naserpour-Farivar T, Azarhoosh K, Peymani, A. Distribution of the *bla*$_{OXA}$, *bla* $_{VEB-1}$, and *bla* $_{GES-1}$ genes and resistance patterns of ESBL-producing *Pseudomonas aeruginosa* isolated from hospitals in Tehran and Qazvin Iran. Rev Soc Bras Med Trop. 2017; 50(3):315–320.
48. Khurana S, Mathur P, Kapil A, Valsan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial Gram-negative pathogens from North and South Indian hospitals. J Med Microbiol. 2017; 66:999–1004.
49. Zeynudin A, Pritsch M, Schubert S, Messerer M, Liegl G, Hoelscher M, Belachew T, Wieser A. Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. BMC Infect Dis. 2018; 18 (1):524-533.

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