GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Investigation Genetic of *Enterobacteriaceae* isolated from sewage of Baghdad Hospital

Samaa Kh. Alhashimy *, Arwa M. Nasser, Bashar Qussay Kadhem and Osamah Alkilidar

Scientific Research Commission, Baghdad, Iraq.

GSC Biological and Pharmaceutical Sciences, 2026, 35(03), 303-310

Publication history: Received on 15 May 2026; revised on 24 June 2026; accepted on 26 June 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.35.3.0239

Abstract

This study aimed to investigate the presence of the *blaTEM* and *blaSHV* genes in 50 environmental isolates obtained from the Tigris River. The isolates were biochemically identified using the VITEK-2 system, which enabled precise species-level identification. The results of biochemical analyses revealed that the predominant species among the isolates were *Klebsiella pneumoniae* (44.44%), *Proteus mirabilis* (31.48%), *Enterobacter aerogenes* (12.96%), *Citrobacter freundii* (7.40%), *Citrobacter koseri* (1.85%), and *Enterobacter* spp. (1.85%). Antimicrobial susceptibility testing against 20 antibiotics indicated a high level of resistance, with 100% of isolates exhibiting resistance to penicillin's and aminopenicillins (ampicillin and amoxicillin). Furthermore, resistance to tetracycline (81.18%) and gentamicin (63.16%) was observed. Molecular detection of resistance genes via PCR revealed that *blaTEM* was present in 48.14% of the isolates, while *blaSHV* was detected in 33.3%. The presence of these genes highlights the potential dissemination of extended-spectrum β-lactamases (ESBLs) in environmental sources, particularly in hospital-associated sewage.

Keywords: *Enterobacteriaceae*; MDR; Hospital sewage; Resistance genes

1. Introduction

A major source of genes and germs resistant to antibiotics, hospital sewage presents a serious risk to public health. The role of sewage as a source of antimicrobial resistance genes (ARGs) and AMR is one area that requires additional research [1]. Hospital sewage systems can harbor drug-resistant bacterial infections in patients' urine and feces, as well as antimicrobial agent metabolites from medication consumption, which can operate as hotspots for antimicrobial resistance (AMR) [2]. Consequently, MDR bacteria are frequently found in hospital sewage [3]. These bacteria travel quickly into the environment through plasmids and transposons, which facilitate horizontal gene transfer [4]. Additionally, the problem worsens when hospital wastewater that has been improperly or inadequately cleaned is dumped into adjacent sewage systems. [5]. The impact of hospital wastewater treatment on antimicrobial resistance (AMR) differs across studies, likely due to the variability in methodologies employed [6]. Research has demonstrated a favorable correlation between the prevalence of antibiotic resistance in the corresponding human population and the AMR rates in bacteria isolated from wastewater [7]. One study identified a potential pathway for the spread of *Enterococcus fecium*, which is resistant to ampicillin and ciprofoxacin, from hospital patients to urban sewage, then via wastewater treatment facilities to surface water and ultimately back to people [8]. Gram-negative bacteria that are extended spectrum β-lactamase (ESBL) producers are of particular significance, causing infections that are particularly difficult to treat [9]. microorganisms produce a group of β-lactamases, which share the ability to hydrolyze penicillin's, first, second and third-generation cephalosporin's, aztreonam and carry genes encoding resistance to additional drug classes such as aminoglycosides [9, 10]. *E. coli and K. pneumonia*, which are also indicator bacteria in AMR surveillance in the environment [11], represent the commonest multidrug-resistant (MDR) pathogens that exhibit ESBL, carbapenem and quinolone resistance [10, 12, 13] .*bla-TEM* has the ability to hydrolyze ampicillin more than Carbenicillin,

* Corresponding author: Samaa Kh. Alhashimy.

Oxacillinand Cefalotin, and has lisle activity against broad-spectrum Cephalosporin and inhibited by clavulanic acid. Common ESBL genes coding for isolates of K. pneumonia and E. coli were identified as CTX-M (cefotaximase, which preferentially hydrolyses cefotaxime), TEM (discovered and isolated in the early 1980s from Teminora, a Greek patient), and SHV (for variable of sulphydryl, initially detected in a single Klebsiella ozaenae strain recovered in Germany). These transposon-, plasmid-, or chromosome-mediated genes are described infrequently worldwide. [14]

2. Material and methods

2.1. Wastewater and Environmental Sampling

Raw water samples were taken from 20 to 30 cm below the surface of the Tigris river, and put in clean, sterilized glass bottles with a capacity of 1000 mL. All of these bottles were meticulously sealed and taken to the lab on ice, where they were stored at 4°C and used separate sterile pipettes, decimal dilutions of 10-1, 10-2, 10-3 were prepped, of raw water by moving 10 mL of sample to 90 mL of sterile water. To identify Enterobacteriaceae bacteria, taken 1 mL from water diluted and used a sterile-shaped glass rod (spreader) was used to disseminate different dilutions of water samples and distributed on MacConkey, after that working a sub-culture on the Nutrient agar, and Eosin-methylene blue agar (EMB), Xylose – Lysin–Deoxycholate (XLD) and(SS) agar to determine the bacteria. The dishes were put in an incubator overnight at 37°Cfor 24 hrs., following incubation, cultures were inspected for different colonies, and incubated at 44°C for 24–48 h to fecal coliform bacteria (FCB).

2.2. Morphological description

Shape, color, consistency, size, and lactose fermentation have all been used to identify specific colonies. to on divers media (Nutrient agar, EMB, and MacConkey agar) The following have performed gram staining company instructions.

2.3. Biochemical tests

According to, various biochemical tests have been conducted. As directed by the manufacturing business, the API 20E strep (Biome Rieux, France) has been applied for additional identification.

2.4. Vitek2 compact system

The Vitec 2 compact has been utilized to verify the collaboration of Enterobacteriaceae isolates. Vitek2 is an autonomous technology that represents a smarter technique for diagnostic testing in microbiology. It allows automated, quick, and consistent validation of test findings.

2.5. Genetic identification

2.5.1. Extraction of genomic DNA

Isolate cultures were incubated on nutritional broth for 18 to 24 hours, centrifuged at 7000 to 8000 cycles per minute for 10 to 15 minutes, and then rinsed with distilled water while getting ready to use the genomic DNA mini kit (Geneaid, Taiwan) as closely as possible, varying the amounts and timing of the addition of materials. Genomic DNA detection via gel electrophoresis. The 0.8% agarose electrophoresis method has been used. Genomic DNA detection via gel electrophoresis. Sam brook and Russell state that the use of 0.8% agarose in the electrophoresis method has been made feasible by the available facilities.

2.6. Determination of TEM (*blaTEM*) and SHV (*blaSHV*) the genes of *Enterobacteriaceae* in the DNA sample

Multiplex PCR was employed to determine the TEM (*blaTEM*) and SHV *(blaSHV*) genes of the Enterobacteriaceae isolates from their DNA samples.

Table 1 PCR reaction after optimization used in genetic diagnosis using the initiator *blaTEM-blaSHV*

Step	Temperature	Time	NO.of cycle
Initial denaturation	95	3 min	30
Denaturing	95	30 sec	
Annealing	58	30 sec	
Extension	72	60 sec	
Last-minute extension	72	10 min	
Hold	4 c	10 min	

Table 2 Primers sequence used in this study

Genes	Sequence	Size(bp)	Reference
blaSHV	GATGAACGCTTTCCCATGATG-F CGCTGTTATCGCTCATGGTAA-R	471	(Kim *et al.,* 2009
blaTEM	AGTGCTGCCATAACCATGAGTG-F CTGACTCCCCGTCGTGTAGATA-R	459	(Kim *et al*., 2009)

TEM-2 and TEM-3 have similar hydrolytic profiles

3. Results

Isolation and cultivation: three isolate have been selected and identified by morphological and biochemical test and the microscopic examination, then the diagnosis was utilizing the VITECK-2 system.

Morphology: Using the Gramme stain, one may differentiate between bacteria Gram-positive and those that are Gram-negative. reaction as described in the Gramme stain procedure, all isolated bacteria are subjected to Gramme stain for microscopic examination. Under a light microscope (100X), the bacteria appeared as bacilli, short rods, single-cell, and gram-negative bacteria. The Gramme stain results also supported the gram-negative classification of the bacteria.

Colonies characteristics: E. coli type was specified relative to its physical characteristics. When the isolate is grown on MacConkey agar, the colonies were bright pink, whereas the colonies on EMB medium appeared with a green metallic sheen. This is because the crystal violet and bile salts in MacConkey agar promote GN bacteria growth while inhibiting GP bacteria growth. Also located that the bacteria fermented sugar as displayed While on Eosin Methylene Blue (EMB). It was a selective medium for E. coli that was utilized to distinguish it from other Enterobacteriaceae individuals. Sheen green metallic colonies were discovered, indicating that the colonies made organic acids as a result of glucose and lactose fermentation, which gave the colonies a sheen green metallic. The study's findings showed that several locations, including the first one next to Baghdad Medical City, the second one, close to Karkh Maternity Hospital, and the three site close to Nuaman General Hospital, had significant pollution levels. The most pollution was found in location 1, while the lowest average pollution was found in locations 2, 3

Table 3 The three hospitals sources for the sample

Hospital	Isolate number
Baghdad medical city	E1
Karkh maternity hospital	E2
Nuaman General Hospital	E3

Table 4 Different bacterial isolates

NO	Types of bacteria	Set NO.1	Set NO.2	Set NO.	Total bacterial	%
1	*E.coli*	11	8	5	24	48.8
2	*Klebsiella pneumonia*	5	7	2	14	% 28.2
3	*Enterobacter aerogenes*	4	1	1	6	%12.9
4	*Proteus mirabilis*	2	-	2	4	%8
5	*Citrobacter Kosri*	1	-	1	2	%4.6
	TOTAL				50	

The results of this study showed high levels of pollution in different locations: the first location near Baghdad Medical City, the second location near Al-Karkh Maternity Hospital, and the third location near Al-Nu'man General Hospital. The highest pollution level was recorded at the first location, while the lowest average level was recorded at the third location

Table 5 Showing the results of bacterial tests for wastewater samples

Sample type	Type of examination	Number of bacteria per 100 ml of sample	Sample condition
Waste water	MacConkey agar	101	contaminated
Waste water	Nutrient agar	101	contaminated
Waste water	Nutrient broth	Full	contaminated
Waste water	SSA	35	contaminated

All samples showed clear bacterial contamination. 2. Bacterial counts in most media (MacConkey and nutrient agar) indicated very high concentrations compared to the water permitted for discharge into rivers. Nutrient broth showed complete bacterial growth, indicating that the sample contained very high numbers of microorganisms. The SSA medium used for Salmonella and Shigella detection showed 35 units/100 ml, indicating possible contamination

3.1. *Enterobacteriaceae* for Identification of VITEK-2 System

Enterobacteriaceae has been definitively identified using this approach. By using identification gram-negative bacteria (IDGNB) cards, the system efficiently and quickly revealed bacteria out of contamination that might prevent the pathogen from being disclosed. The results showed that (27) isolates belonged to Enterobacteriaceae, from environmental samples, The GN card, which consists of 47 biochemical assays, was used to identify gram-negative bacteria.

3.2. Antibiotics resistance of environmental samples

The percentages of antibiotic resistance in the bacterial isolates that were taken from the water varied, according to the results. Out of the 34 environmental isolates of Enterobacteriaceae, 27 isolates shown resistance to a class of beta-lactam antibiotics. The isolates apparent range in response to the antibiotics used in the study is displayed in table Enterobacteriaceae family showed the highest levels of resistance to Penicillin 100, Amoxicillin 100, Cephalexin 100, Ceftazidime 100, Ceftazidime 100 , and Tetracycline 100 followed by Amoxicillin -clavulanic acid 77.27 and Cefotaxim77.2 for a few antibiotics, such as cefixime (63.6), cefoxitin (59.09) ,and azitreonam (54.5),moderate resistance was seen. The resistance to imipenem (13.6) and meropenem (18.18) was the lowest.

Increased irrational and careless antibiotic use sales of substandard antibiotics and the spread of drug -resistant microorganisms among people are all potential causes of antibiotic resistance in bacteria. Antimicrobial resistance has emerged as a significant global public health issue. One of the main causes of morbidity and death is infections brought on by bacteria that are resistant to drugs [15]. The isolates of E. coli exhibit 100% resistance to Tetracycline, Amoxicillin, Cephalexin, Ceftazidime, Cefotaxime, and Penicillin. The remaining antibiotics, which grade Amoxicillin–clavulanic acid

at 83.3%, Cefixime at 83.3%, Aztreonam at 66.6%, Cefoxitin at 50%, Imipenem, and Meropenem at 18.18%, had varying percentages of resistance.

3.3. Multiple Antibiotic Resistance

The results show that 2 total isolates of environmental multi-drug resistant (MDR) to three different groups of antibiotic the sum of the different antibiotics used in the study, which includes biologics as a group of beta-lactam antagonists as a Penicillin and group of Antibiotic such as Monobactams the majority of the isolates under study showed the character of multiple resistance to the antibiotics used in the treatment. Due to the indiscriminate use of antibiotics and a lack of health awareness, bacteria have developed multiple mechanisms of antibiotic resistance, which has led to the development of resistant strains with their own flow

and systems, the ability to alter the permeability barrier, and the production of beta-lactamase enzymes. This has created a persistent problem that makes it challenging to select the best treatment for it.

3.4. Genetic identification:

3.4.1. Detection of the blaTEM gene and blaSHV in environmental isolates of Enterobacteriaceae

The results show that 27 environmental isolate of *Enterobacteriaceae* contained the gen *blaTEM* and depending on the appearance of a band of size 800 base pairs and gen *blaSHV* appearance of a band of size 730 base pairs.

Figure 1 PCR amplification of *blaTEM genes* in environmental *Enterobacteriaceae* isolates. Electrophoresis was done in agarose gel (1.5%) at (75V/cm) for 90 min

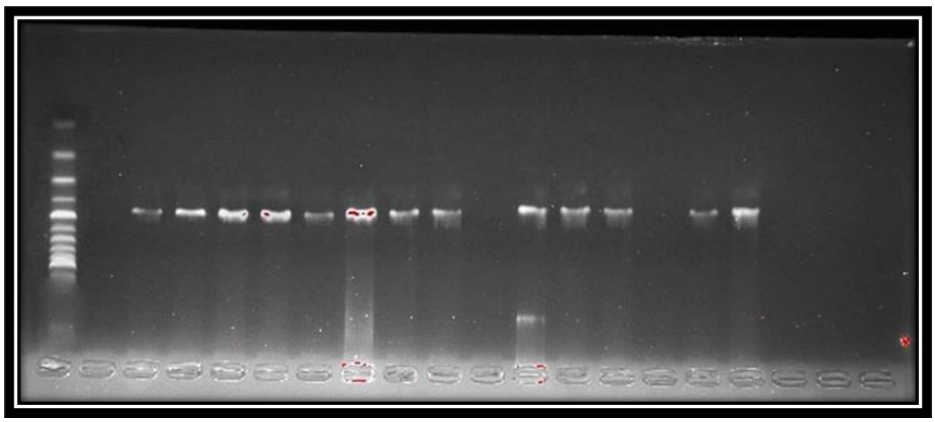

Figure 2 PCR amplification of *blaSHV* genes in environmental *Enterobacteriaceae* isolates. Electrophoresis was done in agarose gel (1.5%) at (75V/cm) for 90 min.

The results show that contained the genes of the *blaTEM* percentage of 81.14% depending on the appearance of a band size of 730 and 800 base pairs.

4. Discussion

The results in Table **3.1** show high levels of pollution in all three locations, with the highest pollution level recorded at the first location. This is attributed to the high levels of hospital wastewater. Hospital sewage poses a risk to public health. These effluents release dangerous microorganisms including as viruses, bacteria, fungi, protozoans, and helminthes into the environment along with medicines, heavy metals, antibiotics disinfectants, anesthetics, and no metabolized medications [16]

The results shown in Table **4** indicate that *Escherichia coli* was the most prevalent species, with a total of 24 isolates (48.8%), followed by *K. pneumoniae* (28.2%), and *C. kosri* (1.85%). This demonstrates a highly significant difference ($P < 0.01$) between *Escherichia coli* and the other isolates. These findings are consistent with a study conducted in India on hospital water samples, where various bacterial species were isolated, including *Escherichia coli* (28.6%) and *K. pneumoniae* (25.7%), and with a previous local study in Baghdad Governorate. The Enterobacteriaceae family constituted the highest percentage of the total identified and isolated isolates from the Tigris River waters. The percentages of these genera were *Escherichia coli* at 27%, *Clostridium pneumoniae* at 30%, and *Citrobacter freundii* at 14%.

The normal flora of the intestines of humans and other warm-blooded animals includes *E.coli* bacteria ,which contributes to wastewater high proportion .most of these bacteria are able to adapt to varying temperatures and Ph. Levels certain species may be harmful, particularly those that cause digestive system disorders [17, 18].

The results showed variations in antibiotic resistance rates among the bacterial isolates taken from water. Twenty-seven out of 50 environmental isolates from the Enterobacteriaceae family showed resistance to a range of beta-lactam antibiotics. There was a clear variation in the degree of response to the antibiotics used in the study. The highest resistance rates among Enterobacteriaceae were recorded against penicillin (100%), amoxicillin (100%), cephalexin (100%), ceftazidime (100%), and tetracycline (100%), followed by amoxicillin-clavulanic acid (77.27%). The results of this study are consistent with those of [19], where the isolates exhibited resistance to ampicillin in 95% of cases and to ceftazidime in 100% of cases. However, they differ from those of whose isolates showed resistance to ampicillin in 52.8% of cases, to ceftazidime in 5.1%, and to tetracycline in 4.2%. They also differ from the results for cefotaxime (11.9%). Antibiotic resistance in bacteria may result from the increased irrational and indiscriminate use of antibiotics, the sale of substandard antibiotics, and the transmission of drug-resistant microorganisms between individuals. Antibiotic resistance has become a major public health concern worldwide. Infections caused by drug-resistant bacteria are a leading cause of illness and death. Antibiotics for treating bacterial infections should be selected based on culture data and bacterial susceptibility (Nuban *et al*., 2016).

The findings indicate in figures (1), figure (2) that the percentage of *blaTEM* and *blaSHV* genes was 81.74%, depending on the appearance of a band size of 730 and 800 base pairs. The results of the current study were in line with [20], which reported a 47% *blaTEM* proportion in Enterobacteriaceae and a 17.4% *blaSHV* result. In contrast to the current findings, a study by [21] found that 24.1% of Enterobacteriaceae isolates from wastewater had the ubiquitous gene blaTEM**.** The results of the current study were consistent with [22], which found that the percentage of *blaTEM* in Enterobacteriaceae was 47%, while the *blaSHV* results showed a difference of 17.4%. In contrast to the current findings, a study by [21] found that 24.1% of Enterobacteriaceae isolates from wastewater had the ubiquitous gene *blaTEM* The quantity of isolates, the primers used, and the location and kind of the various isolates are the reasons why the results of the current investigation differ from those of previous studies.

5. Conclusion

- The most prevalent species of bacteria found in environmental samples were found to be *E. coli* and *K. pneumoniae*.
- The environmental isolates exhibited the highest percentage of resistance to penicillin, amoxicillin, cephalexin, ceftazidime, and tetracycline, and moderate resistance to cefixime, cefoxitin, Aztreonam, and it exhibited the least amount of resistance to Imipenem and Meropenem.
- The resistance *blaTEM* and *blaSHV* genes of Enterobacteriaceae were found in environmental isolates using Polymerase Chain Reaction (PCR). Isolates from the environment revealed their resilience the *blaTEM* and *blaSHV* genes.

Recommendations

- Controlling the release of Hospital sewage into the river.
- Controlling the use of antibiotic.
- study of methods of treating river water from pollution.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Hendriksen RS, Munk P, Njage P, Van Bunnik B, McNally L, Lukjancenko O, et al. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. Nature communications. 2019;10(1):1124.

[2] Verburg I, García-Cobos S, Hernández Leal L, Waar K, Friedrich AW, Schmitt H. Abundance and antimicrobial resistance of three bacterial species along a complete wastewater pathway. Microorganisms. 2019;7(9):312.

[3] Zagui GS, de Andrade LN, Moreira NC, Silva TV, Machado GP, da Costa Darini AL, et al. Gram-negative bacteria carrying β-lactamase encoding genes in hospital and urban wastewater in Brazil. Environmental Monitoring and Assessment. 2020;192(6):376.

[4] Korzeniewska E, Harnisz M. Beta-lactamase-producing Enterobacteriaceae in hospital effluents. Journal of environmental management. 2013;123:1-7.

[5] Hocquet D, Muller A, Bertrand X. What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. Journal of Hospital Infection. 2016;93(4):395-402.

[6] Buelow E, Bayjanov JR, Majoor E, Willems RJ, Bonten MJ, Schmitt H, et al. Limited influence of hospital wastewater on the microbiome and resistome of wastewater in a community sewerage system. FEMS microbiology ecology. 2018;94(7):fiy087.

[7] Reinthaler FF, Galler H, Feierl G, Haas D, Leitner E, Mascher F, et al. Resistance patterns of Escherichia coli isolated from sewage sludge in comparison with those isolated from human patients in 2000 and 2009. Journal of water and health. 2013;11(1):13-20.

[8] Iversen A, Kühn I, Rahman M, Franklin A, Burman LG, Olsson-Liljequist B, et al. Evidence for transmission between humans and the environment of a nosocomial strain of Enterococcus faecium. Environmental Microbiology. 2004;6(1):55-9.

[9] Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet. 2016;387(10014):176-87.

[10] Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-antimicrobial resistance. 2021;3(3):dlab092.

[11] Anjum MF, Schmitt H, Börjesson S, Berendonk TU, Donner E, Stehling EG, et al. The potential of using E. coli as an indicator for the surveillance of antimicrobial resistance (AMR) in the environment. Current Opinion in Microbiology. 2021;64:152-8.

[12] Cheng VC, Wong S-C, Wong SC, Ho P-L, Yuen K-Y. Control of Carbapenemase-producing Enterobacteriaceae: Beyond the hospital. EClinicalMedicine. 2018;6:3-4.

[13] Harmon DE, Miranda OA, McCarley A, Eshaghian M, Carlson N, Ruiz C. Prevalence and characterization of carbapenem-resistant bacteria in water bodies in the Los Angeles–Southern California area. Microbiologyopen. 2019;8(4):e00692.

[14] Akpaka P, Legall B, Padman J. Molecular detection and epidemiology of extended-spectrum beta-lactamase genes prevalent in clinical isolates of Klebsiella pneumoniae and E coli from Trinidad and Tobago. West Indian Medical Journal. 2010;59(6):591.

[15] Neupane S, Pant ND, Khatiwada S, Chaudhary R, Banjara MR. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic Escherichia coli isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. Antimicrobial resistance and infection control. 2016;5(1):5.

[16] Santoro D, Cardoso A, Coutinho F, Pinto L, Vieira R, Albano R, et al. Diversity and antibiotic resistance profiles of Pseudomonads from a hospital wastewater treatment plant. Journal of applied microbiology. 2015;119(6):1527-40.

[17] Anderson KL, Whitlock JE, Harwood VJ. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Applied and environmental microbiology. 2005;71(6):3041-8.

[18] Ibrahim IA, Al-Shwaikh RM, Ismaeil MI. Virulence and antimicrobial resistance of Escherichia coli isolated from Tigris River and children diarrhea. Infection and Drug Resistance. 2014:317-22.

[19] Ngene AC, Ohaegbu CG, Awom IE, Egbere JO, Onyimba IA, Coulthard OD, et al. High prevalence of multidrug resistant enterobacteriaceae isolated from wastewater and soil in Jos Metropolis, Plateau State, Nigeria. African Journal of Bacteriology Research. 2021;13(2):22-9.

[20] Ojer-Usoz E, González D, Vitas AI. Clonal diversity of ESBL-producing Escherichia coli isolated from environmental, human and food samples. International journal of environmental research and public health. 2017;14(7):676.

[21] Amador PP, Fernandes RM, Prudêncio MC, Barreto MP, Duarte IM. Antibiotic resistance in wastewater: Occurrence and fate of Enterobacteriaceae producers of Class A and Class C β-lactamases. Journal of Environmental Science and Health, Part A. 2015;50(1):26-39.

[22] Ojer-Usoz E, González D, García-Jalón I, Vitas AI. High dissemination of extended-spectrum β-lactamase-producing Enterobacteriaceae in effluents from wastewater treatment plants. Water research. 2014;56:37-47.