Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

FREQUENCY AND DISTRIBUTION OF TEM AND CTX-M GENES IN ESBL PRODUCING ENTEROBACTER CLOACAE

Kiran Zahra[1], Muhammad Ahsan Murtaza[2], Saad Ullah[3], Mudassir Ahmad[4], Ruqia Ibrahim[5], Shahab Imtiaz[6], Zainab Masood[7], Talha Rehman[8], Kashif Ahmad[9], Kaleem Ullah[*10], Farman Ali[*11], Latifullah[12]

[1]Department of Microbiology Hazara University Mansehra
[2]Department of Biological Sciences, The Superior University Lahore
[3]Institute of Biotechnology and Microbiology, Bacha Khan University Charsadda
[4]Department of Cardiology, University of Peshawar
[5]Department of Biotechnology, COMSATS University Abbottabad
[6]Department of Molecular Biology, Islamia College University Peshawar
*[7,8, *11]Department of Microbiology, Abbottabad University of Science & technology*
[9]Department of Zoology, University of Buner
*[*10]Department of Medical Lab Technology, Abbottabad University of Science & Technology*
[12]Department of Nursing, Khyber Medical University Peshawar

[*1]k07256199@gmail.com, [2] ahsanmurtaza9817@gmail.com, [3]saadullah.fsons@gmail.com, [4]www.mudassirahmad.2006@gmail.com, [5]riyakhanswati25@gmail.com, [6]kshahab9575@gmail.com, [7]zainabmasood224@gmail.com, [8]talhakhan12218@gmail.com, [9]kashifahmad9217544@gmail.com, [*10]ku1948477@gmai.com, [*11]farmanalibk7@gmail.com, [12]latifullahswat44@gmail.com

DOI: https://doi.org/10.5281/zenodo.16303503

Keywords

Article History
Received on 21 April 2025
Accepted on 06 July 2025
Published on 21 July 2025

Corresponding Author: *
Kaleem Ullah,
Farman Ali

Abstract

The Enterobacteriaceae family of bacteria includes naturally occurring facultative anaerobic gram-negative bacteria. These bacteria are saprophytic in nature and reside in soil and sewage. Most importantly, these bacteria also part of the symbiotic gut microbial community residing in human gastrointestinal system. In the last several decades, Enterobacter has become clinically significant, as it has emerged as a leading nosocomial infection causing agent in patients of critical care units. The problem has further aggravated by the accumulating evidence of resistance of E. cloacae to routinely prescribed antibiotics, specifically those caused extended spectrum beta-lactamases. Keeping this in view, it's critical to keep track of antibiotic resistance and the frequency of causative genes in a population on a regular basis. As a result, the current study focused on determining the frequency of E. cloacae TEM and CTX-M genes isolated from clinical samples. A total of 150 Clinical samples (30 urine, 30 pus, 30 sputum, 30 bloods, and 30 wounds) were collected from patients admitted or visiting the Khyber Teaching Hospital in Peshawar to isolate ESBL producing E. cloacae. The antibiotic resistance analysis of positive samples revealed highest resistance to Aztreonam (92%) followed by Ceftazidime (82%), Ciprofloxacin (56%), Piperacillin (50%), Imipenem (32%), Gentamycin and Cefepime (30%), Amikacin (28%), Meropenem (22%), and Ertapenem (22%). Colistin, on the other hand, showed the least amount of resistance (2%). ESBL genes frequency investigation using Polymerase Chain Reaction (PCR) revealed that the majority of resistant isolates (47%) contained

Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

the TEM gene, while 38% had the CTX-M gene, and 15% were negative for both.

It is concluded that ESBL based antiobiotic resistance is at rise (1/3 of the samples resulted positive) which is mainly (85%) caused by presence of either TEM or CTX-M genes. The medical practitioner should consider this alarming rise of resistance while prescribing the antibiotics.

INTRODUCTION

The prevalence of extended-spectrum beta-lactamase is rising rapidly worldwide. Bacterial resistance is manifested by a variety of methods, including drug modification or inactivation, target changes, metabolic pathways, or excretion mechanisms (1). The emergence of this danger is the result of the reckless use of antibiotics in animals, humans, and agriculture. The World Health Organization (WHO) and the World Organization for Animal Health (OIE) both recommend that antibiotics be used properly in agriculture and animals (2).

According to the World Health Organization, antibiotic resistance is the ability of microorganisms to withstand antibiotics that were once effective against them. Initially, these drugs successfully treat infections (3). Microbiologists define resistance as a microorganism's capacity to survive even at the highest antibiotic concentrations, often due to misuse or overuse of antibiotics (4). Resistance can spread when resistant bacteria transfer their resistance genes to susceptible bacteria. These genes are carried by various DNA elements such as plasmids, phages, transposons, and integrons (5, 6). Antibiotics typically enter bacterial cells via active transport across the cell wall or membrane. Changes in the bacterial cell wall composition play a crucial role in antibiotic susceptibility. Gram-positive bacteria have a simpler peptidoglycan structure, while Gram-negative bacteria have a complex lipopolysaccharide-rich cell wall, making them harder to treat (6).

Antibiotic resistance arises vertically through chromosomal mutations or horizontally when susceptible bacteria acquire resistance elements from resistant bacteria via genetic exchange mechanisms such as conjugation, transformation, or transduction (7). In conjugation, two bacteria form a pilus to transfer DNA; in transformation, bacteria take up DNA from their environment. Resistance can persist in both intracellular and extracellular bacteria and knows no borders. Studies have shown that rural rodents in England carry multi-drug-resistant bacteria, indicating that resistant bacteria spread across communities, hospitals, and countries (8).

Many bacteria produce beta-lactamases, which deactivate antibiotics like penicillins and cephalosporins by breaking their four-membered beta-lactam ring. Extended-spectrum beta-lactamases (ESBLs) can hydrolyze cephalosporins, penicillins, and aztreonam but not cephamycins (FOX) and carbapenems (MEM and IPM). These enzymes are also often resistant to aminoglycosides, trimethoprim-sulfamethoxazole, and quinolones, and they spread between bacteria via plasmids (9). Globally, 10–40% of Enterobacteriaceae strains produce ESBLs. Mutations in TEM-2, TEM-1, and SHV-1 genes (including SHV, CTX-M-1, CTX-M-9, and TEM) generate these enzymes, which can be inhibited by clavulanic acid, tazobactam, or sulbactam (10).

Beta-lactam antibiotic resistance, once rare for this widely used class, is now a major concern because beta-lactamases inactivate many beta-lactams, including monobactams and third- and fourth-generation extended-spectrum cephalosporins (ESCs) (11). ESBLs are the main mechanism of cephalosporin resistance, especially in gram-negative bacteria (12). ESBL-producing bacteria often show multidrug resistance, limiting treatment options and causing drug failure (13-15) The main ESBL genes are blaCTX-M, blaSHV, and blaTEM. The blaCTX-M family has five subgroups numbered as blaCTX-M-1, -2, -8, -9, and -25. CTX-M ESBLs have become the dominant type globally in humans (16) and are also found in food animals (14, 15, 17).

Identified in 1983, ESBLs disable monobactams and oxyimino-cephalosporins, but not carbapenems and cephamycins (Bradford, 2001). They are mainly produced by *Escherichia coli* and *Klebsiella pneumoniae*,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

but other Enterobacteriaceae and *Pseudomonas* species worldwide also carry them (18).

Previous studies in Lahore confirmed ESBL-expressing *Enterobacter* spp. (19), while a study in Peshawar found blaCTX-M-1 and blaTEM-1 in *E. coli* (20). Although ESBL-producing *Enterobacter* spp. have been associated with urinary tract infections in Peshawar, the prevalence of TEM and CTX-M genes in resistant *E. cloacae* isolates remains unclear. Therefore, this study aims to determine the antibiotic resistance pattern and gene frequency in *E. cloacae* clinical isolates to assess gene spread in the community.

2. MATERIALS AND METHODS

2.1. Study Area

Clinical samples (urine, pus, blood, and swabs including wound, vaginal, throat swabs) from patients admitted or visiting the Khyber Teaching Hospital in Peshawar were used in this investigation.

2.2. Ethical Consideration

First and foremost, ethical approval was acquired from the hospital's Ethics Committee as well as the hospital's Administrators before the study began. Similarly, all of the patients involved in the trial gave their consent.

2.3. Sample collection

Around 150 clinical samples were gathered in sterile settings from patients admitted or visiting the Khyber Teaching Hospital in Peshawar (30 urine, 30 pus, 30 blood, 30 wound swabs, and 30 sputum).

Table 2.1: Displays the number of samples

S. No	Hospital	Clinical Sample	Number of samples
1	KTH	Urine	30
		Wound	30
		Pus	30
		Sputum	30
		Blood	30

2.4. Sterilization of Glassware

All glassware and plastic equipment used in the experiment were autoclaved for 15 minutes at 121°C and sterilized in a hot oven for 20 minutes.

2.5. Isolation of Enterobacter cloacae

By streaking the samples on the appropriate culture media, the bacteria were extracted and identified using the previously reported procedures (Afridi *et al.*, 2011).

2.6. Identification of bacterial isolates

2.6.1. Macroscopic and Microscopic Examination:

Physical characteristics such as smell, consistency, and colour were first noted. For microscopic confirmation, Gram staining was performed following standard procedure: a bacterial colony from an agar plate was emulsified on a sterilized slide, heat-fixed, stained with crystal violet for 60 seconds, rinsed, treated with Gram's iodine for 1 minute, decolorized with 95% ethanol, counterstained with safranin for 90 seconds, air-dried, and examined under oil immersion (100×). *E. cloacae* appeared as pink rod-shaped bacteria, and images were documented (21).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

2.6.2. Biochemical Identification (API 10-S) and Preservation:

Biochemical characterization was conducted using the API 10-S system (Biomerieux, France), which contains ten microtubes with dehydrated substrates. A bacterial suspension was prepared in 0.85% API NaCl or API Suspension Medium as per the kit's protocol. Clinical specimens were not used directly; instead, pure cultures were obtained per CLSI guidelines.

To ensure a moist incubation environment, 3 ml of demineralized water was added to the tray's wells, and the API strip was placed in the incubation box. A single colony from a purified culture was mixed with sterile saline or API medium to prepare the inoculum, which was carefully transferred into the strip's tubes to avoid bubbles. For the citrate (CIT) test, both tube and cupule were filled, whereas for tests requiring anaerobic conditions (LDC, ODC, H_2S, URE), mineral oil was added to prevent oxygen exposure. The strips were incubated at 37 °C for 18–24 hours.

Reading and Interpretation:

Post-incubation, color changes were observed and recorded using the identification key (Padmavathy et al., 2013). Specific reagents were added to certain tests: TDA reagent for tryptophan deaminase (red-brown color indicates positive), JAMES reagent for indole (pink color indicates positive), and NIT 1 + NIT 2 reagents for nitrate reduction (red color indicates positive).

Reactions were noted on the result sheet, and tests were grouped and assigned values (1, 2, or 4). These were summed to generate a 4-digit numerical profile, which, along with oxidase and nitrate test results, was compared to the API 10-S index for final identification, as detailed in the results and discussion.

2.7. Antibiotic susceptibility pattern analysis

For the antibiotic susceptibility pattern of *Enterobacter cloacae* against a battery of antibiotics, including Augmentin, Cefepime, Cefotaxime, Ceftazidime, and Azeteronem were used by applying the disc diffusion method. The bacteria were cultivated on an MHA medium that has been autoclaved, and the antibiotic zone of inhibition will be determined. The outcome will be classified as either resistant, intermediate, or sensitive (20).

Table 2.2: Measurements of diameter (Zone of inhibition) produced by antibiotics against for *E. cloacae* (CLSI, 2016)

S. No	Antibiotics	Conc(µg)	Resistant zone (mm)	Intermediate zone (mm)	Susceptible zone (mm)
1	Amikacin	(30µg)	≤14	15-16	≥17
2	Colistin	MIC	≤4	____	≥2
3	Meropenem	(10µg)	≤19	20-22	≥23
4	Ciprofloxacin	(5µg)	≤21	22-25	≥26
5	Ertapenem	(10µg)	≤18	19-21	≥22
6	Imipenem	(10µg)	≤19	22-22	≥23
7	Piperacillin)	(100µg)	≤17	18-20	≥21
8	Gentamycin	(10µg)	≤12	13-14	≥15
9	Cefipime	(30µg)	≤18	19-24	≥25
10	Aztreonam	(30µg)	≤17	18-20	≥21
11	Ceftazidime	(30µg)	≤17	18-20	≥21

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

2.8. Detection of extended spectrum β -lactamases (ESBL) producing Enterobacter spp.

The phenotypic detection of ESBL was done using a double-disc synergy test. Muller Hinton agar plates were infected with probable *Enterobacter* in this test. Then, in the middle of the previously prepared plate, a disc of clavulanic acid (10 mg) + amoxicillin (20 mg) was placed. Testing antibiotics i.e., Cefipime (30 mg), ceftazidime (30 mg), cefotaxime (30 mg), and aztreonam (30 mg) were placed on the plate, ~20-25 mm from the center. After a 24-hour incubation period, each plate was checked. The tested organism was declared positive for ESBL production if the zone of inhibition of aztreonam and one or more cephalosporins extended on the side closest to the clavulanic acid + amoxicillin (22).

2.9. DNA extraction

The DNA was extracted using the directions included in a commercially available DNA extraction kit (BioMerieux, France). Electrophoresis was used to confirm the DNA's integrity, and the isolated DNA was then kept at -20°C.

2.10. Molecular detection of selected antibiotic resistance genes (CTX-M and tem)

Using specific primers, the antibiotic resistance genes CTX-M and tem *of Enterobacter cloacae* were amplified (Shah *et al.*, 2004). The amplified PCR products, as well as ladder, positive, and negative controls, were run on a 1.5 percent agarose gel. A gel documentation system was used to visualize the electrophoresed products.

Table 2.3: Primers of ESBL genes

Primer	PCR Target	Sequence	Annealing Temperature	Product size	Reference
CTX-M-F **CTX-M-R**	CTX-M	CGG TCA GTC CGT TTG TTC CTT GGT CGG TCT GTA	50°C	547-bp	(Villegas *et al.*, 2004)
Tem-F **Tem-R**	tem	TGT TGC TTG TGC CGA TTG GA AGA TGG TAT TGT TGG TTG CTG	56 °C	245-bp	(Yao *et al.*, 2007)

2.10.1. PCR Condition

The Polymerase chain reaction was used to examine Esbl genes. The experiment was carried out with a $12\mu l$ combination. $6\mu l$ master mix, $1\mu l$ forward primer, $1\mu l$ reverse primer, $2\mu l$ lysate, and $2\mu l$ nuclease-free water to prevent loss and maintain the volume of the mixture, 0.5 μl of double distal water was added. All of the components were placed in PCR tubes and run through a thermal cycler. The reaction was carried out under conditions as shown in figure 2.1.

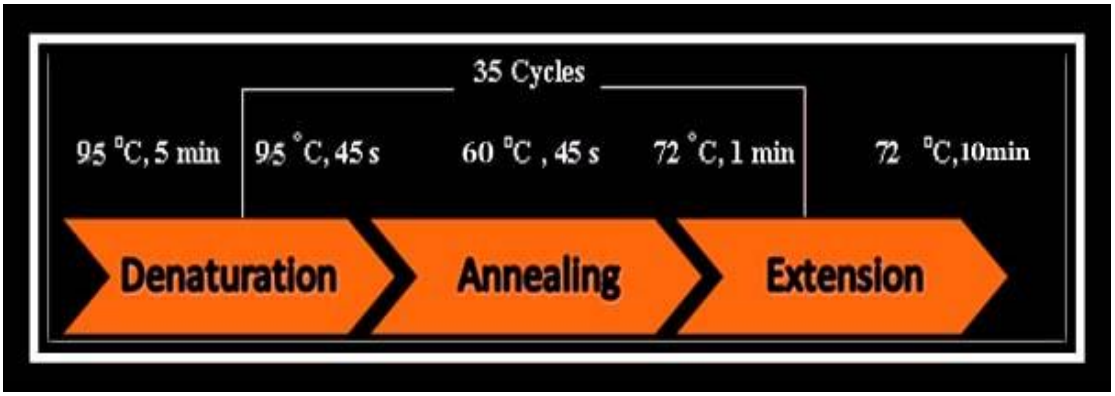

Figure 2.1: Polymerase chain reaction steps

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

2.11. Gel Electrophoresis

For detection of PCR amplified products, the product was run on gel and electrophoresed. One percent agarose was made by boiling 0.4 grams of agarose in 40 milliliters of TAE/TBE buffer for one minute. After cooling, two liters of ethidium bromide was added. Comb was placed in order to produce wells for sample loading on a side-by-side gel trey. The agarose mixture was then added into the trey and allowed to solidify. After solidification, the gel was transferred to a gel tank filled with TAE buffer. Then, in the first well, 5 liters of the ladder were loaded, followed by 3.5 liters of PCR product, and 1.5 liters of loading dye in the other wells. For 30 minutes, the gel tank's positive and negative electrodes were connected to 60 volts delivered at 100 mA power (23).

3. RESULTS AND DISCUSSION

Enterobacter is a genus of naturally occurring facultative anaerobic gram-negative bacteria of the Enterobacteriaceae family. It's a rod-shaped Gram-negative bacterium with peritrichous flagella that's facultatively anaerobic. It is catalase-positive and oxidase-negative. These bacteria are saprophytic in the environment, since they live in soil and sewage, and they are also a part of the human gastrointestinal system's symbiotic gut flora (24).

The National Nosocomial Infections Surveillance System reported data on nosocomial bacteremia in the United States from 1976 to 1989, and the National Healthcare Safety Network (2008) reported that Enterobacter spp. account for approximately 5% of nosocomial bacteremia cases; these figures have not changed (25). Another multicenter study examined 24,179 cases of nosocomial bloodstream infections between 1995 and 2002 and discovered that Enterobacter spp. was one of the top ten most often diagnosed nosocomial bacteria, with a greater prevalence in critical care unit wards (26). The Enterobacter cloacae complex now includes six species: E. cloacae, Enterobacter asburiae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii, and Enterobacter nimipressuralis. Its most prominent representative, E. cloacae, has emerged as a problematic infection for healthcare facilities worldwide. E. cloacae is responsible for up to 5% of hospital-acquired sepsis, 5% of nosocomial cases of pneumonia, 4% of nosocomial urinary tract infections, and 10% of postsurgical peritonitis cases (27, 28).

In the Lahore region, previous studies on clinical isolates expressing extended-spectrum-lactamase (ESBL) genes including TEM and CTX-M demonstrated the presence of resistant *Enterobacter* spp. (29). These genes were also discovered in a Peshawar study to investigate the frequency of $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}1}$ beta lactamases in *Escherichia coli* (30). Despite the fact that ESBL-producing *Enterobacter* spp. have been linked to urinary tract infections in the Peshawar region, the frequency of resistance-associated genes like TEM and CTX-M in resistant isolates has yet to be determined.

Therefore, the goal of this study is to determine *Enterobacter cloacae's* antibiotic resistance pattern as well as the frequency of important genes in clinical isolates. This research also pinpoints genes in *E. cloacae* that cause resistance to ß-lactam antibiotics.

3.1. Isolation and confirmation of *E. cloacae*

This study evaluated 150 clinical samples (30 urine, 30 pus, 30 blood, 30 wound swabs, and 30 sputum) from patients admitted or visiting Khyber Teaching Hospital Peshawar. MacConkey agar was used to isolate *E. cloacae*, which resulted in pink colonies (Figure 3.1). Isolates were confirmed microscopically and biochemically (Figure 3.2).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

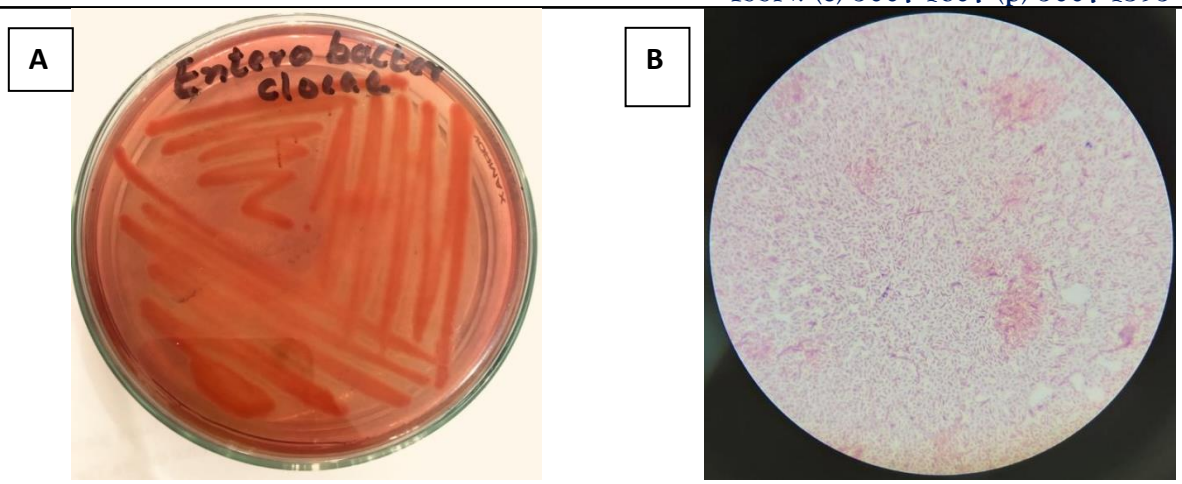

Figure 3.1: *E. cloacae* appearance on maCconkey agar (A) & under microscope (B).

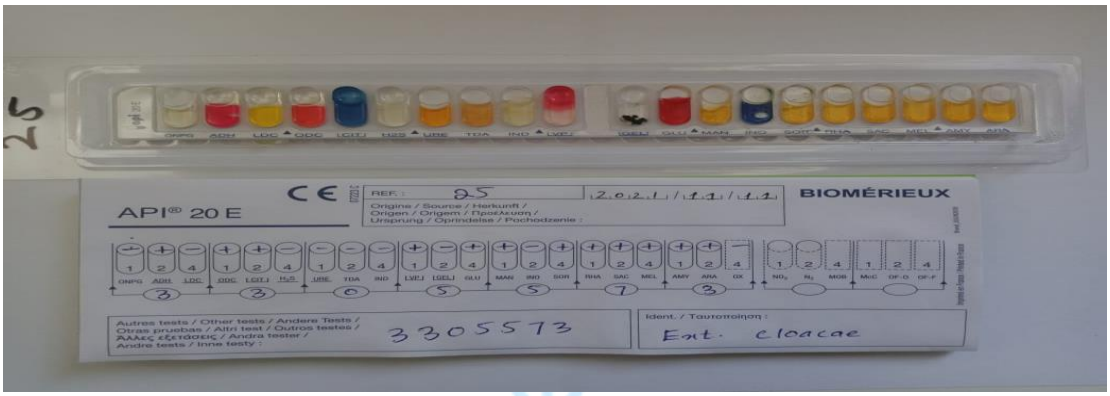

Figure 3.2: API profile of E. cloacae

3.2. Frequency of E. cloacae

Among 150 clinical samples tested for presence of *E. cloacae* 70 samples were found positive (Figure 3.3). This showed that approximately 47% of total samples were containing *E. cloacae*. The recovered isolates were examined for ESBL, and 50 of them were found to be *E. cloacae* that produced the enzyme. A sample wise distribution is shown in figure 3.4. *E. cloacae* was shown to be present in 47% of clinical isolates, while (31) discovered that 44% of clinical isolates expressed Extended-spectrum-lactamase (ESBL). Different sample times and geographic locations could account for the little difference in prevalence.

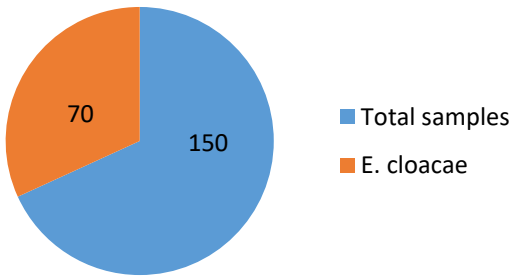

Figure 3.3: *E. cloacae* positive culture in clinical samples

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

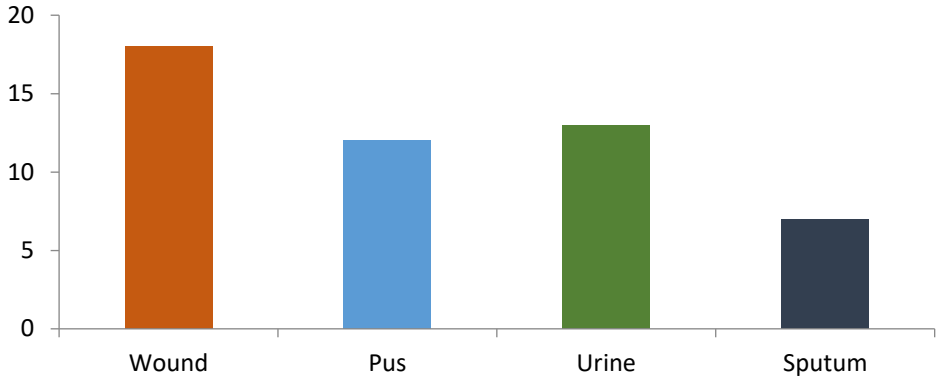

Figure 3.4: Frequency of ESBL producing *E. cloacae* among samples

3.3. Detection of ESBL producing E. cloacae

The recovered isolates (n=70) were examined for ESBL. The double disc synergy test as described in material and methods and shown as figure 3.5 was used to test a total of 70 *E. cloacae* for their ability to produce ESBLs. Among 70 tested samples 50 of the isolates were found to be *E. cloacae* for the production of B-lactamase enzyme.

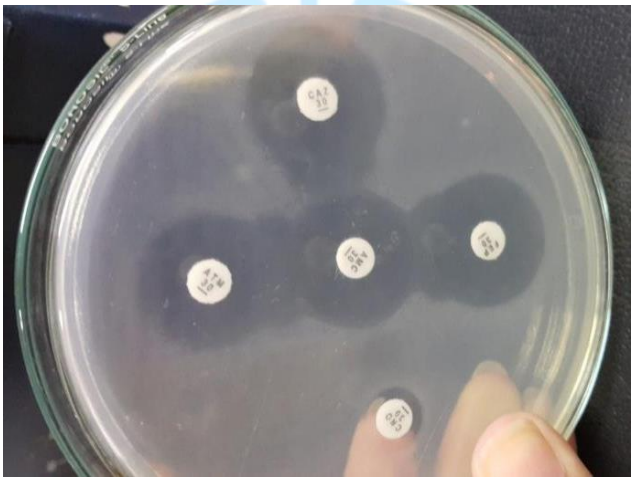

Figure 3.5: Detection ESBL producing *E. cloacae*

3.4. Profile of Antibiotics

Antibiotic resistance, susceptibility, and intermediation were investigated in ESBL positive samples (50) using disc diffusion method (Figure 3.6). The majority of ESBL-producing *E. cloacae* showed multidrug resistance, however, sensitivity, resistance, and intermediate resistance to the 11 antibiotics used varied (Table 3.1). (32) looked at an outbreak of ESBL-producing *Enterobacter cloacae* caused by a contaminated ureteroscope causing urinary tract infections. Participants in this study had undergone ureteroscopy previous to the illness. The bacteria *Enterobacter cloacae* were discovered in the urine of 15 patients who had previously undergone ureteroscopy. 70 specimens were obtained during the initial monitoring operation. All 15 patient isolates and three ureteroscope isolates followed the same pattern with minor differences. Furthermore, antibiotic sensitivity was evaluated, and the results indicate that Ertapenem, Amikacin, Gentamycin, and Meropenem have the highest sensitivity to antibiotics, which is consistent with our findings. This research backs up our findings.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

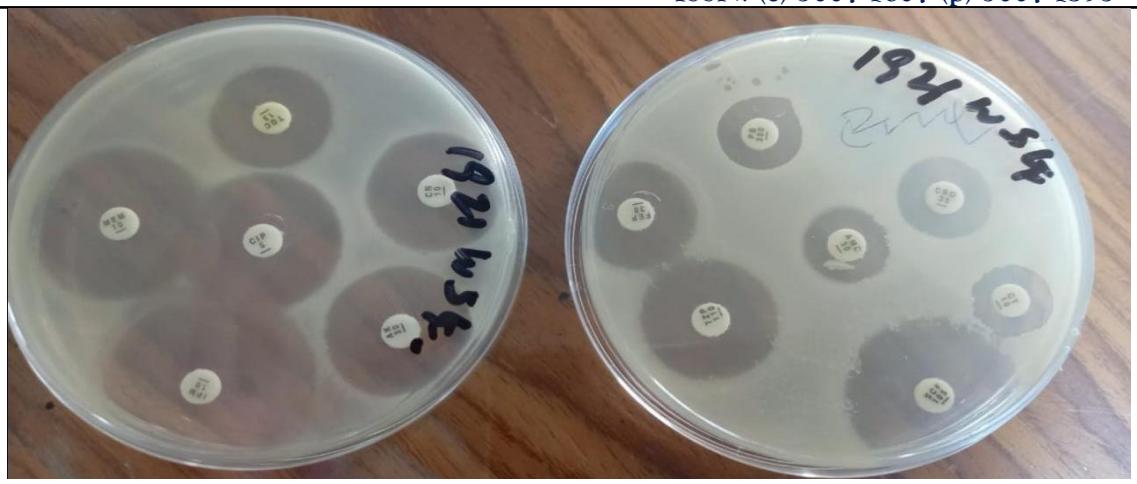

Figure 3.6: Antibiotic resistances profiling on MHA agar plate

Table 3.1: Antibiotic susceptibility profile of ESBL producing isolates

yujS. N	Antibiotic	Conc(µg)	Susceptible %	Intermediate %	Resistance %
1.	Colistin	MIC	49(98)	0(0)	1(2)
2.	Ertapenem	10	45(90)	0(0)	5(10)
3.	Imipenem	10	34(68)	0(0)	16(32)
4.	Meropenem	10	39(78)	0(0)	11(22)
5.	Piperacillin	100	25(50)	0(0)	25(50)
6.	Cefepime	30	34(68)	1(2)	15(30)
7.	Amikacin	30	35(70)	1(2)	14(28)
8.	Aztreonam	30	4(8)	0(0)	46(92)
9.	Ciprofloxacin	05	19(38)	3(6)	28(56)
10.	Ceftazidime	30	9(18)	0(0)	41(82)
11.	Gentamycin	10	35(70)	0(0)	15(30)

3.4.1. Susceptibility

As depicted in Figure 3.7, Colistin was shown to be the most susceptible (98%) antibiotic, followed by Ertapenem (90%), Meropenem (78%), Amikacin and Gentamycin (70%), Cefepime and Imipenem (68%), Piperacillin (50%), Ciprofloxacin (38%), and Ceftazidime (38%). Aztreonam, on the other hand, exhibited the least susceptibility (8%). These results back up finding of (32).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

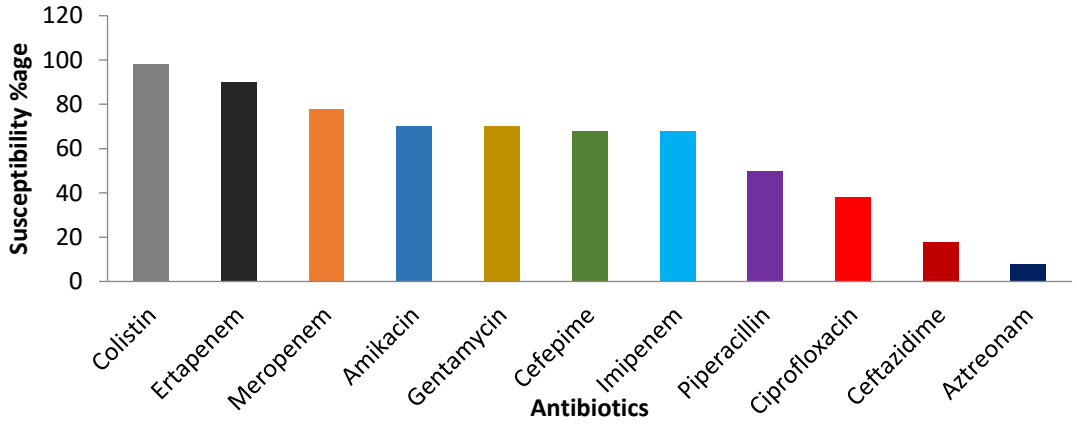

Figure 3.7: Susceptibility pattern of *E. cloacae* against tested antibiotics

3.4.2. Resistance

As Figure 3.8 shown, Aztreonam exhibited the highest resistance (92%) followed by Ceftazidime (82%), Ciprofloxacin (56%), Piperacillin (50%), Imipenem (32%), Gentamycin and Cefepime (30%), Amikacin (28%), Meropenem (22%), and Ertapenem (22%). Colistin, on the other hand, showed the least amount of resistance (2%). It is implicated from the study that colistin is best antibiotic as it showed least resistance. Other antibiotics including Amikacin, Meropenem and Gentamycin are also better choice antibiotics.

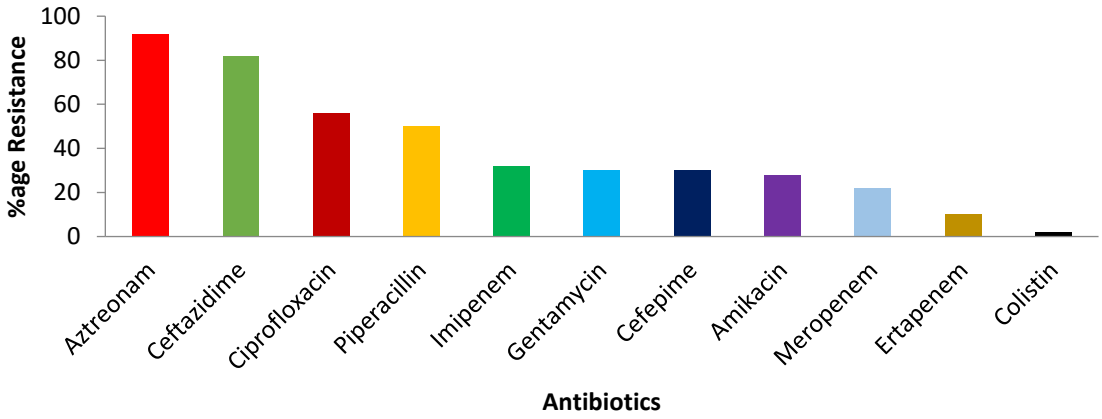

Figure 3.8: Resistance pattern of *E. cloacae* against antibiotics

3.4.3. Intermediate

Some antibiotics, such as Ciprofloxacin (6%), Amikacin, and Cefipime, showed mild resistance (2%) as described in Figure 3.9.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

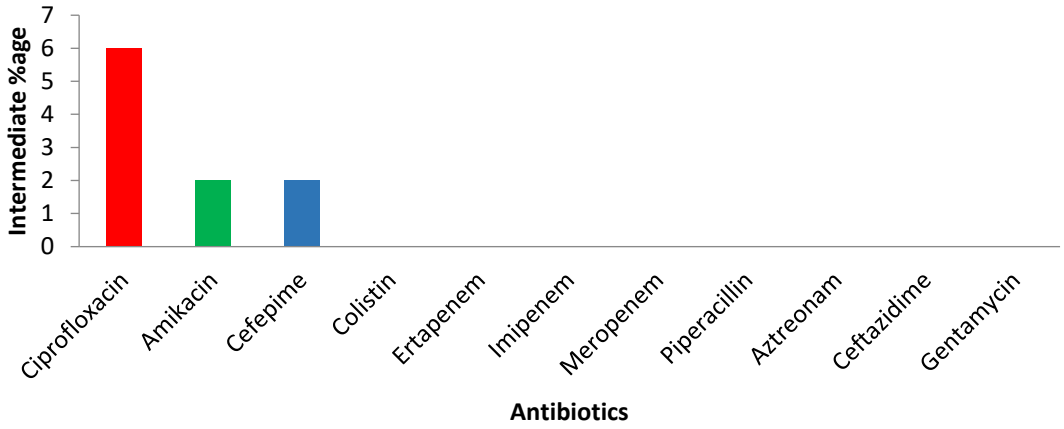

Figure 3.9: Intermediate resistance pattern of *E. cloacae* against antibiotic

3.5. Amplification and Frequency of CTX-M and TEM in ESBL positive Isolates

The CTX-M and TEM were detected in those 50 isolates which were capable of producing ESBL through PCR and using specific primers bla_{TEM} and bla_{CTX} genes were amplified (Figure 3.10 & 3.11). Along with ladder, the amplified products were run separately on agarose gel. Presence of a band of 545 bp was detected and compared with the marker (Figure 3.9, lane 2-6). The comparison of size of the product confirmed the amplification of CTX-M gene. A negative control was also run for the authenticity of amplification (lane 7). Likewise, the PCR amplification of TEM gene was also performed. Presence of a 247bp amplified product was on agarose gel confirmed the isolate's positivity for this gene (Figure 3.10, lane 2-7). The negative control was run as done previously.

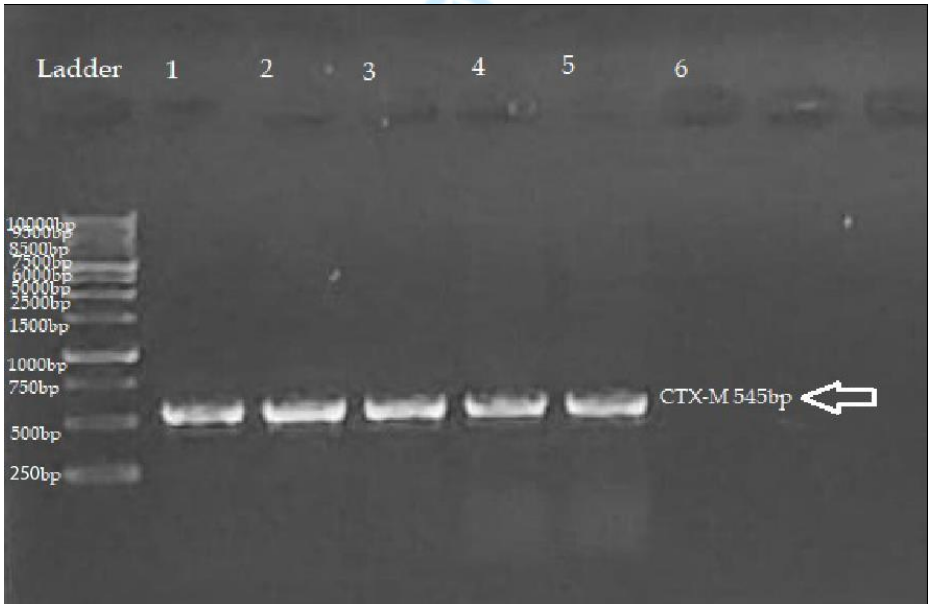

Figure 3.10: Amplification of CTX-M gene (545bp)

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Figure 3.11: Amplification of TEM gene (247bp)

TEM and CTX-M were found to be present in 47 percent and 38 percent of the population, respectively (Table 3.2). (33) discovered 171 *E. coli*, *K. pneumoniae*, and *E. cloacae* isolate from clinical samples, corroborating our findings. The isolates' ability to produce B-lactamases was examined. PCR amplification of the bla_{SHV}, bla_{TEM}, and bla_{CTX} genes revealed that a total of 53%, 81%, and 43% genes were amplified, respectively, using specific primers. The minor variations in these studies could be related to different sample collection times and conditions.

Table 3.2: Frequency of ESBL genes among all isolates from clinical samples (n=50)

Genes	No of +ive samples	No of –ive samples	%age of +ive samples	%age of –ive samples
CTX-M	19	31	38	62
TEM	23	27	47	53

According to another study, *Enterobacter cloacae* had the greatest fatality rate among *Enterobacter* infections. The goal of this study was to see how often the class I integron, qnr genes, and CTX-M ESBLs genes were in clinical isolates, as well as how they were transmitted. In the 100 clinical isolates tested, the class I integron positive rate was 65%. The positive rates for *qnrA*, *qnrB*, and *qnrS* were 6%, 23%, and 8%, respectively; CTX-M ESBLs had a 34% positive rate (34). In our study, we detected 38% of CTX-M ESBL-positive isolates. The slight difference in the prevalence may be due to different timings of samples collection and different geographic region.

4. CONCLUSION AND RECOMMENDATIONS

Bacterial resistance to beta-lactams, a common, safe, and effective antibiotic, is becoming a shocking problem. Because beta-lactamases are capable of inactivating most -lactam antibiotics containing monobactams and the 3rd and 4th generation of ESCs, they cause resistance in bacteria. ESBLs are generated mostly in gram-negative bacteria and are considered as the primary mechanism for cephalosporin resistance. Most ESBL-producers have been found to have multi-drug resistance (MDR), and even more astonishingly, co-resistance to other commonly used antibiotics such as aminoglycosides,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

four quinolones, and tetracycline has been identified. This demonstrates that these bacteria have limited treatment choices and are resistant to a wide spectrum of medications. Therefore, this study is designed to detect the ESBL resistance pattern of *Enterobacter cloacae* and the frequency of responsible genes in the tested clinical isolates. This work reports genes responsible for resistance against β- lactam antibiotics in *E. cloacae*. It was found that 47% of the isolates were able to produce ESBLs. CTX-M and TEM were detected in isolates of clinical samples. The rate of CTX-M and TEM was 38% and 47% respectively. It is concluded that humans may be a source of resistant bacteria that can easily spread to other individuals and the environment. An immediate response is essential, as well as education about the appropriate antimicrobial's usage in humans.

FUTURE RECOMMENDATION

- The *β*-lactam antibiotics are used commonly in clinics due to their higher effectiveness against gram negative bacteria and this ill use is leading to an increase in development of resistance against these antibiotics.
- Our research recommends the appropriate usage of antibiotics.
- Preventing bacteria with resistance genes from propagating is the need of time.
- Antibiotic use in dairy animals and humans should be decreased.

REFERENCES

1. Connell SR, Tracz DM, Nierhaus KH, Taylor DE. Ribosomal protection proteins and their mechanism of tetracycline resistance. Antimicrobial agents and chemotherapy. 2003;47(12):3675-81.
2. Organization WH. World antibiotic awareness week, 2017.
3. Selection WECot, Medicines UoE, Organization WH. The Selection and Use of Essential Medicines: Report of the WHO Expert Committee, 2013 (including the 18th WHO Model List of Essential Medicines and the 4th WHO Model List of Essential Medicines for Children): World Health Organization; 2014.
4. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiology and molecular biology reviews. 2010;74(3):417-33.
5. Normark BH, Normark S. Evolution and spread of antibiotic resistance. Journal of internal medicine. 2002;252(2):91-106.
6. Greenwood D, Finch R, Davey P. Antimicrobial chemotherapy: Oxford University Press; 2007.
7. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. The American journal of medicine. 2006;119(6):S3-S10.
8. Bartoloni A, Pallecchi L, Rodríguez H, Fernandez C, Mantella A, Bartalesi F, et al. Antibiotic resistance in a very remote Amazonas community. International journal of antimicrobial agents. 2009;33(2):125-9.
9. Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. Trends in microbiology. 2011;19(12):588-95.
10. Upadhyay S, Sen MR, Bhattacharjee A. Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta-lactamase enzyme. The Journal of Infection in Developing Countries. 2010;4(04):239-42.
11. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrobial agents and chemotherapy. 2010;54(3):969-76.
12. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrobial agents and chemotherapy. 2007;51(10):3471-84.
13. Chen H, Shu W, Chang X, Chen J-a, Guo Y, Tan Y. The profile of antibiotics resistance and integrons of extended-spectrum beta-lactamase producing thermotolerant coliforms isolated from the Yangtze River basin in Chongqing. Environmental pollution. 2010;158(7):2459-64.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

14. Timofte D, Maciuca IE, Evans NJ, Williams H, Wattret A, Fick JC, et al. Detection and molecular characterization of Escherichia coli CTX-M-15 and Klebsiella pneumoniae SHV-12 β-lactamases from bovine mastitis isolates in the United Kingdom. Antimicrobial agents and chemotherapy. 2014;58(2):789-94.
15. Xu G, An W, Wang H, Zhang X. Prevalence and characteristics of extended-spectrum β-lactamase genes in Escherichia coli isolated from piglets with post-weaning diarrhea in Heilongjiang province, China. Frontiers in microbiology. 2015;6:1103.
16. D'Andrea MM, Arena F, Pallecchi L, Rossolini GM. CTX-M-type β-lactamases: a successful story of antibiotic resistance. International Journal of Medical Microbiology. 2013;303(6-7):305-17.
17. Liu H, Wang Y, Wang G, Xing Q, Shao L, Dong X, et al. The prevalence of Escherichia coli strains with extended spectrum beta-lactamases isolated in China. Frontiers in microbiology. 2015;6:335.
18. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical microbiology reviews. 2001;14(4):933-51.
19. Afridi FI, Farooqi BJ, Hussain A. Frequency of extended spectrum beta lactamase producing enterobacteriaceae among urinary pathogen isolates. J Coll Physicians Surg Pak. 2011;21(12):741-4.
20. Mumtaz S, Ahmed J, Ali L, Hussain H. Prevalence of extended spectrum beta lactamases (ESBL) in clinical isolates from a teaching hospital in Peshawar, Pakistan. African Journal of Microbiology Research. 2011;5(19):2880-4.
21. Merchant I, Packer R. The genus corynebacterium. Veterinary Bacteriology and Virology The Iowa State University Press, IA, USA. 1967:425-40.
22. Shah A, Hasan F, Ahmed S, Hameed A. Extended-spectrum β-lactamases (ESBLs): Characterization, epidemiology and detection. Critical reviews in microbiology. 2004;30(1):25-32.
23. Al-Agamy MH, Shibl AM, Tawfik AF. Prevalence and molecular characterization of extended-spectrum β-lactamase-producing Klebsiella pneumoniae in Riyadh, Saudi Arabia. Annals of Saudi medicine. 2009;29(4):253-7.
24. Dalben M, Varkulja G, Basso M, Krebs V, Gibelli M, Van Der Heijden I, et al. Investigation of an outbreak of Enterobacter cloacae in a neonatal unit and review of the literature. Journal of Hospital Infection. 2008;70(1):7-14.
25. Streit JM, Jones RN, Sader HS, Fritsche TR. Assessment of pathogen occurrences and resistance profiles among infected patients in the intensive care unit: report from the SENTRY Antimicrobial Surveillance Program (North America, 2001). International journal of antimicrobial agents. 2004;24(2):111-8.
26. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infection Control & Hospital Epidemiology. 2008;29(11):996-1011.
27. Paauw A, Caspers MP, Schuren FH, Leverstein-van Hall MA, Delétoile A, Montijn RC, et al. Genomic diversity within the Enterobacter cloacae complex. PLoS one. 2008;3(8):e3018.
28. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clinical infectious diseases. 2004;39(3):309-17.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

29. Morand PC, Billoet A, Rottman M, Sivadon-Tardy V, Eyrolle L, Jeanne L, et al. Specific distribution within the Enterobacter cloacae complex of strains isolated from infected orthopedic implants. Journal of clinical microbiology. 2009;47(8):2489-95.
30. Wang G-F, Xie G-L, Zhu B, Huang J-S, Liu B, Kawicha P, et al. Identification and characterization of the Enterobacter complex causing mulberry (Morus alba) wilt disease in China. European journal of plant pathology. 2010;126(4):465-78.
31. Peymani A, Farivar TN, Sanikhani R, Javadi A, Najafipour R. Emergence of TEM, SHV, and CTX-M-extended spectrum β-lactamases and class 1 integron among Enterobacter cloacae isolates collected from hospitals of Tehran and Qazvin, Iran. Microbial Drug Resistance. 2014;20(5):424-30.
32. Chang C-L, Su L-H, Lu C-M, Tai F-T, Huang Y-C, Chang K-K. Outbreak of ertapenem-resistant Enterobacter cloacae urinary tract infections due to a contaminated ureteroscope. Journal of Hospital Infection. 2013;85(2):118-24.
33. Chang C, Wang Y-F, Kanamori Y, Shih J-J, Kawai Y, Lee C-K, et al. Etching submicrometer trenches by using the Bosch process and its application to the fabrication of antireflection structures. Journal of micromechanics and microengineering. 2005;15(3):580.
34. Leski TA, Taitt CR, Bangura U, Stockelman MG, Ansumana R, Cooper WH, et al. High prevalence of multidrug resistant Enterobacteriaceae isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone. BMC infectious diseases. 2016;16(1):1-9.