Molecular Identification of CTX-M-9 and PER gene in Extended Spectrum Beta lactamase (ESBL) Producing Bacteria from Clinical Isolates

Alina Maharjan[1*], Laxmi Sen[2], Anil Kumar Shah[2] and Rajani Shrestha[1]

[1] St. Xavier's College, Maitighar, Kathmandu, Nepal.

[2] Annapurna Neurological Institute and Allied Sciences, Kathmandu, Nepal

*corresponding author

IJSER

IJSER © 2021
http://www.ijser.org

Abstract

Introduction: Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. ESBL has spread threat in many regions of the world. This study is carried out to detect plasmid-mediated *bla*$_{\text{CTXM-9}}$ and *bla*$_{\text{PER}}$ genes among the ESBL producing bacteria from different clinical specimens. **Methods:** A total of 397 clinical samples (urine, sputum, blood, pus, wound swab, tracheal aspirates, catheter tips, IVP tips) were collected and ESBL producers were screened. It was confirmed using the modified Kirby Bauer disc diffusion according to CLSI guidelines. Plasmids extracted from the confirmed ESBL positives were the template for PCR. *bla*$_{\text{CTXM-9}}$ and *bla*$_{\text{PER}}$ genes were amplified using specific primers of respective genes by uniplex PCR. The presence of these genes was confirmed by gel electrophoresis. **Results:** Out of the total 397 samples, 243 samples showed positive culture results, and 98 isolates were found to be ESBL producers. Of confirmed 98 ESBL producing isolates *bla*$_{CTX\text{-}M\text{-}9}$ gene was most predominantly found in *E. coli* (33.33%) followed by *Enterobacter* spp. (25%). Similarly, *bla*$_{PER}$ gene was most predominantly found in *Acinetobacter* spp. (81.81%) followed by *E. coli* (50%). **Conclusion:** ESBL *bla*$_{\text{CTX-M-9}}$ and *bla*$_{\text{PER}}$ genes are predominantly present in a tertiary care hospital in Kathmandu, Nepal.

Keywords: ESBLs, *bla*$_{\text{CTX-M-9}}$, *bla*$_{\text{PER}}$, PCR, Gel electrophoresis.

IJSER © 2021
http://www.ijser.org

1. Introduction

The world is facing a serious problem with an antibiotic-resistant microorganism that has created a great problem in health sectors. There's an urgent need to investigate various treatment choices when there are still some antibiotics left. New resistance mechanisms emerge and unfold globally threatening the ability to treat common infectious diseases, leading to death and incapacity of people. Antibiotic resistance has become a pressing issue these days, each globally and in the Asian country. Totally different microorganisms have expressed different mechanisms with an associate expression of resistance factor. Extended-spectrum beta-lactamases (ESBLs) mediate resistance to antibiotics has become a widespread problem[1].

Beta-lactams are the most used antibiotics in clinical settings for the treatment of varied infections due to their bactericidal action, low toxicity, and various spectrums. Beta-Lactam antibiotics significantly, cephalosporins are the foremost prescribed antibiotics. The employment of oxyimino-cephalosporins like ceftazidime and ceftriaxone has resulted within the choice of β-lactamase producing isolates that hydrolyze the new cephalosporins[2]. ESBLs are a heterogeneous cluster of plasmid-mediated microorganism enzymes. The production of β-lactamases is the predominant explanation for resistance to β-lactam antibiotics in the gram-negative microorganisms and is responsible for mediating resistance to extended-spectrum cephalosporins and monobactam, aztreonam[3]. These enzymes cleave the amide bond in the β-lactam ring, rendering β-lactam antibiotics harmless to bacteria. Several genera of gram-negative bacteria possess a naturally occurring, chromosomally mediate β-lactamase[4]. At intervals some years when its initial isolation, β-lactamase enzymes had spread worldwide and are currently found in many alternative species of the members of the Enterobacteriaceae family as well as *Pseudomonas aeruginosa, Acinetobacter spp*[5].

Long hospitalization, diabetes, age over 60 and former antibiotic treatment patients have been reported as the risk factors to acquire infections with ESBL strains[6]. The results show that substitution cephalosporins with antibiotic-containing β- lactamase inhibitors (piperacillin-tazobactam) might facilitate to reduce the

IJSER © 2021
http://www.ijser.org

occurrence of ESBLs producing organisms[3]. It has been worrying that microorganism that harbors multiple β-lactamase genes are being reported with increasing frequency and described the occurrence of Sulfhydryl variable extended spectrum cephalosporinase (SHV), Pseudomonas Extended Resistance (PER) and Ceftriaxone hydrolyzing extended spectrum beta-lactamase (CTX-M) genes in ESBLs produced by single strains[7]. These enzymes can be chromosomal or plasmid- mediated[8]. Intense pressure by patients to use PER-1 antimicrobial drugs has resulted in the eradication of normal flora and substitution with MDR isolates[9].

In a developing country like Nepal, where there is rapid irrational use of antibiotics, the main reason is that antibiotics can be obtained and used without medical authorization which leads to an increase in antimicrobial resistance[10]. Many studies have been performed in the field of ESBL in Nepal, but the studies associated with the molecular characterization of ESBL genes like CTX-M-9 and PER in Nepal have solely restricted studied. There is a rapid need to study the occurrence of major types of genes causing the widespread ESBL that explains the epidemiological features of ESBL producing microorganisms. The spread of ESBL-producing bacteria has been spreading rapidly worldwide; indicate that continuous monitoring systems and effective infection control measures are absolutely required. Also, therapeutic options for infections due to ESBL producers have become increasingly limited. Therefore, molecular detection and identification of beta- lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. This study mainly focuses on the identification of ESBL producing bacteria from patients' sample and detection of CTX-M-9 and PER gene by using molecular techniques. It may also play a significant role in the treatment of patients with ESBL infection.

IJSER

2. Material and Methods

2.1 Specimen collection and Identification of Organism

IJSER © 2021
http://www.ijser.org

A cross-sectional study was carried out in Annapurna Neurological Institute and Allied Science Hospital (ANIASH) and Annapurna Research Center, Maitighar, Kathmandu, Nepal. A total of 397 samples were processed out. Different samples include urine, sputum, CSF, blood, pus, swab, catheter tip, CPV, tracheal aspirates were collected from the general ward, Intensive care unit (ICU), outpatient department, and gynecology (Gyneo) ward of ANIASH. All the samples were performed in the microbiology laboratory and cultured by standard microbiological techniques. The organism was identified by Gram staining, cultural characteristics in MacConkey agar (Hi media, India) and Blood agar (Hi media, India), in addition to these urine samples was also subculture on the CUTI agar (Hi media, India) and biochemical tests such as IMViC, catalase test, oxidase test, urease test and oxidative/fermentative test (HiMedia, India). The ethical approval for the study was obtained from Nepal health research council (NHRC). The written and signed inform consents were received from the patients and the guardian of younger patients.

IJSER

2.2 Antibiotic Susceptibility Tests

Antibiotic susceptibility testing (AST) was performed using the disc diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI 2014) using the Kirby-Bauer method. Antibiotics tested were Ceftriaxone (30 μg), ceftazidime (30 μg), nalidixic acid (30 μg), amoxy-clavulanic acid (30 μg), ampicillin (25 μg), co-trimoxazole (25 μg), cefotoxime (30 μg), imipenem (30 μg), gentamicin (30 μg), amikacin(30 μg), cefepime (30 μg), aztreonam (30 μg), nitrofurantoin (300 μg) and piperacillin/tazobactam (100/10 μg) (HiMedia, India).

2.3 Screening and Confirmation of ESBL

The screening was done by disc diffusion method using cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), aztreonam (30 μg). For confirmation, combined disc test was performed using cefotaxime (30 μg) and ceftazidime (30 μg) alone, and cefotaxime + clavulanic acid (20μg/10 μg) and ceftazidime + clavulanic acid (20μg/10 μg).

IJSER © 2021
http://www.ijser.org

2.4 Plasmid DNA extraction

Confirmed ESBL producers, was preserved on Tryptic soya broth for the plasmids extraction process. The plasmid was extracted by using standard alkaline hydrolysis method[11]. These plasmids served as the template for PCR. PCR amplification was carried out using $bla_{\text{CTX-M-9}}$, bla_{PER} specific primers (Macrogen, Korea). Finally, the plasmid preparation was stored at -20°C.

2.5 Amplification of genes by Polymerase Chain Reaction (PCR)

The reaction mixture for the PCR was prepared as blank, positive control and sample. All these mixtures are added on individual amplification tube to makes the total volume of 25 µl. Amplification reaction was carried out under the following thermal cycling conditions:

2.5.1 Condition for CTX-M-9 gene:

Initial denaturation: 94°C for 10 minute, Denaturation: 94°C for 40 second, Annealing: 60°C for 40 second, Extension: 72°C for 1 minute, Final extension: 72°C for 7-10 minute. CTX-M-9 F was 5'TCAAGCCTGCCGATCTGGT3' and CTX-M-9 R was 5'TGATTCTCGCCGCTGAAG3' with the amplicon of 561bp.

2.5.2 Condition for PER gene:

Initial denaturation: 94°C for 10 minute, Denaturation: 94°C for 40 second, Annealing: 57°C for 40 second, Extension: 72°C for 1 minute, Final extension: 72°C for 7-10 minute. PER F was 5'GCTCCGATAATGAAAGCGT3' and PER R was 5'TTCGGCTTGACTCGGCTGA3' with the amplicon of 520bp.

2.6 Detection of PCR Products by Gel Electrophoresis

The amplified products were characterized by performing gel electrophoresis with 1.5 % agarose gel made in w/v tris-acetate-EDTA. 0.5 µl of Ethidium bromide was used in the gel as a tracking dye. The gel was allowed to solidify on the plastic cast with a comb. After proper solidification, the wells on the gel were loaded with 1

IJSER

IJSER © 2021
http://www.ijser.org

µl of 100bp DNA ladder, 3 µl of positive control and 3 µl negative control/ blank and 3 µl of PCR amplicons respectively.

2.7 Controls

The optimization Positive control of *E. coli, Klebsiella* spp., *Pseudomonas* spp., *Citrobacter* spp., *Enterobacter* spp., *Acinetobacter* spp., isolates carrying $bla_{CTX\text{-}M\text{-}9}$ gene and bla_{PER} gene, were used for PCR. Also, blank or negative controls were prepared without the application of any DNA (plasmid).

2.8 Statistical analysis

All the results were entered into the worksheet of Statistical Package for Social Science (SPSS) version 16 software package. The chi-square test was used to check the significance level among the parameters. The P-value <0.05 was assumed to be significant for the analyses.

IJSER

3. RESULTS

3.1 Distribution of Bacterial Isolates

Among 243 (61%, 243/397) bacterial isolates obtained from different clinical samples, *E. coli* were predominant isolates with a percentage of (23.05%, 56/243) followed by *Pseudomonas* spp. (12.35%, 29/243), *Klebsiella* spp. (18.93%, 46/243), *Citrobacter* spp. (9.05%, 22/243), *Enterobacter* spp. (3.29%, 8/243), *Acinetobacter* spp. (11.11%, 27/243) (Table1). Out of 243 positive samples, the most samples were obtained from the general ward 118 (49%) followed by the ICU ward 63 (26%), OPD 47 (18%), and Gyno ward 15 (7%). In case of different wards, *E. coli* (23.72%) was most predominant followed by *Klebsiella* spp. (20.33%) and *Acinetobacter* spp. (12.71%) in general ward. From ICU ward *Klebsiella* spp. (19.04%) and *Pseudomonas spp.* (17.46%) were mostly found. Similarly, from OPD *E. coli* (38.29%) and from Gyno ward *Acinetobacter* spp. (26.67%) were mostly found. Regarding gender distribution (51.44%, 125/243) were male and (48.55%, 118/243) were female (Table 1).

IJSER © 2021
http://www.ijser.org

3.2 Antimicrobial Susceptibility Pattern of bacterial isolates

Out of 243 bacterial isolates, 56 *E. coli,* 46 *Klebsiella* spp., 30 *Pseudomonas* spp., 22 *Citrobacter* spp., 8 *Enterobacter* spp. and 27 *Acinetobacter* spp. were processed for antibiotic susceptibility tests. Among 189, (13.75%, 26/189) were resistance to amikacin, followed by (5.82%, 11/189) Piperacillin/Tazobactam, (18.51%, 35/189) Ofloxacin, (12.16%, 23/189) Gentamicin, (48.67%, 92/189) Ceftazidime, (47.97%, 90/189) Cefotaxime, (44.97%, 85/189) Ceftriaxone, (1.58%, 3/189) Cefepime, and (1.1%, 2/189) Imipenem. (34.5%, 29/84) of the urinary isolates showed resistance towards nitrofurantoin in the case of a urine sample and catheter tip (Figure 1).

3.3 Prevalence of ESBL Positive Bacteria

Of 189 bacterial isolates obtained from clinical samples, (51.85%, 98/189) isolates were ESBL positive; the highest proportion of ESBL was found in *Acinetobacter* spp. (81.48%, 22/27) followed by *Pseudomonas* spp. (60%, 18/30), ESBL- *Klebsiella* spp. (54.34%, 25/46) and (42.85%, 24/56) ESBL-*E. coli* (Figure 2).

IJSER

3.4 MDR Profile in Bacterial isolates

Among total of 189 isolates, (15.87%, 30/189) were MDR; the highest proportion of MDR was found in *E. coli* (30%, 9/30), followed by *Klebsiella* spp. (23%, 7/30) and *Citrobacter* spp. (23%, 7/30) (Figure 3).

3.5 Prevalence of ESBL Isolates According to Age and Hospital Units

Of 189 bacterial isolates obtained from clinical samples, (51.85 %, 98/189) isolates were ESBL positive. Among these 98 ESBL bacterial isolates, (24.48%, 24/98) were *E. coli,* (18.37%, 18/98) *Pseudomonas* spp., (25.51%, 25/98) *Klebsiella* spp., (5.1%, 5/98) *Citrobacter* spp., (4.08%, 4/98) *Enterobacter* spp. and (22.44%, 22/98) *Acinetobacter* spp. Among 98 ESBL bacteria were isolated from the General ward (42.86%, 42/98) followed by ICU ward (32.65%, 32/98). The highest percentage of ESBL producing bacteria was isolated from the patients in the age group 50-70 years (43.87%, 43/98) followed by the age group 30-50 years (30.61%, 30/98) (Table 2).

IJSER © 2021
http://www.ijser.org

3.6 Prevalence of *bla CTXM-9* and *PER genes* in ESBL producers

All phenotypically confirmed 98 ESBL producing isolates were subjected to PCR for molecular detection of $bla_{CTX\text{-}M\text{-}9}$ gene and bla_{PER} gene. Among the 98 ESBL producing isolates (14.28%, 14/98) had $bla_{CTX\text{-}M\text{-}9}$ gene and (46.93%, 46/98) had bla_{PER} gene detected by PCR (Table 2, Figure 4). $Bla_{CTX\text{-}M\text{-}9}$ gene was most predominantly found in *E. coli* (57.14%, 8/14) followed by *Pseudomonas* spp. (21.42%, 3/14), and *Klebsiella* spp. (14.29%, 2/14). However, $bla_{CTX\text{-}M\text{-}9}$ gene was absent in *Citrobacter* spp. and *Acinetobacter* spp. In the case of hospital units $bla_{CTX\text{-}M\text{-}9}$ gene was found mostly in general and OPD ward (35.71%, 5/14) and in the age group between 50-70 years (64.29%, 9/14) (Table 2). Similarly, bla_{PER} gene was most predominantly found in *Acinetobacter* spp. (39.13%, 18/46) followed by *E. coli* (26.08%, 12/46), *Klebsiella* spp. (19.56% 9/46), *Pseudomonas* spp. (15.21%, 7/46). In the case of hospital units, bla_{PER} gene was found mostly in the general ward (47.83%, 22/46) and in the age group between 50-70 years (39.13%, 18/46) (Table 2).

IJSER

4. Discussion

This study provides essential information relating to the distribution of extended- spectrum beta-lactamases (ESBL) producing clinical isolates and their major sorts (CTX-M-9 and PER gene) among patients visiting Annapurna Neurological Institute and Allied Sciences, Maitighar, Kathmandu. The aim of the study was to observe numerous ESBL expressing genes in ESBL producing clinical isolates. Beta-lactamase mainly extended-spectrum cephalosporins and carbapenems represent the main therapeutic choice to treat infections caused by family Enterobacteriaceae. During this study, different clinical specimens were collected from different age groups and subjected to the process for culture and sensitivity tests.

In this study, high rate of sample growth (61%) was reported which is in accordance with the study reported by Pokhrel *et.al* 2014. Out of total bacteria, *E. coli* (23.05%, 56/243) were found in the highest number in the urine sample and most of the pathogenic isolates were isolated from a urine sample. Urine has been

IJSER © 2021
http://www.ijser.org

considered a good growth medium for *E. coli*, as it contains a variety of inorganic salts and organic compounds and is regularly replenished by fresh urine production[12]. *E. coli* was found to be most common in patients suffering from UTI. The Flow rate of Urinary Tract Infection (UTI) patients were high during the study period. Further, *Klebsiella spp, Acinetobacter spp* were also a common cause of UTI. Other studies from Nepal and other parts of the world have also reported *E. coli* as the common urinary isolates[13,14].

The single tracheal aspirate sample was culture-positive from which *E. coli* was isolated. In our study, *E. coli* and *Klebsiella* spp. were also found in Foley's tip and catheter tip. Maximum colonization as well as definite bacteremia took place after the catheter left in situ for more than 72 hours[15].

Urinary isolates (isolated from urine and catheter tips) were exposed to nitrofurantoin. Nitrofurantoin is most commonly used in treating UTI than other sites as it has better pharmacokinetics[16]. 34.5% of the urinary isolates showed resistance towards nitrofurantoin. Higher number of bacteria were resistant towards third generation cephalosporins as well (ceftazidime (CAZ) = 48.67%, cefotaxime (CTX) = 47.97%, ceftriaxone (CTR) = 44.97%). Bacterial isolates also showed resistance towards another group of antibiotics such as ofloxacin, gentamicin, amikacin, piperacillin/tazobactam, imipenem, and cefepime. This is similar to reports of different other studies[10,17,18]. Identifying ESBL producing organisms is a major challenge in clinical settings. Due to the selective pressure caused by heavy use of extended spectrum cephalosporins, lapses ineffective infection control measures, and affinity of these enzymes for different substrates, outbreaks are increasing. The prevalence of ESBL producing Enterobacteriaceae varies greatly among community settings and hospital settings within the country[19].

MDR is a global issue; their impacts vary among different countries, with a relatively high burden in developing countries and are often difficult to treat and are associated with a nosocomial origin[20]. In this study, 15.87% of the isolates were MDR; the highest proportion of MDR was found in *Citrobacter* spp.

IJSER

followed by *E. coli* and *Klebsiella* spp. A high prevalence of bacteria with MDR has been reported frequently in other studies conducted in Nepal and in world[12].

A Molecular screening was done by PCR using gene-specific primers genes. All 98 ESBL positive isolates were performed to detect *bla* genes. This gene was detected in numerous *E. coli, Pseudomonas* spp*.,* *Klebsiella* spp.*,* and *Enterobacter* spp*.* Among 98 ESBL producing isolates, 14.28% carried $bla_{\text{CTX-M-9}}$ gene. This value is comparatively higher than previously stated values (10%)[21]. *The* $bla_{CTX\text{-}M\text{-}9}$ gene was most predominantly found in *E. coli* followed by *Pseudomonas* spp. However, $bla_{CTX\text{-}M\text{-}9}$ gene was absent in *Citrobacter* spp. and *Acinetobacter* spp. The wider spread of $bla_{CTX\text{-}M\text{-}9}$ is also due to the overuse of the third generation cephalosporins. However, the location of $bla_{CTX\text{-}M\text{-}9}$ gene was not studied and needs further investigation.

In the present study, bla_{PER} gene was most predominantly found in *Acinetobacter* spp. (39.13%,) *Acinetobacter* strains, particularly *A. baumannii*, have emerged as multiply resistant nosocomial isolates, and *Acinetobacter* strains remained susceptible in variable degrees to some β-lactams and β-lactam–sulbactam combinations[22]. Amirkamali *et al* 2017 reported that 17% of ESBL producing *P. aeruginosa* isolates carried the bla_{PER} gene[23]. Thus, these studies also revealed that molecular detection is more sensitive and specific than phenotypic screening.

Early detection of those sorts of resistance genes like CTX-M-9 and PER would be a useful tool for the identification of infection thereby helping in controlling and prevention their spread. Thus, it is vital for every laboratory to become alert about the infection which may be a threat leading to the epidemic condition if not treated and controlled at the time. Therefore, phenotypic, and genotypic identification techniques should be performed for the specific diagnosis of diseases.

Although every factor was included as far as concerned about the study, few limitations are found in the study. The study was limited due to a short duration of time framework which not only insisted to limit the

IJSER © 2021
http://www.ijser.org

IJSER

sample size but also reduced the ability of proper analysis of methodology. The small sample size may not have represented the whole population while the seasonal trend may also have affected the result. Hence, the relationship shown among different factors on the study may not be conclusive thus needing further proper evaluation.

5. Conclusion

It gives fair data regarding the increased predominance of the major ESBL bla_{CTXM-9} and bla_{PER} gene types in a tertiary care hospital in Kathmandu. *E. coli, Pseudomonas* spp., *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp. *Acinetobacter* spp. were found as ESBL producer gram negative bacteria in clinical samples which were found most effective towards third generation cephalosporins. In this study, amikacin, ofloxacin, and gentamicin were found to be the most effective drugs for the treatment of the ESBL infection. More than 25% of those ESBL producer bacteria harbor $bla_{CTX-M-9}$ genes whereas 39.13% of *Acinetobacter* species and 26.08% *E. coli* carried bla_{PER} gene. Thus, it requires the regular monitoring and surveillance of antibiotic resistance and the gene encoding them across Nepal to identify better treatment options and de-escalate the use of antibiotics to reduce the chance of spreading antibiotic-resistant organisms and to preserve the antibiotics for their efficient use in the future generation.

Acknowledgments

We would like to acknowledge NHRC, Kathmandu, Nepal for providing us with ethical approval. We would like to thank the Department of Microbiology, St. Xavier's College, Maitighar and Annapurna Neurological Institute & Allied Sciences, Hospital, and Research center for providing us the chance to accomplish the research work. We would like to extend our deepest gratitude to all the participant patients and their guardians

IJSER © 2021
http://www.ijser.org

without whom this dissertation would never have been completed and to teachers and family for supporting and encouraging our spirit of developing confidence in us and always being there for us.

Disclosure

All the authors declare that they have no competing interests.

Funding

Not Funding

Consent for publication

Not applicable

Availability of data and materials

On request to corresponding author

Data Sharing Statement

All data pertaining to this study are presented in the manuscript.

IJSER

Ethics Approval and Consent to Participate

The ethical approval was taken from the Ethical Review. Board of Nepal Health Research Council (NHRC), Kathmandu, Nepal (Reg. no. 316/2018).

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

References

IJSER © 2021
http://www.ijser.org

1. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi journal of biological sciences.* 2015;22(1):90-101.
2. Shahcheraghi F, Nikbin V-S, Feizabadi MM. Prevalence of ESBLs genes among multidrug-resistant isolates of Pseudomonas aeruginosa isolated from patients in Tehran. *Microbial Drug Resistance.* 2009;15(1):37-39.
3. Rawat D, Nair D. Extended-spectrum β-lactamases in Gram Negative Bacteria. *Journal of global infectious diseases.* 2010;2(3):263.
4. Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrobial agents and chemotherapy.* 2004;48(1):1-14.
5. Islam SS, Malek MA, Haque AF, Talukder KA, Akhter MZ. Beta lactamase genes of extended spectrum beta lactamase producing Escherichia coli from anorectal sepsis cases in Bangladesh. *Bangladesh Journal of Microbiology.* 2013;30(1-2):23-29.
6. Nasehi L, SHAH CF, Nikbin V, Nematzadeh S. PER, CTX-M, TEM and SHV Beta-lactamases in clinical isolates of Klebsiella pneumoniae isolated from Tehran, Iran. 2010.
7. Chanawong A, M'Zali FH, Heritage J, Xiong J-H, Hawkey PM. Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among Enterobacteriaceae in the People's Republic of China. *Antimicrobial agents and chemotherapy.* 2002;46(3):630-637.
8. Ahmed OI, El-Hady SA, Ahmed TM, Ahmed IZ. Detection of bla SHV and bla CTX-M genes in ESBL producing Klebsiella pneumoniae isolated from Egyptian patients with suspected nosocomial infections. *Egyptian Journal of Medical Human Genetics.* 2013;14(3):277–283-277–283.
9. Fazeli H, Kamali Dolatabadi R, Taraghian A, Nasr Isfahani B, Moghim S. Genetic Characterization of blaSHV/VEB/PER Genes in ESBL-producing MDR Klebsiella Pneumonia Strains Isolated from

IJSER © 2021
http://www.ijser.org

Patients in Isfahan, Iran. *European Online Journal of Natural and Social Sciences.* 2015;4(1):pp. 191-202.

10. Pokhrel RH, Thapa B, Kafle R, Shah PK, Tribuddharat C. Co-existence of beta-lactamases in clinical isolates of Escherichia coli from Kathmandu, Nepal. *BMC Research Notes.* 2014;7(1):694.
11. He F. Plasmid DNA extraction from E. coli using alkaline lysis method. *Biology Protocol.* 2011;30:1-3.
12. Haugan MS, Hertz FB, Charbon G, Sahin B, Løbner-Olesen A, Frimodt-Møller N. Growth Rate of Escherichia coli During Human Urinary Tract Infection: Implications for Antibiotic Effect. *Antibiotics.* 2019;8(3):92.
13. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, Shrestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC Research notes.* 2012;5(1):38.
14. Chander A, Shrestha CD. Prevalence of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae urinary isolates in a tertiary care hospital in Kathmandu, Nepal. *BMC Research notes.* 2013;6(1):487.
15. Deep A, Ghildiyal R, Kandian S, Shinkre N. Clinical and microbiological profile of nosocomial infections in the pediatric intensive care unit (PICU). *Indian Pediatr.* 2004;41(12):1238-1246.
16. Munoz-Davila MJ. Role of old antibiotics in the era of antibiotic resistance. Highlighted nitrofurantoin for the treatment of lower urinary tract infections. *Antibiotics.* 2014;3(1):39-48.
17. Andrew B, Kagirita A, Bazira J. Prevalence of extended-spectrum beta-lactamases-producing microorganisms in patients admitted at KRRH, Southwestern Uganda. *International journal of microbiology.* 2017;2017.
18. Nepal K, Pant ND, Neupane B, et al. Extended spectrum beta-lactamase and metallo beta-lactamase production among Escherichia coli and Klebsiella pneumoniae isolated from different clinical samples

IJSER

in a tertiary care hospital in Kathmandu, Nepal. *Annals of clinical microbiology and antimicrobials.* 2017;16(1):62.

19. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL production among E. coli and Klebsiella spp. causing urinary tract infection: a hospital based study. *The open microbiology journal.* 2017;11:23.

20. Shrestha B, Sah AK, Thakuri LS, et al. Detection Of Kpc And Oxa-48 Gene In Clinical Isolates Of Carbapenem Resistant Klebsiella Pneumoniae. 2019.

21. Chanawong A, Lulitanond A, Kaewkes W, Lulitanond V, Srigulbutr S, Homchampa P. CTX-M extended-spectrum beta-lactamases among clinical isolates of Enterobacteriaceae in a Thai university hospital. *Southeast Asian journal of tropical medicine and public health.* 2007;38(3):493.

22. Vahaboglu H, Oztürk R, Aygün G, et al. Widespread detection of PER-1-type extended-spectrum beta-lactamases among nosocomial Acinetobacter and Pseudomonas aeruginosa isolates in Turkey: a nationwide multicenter study. *Antimicrobial agents and chemotherapy.* 1997;41(10):2265-2269.

23. 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. *Revista da Sociedade Brasileira de Medicina Tropical.* 2017;50(3):315-320.

IJSER

Figure Legends

Figure 1 Antimicrobial susceptibility test. The graph representing the antibiotic resistance to different class of antibiotics.

Figure 2. Prevalence of ESBL positive bacteria. Bacterial isolates obtained from clinical samples that are ESBL producers.,

Figure 3. MDR profile in bacterial isolates. Proportion of MDR bacteria isolates obtained f4om the clinical samples.

Figure 4. Gel electrophoresis of PCR amplicons. (A) $bla_{CTX\text{-}M\text{-}9}$ gene **(B)** bla_{PER} gene (1: Ladder, 2: Blank, 3: Positive control, 4-8: Samples)

IJSER

Tables

Table 1: Bacterial isolates obtained from different clinical samples

Species	*E. coli* (%)	*Klebsiella* spp. (%)	*Pseudomonas* spp. (%)	*Citrobacter* spp. (%)	*Enterobacter* spp. (%)	*Acinetobacter* spp. (%)	Others (%)
Blood	6 (10.71)	3(6.52)	5(16.67)	2(9.09)	1(12.5)	7(25.93)	8(14.81)
Urine	26(46.42)	22(47.82)	12(40)	12(54.54)	0(0)	11(40.7)	28(51.85)
Sputum	10(17.85)	16(34.78)	9(30)	6(27.27)	2(25)	6(22.22)	10(18.51)
CSF	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Swab	2(3.57)	2(4.34)	1(3.33)	0(0)	1(12.5)	0(0)	3(5.55)
CVP Tips	3(5.35)	1(2.17)	2(6.67)	0(0)	1(12.5)	1(3.7)	1(1.85)

IJSER © 2021
http://www.ijser.org

Pus	2(3.57)	0(0)	1(3.33)	1(4.54)	2(25)	1(3.7)	2(3.7)
Tracheal Aspirates	1(1.78)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Foley's Tip	1(1.78)	1(2.17)	0(0)	0(0)	0(0)	0(0)	1(1.85)
Catheter Tips	5(8.93)	1(2.17)	0(0)	1(4.54)	1(12.5)	1(3.7)	1(1.85)
Total No.	**56(23.04)**	**46(18.93)**	**30(12.34)**	**22(9.05)**	**8(3.29)**	**27(11.11)**	**54(22.22)**

IJSER

Table 2: Prevalence of ESBL, CTX-M-9 and PER gene in clinical isolates, hospital units and age groups

Characteristics	ESBL (%)	CTX-M-9(%)	PER (%)
Bacterial Isolates			
E. coli (n=56)	24 (24.48)	8(57.14)	12(26.08)
Pseudomonas spp.(n=30)	18 (18.37)	3(21.42)	7(15.22)
Klebsiella spp. (n=46)	25(25.51)	2(14.29)	9(19.57)
Citrobacter spp. (n=22)	5 (5.1)	0(0)	0(0)
Enterobacter spp. (n=8)	4 (4.08)	1(7.14)	0(0)
Acinetobacter spp.(n=27)	22 (22.44)	0(0)	18(39.13)
p-value	p>0.05	P=0.0246 P<0.05	P=0.0010 P<0.05
Hospital Units			
General (n=116)	42(42.86)	5 (35.71)	22 (47.83)
ICU (n=63)	32 (32.65)	3(21.43)	7 (15.22)
OPD (n=49)	16 (16.32)	5 (35.71)	13 (28.26)

IJSER © 2021
http://www.ijser.org

Gynecology (n=15) p-value	8 (8.16) P=0.1145 p>0.05	1 (7.14) P=0.9136 p>0.05	4 (8.70) P=0.77537 p>0.05
Age Groups (years) 10-30 (n=33) 30-50 (n=88) 50-70 (n=93) 70-90 (n=29) p-value	 17 (17.34) 30 (30.61) 43 (43.87) 8 (8.16) P=0.091 p>0.05	 2 (14.28) 3 (21.43) 9 (64.29) 0 (0) P=0.6322 p>0.05	 12 (26.09) 13 (28.26) 18 (39.13) 3 (6.52) P=0.19818 p>0.05

IJSER

Figures

Figure 1

IJSER © 2021
http://www.ijser.org

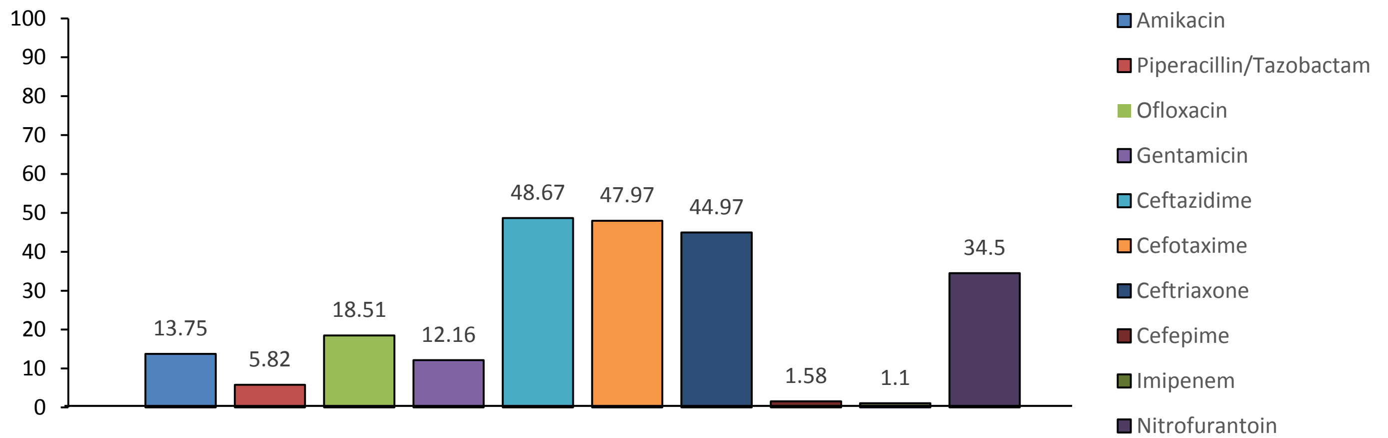


Figure 2

IJSER

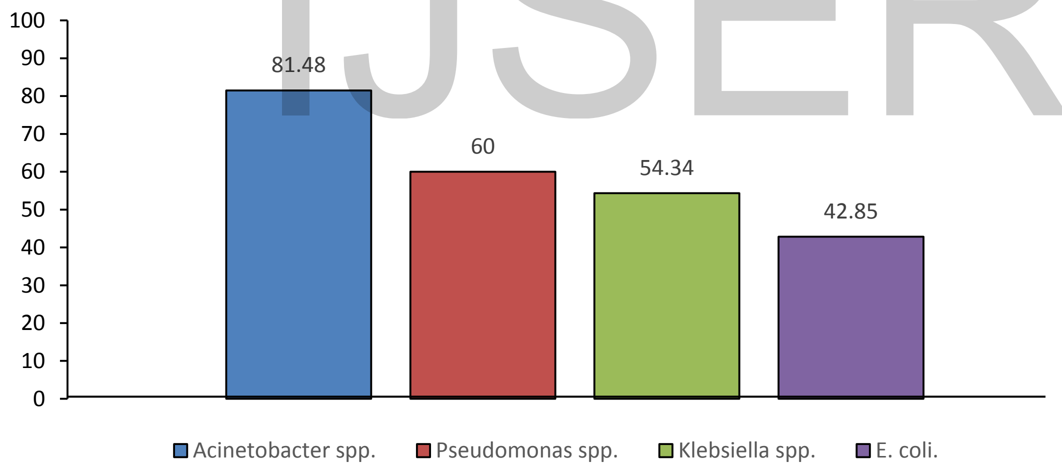


Figure 3

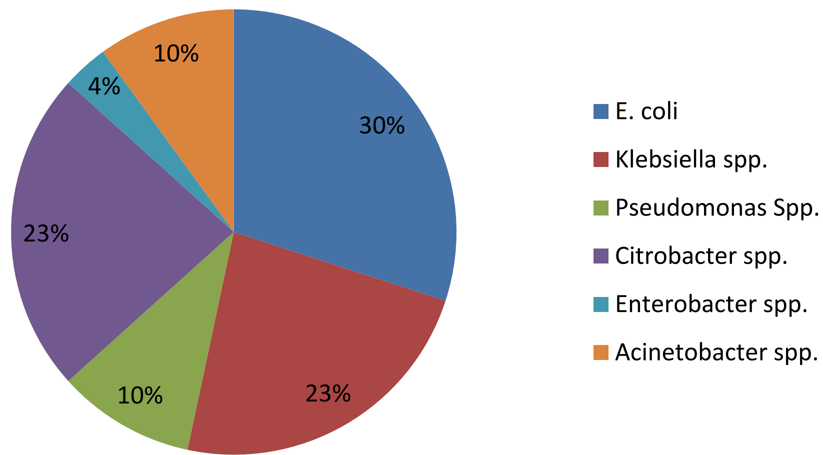
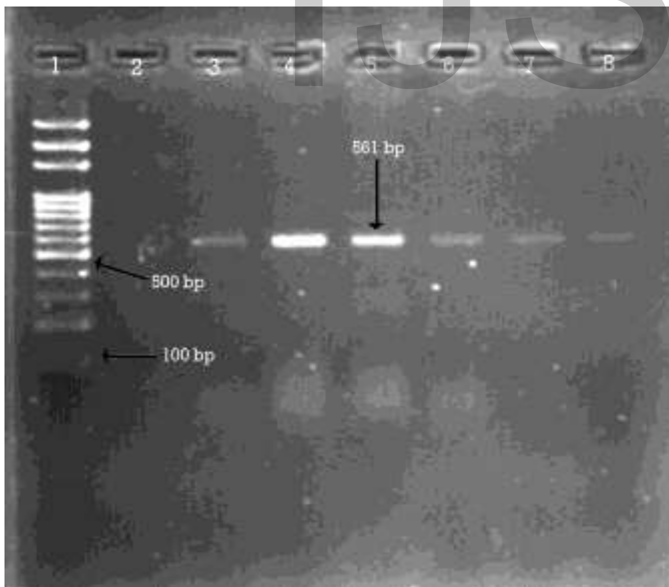


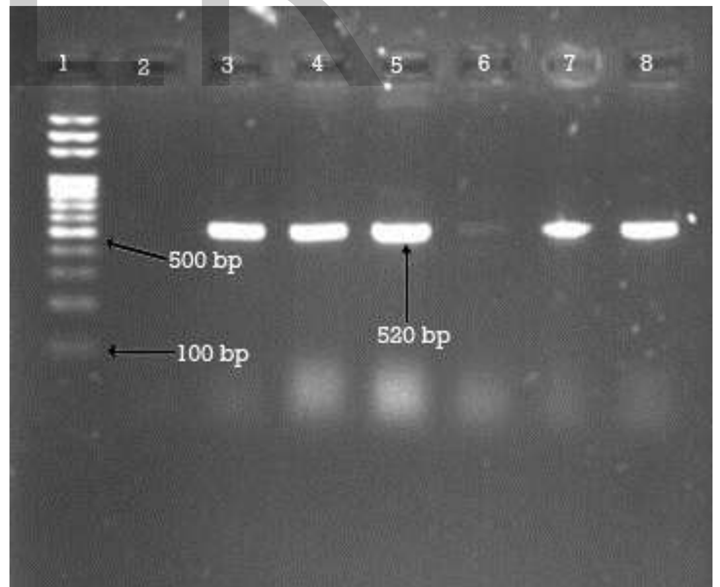
Figure 4

IJSER

(A)



(B)



IJSER © 2021
http://www.ijser.org