Frequency and Impact of Extended-Spectrum ß-Lactamase (ESBL) Isolates in Clinical Specimens from Intensive Care Units: A Comprehensive Study

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ABSTRACT

In the middle of the 1980s, the first ESBL isolates were found in Western Europe, and in the late 1980s, they were found in the US. ESBLs are the enzymes that confer resistance against all ß-Lactam drugs (penicillin, 1st, 2nd, 3rd generation cephalosporin's, monobactams except carbapenems and cephamycin). These enzymes can breakdown the active ingredients in many common antibiotics making them ineffective. ESBL have only been reported in Gram negative bacterial infections. The objective of this study was to determine the frequency of extended-spectrum ß-lactamase (ESBL) producing gram-negative bacilli recovered from clinical specimens in Intensive Care Unit. The Cross-sectional descriptive study was performed from January 2024 to April 2024. The study was carried out at Department of Microbiology Armed Forces Institute of Pathology (AFIP) Rawalpindi. A total of 306 ESBL producing isolates from various clinical specimens sent to AFIP for culture and sensitivity were identified using standard microbiological techniques and tested for antimicrobial susceptibility. At the same time screening for ESBL production was also done. ESBL production was confirmed by combination disc synergy method. A total of 306 specimens that were ESBL producers are included in this study. Urine was the major source of bacterial isolates collected, comprising 76.10% of total samples, Pus 19.60%, Sputum 2%, NBL 1%, Blood 0.70%, CVP Tip 0.70%. E. coli was the most common species isolated from these specimens, comprising 230 (75.2%), Klebsiella Pneumonia was the second 34 (11.1%), Serratia Marcescens 16(5.2%), Proteus Mirabilis 7(2.3%), Serratia ordorifera5(1.6%), Enterobacter Aerogenes 3(1%), Klebsiella oxytoca 3(1%), Enterobacter cloacae 3(1%), Citrobacter freundii 3(1%), Citrobacter koseri 1(.3%).

INTRODUCTION:

Antibiotic resistance arises from bacterial mutations that diminish or completely eradicate the medication's efficacy. Antimicrobial medications can fight resistant germs, rendering conventional therapy useless in certain cases. Extremely resilient germs endure and proliferate, spreading further damage. A significant risk to the effective treatment of infections in hospitals is antibiotic resistance in Enterobacteriaceae isolates and other Gram-negative rods. Antibiotic resistance raises the expense of healthcare, lengthens hospital stays, and increases mortality(1).

ESBLs are the enzymes that confer resistance against all ß-Lactam drugs (penicillin, 1st, 2nd, 3rd generation cephalosporin's, monobactams except carbapenems and cephamycins). These enzyme can breakdown the active ingredients in many common antibiotics making them ineffective. ESBL have only been reported in Gram negative bacterial infections. Bacterial infection involving ESBL are known to cause several associated health conditions. Infection caused by *ESBL* –producing bacteria can be more difficult to treat because of antibiotic resistance as there are fewer effective antibiotics to use. ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins (2,3). ESBL testing involves two important steps. The first is a screening test with an indicator cephalosporins which detects resistance or diminished susceptibility, thus identifying isolates likely to be harbouring ESBLs. The second one test for synergy between an oxyimino cephalosporin and clavulanate distinguishing isolates with ESBLs from those that are resistant for other reasons. (4)

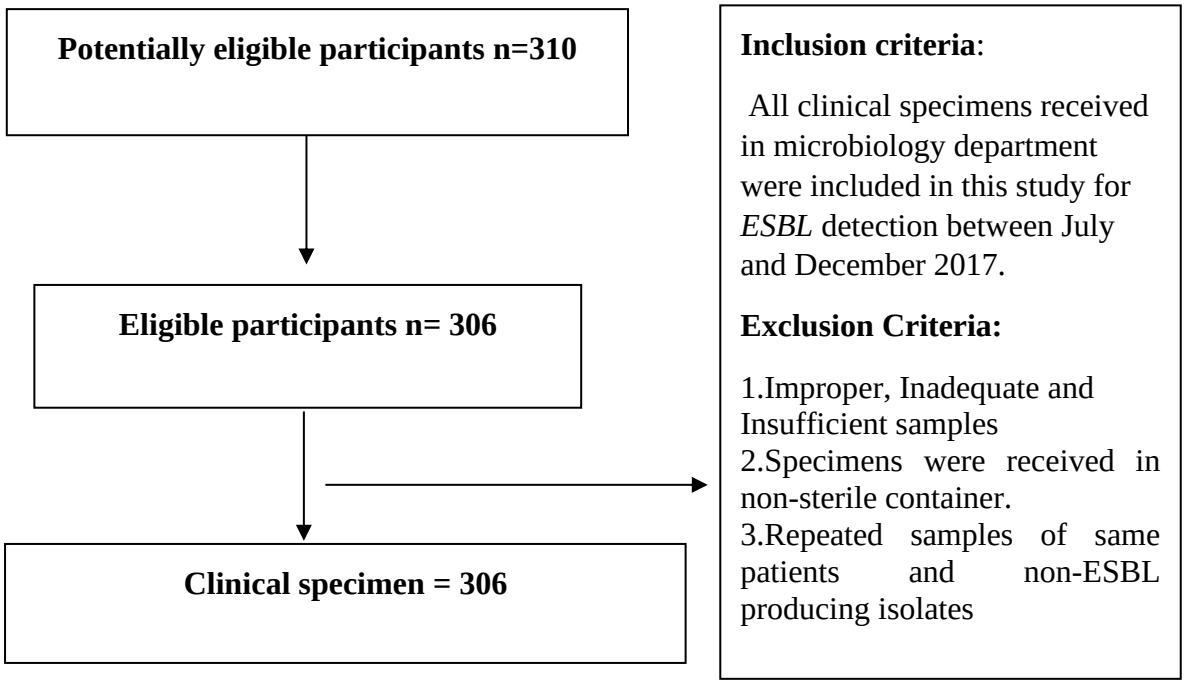
ESBL production among potential ESBL-producing isolates was confirmed phenotypically using combined disc method. Comparison of the zone of inhibition was made for the ceftazidime (30µg) and cefotaxime (30µg) discs alone vs. that of the ceftazidime and cefotaxime discs containing clavulanic acid (10µg), when placed 25mm apart (centre to center). Isolates showing an increase in zone diameter of ≥5 mm around either of the clavulanate combined discs compared to that of the disc alone was considered ESBL producer. *K.* pneumoniae ATCC 700603 was used as control strains. (5,6)

The objective of this study was to determine the frequency of extended-spectrum ß-lactamase (ESBL) producing gram-negative bacilli recovered from clinical specimens in our set up.

MATERIAL AND METHOD:

From January to April 2024, a cross-sectional descriptive study was conducted. At Rawalpindi's Armed Forces Institute of Pathology (AFIP), the Department of Microbiology conducted the study. Using conventional microbiological methods, 306 ESBL-producing isolates were identified from a variety of clinical specimens submitted to AFIP for sensitivity and culture. These isolates were then examined for antibiotic susceptibility. Screening for the manufacture of ESBLs was also conducted concurrently. Using the combination disc synergy approach, the generation of ESBL was verified. Institutional consent was taken from Institutional ethical committee, Armed Forces Institute of Pathology (AFIP), Rawalpindi. Data

was intended to keep confidential and strictly for academic purpose. Clinical Specimens were given specific identification number to maintain anonymity**.**



Methods: -

The isolates were identified on basis of colony morphology, Gram staining, certain rapid tests like catalase test, oxidase test, motility and biochemical reactions. They were simultaneously tested for anti-microbial susceptibility by modified Kirby Bauer disc diffusion technique by inoculating onto Mueller Hinton agar (Mast diagnostics UK) according to CLSI 2017. Confirmation to the species level was done by API 20 E & API 20 NE where required. ESBL production was detected by placing a susceptibility disk containing amoxicillin-clavulanate (20/10 ug) as the inhibitor of beta lactamase in the center of the plate and cefotaxime (30ug), ceftazidime (30ug), ceftriaxone (30ug) and aztreonam (30ug) disks at 30 mm (center to center) from the amoxicillin-clavulanate disk. Enhancement of the zone of inhibition of the oxyimino-β-lactam caused by the synergy with clavulanate in the amoxicillin-clavulanate disk was considered as evidence of ESBL production. Escherichia coli ATCC25922 and Klebsiella pneumoniae ATCC 700603 were used as control strains

Double-disc test for ESBLs

Third-generation cephalosporin and Augmentin test discs are positioned 30 mm apart from one another on inoculated Mueller-Hinton Agar (MHA). The edge of the cephalosporin inhibition zone clearly extending towards the Augmentin disc is considered as positive for the formation of ESBLs.

RESULTS

Complication and statistical analysis of the data was performed using IBM SPSS statistics ver.22 and MS excel. Data was analysed using descriptive statistics such as frequency, mean, standard deviation (SD) and presented in the form of percentages, graphs and charts. Urine was the major source of bacterial isolates collected, comprising 76.10% of total samples and E. coli was the most common species isolated from these specimens, comprising 230 (75.2%).

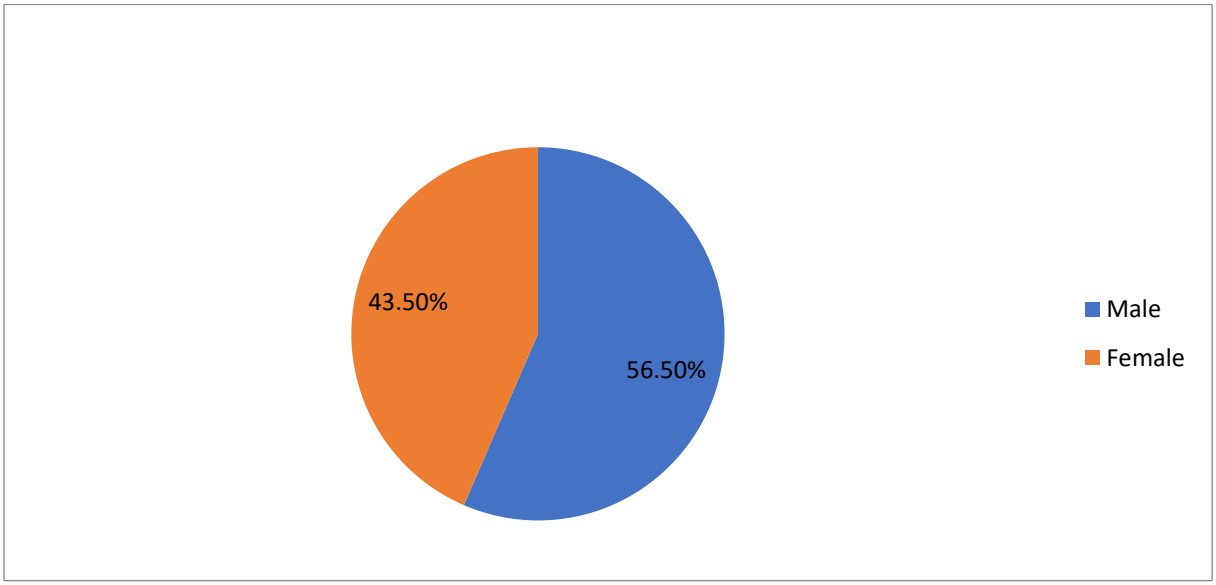


Figure 1: Graphical representation of Gender distribution. According to this study the ESBLS are highly produces in men that counts 56.50% and 43.50% is produces in females.

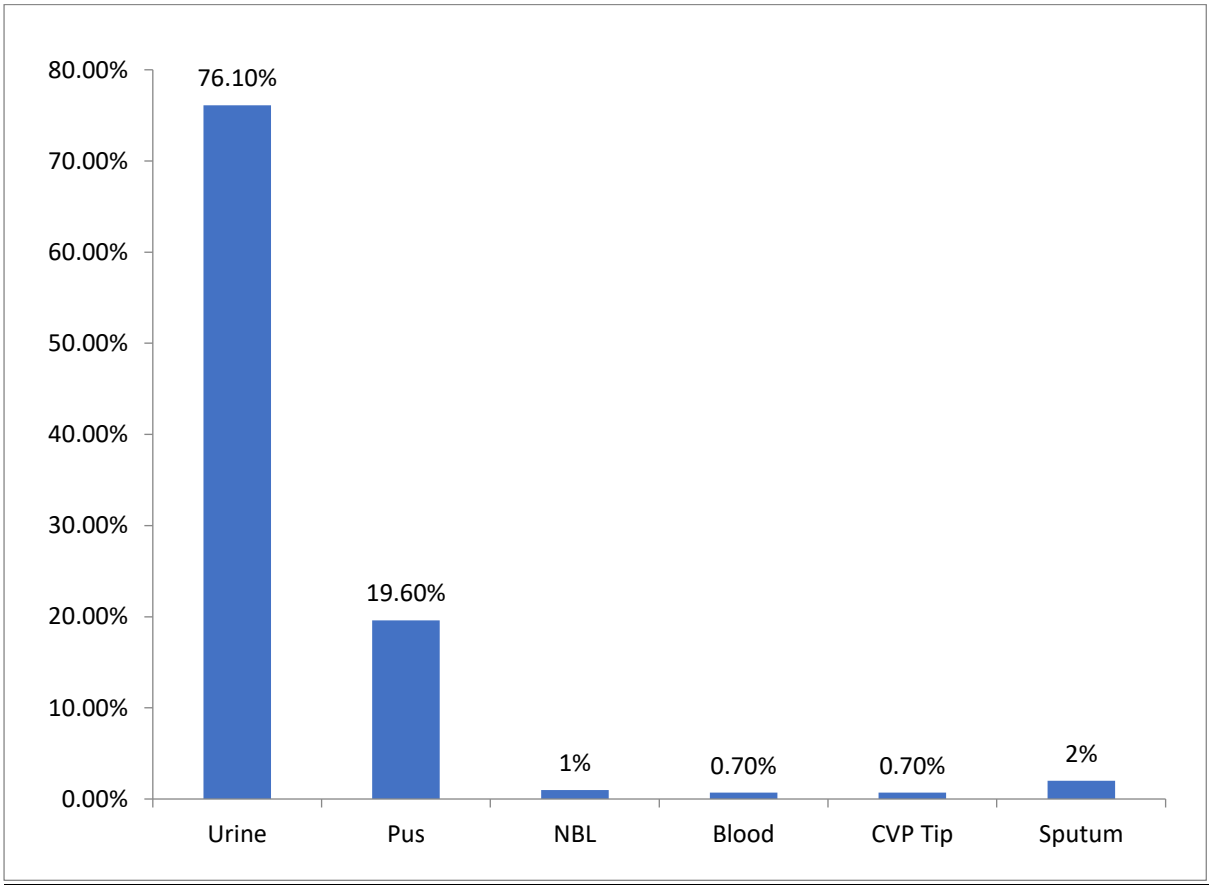


Figure 2: Graphical representation of Samples. Urine was the major source of bacterial isolates collected, comprising 76.10% of total samples, Pus 19.60%, Sputum 2%, NBL 1%, Blood 0.70% and CVP Tip 0.70%.

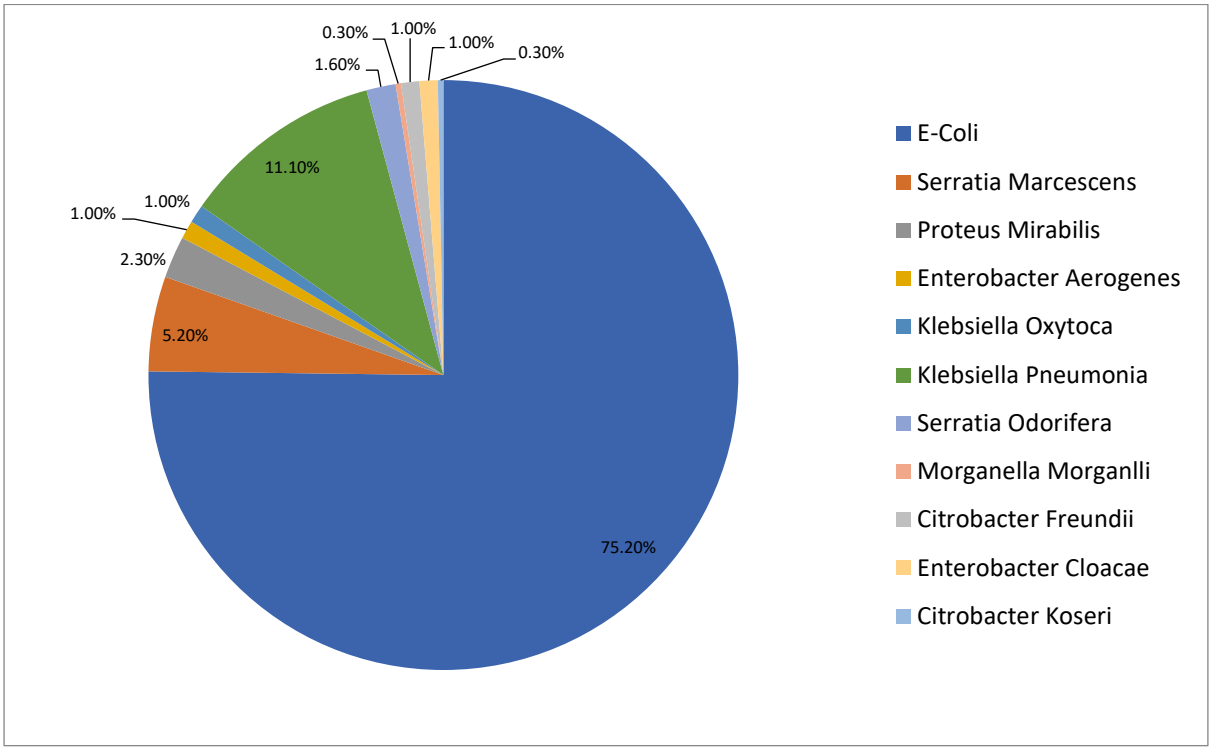


Figure 3: Graphical representation of Isolates. E. coli was the most common species isolated from these specimens, comprising 230 (75.2%), Klebsiella pneumonia was the second 34 (11.1%), Serratia marcescens 16(5.2%), Proteus mirabilis 7(2.3%), Serratia ordorifera5(1.6%), Enterobacter aerogenes 3(1%), Klebsiella oxytoca 3(1%), Enterobacter cloacae 3(1%), Citrobacter freundii 3(1%) and Citrobacter koseri 1(.3%).

Discussion

ESBL producing organisms are among the faster growing problems in the area of infectious diseases. Extended spectrum beta lactamase refers to beta lactamase enzymes produced by gram negative bacteria that show resistance against beta-lactam drugs. The objective of present study was to assess the frequency of ESBL in clinical specimens of intensive care unit. This study shows that E. coli was the most common species that is ESBL producer comprising (75.2%). Our study is comparable with the study conducted in Hyderabad MS Kumar et al reported that E. coli (63.7%) was the largest group producing ESBL. Another study is comparable with our study that was conducted in Northern Jorden Raymond G Batchoun et al reported that E. coli (41.4%) was the most common species isolated. Klesbiella was the second isolated pathogen constituting (28%) of total isolates.

In 2002 Ali AM et al observed the highest positivity for ESBL production in Klebsiella spp (87.2%) followed by the Enterobacter spp (65.3%) in Army Medical College Rawalpindi. Amita Jain et al reported that (87.2%) of Klebsiella pneumoniae isolates were ESBL producers in India. A study was conducted at Agha Khan University Karachi Pakistan reported the highest positivity of ESBL in Enterobacter (50%) followed by the E. coli (41%) was the second common ESBL producer. All differences between countries and laboratories may be explained by microbiological methods (from sample technique to culture media) local infection control standards and the local prevalence of ESBL.

The prevalence of ESBL varies between institutions and geographic areas. The difference in the design of the study such as the sample size and the method of ESBL detection. In our study the ESBL production was particularly high among E. coli (75.2%), followed by the Klebiella Pneumoniae (11.1%) and Serratia marcescens (5.2%).

Conclusion:

The present study indicates high prevalence of ESBL producing E-coli and klebsiella pneumoniae in all clinical specimens. Due to limited resources, several laboratories in developing countries do not routinely detect ESBL production. However, our data supports an urgent need for regular screening and surveillance for these organisms in this region. Strict hospital infection control policies and a prudent anti-microbials use regimens are to be adopted by the physicians. It is essential and mandatory to have a regular and routine monitoring of ESBL producing clinical isolates in clinical laboratories.

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