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Original Research Article

Prevalence of Extended Spectrum β-lactamase and Carbapenemase producing Gram-Negative Bacilli in Rural Tertiary Care Hospital

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Abstract

Background: Multidrug resistance among Gram-negative bacilli (GNB) is rising rapidly, contributing to both hospital-acquired and community acquired infections. Microorganisms are constantly finding new resistance mechanisms to survive the effects of antibiotics. One of the most predominant mechanisms is resistance to the beta lactam antibiotics in gram negative bacilli (GNB) is the production of extended spectrum β-lactamase (ESBL), which is responsible for the resistance to the 3rd generation of cephalosporin.

Aims & Objectives: To determine prevalence, antibiotic profile and associate factors of ESBL and carbapenemase producing gram-negative bacilli from various samples received at Microbiology laboratory in tertiary care hospital.

Methodology: The present study was conducted at rural tertiary care hospital. The study was done from Jan– 2024 to December– 2024. Isolates from clinical specimen such as blood, urine, sputum, pus, endotracheal aspirate, sterile body fluid were analysed.

Results: Total 1631 samples were received, among them 607 were positive. Out of 607 samples, 150(24.71%) samples were gram positive and 450(74.13%) were gram negative bacilli. Among the isolates of Klebsiella species, 39.3 % of the isolates were ESBL producers and 16.5% of the isolates were Carbapenemase producer. Among the isolates of E. coli, 26.2% of the isolates were ESBL producers and 9.9 % of the isolates were Carbapenemase producer. Ward has the highest total number of CRE (62.80%) isolates, with Pseudomonas spp. being the most dominant species here. The ICU shows a high prevalence of Klebsiella spp., making it the most frequently isolated CRE organism in this area. Carbapenems and aminoglycosides were the most sensitive drug for all the gram-negative isolates. Fluoroquinolones like levofloxacin remain effective against Pseudomonas (71%) and Acinetobacter (98%), but less so for E. coli and Klebsiella.Colistin shows 100% sensitivity.

Conclusion: The prevalence of beta-lactamase and carbapenemase production among gram negative bacilli isolates from clinical infections, highlighting significant findings in both the distribution of resistant strains and their mechanisms of resistance, and the broader implications for clinical treatment and healthcare systems. A total of 607 participants were included, with a higher representation of males (52.71%) compared to females (47.28%). The study shows high prevalence of ESBL (50.25%) and carbapenemase (19.94%) producing gram-negative bacilli, particularly among Klebsiella spp., E. coli, and Pseudomonas spp., underscores a serious threat to effective treatment of infections.

Keywords: β-lactamase, Carbapenemase, Gram Negative bacilli, Mutlidrug Resistance, Microorganism.

Introduction

Multidrug resistance among Gram-negative bacilli (GNB) is rising rapidly, contributing to both hospital-acquired and community acquired infections. Microorganisms are constantly finding new resistance mechanisms to survive the effects of antibiotics. One of the most predominant mechanisms is resistance to the beta lactam antibiotics in gram negative bacilli (GNB) is the production of extended spectrum β-lactamase (ESBL), which is responsible for the resistance to the 3rd generation of cephalosporin [1,2]. They cannot hydrolyse cephamycin and are inhibited by clavulanic acid. [2] The use of carbapenem into patients represents a great development for the

treatment of β-lactam resistant bacteria. Carbapenem has broad spectrum of activity and stable to hydrolysis by most beta lactamases, so the carbapenem is the drug of choice for treatment of infections caused by penicillin or cephalosporin resistant GNB. [3]

The metallo β-lactamase (carbapenemase) in GNB is becoming a treatment challenge, as these enzymes hydrolyse all β-lactam antibiotics including carbapenems. [4] Accurate and timely detection of resistant mechanisms is very important in deciding the appropriate treatment plan. Detection of the resistant mechanisms is always a grave task to the clinical laboratories. [5] This study detects the prevalence of extended spectrum β-lactamase (ESBL) and carbapenemase producing gram-negative bacilli in rural tertiary care hospital.

Aims & Objectives:

1. To determine prevalence of ESBL and carbapenemase producing gram-negative bacilli from various samples received at Microbiology laboratory in tertiary care hospital.
2. To determine antibiotic profile of ESBL and Carbapenemase producing gram-negative bacilli.
3. To determine associated factors, e.g. demographic (age, sex, residence), Clinical (Outdoor/indoor patients, site of infections, antibiotic treatment status)

Materials and Method

The present study was conducted at rural tertiary care hospital. The study was done from Jan– 2024 to December– 2024.

All suspected patients for infections referred by clinician were included and Patients who is not willing to provide consent were excluded. Isolates from clinical specimen such as blood, urine, sputum, pus, endotracheal aspirate, sterile body fluid were analysed. The samples were properly labelled with all details of patient & sent to the microbiology laboratory. Samples with completely filled laboratory request form (LRF) were received in lab. Laboratory ID was generated by using software.

All the samples were inoculated on Nutrient agar, MacConkey agar and chocolate agar plates by adopting standard microbiological techniques. The inoculated plates were incubated aerobically in an incubator, and results were read after 24 hours. All inoculated plates were observed next day, and colony characteristics were noted. All the isolates were identified by colony morphology, microscopic appearance, biochemical tests and phenotypic tests for drug resistance. Antimicrobial Susceptibility Testing will be performed by Kirby-Bauer Disc Diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guidelines.[6] The following antibiotics discs were used in the sensitivity test: Imipenem (10 mcg), Colistin (10 mcg), Amikacin (10 mcg),Gentamicin (10 mcg), Cefotaxime (30 mcg), Ceftriaxone (30 mcg), Ceftazidime (30 mcg), Cefepime (30 mcg), Ciprofloxacin(5 mcg), Nitrofurantoin (100 mcg), Cotrimoxazole (25 µg), Pipracillin Tazobactum (100/10mcg), and Ampicillin (10mcg), Cefuroxime (30 µg), Ampicillin-sulbactam(10/10 µg), Amox ycillin-Clavulanate(20/10 µg), Levoflo xacin (5 µg),Tetracycline(30 µg),colistin(MIC).

Screening and confirmation of extended spectrum β-lactamase (ESBL) production:

Screening test: Gram negative bacilli isolate exhibiting a growth inhibition zone size of diameter of ≤ 22 mm for ceftazidime (30µg), ≤ 27 mm for cefotaxime (30µg) will be considered to be screening positive. [6]

Confirmation test: Confirmation will be done by combined disk diffusion methods using ceftazidime (30µg) & ceftazidime+ clavulanic acid (30/10µg) disc, in which increase of ≥ 5mm in the zone of inhibition of the combination discs in comparison to the ceftazidime disc alone will be considered as ESBL positive. [6,7]

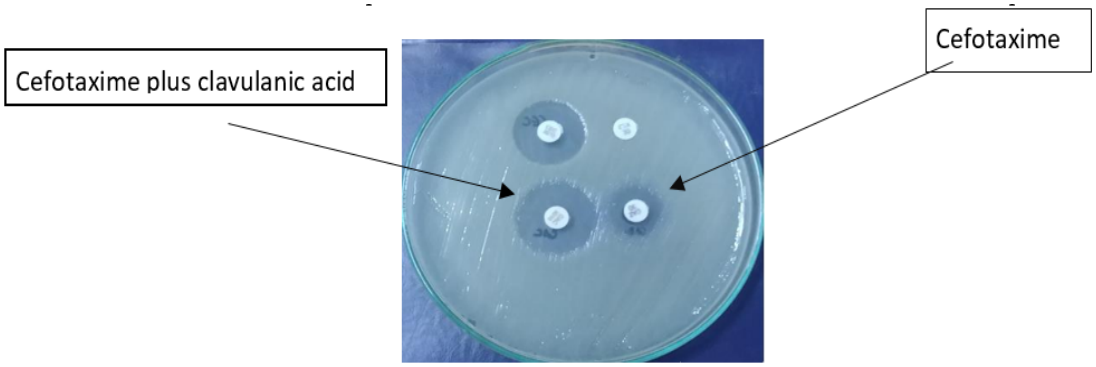

Figure 1:

Screening and confirmation of carbapenemase production:

Screening test: Gram negative bacilli isolates resistance to meropenem /imipenem in the Kirby Bauer disk diffusion test will be considered as positive for carbapenemase screening.

Confirmation test: Metallobetalactamase (MBL) carbapenemase detection done by combined disk diffusion methods using Imipenem and Imipenem+ EDTA, in which ≥ 7-mm increase in the zone diameter with EDTA containing disk will be considered as MBL carbapenemase positive. [8,9,10]

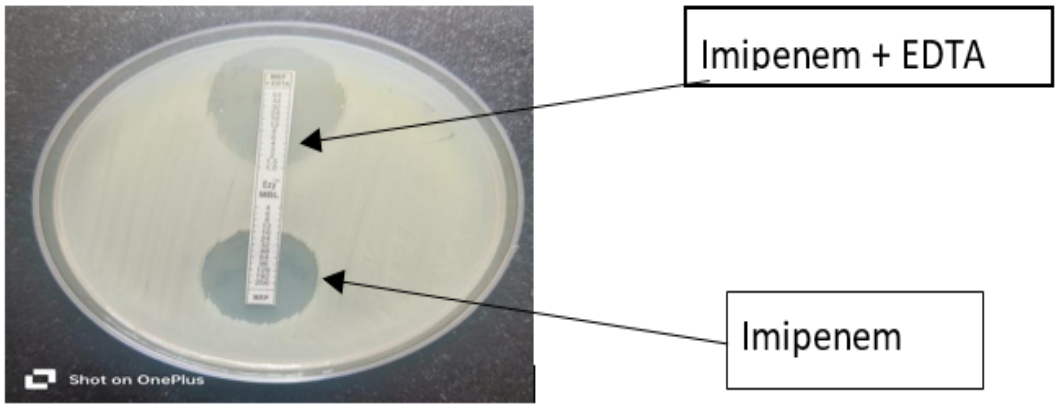

Figure 2:

Result

The present study was conducted at rural tertiary care hospital. The study was done from Jan– 2024 to December– 2024. Isolates from clinical specimen such as blood, urine, sputum, pus, endotracheal aspirate, sterile body fluid were analysed. Total 1631 samples were received, among them 607 were positive. Out of 607 samples, 150(24.71%) samples were gram positive and 450(74.13%) were gram negative bacilli.

Table 1: Age and sex wise distribution:

Age	Male	Female	Total
<20	65(43.9%)	83(56.1%)	148(24.4%)
20-40	67(44.4%)	84(55.6%)	151(24.9%)
41-60	113(66.1%)	58(33.9%)	171(28.2%)
>60	75(54.7%)	62(45.3%)	137(22.6%)
Total	320(52.71%)	287(47.28%)	607(37.21%)

A majority of the patients were male (52.7%) while the remaining 47.28% were female. The 41–60 age group has the highest number of cases (28.2%). [Table 1]

Table 2: Specimen wise isolated organism

Specimen Type	E. coli	Klebsiella spp.	Pseudomonas spp.	Acinetobacter spp.	Other	Total
Urine	80	43	12	03	08	146(24.1%)
Blood	02	19	13	04	05	33(5.4%)
Pus	35	82	120	17	69	323(53.2%)
Other	15	42	38	05	05	105(17.3%)
Total	132(21.8%)	186(30.6%)	173(28.5%)	29(4.8%)	87(14.3%)	607(37.21%)

Predominant isolates were obtained from Pus samples (53.2%) followed by urine sample (24.1%).

Predominant isolates were Klebsiella spp. (30.6%) followed by Pseudomonas spp. (28.5%). [Table 2]

Distribution of ESBL and CRE: 305(50.25%) isolates were ESBL positive, and 121(19.94%) isolates were resistant to carbapenem. In total, 50.25% of the isolates were ESBL producers, 9.37% of the isolates were positive for Carbapenemase production.

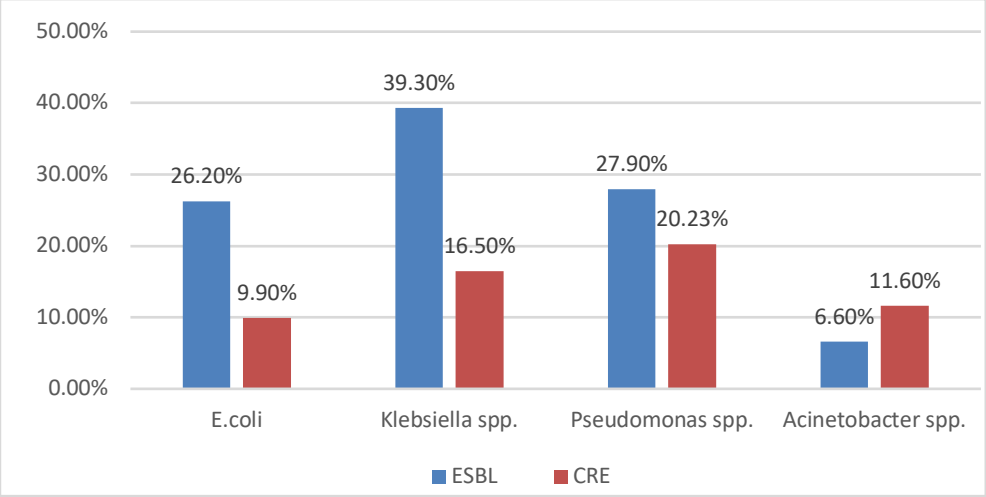

Figure 1: ESBL and CRE Producing Organism

Among the isolates of Klebsiella species, 39.3 % of the isolates were ESBL producers and 16.5% of the isolates were Carbapenemase producer. Among the isolates of E. coli, 26.2% of the isolates were ESBL producers and 9.9 % of the isolates were Carbapenemase producer. [Figure 3]

Table 3: Area wise distribution ESBL Producing organism

Area	E. coli	Klebsiella spp.	Pseudomonas spp.	Acinetobacter spp.	Total
ICU	13(15%)	31(33%)	40(43%)	8(9%)	92(30.2%)
Ward	32(25%)	58(45%)	30(25%)	7(5%)	127(41.6%)
OPD	35(40%)	31(36%)	15(18%)	5(6%)	86(28.2%)

Ward has the highest total number of ESBL (41.6%) isolates, with Klebsiella spp. being the most dominant species here. The ICU shows a high prevalence of Pseudomonas spp., making it the most frequently isolated ESBL organism in this area. [Table 3]

Table 4. Area wise distribution CRE Producing organism

Area	E. coli	Klebsiella spp.	Pseudomonas spp.	Acinetobacter spp.	Total
ICU	7(21%)	11(34%)	9(27%)	6(18%)	33(27.27%)
Ward	4(5%)	6(8%)	61(80%)	5(7%)	76(62.80%)
OPD	1(8%)	3(25%)	5(42%)	3(25%)	12(9.91%)

Ward has the highest total number of CRE (62.80%) isolates, with Pseudomonas spp. being the most dominant species here. The ICU shows a high prevalence of Klebsiella spp., making it the most frequently isolated CRE organism in this area. [Table 4]

Table 5. Antibiotic profile (Percentage of sensitivity) of ESBL and Carbapenemase producing gram-negative bacilli.

Antibiotic	E. coli	Klebsiella spp.	Pseudomonas spp.	Acinetobacter spp.
Ampicillin	7%	-	-	-
Amoxicillin/Clavulanic acid	16%	8%	-	-
Ampicillin/Sulbactam	14%	10%	-	8%
Cefuroxime	14%	20%	-	-
Cefotaxime	34%	28%	-	-
Cefepime	32%	26%	34%	30%
Piperacillin- tazobactam	37%	27%	28%	46%
Ceftazidime	44%	40%	68%	38%
Gentamicin	80%	70%	64%	55%
Amikacin	88%	75%	68%	67%
Ciprofloxacin	40%	42%	62%	44%
Levofloxacin	70%	66%	71%	98%
Tetracycline	42%	61%	-	-
Cotrimoxazole	50%	48%	-	65%
Imipenem	88%	84%	82%	80%
Colistin	100%	100%	100%	100%

Carbapenems and aminoglycosides were the most sensitive drug for all the gram-negative isolates. Fluoroquinolones like levofloxacin remain effective against Pseudomonas (71%) and Acinetobacter (98%), but less so for E. coli and Klebsiella.Colistin shows 100% sensitivity. [Table 5]

Discussion

Total 1631 samples were received from jan-2024 to dec-2024.Among 1631 samples 607 were positive. Out of 607 samples, 150(24.71%) samples were gram positive and 450(74.13%) were gram negative bacilli. A majority of the patients were male (52.7%) while the remaining 47.28% were female. In a study by Murray et al. [11] focusing on cardiovascular disease, it was noted that males were more likely to participate, with 63% of their study population being male. For instance, a systematic review by Freeman et al. [12] on the global prevalence of menopausal symptoms highlighted that the majority of participants were between 40 and 60 years old, similar to our study's age distribution. Predominant isolates were obtained from Pus samples (53.2%) followed by urine sample (24.1%). Predominant isolates were Klebsiella spp. (30.6%) followed by Pseudomonas spp. (28.5%). Similarly, a study by Deshmukhe et al. [13] investigated 53% were ESBL-producers**,** with a majority coming from pus samples, followed by urine and wound swabs. Study by Saumya et al. [14] found CRE isolates were mostly found in the 45–65 age group, with pus (50%) and urine (32%) being the top sources.

Our study found that Klebsiella spp. was the most frequently isolated organism, accounting for 30.6% of the total cases. Pseudomonas spp. (28.5%) and E. coli (21.8%) were also commonly isolated. This distribution highlights the dominance of Klebsiella spp. The study by Johnson J et al., [15] reported that Klebsiella pneumonia was the most frequently isolated organism, representing 40% of cases, with E. coli at 30%. Similarly, Zhang Y et al., [16] found Klebsiella pneumoniae to be the most prevalent, at 35%, with E. coli at 28%.

Our study revealed, ESBL-producing organisms accounted for 50.25% of the total isolates, with Klebsiella spp. showing the highest ESBL production at 39.3%. Carbapenemase-producing organisms made up 19.94% of the isolates, indicating a concerning level of resistance to carbapenems, a critical class of antibiotics. Similarly, Smith et al. [17] conducted a study focusing on carbapenemase producing organisms, noting that 23.5% of isolates were resistant to at least one carbapenem, with Klebsiella pneumoniae being a major contributor. A study by Muti et al. [18] examined the prevalence of ESBL and carbapenemase-producing Enterobacteriaceae in a European hospital setting. The study found that 28% of the isolates were ESBL producers, with E. coli and Klebsiella spp. being the predominant organisms. Further, a study by Wang et al. [19] revealed that 32% of the isolates were ESBL producers, with a notable 27% being carbapenem-resistant.

In our study, Ward has the highest total number of ESBL (41.6%) isolates, with Klebsiella spp. being the most dominant species here. The ICU shows a high prevalence of Pseudomonas spp., making it the most frequently isolated ESBL organism in this area. A study by Sharma et al. [20] examined the distribution of bacterial pathogens in OPD versus IPD settings. The study found that Klebsiella spp. was significantly more prevalent in the IPD setting. Similarly, a study by Khan et al. [21] investigated the patterns of bacterial infections in a tertiary care hospital. They reported that Klebsiella spp. were isolated more frequently from IPD patients (27%) compared to OPD patients (17%).

In our study, Ward has the highest total number of CRE (62.80%) isolates, with Pseudomonas spp. a being the most dominant species here. The ICU shows a high prevalence of Klebsiella spp., making it the most frequently isolated CRE organism in this area. Similar in B. A. Han, et al. study, ICU shows a significantly higher prevalence of Klebsiella spp. (ESBL producers) and P. aeruginosa (CRE), while general wards have a higher rate of ESBL isolates in non-ICU patients. [22] Colistin showed 100% sensitivity to all the ESBL and CRE producing bacteria.

Conclusion

This study examined the prevalence of beta-lactamase and carbapenemase production among gram negative bacilli isolates from clinical infections, highlighting significant findings in both the distribution of resistant strains and their mechanisms of resistance, and the broader implications for clinical treatment and healthcare systems. A total of 607 participants were included, with a higher representation of males (52.71%) compared to females (47.28%). The study shows high prevalence of ESBL (50.25%) and carbapenemase (19.94%) producing gram-negative bacilli, particularly among Klebsiella spp., E. coli, and Pseudomonas spp., underscores a serious threat to effective treatment of infections. These resistant strains show marked resistance to key antibiotics, including third-generation cephalosporin and carbapenems. Colistin showed 100% sensitivity to all the ESBL and CRE producing bacteria. Urgent, coordinated efforts involving infection control, antimicrobial stewardship, and public education are critical to curb the spread of these multidrug-resistant organisms.

References

1. https://www.cdc.gov/hai/organisms/ESBL.html
2. Umadevi S, Kanhakumari G, Joseph NM, Kumar S, Eaow JM, Atephen S, Singh UK. Prevalence and antimicrobial susceptibility pattern of ESBL producing gram negative bacilli. J Clin Diag Res 2011; 5(2): 236-239
3. Shoorashetty RM, Nagarathnamma T, Prathibha J. Comparison of the boronic acid disk potentiation test and cefepime-clavulanic acid method for the detection of ESBL among AmpC-producing Enterobacteroaceae. Indian J Med Microbiol 2011; 29 (3): 297-30.
4. Jesudason MV, Kandathil AL, Balaji V.Comparison of two methods to detect carabapenemase and matallo-β-lactamase production in clinical isolates. Indian J Med Res 2005; 121:780-3
5. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo β-lactamase production in Enterobacteriaceae. J Antimicrob Chemother 2008; 61:548- 53.
6. Clinical and Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI Document M 100-S20, Wayne, PA: CLSI, Wayne, PA, USA, 2022
7. Dallela G. Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, India. Journal of Clinical and Diagnostic Research 2012; 6(2): 182-187
8. 10. Pandya NP, Prajapati SB, Mehta SJ et al. Evaluation of various methods for detection of metallo-ß - lactamase (MBL) production in Gram negative bacilli. Int J Biol Med Res 2011; 2(3):775-777.
9. Pandya NP, Prajapati SB, Mehta SJ et al. Evaluation of various methods for detection of metallo-ß - lactamase (MBL) production in Gram negative bacilli. Int J Biol Med Res 2011; 2(3):775-777.
10. Datta P, Gupta V, Garg S, Chander J. Phenotipic method for differentiation of carbapenemases in Enterobacteriaceae; study from North India. Indian J Pathol Microbiol 2012; 55:157-60.
11. Asthana S, Mathur P, Tak V. Detection of Carbapenemase Production in Gram-negative Bacteria. Journal of Laboratory Physicians 2014; 6(2): 69-75.
12. Murray, C. J., et al. (2014). "Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013." The Lancet, 385(9963): 117-171
13. Freeman, E. W., et al. (2007). "Symptom clusters in menopausal women: A secondary analysis of data from the Seattle Midlife Women's Health Study." Menopause 14(6): 990-999.
14. Deshmukhe et al., Prevalence of ESBL in Klebsiella Sp. And Its Antibiotic Resistance Pattern from Various Clinical Samples in a Tertiary Care Hospital. 34(42A):28-39, 2022; Articleno. JPRI.81554ISSN:2456-9119. Journal of Pharmaceutical Research International
15. Saumya et al., Genotypic Characterization of Carbapenem-Resistant Enterobacteriaceae in a Tertiary Care Hospital in South India. 2024 Dec 3; 16(12):e75032. doi: 10.7759/cureus. 75032. Cureus Journal of Medical Science
16. Johnson J, et al. Prevalence and antimicrobial resistance patterns of Klebsiella pneumoniae and other Gram-negative bacilli. J Clin Microbiol. 2019.
17. Zhang Y, Lee C, et al. Isolated pathogens and their antimicrobial susceptibility profiles in a tertiary care hospital. Infect Drug Resist. 2020
18. Smith R, Johnson L, Williams E, et al. Prevalence and resistance mechanisms of carbapenemase-producing organisms in healthcare settings: A comprehensive review. J Clin Microbiol. 2024; 62. Available from: https://jcm.asm.org/content/62/3/e00321-24.
19. Muti M, Crimi S, Nicastri E, et al. Prevalence of ESBL and carbapenemase-producing Enterobacteriaceae in a European hospital: A multicenter study. Infect Drug Resist. 2024; 17:183-191. Available from: https://www.dovepress.com/articles.php?article_id=62423.
20. Wang H, Sun Z, Huang Z, et al. High prevalence of ESBL and carbapenemase-producing Enterobacteriaceae in a Chinese healthcare setting. BMC Infect Dis. 2024; 24:95. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-024-07845-7.
21. Sharma S, Gupta R, Bhatt P, et al. Comparative analysis of bacterial isolates in OPD and IPD settings in a tertiary care hospital. Indian J Med Microbiol. 2024; 42:54-60. Available from: https://www.ijmm.org/article.asp?issn=02550857;year=2024;volume=42;issue=1;spage=54;epage=60.
22. Khan A, Akhtar A, Qureshi H, et al. Patterns of bacterial infections in OPD and IPD settings: A retrospective study. J Hosp Infect. 2023; 132:67-73. Available from: https://www.journalofhospitalinfection.com/article/S0195670123)00067-1/fulltext.
23. B. A. Han, et al. Epidemiology of Extended-Spectrum Beta-Lactamase-Producing and Carbapenem-Resistant Enterobacteriaceae in

Critical Care Units.2017. Infection Control & Hospital Epidemiology.