International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Isolation, Identification and Prevalence of ESBLs Producing Gram Negative Bacilli from Urine and Exudate Samples in a Tertiary Care Hospital: An Observational Study

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Abstract: ***Introduction**: Antimicrobial resistance (AMR) poses severe threat to public health as it lowers the efficacy of currently available antibiotic therapies for bacterial infections. Extended spectrum β-lactamases are a rapidly emerging class of β-lactamases, hydrolyze penicillin's, aztreonam, and third-generation cephalosporins; however, clavulanic acid inhibits their activity. They are plasmid encoded, mostly produced by Klebsiella pneumoniae along with Escherichia coli, also observed in other Enterobacteriaceae family members. Simple, uncomplicated urinary tract infections to acute, perhaps fatal sepsis can be brought on by ESBL infections. Hence this study was done to isolate, identify and to study the prevalence of ESBLs producing Gram-negative bacilli from urine and exudate samples. **Materials and methods**: A research using prospective observation was done in the Department of Microbiology, GMC, Ongole, Andhra Pradesh from April 2023 to August 2023 with 200 clinical samples-97 urine, 103 exudates from inpatients, after obtaining acceptance from Ethical Committee, and informed consent from the patients and samples are processed for isolation, identification and antibiotic susceptibility testing as per standard microbiological procedures. ESBL screening was performed to the pathogens which were resistant to either ceftazidime or cefotaxime disk by Double-disc diffusion test, Disc-on-disc test and Disc potentiation test according to CLSI guidelines. **Results**: Out of 200 clinical samples-97 (48.5%) were urine samples and 103 (51.5%) were exudate samples. Most of the samples in total showed evidence of bacterial development, 122 (61%) of samples, of them, 92 (75.4%) were Gram negative bacilli whereas 30 (24.6%) were Gram positive organism and remaining 78 (39%) samples were culture negative. Of the total 92 Gram-negative bacilli, 62 was resistant to either cefotaxime or ceftazidime. Out of 62 resistant bacilli 12 (13.1%) ESBLs were detected. **Conclusion**: The current research detected low prevalence of ESBL (13.1%). As ESBL producing organisms poses a significant therapeutic challenge for hospitalized patients today, effective infection control procedure are necessary to prevent outbreaks as well as intervention tactics like antibiotic rotation to prevent the spread of hospital acquired infections.*

Keywords: Gram negative bacilli (GNB), ESBL, Prevalence, Isolation, Phenotypic method.

1. Introduction

Antimicrobial resistance (AMR) poses severe threat to public health as it lowers the efficacy of currently available antibiotic therapies for bacterial infections. Healthcare facilities frequently harbor antimicrobial resistance (AMR) to various microorganisms because of the ecological gap created by patients, hospital adaptation, and antibiotic exposure. [1]

In Gram-negative bacilli (GNB), ESBLs (Extended-Spectrum B-Lactamases) are the main source of β-lactam resistance, which is becoming more prevalent and poses a serious concern, causing AMR, particularly in India. [1, 2] The increasing frequency and prevalence of GNB-producing ESBL infections worldwide in both community and clinical settings presents significant treatment challenges. [3-5] Penicillins, extended-spectrum cephalosporins, and aztreonam are hydrolyzed by ESBLs however, clavulanic acid inhibits their activity, they are plasmid-mediated groups of enzymes. [6, 7] The blaSHV, blaTEM, and blaCTX-M genes, which encode SHV, TEM, and CTX-M, respectively, are responsible for ESBLs production. Approximately 300 unique ESBL variants have been reported. [8]

While SHV and TEM "variants are the most prevalent ESBLs, strains with CTX-M ESBLs have been identified in a number of countries recently. [9, 10] and currently, they are the most prevalent ESBL type that is neither TEM or SHV. Within the Enterobacteriaceae family, ESBLs have been identified mainly in Klebsiella species as well as E. coli, additionally, they have been found in several Enterobacteriaceae" groups including Proteus species, Enterobacter species, Morganella species, Citrobacter species, Salmonella species, Providencia species, and Serratia species. [3, 11, 12]

ESBL producing organisms are isolated from several samples including bodily fluids, sputum, urine, blood, swabs as well as catheter tips. In addition to being resistant to broad-spectrum cephalosporins as well as monobactams, ESBL also shows co-resistance to other antibiotic classes, including quinolones, co-trimoxazole, and tobramycin. This results in a reduction in the range of therapeutic alternatives that are accessible. ESBL growth indicators are an eight-fold decrease in MIC (Minimum Inhibitory Concentration) and accentuation of 3rd generation cephalosporin's zone of inhibition in the existence of clavulanic acid. More prolonged hospital stay, higher rates of morbidity as well as death, & rise in healthcare expenses are all attributed to ESBL-producing Gram-negative bacteria. [13] Recognizing the severity of the condition, the current research was done in order to separate, identify as well as assess the frequency of Gram-negative

bacteria that are produced by ESBLs from urine as well as exudate samples from inpatients.

Aim:
To isolate, identify and to study the prevalence of ESBLs producing Gram-negative bacilli from urine as well as exudate samples.

Objectives:
1) To isolate Gram-negative bacilli from urine as well as exudate samples.
2) To identify ESBL producer from Gram-negative bacilli by Double-disk diffusion test, Disk-on-disk test &Disk potentiation test.
3) To study the prevalence of ESBLs producing Gram-negative bacilli from urine as well as exudate samples.

2. Materials and Methods

- **Study design:** Prospective observational research
- **Study period:** from April 2023 to August 2023.
- **Study location:** Department of Microbiology, GMC, Ongole, Andhra Pradesh.
- **Sample Size:** 200 clinical samples with 97 urine &103 exudates (wound swab-79, vaginal swab–10, pleural fluid-10, throat swab-3 & ascitic fluid-1) from inpatients.

Inclusion Criteria
1) Patients of all age groups as well as both sexes admitted with associated risk factors
2) Patients who gave an informed consent.

Exclusion Criteria
1) Outpatients.
2) Samples other than urine and exudates.
3) Patients those who were not willing to give an informed consent.

3. Methodology

The present research was conducted with 200 clinical samples (97 urine &103 exudates) in the department of Microbiology, GMC, Ongole after obtaining acceptance from Ethical Committee, and informed consent from the patients. Early morning first voided clean catch mid-stream urine samples from individuals suspected of having UTIs (Urinary Tract Infections) and exudates (wound swab, vaginal swab, pleural fluid, throat swab & ascitic fluid) were collected under aseptic conditions. History of patient's demographics and hospitalization status was taken.

After the samples were inspected both macroscopic and microscopic, they "were inoculated on nutrient agar, blood agar, and MacConkey's agar, and they were then incubated aerobically at 37°C for 18 to 24 hours. Following this, the organisms were isolated and identified using conventional microbiological methods. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion technique on Mueller-Hinton agar plate using Himedia (Mumbai) commercially available antibiotic discs for screening of ESBLs. The disks that are utilized are 30µg of ceftazidime, 30µg of cefotaxime, 5µg of ciprofloxacin, 10µg of gentamicin, 30µg of" cefuroxime, and 10µg of ampicillin. Zones of inhibition were determined utilizing a zone scale as well as CLSI criteria were followed for interpretation of results. Confirmatory phenotypic tests were performed on organisms that exhibited resistance to cefotaxime or ceftazidime, the third generation cephalosporins. [14]

Quality control strains used are ESBL Klebsiella pneumonia ATCC 700603 as the positive control whereas non-ESBL Escherichia coli ATCC 25922 as the negative control. [14]

Test for disc potentiation: Two cephalosporin discs, one in presence and one in absence of clavulanic acid, are positioned on opposing sides of a 90mm Mueller-Hinton agar plate swabbed with 0.5 McFarland standardized isolates of the test organism and incubated aerobically at 37°C for 18 to 24hrs. If the zone of inhibition surrounding the combo disc is at least 5 mm greater than that of the cephalosporin alone, then it is considered an ESBL producer. [14]

Double-disk diffusion test (the modified "double-disk synergy test or double-disc approximation approach).0.5 McFarland standardized isolates of the test organism were swabbed onto a 90 mm Mueller-Hinton agar plate. A susceptibility disc consisting amoxicillin as well as clavulanate is" positioned twenty millimeters (from Centre to Centre) apart from ceftazidime and cefotaxime & incubated aerobically at 37°C for18 to24hrs. The inhibitory zone's noticeable elevation towards the Amoxicillin & Clavulanate disc is considered to be ESBL producer. [14]

Disk-on-disk test: A 90mm Mueller-Hinton agar plate is swabbed "with 0.5 McFarland standardized inoculums of the test organism. Cefotaxime and ceftazidime discs are tested against test organisms alone and combined with a cephalosporin disc that is placed on top of it & incubated aerobically at 37°C for" 18 to24hrs. An organism is considered an ESBL producer if the zone diameter surrounding the combination disc is five millimeters larger as compared to that of cephalosporin disc. [14].

4. Results

Out of 200 clinical samples, 97 (48.5%) were urine &103 (51.5%) were exudates. In this study, the majority 124 (62%) of the samples were from females. (Figure 1) Of the total samples, bacterial growth was seen in 122 (61%) of samples, consisting of 52 (53.6%) of urine, and 70 (67.9%) of exudates, while the remaining samples were sterile 78 (39%) (Figure 2)

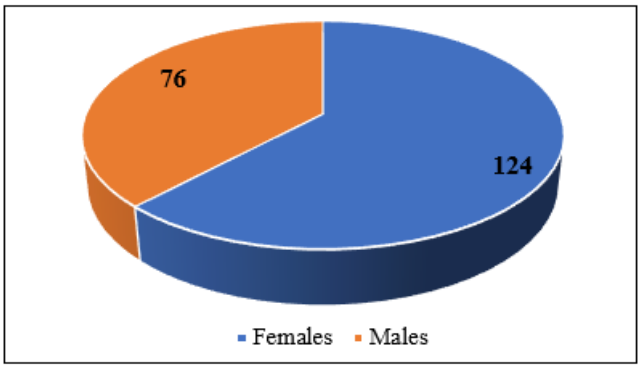

Figure 1: Gender distribution of total samples

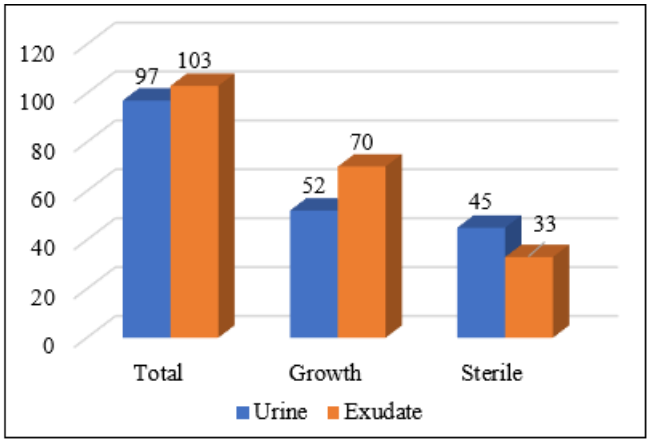

Figure 2: Culture positivity among urine and exudate samples

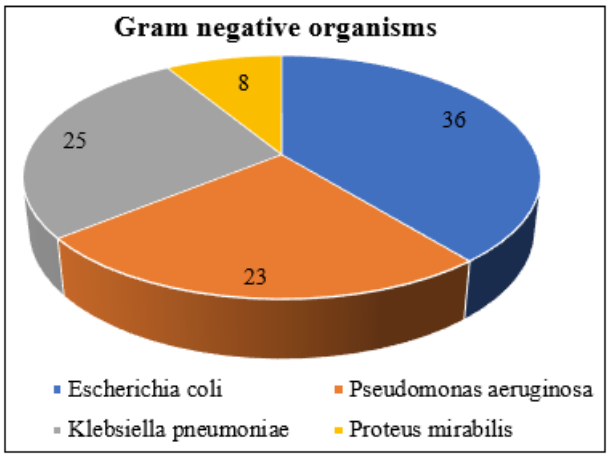

Figure 4: Distribution of Gram-negative organisms

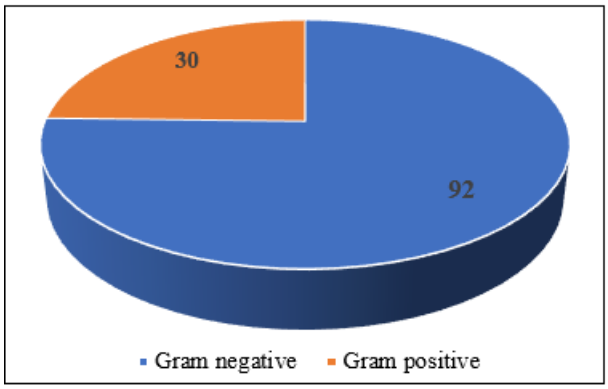

Figure 3: Distribution of Gram-negative and Gram-positive organism

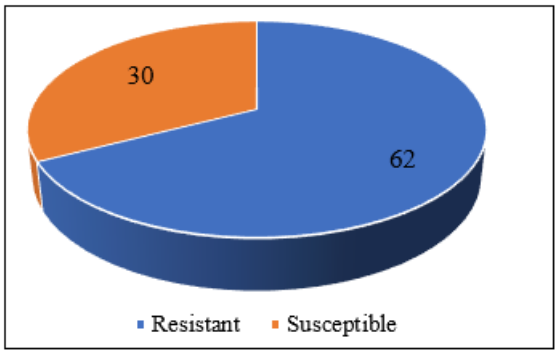

Figure 5: Pattern of antibiotic susceptibility of Gram-negative bacilli to ceftazidime or cefotaxime

Table 1: Distribution of ESBLs among Gram-negative bacilli from urine and exudate samples

Organism	Total	Urine	Resistant	ESBL from urine	Exudate	Resistant	ESBL from exudate
Escherichia coli	36	23	9	2	13	11	1
Pseudomonas aeruginosa	23	3	3	0	20	14	4
Klebsiella pneumoniae	25	6	5	0	19	15	2
Proteus mirabilis	8	0	0	0	8	5	3

Out of the 122 culture positives, 92 (75.4%) were Gram-negative bacilli. Of them, Escherichia coli was seen in most, 36 (39.1%). Out of 92 Gram-negative bacilli, 62 Gram-negative bacilli were resistant to ceftazidime/cefotaxime. Out of 62 resistant bacilli, 12 (13.1%) ESBLs were detected. Most of ESBL10 (83.3%) were from exudates, with the majority 4 (33.3%) being Pseudomonas aeruginosa and the remaining 2 ESBLs were from urine. (Escherichia coli) (Table 1)

Table 2: Distribution of ESBL by age and gender

Age group (in years)	Male	Female	Total
<15	0	0	0
15-29	0	1 (100%)	1 (8.3%)
30-44	2 (66.7%)	1 (33.3%)	3 (25%)
45-59	2 (66.7%)	1 (33.3%)	3 (25%)
≥60	3 (60%)	2 (40%)	5 (41.7%)

Regarding age wise distribution of ESBLs samples, most of the cases were seen in ≥60 years, 5 (41.7%), consisting of 3 (60%) male and 2 (40%) female cases. (Table 2) Regarding gender wise distribution of ESBLs cases, the most, 7 (58.3%) of the cases were seen among males, and Pseudomonas aeruginosa was seen in the majority, 3 (42.9%) of males. (Figure 3)

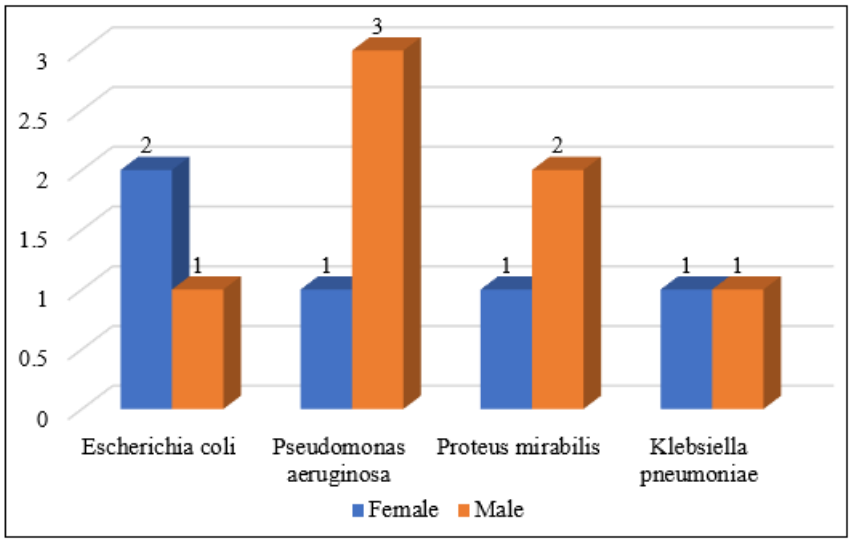

Figure 6: Gender wise distribution of ESBLs

Table 3: Detection of ESBL by different phenotypic methods

Phenotypic method	Detected frequency	Percentage (%)
Total ESBL's detected	12	
Disk potentiation method	11	91.7
Double-disk diffusion method	9	75
Disk-on-disk method	6	50

In this research, a total of 12 ESBL's were identified, while Disk potentiation method detected 11 (91.7%), Double-disk diffusion method detected 9 (75%), and Disk-on-disk method identified only 6 (50%) ESBL's.

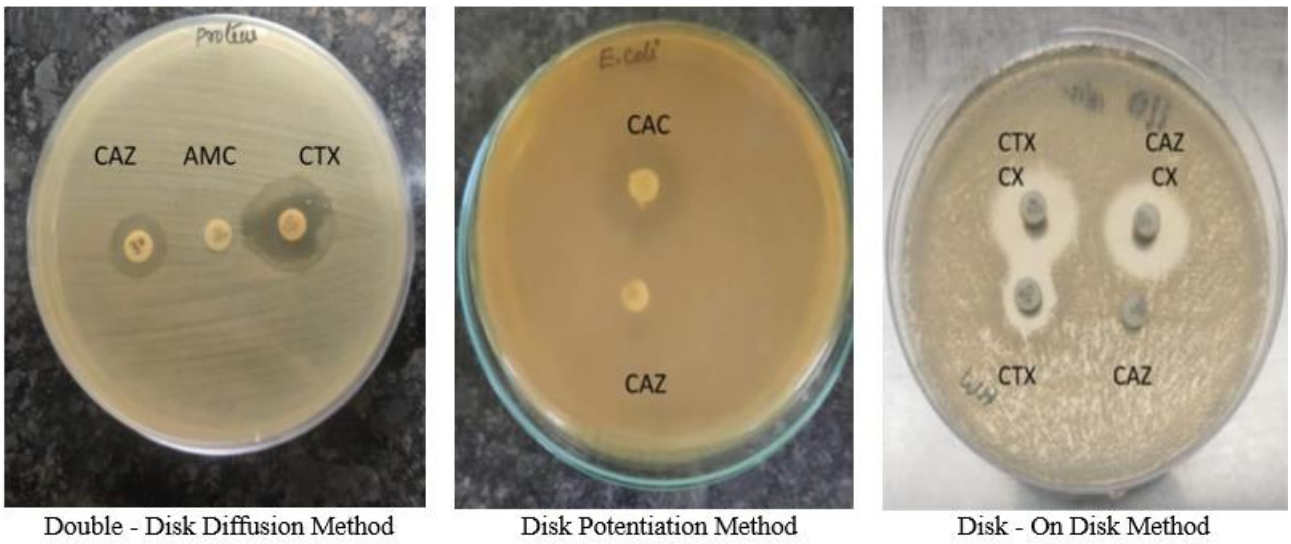

Figure 7: Different phenotypic methods

5. Discussion

The present research was carried out to isolate, identify and study the prevalence of ESBLs producing Gram-negative bacilli from urine as well as exudate samples

Sample distribution

In this study, out of 200 clinical samples, 48.5% were of urine and 51.5% were of exudate samples, while almost same reports were mentioned by Ajimuda OE et al. [16] (50.6% of urine, 20.6% of swab samples). In this study, 53.6% of urine samples were positive, contrast to the studies by Abayneh M et al. [15] (21.6%).

Gender distribution among samples

In this study, the majority, 124 (62%) of the total samples were from females with a female preponderance of 1.63: 1, comparable to the research by Abayneh M et al. [15] (71.4%), Ajimuda OE et al. [16] (55.6%), but contrast to the study by Moges F et al. [20] (54.5% of males)

Bacterial culture

In this study, culture was positive in 122 (61%) of samples, while 78 (39%) of samples were sterile, similar to the studies by Andrews B et al. [18] (52%, 48% respectively), Pavani S et al. [14] (72%, 28% respectively), Abayneh M et al. [15] (21.6%,

79.4% respectively), and Moges F et al. [20] (49.4%, 50.6% respectively)

Bacterial isolates

In this study, Escherichia coli was seen in the majority 36 (39.1%), followed by Klebsiella pneumoniae 25 (27.2%), comparable to the research by Sangeetha KT et al. [22] (46.8%, 31.2% respectively), Abayneh M et al. [15] (85.1%, 14.9% respectively), and Bajpai T et al. [23] (55.3%, 23% respectively).

Ceftazidime or cefotaxime-susceptible and resistant gram-negative bacteria

In the current research, 32.6% of gram-negative organisms were susceptible to ceftazidime or cefotaxime, while Chandramohan et al. showed that Ceftazidime was effective against 5% of the isolates. In the current research, 67.4% of organisms were resistant to ceftazidime or cefotaxime, similar to Abayneh M et al. (70.6%), [15] Ajimuda OE et al. (60.6%). [16]

Sample wise ESBL

In the current research, 10 (83.3%) of ESBLs were from exudate sample and 2 (16.7%) were from urine sample, similar to Sangeetha KT et al. [22] (59.6%, 51.8% respectively), Rudresh SM, and Nagarathnamma T. [27] (70%, 59.1% respectively), but contrast to this finding more ESBLs were reported from urine samples in the studies by Giddi S et al. [28] (12.6%, 65.4% respectively), and Andrews B et al. [18] (32.2%, 44.3% respectively).

Gender wise ESBL

In the current research, most of the ESBL were from males 7 (58.3%), similar to Andrews B et al. [18] (56.4%), Nandagopal B et L. [21] (70.7%), while female preponderance was seen in the studies by Abayneh M et al. [15] (70.6%), Ajimuda OE et al. [16] (56.5%), Patel SC et al. [17] (53.5%), and Shakya P et al. [19] (80.6%).

Age wise ESBL

In the present study, regarding age wise distribution of ESBL cases, the majority 5 (41.7%) of the cases were seen in ≥60 years, similarly most of the cases were distributed in ≥60 years in the studies by Patel SC et al. [17] (63.8%), Andrews B et al. [18] (28.4%), and Shakya P et al. [19] (13.9%).

Sample wise ESBL organisms

In this study, in the exudate sample, the majority of ESBL 4 (40%) was Pseudomonas aeruginosa, contrast to this finding, E. coli was the most found organism in the studies by Giddi S et al. [28] (81.25%), Dalela G [26] (76.9%), and Patel SC et al. [17] (37.5%).

In the urine sample, the majority ESBL found was E. coli (100%), similar to the studies by Giddi S et al. [28] (96.4%), Abayneh M et al. [15] (76.5%), Dalela G [26] (72.2%), and Bajpai T et al. [23] (41.6%), contrast finding was observed in the research by Patel SC et al. [17] (36.4% of E. coli, 47.1% K. oxytoca).

Organism wise ESBL

In the present study, the majority 4 (33.3%) of ESBLs were of Pseudomonas aeruginosa, followed by Escherichia coli 3 (25%), and Proteus mirabilis 3 (25%), while different reports were mentioned in the studies by Abayneh M et al. [15] (76.5% of "E. coli, and 23.5% of K. pneumoniae), Andrews B et al. [18] (45.5% of E. coli, 24.1% of K. pneumoniae, 9.6% of K. oxytoca), Bajpai T et al. [23] (41.6% of E. coli, 36.1% of P. aeruginosa), Dalela G [26] (70.1% of E. coli, 60% of P. vulgaris, 58.1% of K. pneumoniae), and Patel SC et al. [17] (31.5% of E. coli, 10.1% of K. pneumoniae", 0.9% of K. oxytoca)

Detection by different phenotypic methods

In this study, 12 (100%) ESBLs were detected by phenotypic methods. 11 (91.7%) of ESBLs detection was seen by Disk potentiation method, which was comparable to the research carried out by B. Nandagopal et al., [21] 9 (75%) by Double-disk diffusion method, and 6 (50%) by Disk-on-disk method, while Pavani S et al. [14] had different findings (76.4%, 88.2%, 58.8% respectively).

In this study by Double-disk diffusion method 75% detection was shown, while it was different in Sageerabanoo S et al. study., [25] i.e., 53.37% were positive by PCDDT (Phenotypic Confirmatory Disc Diffusion Test) and 45.94% by DDST (Double-Disc Synergy Test).

Prevalence of ESBLs

In the current research, 13.1% of ESBLs were detected, comparable to the research conducted by Subha et al. [24] (6.6%), Shakya P et al. [19] (8%), Abayneh M et al. [15] (23%), but higher prevalence was seen in the studies by Patel SC et al. [17] (32.7%), Pavani S et al. [14] (47.2%), Sageerabanoo S et al. [25] (55.4%), Andrews B et al. [18] (54.8%), and Dalela G [26] (61.6%).

6. Conclusion

Antibiotic susceptibility testing with ESBL detection is essential as this will assist the clinician in prescribing the appropriate antibiotics to prevent the spread of hospital acquired infections. In this study low prevalence of ESBL was observed. This research highlights the requirement for routine monitoring of ESBL production using phenotypic confirmatory tests as it is simple, cost-effective, and less time-consuming. This study also highlights the need for regular as well as ongoing investigation of the resistance against microbes' trend as a key element of the hospital's antibiotic stewardship as well as infection prevention & control programmes to prevent the emergence of antibiotic resistance.

Strength of study

In this study, disk potentiation method, Double-disk diffusion method, and Disk-on-disk methods were done to assess ESBL. This was the strength of the study.

Limitations of the study

In this study the E test was not done, which is the gold standard test and that was the limitation of this study.

Acknowledgement

Thankful to our head of department, other faculty and technical staff for their support

Source of funding
None

Conflict of Interest
None

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