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Research Article

PHENOTYPIC DETECTION OF ESBL AND AMPC BETA LACTAMASES AMONG GRAM-NEGATIVE ISOLATES FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: Drug-resistant Gram-negative bacilli expressing extended-spectrum beta-lactamases (ESBLs) and AmpC pose a serious therapeutic threat in nosocomial infections. Cost-effective screening methods are a boon to patients. This study aims to detect gram-negative bacilli and their antibiotic sensitivity patterns, as well as detect the ESBL and AmpC-producing isolates among Gram-negative bacilli.

Methods: A prospective study was conducted with 150 samples. Gram-negative bacilli were isolated, and their antibiotic sensitivity tests were performed by the Kirby–Bauer disk diffusion method. Potential ESBL producers were screened using Ceftazidime disc, and AmpC producers were screened by Cefoxitin discs by the disc diffusion method. ESBL producers were confirmed by the combined disc diffusion assay method using ceftazidime and ceftazidime/Clavulanic acid disc. AmpC producers were confirmed by the Cefoxitin Cloxacillin Double Disc Synergy Test.

Results: About 38% of 150 samples were gram-negative bacilli, of which 40.35% were *Escherichia coli,* followed by *Pseudomonas aeruginosa* (35.08%). Maximum sensitivity by *E. coli* was found toward imipenem, meropenem, and cotrimoxazole. *P. aeruginosa* showed maximum sensitivity toward piperacillin/tazobactam, imipenem, meropenem, and ceftazidime. 28.07% of Gram-negative isolates were ESBL producers, with *E. coli* (11 isolates) being the maximum, and 15.78% were AmpC producers, with *E. coli* (four isolates) being the maximum. Seven isolates were both ESBL and AmpC producers.

Conclusion: Routine screening and timely reporting of ESBL and AmpC producers help in preventing the spread of multidrug-resistant strains. Antibiotic resistance surveillance helps in the implementation of strict infection control and prevention practices.

Keywords: Extended spectrum beta lactamases, AmpC, Antimicrobial resistance, Infection control.

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INTRODUCTION

Gram-negative bacilli are responsible for causing a wide range of infectious diseases, such as urinary tract infections (UTIs), pneumonia, septicemia, soft-tissue infections, and nosocomial infections [1,2]. Beta lactams were wonder drugs until the emergence of extended-spectrum beta lactamase (ESBL) producers [3].

ESBLs are the most common resistance mechanism among Gram-negative bacteria [4]. ESBLs are plasmid-mediated clavulanate susceptible enzymes hydrolyzing penicillin, expanded-spectrum cephalosporins, and aztreonam [5]. ESBLs are a public health concern as there is increasing colonization among hospitalized and non-hospitalized individuals, highlighting the burden of the problem [4].

AmpC beta-lactamases are clinically important cephalosporinases encoded mostly among members of the enterobacteriaceae [6]. Plasmids not only carry AmpC genes but also ESBL genes in the same plasmid. Organisms producing AmpC beta lactamase will give ESBL screening positive results, but sensitivity to clavulanic acid is decreased. They are resistant to cephamycins but inhibited by cloxacillin instead [3,7]. Overexpression of these enzymes induces resistance to cefoxitin, ceftazidime, and ceftriaxone, posing a threat to therapeutic options for infections caused by *Enterobacter aerogenes* and *Enterobacter cloacae* as these isolates develop resistance upon treatment [6].

These resistance mechanisms are responsible for nosocomial infections around the world [5]. Drug-resistant GNB-expressing ESBLs and AmpC pose a serious therapeutic challenge in treating nosocomial infections due to selection pressure [8]. With added AmpC being prevalent, it is an additional challenge as not only is detection complex, but they also confer resistance to carbapenems with combined decreased outer membrane permeability, leaving therapeutic options sparse for the patient [3].

Although advanced automated and molecular detection methods to identify AmpC enzymes are available, economical and cost-effective methods are still not yet optimized in routine laboratories. Such techniques, if in use, are a boon to patient care by alerting the resistance mechanism at an early stage, with the added benefit of affordability [5].

Hence, this study aims at detecting the ESBL and AmpC-producing gram-negative isolates from clinical samples of a tertiary care hospital.

METHODS

Ethical consideration

Institutional ethical clearance was obtained before the study.

Source of clinical samples

All clinical samples are received in the microbiology laboratory for culture and sensitivity testing.

Study design

This was a prospective study over a period of 2 months.

Sample size

The sample size was 150 samples.

Inclusion criteria
Gram-negative bacilli isolated from clinical samples were included in the study.

Exclusion criteria
Isolates other than gram-negative bacilli were excluded from the study.

Methods
Isolation of gram-negative bacilli from clinical samples

- Various clinical samples received in the microbiology laboratory for culture and sensitivity testing were inoculated on nutrient agar, blood agar, Mac Conkey agar, and CLED agar. The culture plates were incubated at 37°C for 24–48 h.
- Once the growth is obtained, gram-negative bacilli are isolated based on morphology and gram stain.

Detection of the antibiotic sensitivity pattern of gram-negative bacilli

- Identification and antibiotic susceptibility tests were performed by the Kirby Bauer Disc Diffusion Method based on Clinical and Laboratory Standards Institute guidelines as shown in Figs 1-6.
- The following antibiotics were tested for antibiotic susceptibility testing:
  - Enterobacteriaceae: ceftazidime, cefoxitin, ciprofloxacin, gentamicin, amikacin, piperacillin/tazobactam, imipenem, meropenem, cotrimoxazole, nitrofurantoin, and norfloxacin
  - Non–fermenters: ceftazidime, cefoxitin, ciprofloxacin, gentamicin, amikacin, piperacillin/tazobactam, imipenem, meropenem, tobramycin, aztreonam, and nitrofurantoin.
- Isolates resistant to ceftazidime (inhibition zone <17 mm) by the disc diffusion method was considered as potential ESBL producers and tested further.
- Isolates resistant to cefoxitin (inhibition zone <14 mm) by the disc diffusion method were considered as potential AmpC producers and tested further.

Detection of ESBL production [9]

- The ceftazidime-resistant strains were tested for ESBL production by a combined disc diffusion assay using a ceftazidime disc and a ceftazidime/clavulanic acid disc
- The zone diameter difference of >5 mm around the ceftazidime/clavulanic acid disc in comparison to the zone size of the ceftazidime disc was confirmed as an ESBL producer.

Detection of AmpC production [7]

- The cefoxitin-resistant strains were tested for AmpC production by a cefoxitin-cloxacillin double-disc synergy test
- The zone diameter difference of >4 mm around the cefoxitin/cloxacillin acid disc in comparison to the zone size of the cefoxitin disc was confirmed as an AmpC producer.

RESULTS AND DISCUSSION

Table 1 shows that out of 150 samples, 57 (38%) gram-negative bacilli were isolated as shown in Figure 7. 17 isolates (11.33%) were gram-positive cocci, and no bacterial growth was observed in 76 samples (50.66%). Table 2 shows that out of 57 isolates, 32 (56.14%) were males and 25 (43.85%) were females.

Table 3 shows that of the 57 isolates, the maximum isolates were from the age group 41–60 years, followed by 21–40 years (28.07%), and 61–80 years (26.31%).

Table 4 illustrates that out of 57 Gram-negative bacilli, *Escherichia coli* were the maximum isolates (40.35%), followed by *Pseudomonas aeruginosa* with 20 isolates (35.08%). Other gram-negative bacilli isolated were *Klebsiella pneumoniae* 8 (14.03%), *Klebsiella oxytoca* 2 (3.50%), *Escherichia hermanii* 2 (3.50%), *Escherichia vulneris,* and *Proteus vulgaris* 1 each (1.75%). Fig 8 presents Organism wise distribution of Gram-Negative Bacilli.

Table 5 gives the sample-wise distribution of gram-negative bacilli. Of the 57 isolates, 33.33% were urinary isolates, followed by pus or discharge (28.07%). 15.7% of total isolates were from sputum samples,

Table 1: Culture positivity

Isolate	Number (%)
No growth	76 (50.66)
Gram-positive cocci	17 (11.33)
Gram-negative bacilli	57 (38)
Total	150 (100)

Table 2: Gender distribution of gram-negative bacilli

Gender	Number (%)
Male	32 (56.14)
Female	25 (43.85)
Total	57 (100)

Table 3: Age Distribution of gram-negative bacilli

Age (in years) distribution	Number (%)
1–20	7 (12.28)
21–40	16 (28.07)
41–60	17 (29.82)
61–80	15 (26. 31)
81–100	2 (3.5)
Total	57 (100)

Table 4: Organism-wise distribution of gram-negative bacilli

Organism	Number (%)
Escherichia coli	23 (40.35)
Pseudomonas aeruginosa	20 (35.08)
Klebsiella pneumonia	8 (14.03)
Klebsiella oxytoca	2 (3.50)
Escherichia hermanii	2 (3.50)
Escherichia vulneris	1 (1.75)
Proteus vulgaris	1 (1.75)
Total	57 (100)

Table 5: Sample wise distribution of gram-negative bacilli

Sample	Number (%)
Urine	19 (33.33)
Pus/discharge	16 (28.07)
Sputum	9 (15.7)
Swab	7 (12.28)
Tissue	5 (8.77)
ET tube/Secretions	1 (1.75)
Total	57 (100)

Table 6: Department-wise distribution of Gram-negative bacilli

Department	Number (%)
General surgery	16 (28.07)
General medicine	13 (22.80)
ENT	11 (19.29)
ICU	9 (15.78)
Orthopedics	4 (7.01)
Obstetrics and gynecology	4 (7.01)
Total	57 (100)

Table 7: Antibiotic resistance pattern of gram-negative isolates

Antibiotics	*Escherichia* species (%) (n=26)	*Klebsiella* species (%) (n=10)	*Pseudomonas aeruginosa* (%) (n=20)	*Proteus vulgaris* (%) (n=1)
Ceftazidime	18 (69.23)	3 (30)	4 (20)	0 (0)
Cefoxitin	22 (84.61)	3 (30)	16 (80)	0 (0)
Ciprofloxacin	10 (38.46)	1 (10)	10 (50)	0 (0)
Gentamicin	12 (46.15)	4 (40)	8 (40)	0 (0)
Amikacin	13 (50)	7 (70)	9 (45)	0 (0)
Piperacillin/Tazobactam	10 (38.46)	2 (20)	2 (10)	1 (100)
Imipenem	9 (34.61)	2 (20)	4 (20)	1 (100)
Meropenem	9 (34.61)	2 (20)	4 (20)	1 (100)
Cotrimoxazole	9 (34.61)	1 (10)	NA	0 (0)
Tobramycin	NA	NA	8 (40)	NA
Aztreonam	NA	NA	9 (45)	NA
Nitrofurantoin	0 (0)	1 (10)	-	NA
Norfloxacin	7 (26.92)	-	NA	-

Nitrofurantoin and norfloxacin only for urinary isolates

Table 8: ESBL and AmpC isolates distribution among gram negative bacilli

Drug resistant strain	Number (%)
ESBL	16 (28.07)
AmpC	9 (15.78)
Total	25 (43.85)

ESBL: Extended spectrum beta lactamases

Table 9: Sample wise ESBL and AmpC isolates distribution among Gram-negative bacilli

Sample	ESBL: n=16 (%)	AmpC: n=9(%)
Urine	7 (43.75)	2 (22.22)
Pus/discharge	3 (18.75)	4 (44.44)
Sputum	3 (18.75)	2 (22.22)
Swab	3 (18.75)	1 (11.11)
Total	16 (100)	9 (100)

ESBL: Extended spectrum beta lactamases

7 (12.28%) from swabs, and 5 (8.99%) from tissue samples. Table 6 presents the department-wise distribution of gram-negative bacilli. 16 isolates (28.07%) were from the Department of General Surgery, followed by General Medicine 13 (22.80%), ENT 11 (19.29%), ICU 9 (15.78%), Orthopedics 4 (7.01%), and OBGYN 4 (7.01%).

Table 7 presents the antibiotic resistance pattern of Gram-negative isolates. *Escherichia* species showed maximum resistance to cefoxitin (84.61%), followed by ceftazidime (69.23%), amikacin (50%), gentamicin (46.15%), ciprofloxacin, piperacillin/tazobactam (38.46%), imipenem, meropenem, and cotrimoxazole (34.61%). *Klebsiella* species showed maximum resistance to Amikacin (70%), followed by 40% resistance to Gentamicin and 30% of isolates resistant to Ceftazidime and Cefoxitin. The least resistance was toward Ciprofloxacin and Cotrimoxazole (10%).

About 80% of *P. aeruginosa* isolates were resistant to cefoxitin followed by 50% resistant to ciprofloxacin. 45% of isolates were resistant to amikacin and azatreonam. 20% of isolates were resistant to Imipenem, Meropenem, and Ceftazidime. The least resistance of 10% was seen toward piperacillin and tazobactam.

Table 8 and Fig. 9 shows the ESBL and AmpC distribution among Gram – Negative Bacilli. Fig. 10 shows detection of ESBL by combined disk test and Fig. 11 shows AmpC detection by Cefoxitin / Cloxacillin double Disk synergy Test.

Table 9 presents the sample wise distribution of ESBL and AmpC isolates. About 43.75% of ESBL producers were urinary isolates,

Table 10: Organism-wise ESBL and AmpC isolates distribution among Gram-negative bacilli

Organism	ESBL: n (%)	AmpC: n (%)	ESBL+AmpC n=7 (5)
Escherichia coli	11 (68.75)	4 (44.44)	4 (7.01)
Klebsiella species	3 (18.75)	2 (22.22)	2 (3.50)
Pseudomonas aeruginosa	2 (12.5)	3 (33.33)	1 (1.75)
Total	16 (100)	9 (100)	57 (100)

ESBL: Extended spectrum beta lactamases

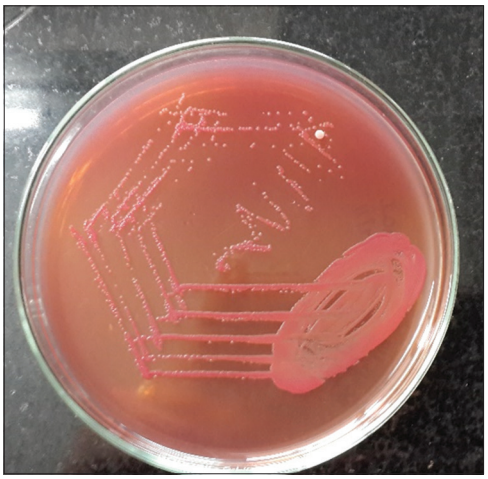

Fig. 1: *Escherichia coli* on MacConkey agar

Fig. 2: Biochemical reactions of *Escherichia coli*

Fig. 3: *Klebsiella* species on MacConkey agar

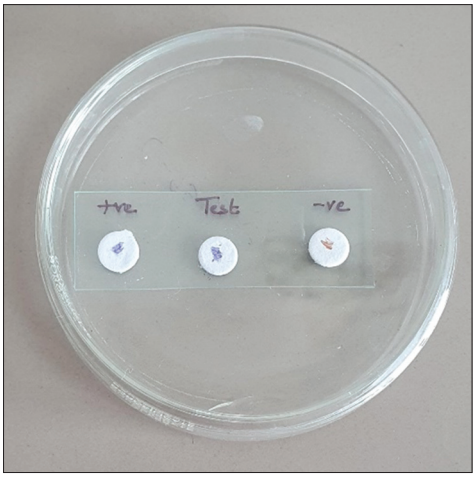

Fig. 6: Oxidase test

Fig. 4: Biochemical reactions of *Klebsiella* pneumonia

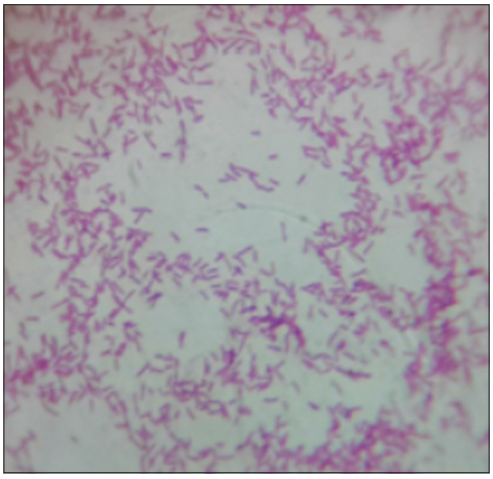

Fig. 7: Gram-negative bacilli

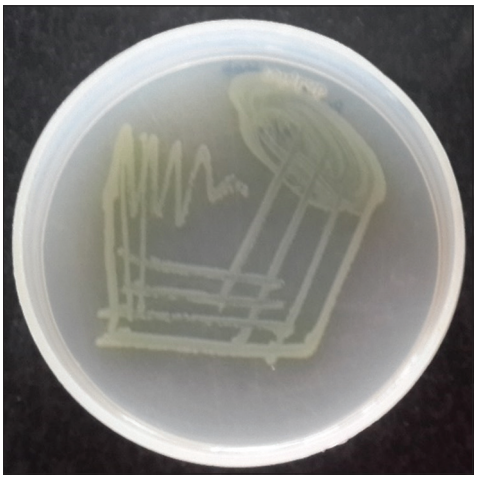

Fig. 5: *Pseudomonas aeruginosa* on nutrient agar

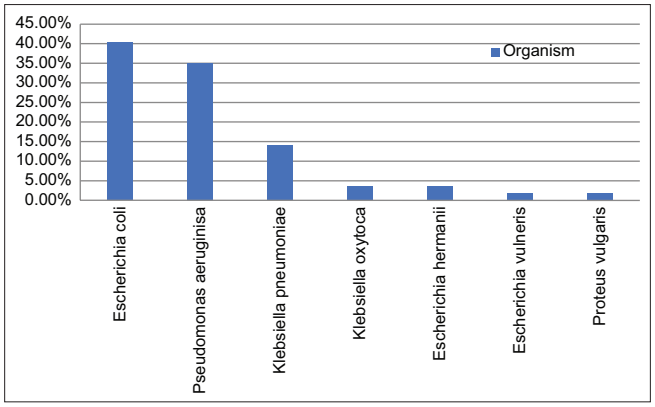

Fig. 8: Organism-wise distribution of Gram-negative bacilli

followed by isolates from pus, discharge, sputum, and swabs (18.75%). About 44.44% of AmpC producers were isolated from pus and discharge, followed by urinary isolates and sputum (22.22%). Sample wise distribution of ESBL and AmpC have been illustrated in Fig. 12.

Table 10 presents the organism-wise ESBL and AmpC isolates. Among the 16 ESBL producers, 11 isolates (68.75%) were *Escherichia* species, 3 (18.75%) were *Klebsiella* species, and 2 (12.5%) were *P. aeruginosa*. Among the 9 AmpC producers, 4 isolates (44.44%) were *Escherichia* species, 3 (33.33%) were *P. aeruginosa*, and 2 isolates (22.22%) were *Klebsiella* species. Among the 57 Gram-negative isolates, 7 isolates (12.28%) were both ESBL and AmpC producers.

The present study was carried out in the Department of Microbiology over a period of 2 months. Out of 150 samples received, 57 (38%) gram-negative bacilli were isolated. Similar isolation rates were reported by Nair and Vaz [10] (36.27%), but higher isolation rates were reported by Mishra *et al.* [11] (84%). There was a higher male pre-ponderance

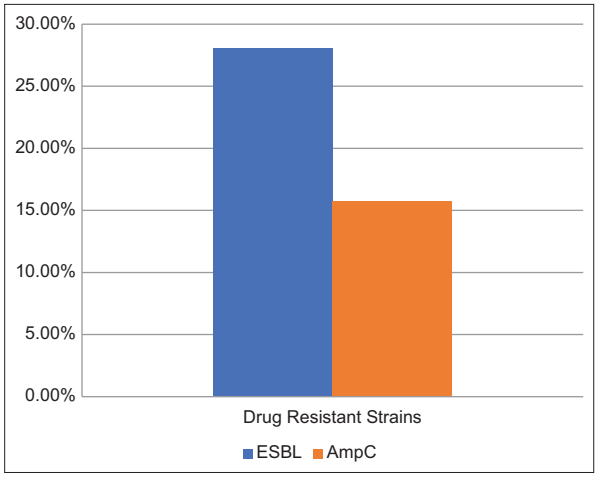

Fig. 9: Extended spectrum beta lactamases and AmpC isolates distribution among Gram-negative bacilli

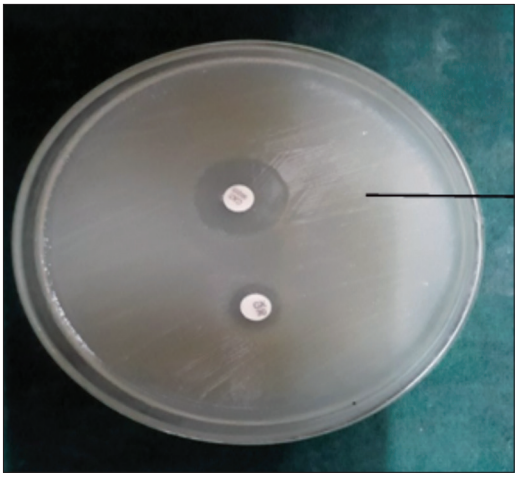

Fig. 11: Amp C detection by cefoxitin/cloxacillin double disc synergy test

Fig. 10: Extended spectrum beta lactamases detection by combined disk test

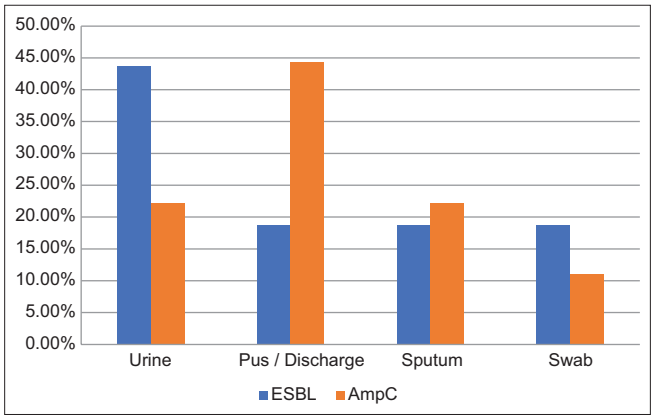

Fig. 12: Sample-wise extended spectrum beta lactamases and AmpC isolates distribution among Gram-negative bacilli

in our study of 56.14%. Of the 57 isolates, maximum isolates were from the age group 41–60 years, followed by 21–40 years (28.07%), and 61–80 years (26.31%).

Out of 57 Gram-negative bacilli, *E. coli* had the maximum isolates (40.35%), followed by *P. aeruginosa* with 20 isolates (35.08%). Other Gram-negative bacilli isolated were *Klebsiella* species 10 (17.03%), similar to findings by Inamdar and Anuradha [7], who reported *E. coli* as the most common isolate. Similar findings were reported by Shipra and Chaudhary [9] with *E. coli* (41.6%) and *K. pneumoniae* (32%). Garbati *et al*. [12], on the contrary, reported *K. pneumoniae* as the most common Gram-negative bacilli isolated (52.8%), followed by *E. coli* (22.98%).

Of the 57 isolates, 33.33% were urinary isolates, followed by pus or discharge (28.07%). 15.7% of total isolates were from sputum samples, 7 (12.28%) from swabs, and 5 (8.99%) from tissue samples.

In our study, Escherichia species showed maximum resistance to Cefoxitin (84.61%), followed by Ceftazidime (69.23%), Amikacin (50%), Gentamicin (46.15%), Ciprofloxacin, and Piperacillin/Tazobactam (38.46%). The least resistance was for imipenem, Meropenem, and Cotrimoxazole (34.61%). *Klebsiella* species showed maximum resistance to Amikacin (70%), followed by 40% resistance to Gentamicin and 30% of isolates resistant to Ceftazidime and Cefoxitin. The least resistance was toward Ciprofloxacin and Cotrimoxazole (10%).

About 80% of *P. aeruginosa* isolates were resistant to cefoxitin followed by 50% resistant to ciprofloxacin. About 45% of isolates were resistant to amikacin and azatreonam. About 20% of isolates were resistant to Imipenem, Meropenem, and Ceftazidime. The least resistance of 10% was seen toward Piperacilli/Tazobactam. Resistance to third-generation cephalosporins by *P. aeruginosa* was 20%, compared to 46.11% reported by Vinita *et al*. [13] Resistance to the antipseudomonal penicillins+β-lactamase inhibitor combination was reported as low as 10%, in contrast to 53% reported by Vinita *et al*. [13].

In our study, 25 out of 57 isolates (43.85%) tested positive on screening for ESBL, similar to Shipra and Chaudhary [9] (45.2%). Only 28.07% of total isolates were confirmed as ESBL producers by the Combined Disc Test using Ceftazidime and Ceftazidime/Clavulanic Acid discs. Similar rates were reported by Chanu *et al*. [14] (26.3%) and Ibadin *et al*. [15] (21%). In contrast, high ESBL rates were reported by Shipra and Chaudhary [9] (45.2%), Pramodhini *et al*. [16] (47.6%), Shayan and Bokaeian [8] (41.11%), Oberoi *et al*. [17] (35.1%), and Kolhapure *et al*. [18] (38.5%).

In our study, 44 isolates (77.19%) tested positive by screening for AmpC using a cefoxitin disc. On confirmation with the CC–DDS method, only 9 isolates (15.78%) were found to be confirmatory for AmpC production. Inamdar and Anuradha [7] reported that 57% of isolates were resistant to cefoxitin, and on confirmation by the CC–DDS method, 47.5% of isolates were confirmed as AmpC producers. Similar rates to our study were reported by Pramodhini *et al*. [16] (20.4%), Shayan and Bokaeian [8] (13.6%), Kolhapure *et al*. [18] (10.3%), and Chanu *et al*. [14] (9.7%). Lower AmpC rates were reported by Ibadin *et al*. [15] (7.8%). Oberoi *et al*. [17] (5.4%).

Among the 57 Gram-negative isolates, 7 isolates (12.28%) were both ESBL and AmpC producers. Lower rates of co-production of ESBL and AmpC were reported by Kolhapure *et al*. [18] (9.7%), Chanu *et al*. [14] (5.7%), Oberoi *et al*. [17] (6.5%), Pramodhini *et al*. [16] (3.9%), and Ibadin *et al*. [15] (2.9%).

Among the 16 ESBL producers in our study, 11 isolates (68.75%) were Escherichia species, 3 (18.75%) were *Klebsiella* species, and 2 (12.5%) were *P. aeruginosa*. Shayan and Bokaeian [8] reported 62.7% of ESBL isolates as *E. coli*; Vinita *et al*. [13] reported 43.47% of ESBL producers among *P. aeruginosa*.

Among the 9 AmpC producers, 4 isolates (44.44%) were Escherichia species, 3 (33.33%) were *P. aeruginosa*, and 2 isolates (22.22%) were *Klebsiella* species in our study. Inamdar and Anuradha [7] reported higher 76.2% AmpC producers as *E. coli* and similar (20.1%) *K. pneumoniae* AmpC producers. A study by Shayan and Bokaeian [8] reported lower rates of ESBL producers among *E. coli* (13.6%). Vinita *et al*. [13] reported 21.73% of *P. aeruginosa* isolates as both ESBL and AmpC producers.

Despite the discovery of ESBLs and AmpC β lactamase in the past few decades, the problem still exists in the optimal phenotypic detection methods in routine laboratory testing. The failure to detect the enzymes, along with the various risk factors, has led to the uncontrolled spread of AMR, with therapeutic failure as a bad outcome [5].

Antimicrobial drug resistance with negative side effects and multiple toxicities has paved the way for novel drugs. Nanoantibiotics are a new type of antibiotic to combat the drug resistance problem [19]. With novel drugs like BAL30072 (monosulfactam antibiotic), Ceftolozane with tazobactam, and Delafloxacin (fluoroquinolones), which destroy gram-negative bacilli and are effective against ESBL producers, they help in treating ventilator-associated pneumonia, complicated UTIs, and complicated intra-abdominal infections [20].

CONCLUSION

Timely reporting of such ESBL and AmpC strains will help in preventing the spread of multidrug resistance isolates and also improve the clinical management of patients suffering from infections caused by drug-resistant organisms.

Antibiotic resistance surveillance plays a major role among the strategies to curb the problem of antimicrobial resistance worldwide [9]. Routine screening will help in the implementation of strict infection control and prevention practices. Regular environmental surveillance of ESBL and AmpC-producing strains should be done to know the prevalence of such strains and their changing trends. Such promising antimicrobials are under clinical development, and the scope of combination regimens opens up the way to delay the development of antimicrobial resistance [20].

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AUTHORS' CONTRIBUTIONS

Dasari Bhavana worked as the principal investigator and contributed to the literature survey, acquiring approval, drafting the proposal, conducting the study, and data collection. Shabnum Musaddiq has contributed as a co-investigator and played a key role in methodology, data analysis, reviewing the project, manuscript preparation, revision, and submission of the article. K. Saileela has played a key role in conceptualizing and reviewing the project and final endorsement of the version to be published.

CONFLICT OF INTERESTS

None.

AUTHORS FUNDING

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