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Original Research Article

Phenotypic detection of ESBL and Amp C beta lactamase among clinical isolates of *Enterobacteriaceae*

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ABSTRACT

Introduction: Emergence of ESBL & amp C β-Lactamase enzymes among clinical isolates of *Enterobacteriaceae* poses a significant public health concern. Antimicrobial agents like 3^{rd} generation cephalosporins, clavulanic acid, imipenem etc. can induce amp C enzyme production. Sometimes the automated antimicrobial susceptibility testing systems fails to detect Amp C phenotype due to presence of multiple resistance mechanisms in gram negative bacilli. To overcome such difficulty, the study was undertaken to detect the presence ESBL and Amp C enzymes among clinical isolates of *Enterobacteriaceae* by phenotypic methods.

Materials and Methods: *Enterobacteriaceae* strains isolated from various clinical samples were included in the study. Strains resistant to two or three groups of antibiotics (MDR) were further tested for the presence of ESBL enzyme by combination disk method. Amp C enzymes were detected by cefoxitin-cloxacillin disc method.

Results: A total 1059 *Enterobacteriaceae* strains were isolated from various clinical samples. Out of these, 170 MDR strains were further processed. ESBL enzymes were detected in 104 (61%) strains and Amp C in 35 (20.5%) strains. 26(15.2%) strains were co-producers.

Conclusion: Detection of ESBL and Amp C enzymes will help clinician in choosing the right antimicrobial treatment for the patient. Routine reporting of presence of Amp C & ESBL enzymes by phenotypic methods can be easily implemented by clinical microbiology laboratory. Cefoxitin-cloxacillin disk test is simple & rapid method for detection of Amp C β- lactamase enzyme.

1. Introduction

Organisms that are resistant to one agent from any three or more antibiotics classes are considered as multi drug resistant organisms (MDRO).[1] The infections caused by MDR organisms are increasing gradually and pose a threat to treatment thereby leading to extended period of hospitalization, increased cost of health care and increased rate of morbidity and mortality.[2]

Common resistance mechanisms found in gram negative bacilli are production of β – lactamase enzymes like Amp C β-lactamase and extended spectrum β - lactamase (ESBL).[3].Extended spectrum beta lactamases (ESBL) can hydrolyze antibiotic groups like first, second, third generation cephalosporins and monobactams (aztreonam). These enzymes are inhibited by beta-lactam inhibitors such as clavulanic acid or tazobactam. They do not affect carbapenems or cephamycin.[4]

Plasmid mediated Amp C β-lactamase enzymes are resistant to penicillin, narrow spectrum of cephalosporins and cephamycins.[5]

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Infections associated with ESBL producing organisms can be treated with third or fourth generation cephalosporins combined with clavulanic acid.[2] However, if organism is an Amp C enzyme producer along with ESBL, patient fails to respond to clavulanic acid combination. In fact, clavulanic acid induces the production of Amp C enzymes and causes more harm. Such patients should be treated with cefepime or carbapenems.[1]

Sometimes the automated antimicrobial susceptibility testing systems fail to detect the presence of Amp C phenotype as gram negative bacilli possess several different resistance mechanisms. Hence it becomes difficult to guide clinicians regarding appropriate antimicrobial therapy. To overcome such difficulty, this study was undertaken to detect the presence of ESBL and Amp C enzymes among clinical isolates of *Enterobacteriaceae* by phenotypic methods.

2. Materials and Methods

2.1. Study design

A descriptive cross-sectional study was performed in the department of microbiology of a tertiary care hospital in Pune from March 2022 to April 2023. Clinical samples such as body fluids, pus, sputum, urine, blood culture, CSF, etc yielding growth of gram- negative bacilli from *Enterobacteriaceae* family were included in the study.

2.2. Sample size

Sample size was calculated based on the prevalence of ESBL among Enterobacteriaceae as reported by Kaup S et al (54.79%).[2] Total 170 strains of *Enterobacteriaceae* showing resistance to two or more classes of antimicrobials were further processed.

2.3. Method

Body fluids, CSF, blood samples were received in automated blood culture bottles (automated BD Bactec FX 40). Routine examinations of urine samples and cytology of CSF/ body fluids were reported. Gram's stain findings of pus, sputum samples were noted. Samples were inoculated on blood agar and MacConkey's agar. Colonies were further processed as per standard microbiological methods**.** The identification of organism was done by automated identification system (Vitek 2 compact- Biomerieux, Pvt Ltd-GN 405 card). Antimicrobial susceptibility (AST) was done by Kirby Baur's method as per CLSI guidelines (2022) and by automated AST (Vitek2 compact system).[6] Antibiotics like ceftazidime (30 μg), ceftazidime- clavulanic acid (30 μg / 10 μg), ceftriaxone (30 μg), cefoxitin (30 μg), cefoxitin-cloxacillin (30 μg/200 μg) and cefepime (30 μg) were tested by disc diffusion method. Strains showing resistance to Second/ third generation cephalosporins and any other class of antimicrobial were further tested for presence of ESBL and Amp C enzymes. All strains were preserved in Trypticase Soy Broth at -20°c for future studies.

2.3.1. Screening test for ESBL

The isolate was tested further for ESBL if . . .

1. Zone size of ceftazidime <22mm
2. Zone size of ceftriaxone < 25mm [as per CLSI guidelines 2022].[6]

2.3.1.1. Phenotypic confirmation of ESBL by double disk synergy test-. As per CLSI guidelines, 0.5 McFarland's suspension of test organism was inoculated on Muller Hinton agar. Antibiotic discs like ceftazidime-clavulanic acid (30 μg/10 μg) and ceftazidime (30 μg) were placed on the agar 30mm apart.[6,7] After incubation for 24 hours at 37°C, zone size was measured. An increase in zone diameter between ceftazidime and ceftazidime-clavulanic acid by >5mm was considered positive for ESBL production (Figure 1).[8]

2.3.2. Screening test for AmpC

The isolate was further tested for AmpC if . . .

1. Zone size of cefoxitin <18mm(Resistant).
2. Zone size of cefepime >25mm (susceptible).[9]

2.3.2.1. Phenotypic confirmation of AmpC by cefoxitin-cloxacillin double disk synergy test-. A 0.5 McFarland's suspension of test organism was inoculated on Muller Hinton agar. Cefoxitin (30 μg) and cefoxitin-cloxacillin (30 μg/200 μg) disks were placed 30mm apart. After 24 hours of incubation the zone diameter was measured. If there was increase in zone diameter between cefoxitin and cefoxitin-cloxacillin by >4mm then the test was considered positive for Amp C production (Figure 2).[10]

2.3.2.2. Modified three dimensional tests (MTDT)-. All Amp C positive strains were confirmed by MTDT. A 0.5 McFarland's suspension of ATCC *E.coli* (25922) strain was inoculated on Muller Hinton agar. Cefoxitin disk (30 μg) was placed in the middle and linear slits of 3cm were made 3mm away from the disk. Around 8-10 similar colonies of test organism were inoculated in the slits with a sterile wire loop and kept for incubation at 37°C for 24 hours. Distortion of zone surrounding the cefoxitin disk it was taken as positive for Amp C production (Figure 3).[11]

2.3.3. Phenotypic detection of ESBL and AmpC Co-production:

Presence of co-producers was confirmed if both observations were present in a strain.

1. Increase in zone diameter between ceftazidime and ceftazidime-clavulanic acid by >5mm.

2. Increase in zone diameter between cefoxitin and cefoxitin- cloxacillin by >4mm (Figure 4).[8,10]

2.3.3.1. Control strains used were- . Negative control: ATCC *E.coli* 25922.

Positive control: ATCC *K.pneumoniae* 700603.

In-house positive strain was used as positive control for Amp C.

2.4. Ethical approval

The study was approved by Institutional ethical committee (Approval number- BVDUMC/IEC/122)

2.5. Statistical analysis

Statistical analysis was done and data are presented as numbers and their percentages.

3. Results

A total 1059 *Enterobacteriaceae* organisms were isolated from various clinical samples within one year period of study. Out of these, 170 strains were MDR. These strains were further tested for presence of further tested for presence of enzymes.

The majority of samples were received from hospitalized patients (112/170 i.e. 65.8%). The most common isolated organism was *E.coli* (69.4%), followed by *K.pneumoniae* (22.9%). Sample wise distribution of MDR *Enterobacteriaceae* is shown in Graph 1.

3.1. Antimicrobial susceptibility testing

E.coli strains were most susceptible to colistin (100%) followed by tigecycline (96.6%), fosfomycin (94.9%), amikacin (88.1%), and meropenem (85.5%). *K pneumoniae* strains were most susceptible to colistin (94.8%), followed by tigecycline (84.6%), amikacin (53.8%), and fosfomycin (51.2%). Table 1 shows AST pattern of isolates.

3.2. Detection of enzymes

ESBL was detected in total 104 (61%) strains by confirmatory method as mentioned by CLSI.[8]

Out of 170 MDR strains, Amp C production was observed in 35 (20%) strains. All Amp C producing strains gave positive results by Modified Three-Dimensional test. A total 26 (15.2%) strains were co-producers (produced Amp C as well as ESBL).

Common ESBL producing organisms were E.coli (74%) followed by *K.pneumoniae* (19%). [Graph 2] AST pattern of ESBL producing strains is shown in Table 2.

The commonest organism producing Amp C was *E.coli* (68.5%), followed by *Enterobacter cloacae* 4% [Graph 3] AST pattern of Amp C producing strains is shown in Table 3.

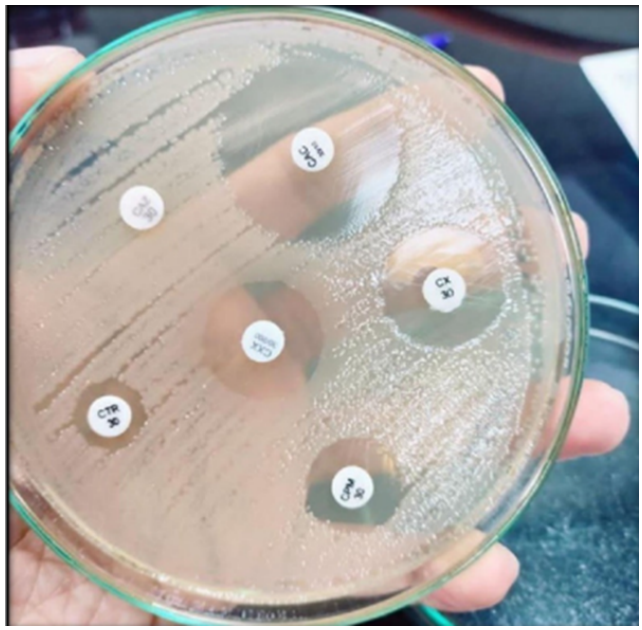


Figure 1: Detection of ESBL by disk potentiation method. Increase in zone size of ceftazidime -clavulanic acid (CAC) >5mm as compared to ceftazidime (CAZ).

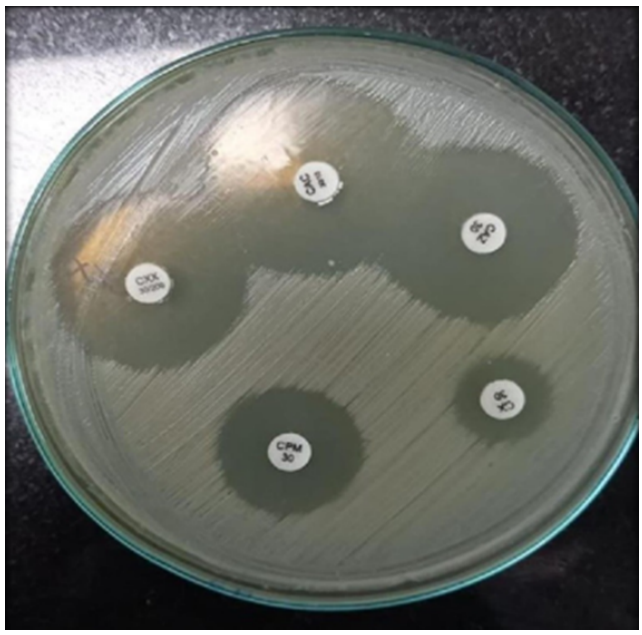


Figure 2: Detection of AmpC enzyme by cefoxitin-cloxacillin disk method, Increase in zone size of cefoxitin-cloxacillin (CXX) > 4mm as compared to cloxacillin disk (CX)

Table 1: Antimicrobial susceptibility pattern of MDR isolates (% susceptible)

Antimicrobial agents	*E.coli* (n=118)	*K.pneumoniae* (n=39)	*Enterobacter cloacae* (n= 7)	*Morganella morganii* (n=1)	*Citrobacter freundii* (n=2)	Proteus mirabilis (n=1)	Serratia marcescens (n=2)
Amoxicillin-clavulanic acid	50 (42.8%)	8 (20.5%)	0	0	0	1	IR
Piperacillin-tazobactam	78 (66.1%)	12 (30.7%)	3	1	1	1	0
Cefuroxime	0	0	0	0	0	0	0
Ceftriaxone	0	0	0	0	0	0	0
Cefoperazone-sulbactam	82 (69.4%)	14 (35.8%)	4	1	2	1	1
Cefepime	12 (10.1%)	0	3	0	2	0	1
Meropenem	101 (85.5%)	16 (41%)	4	1	2	1	1
Amikacin	104 (88.1%)	21 (53.8%)	4	1	2	0	1
Gentamicin	81 (68.6%)	21 (53.8%)	4	1	2	0	1
Ciprofloxacin	4 (3.38%)	2 (5.1%)	3	0	2	0	1
Tigecycline	114 (96.6%)	33 (84.6%)	7	IR	2	IR	IR
Fosfomycin	112 (94.9%)	20 (51.2%)	4	0	2	1	2
Colistin	118 (100%)	37 (94.8%)	7	IR	2	IR	IR
Cotrimoxazole	37 (31.3%)	8 (20.5%)	4	0	2	0	1

*IR – Intrinsic Resistance

Table 2: AST pattern of ESBL producing *E.coli* and *Klebsiella pneumoniae* (% susceptible)

Anti-microbial agents	*E.coli* (77)	K. pneumoniae (20)
Amoxicillin-clavulanic acid	39 (50.6%)	6 (30%)
Piperacillin-tazobactam	59 (76.6%)	8 (40.%)
Cefuroxime	0	0
Ceftriaxone	0	0
Cefoperazone-sulbactam	61 (79.2%)	8 (40.%)
Cefepime	8 (10.3%)	1 (5%)
Meropenem	74 (96.1%)	12 (60%)
Amikacin	73 (94.8%)	14 (70.%)
Gentamicin	56 (72.7%)	11 (55.%)
Ciprofloxacin	3 (3.8%)	2 (10%)
Tigecycline	74(96%)	16 (80%)
Fosfomycin	76 (98.7%)	11 (55.%)
Colistin	77 (100%)	19 (95%)
Cotrimoxazole	27 (35.06%)	6 (30%)

4. Discussion

β-lactam antibiotics are frequently prescribed class of antibiotics.[12] Bacteria have developed resistance to these antibiotics due to synthesis of β-lactamases.[13] Most penicillins and cephalosporins belonging to the third and second generation were precisely developed to withstand the hydrolyzing activity of significant β-lactamases. But as different classes of β-lactams were developed, resistance mechanisms also evolved through emergence of new β-lactamases.[14]

The prevalence of multi drug resistant *Enterobacteriaceae* in the present study was 16.05%. Similar findings were observed by Eline et al in Netherlands (18.2%) and Rehab et al in Kuwait (13.6%).[15,16]

In the present study, commonly isolated organisms were *E.coli* (69.4%) and *K.pneumoniae* (22.9%). Setgen et al, have observed that, *E.coli* (61.2%) and *K.pneumoniae* (15.8%) were common MDR organisms causing urinary tract infections in Ethiopia.[17]

E.coli strains were susceptible to colistin (100%), tigecycline (96.6%), fosfomycin (94.9%), amikacin (88.1%)

Table 3: Antimicrobial susceptibility pattern of AmpC producing strains (% susceptible)

Anti-microbial agents	*E.coli* (24)	K. pneumoniae (2)	*Enterobacter cloacae* (4)
Amoxicillin- clavulanic acid	4 (16.6%)	0	3
Piperacillin- tazobactam	12 (50%)	1	3
Cefuroxime	0	0	0
Ceftriaxone	0	0	0
Cefoperazone- sulbactam	15 (62.5%)	1	4
Cefepime	6 (25%)	0	2
Meropenem	23 (95.8%)	1	4
Amikacin	22 (91.6%)	2	4
Gentamicin	15 (62.5%)	2	3
Ciprofloxacin	0	0	3
Tigecycline	21 (87.5%)	1	4
Fosfomycin	23 (95.8%)	1	3
Colistin	24 (100%)	2	4
Cotrimoxazole	9 (37.5%)	1	3

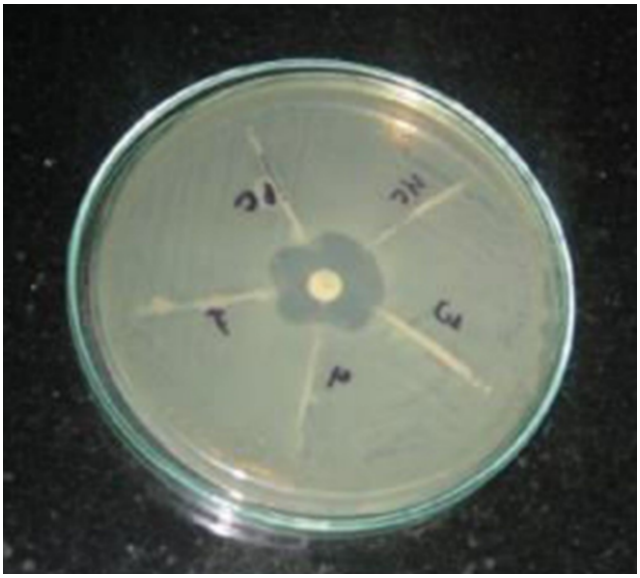


Figure 3: Modified three dimensional test for AmpC enzyme, Strain 1 & 2 positive 3-borderline positive

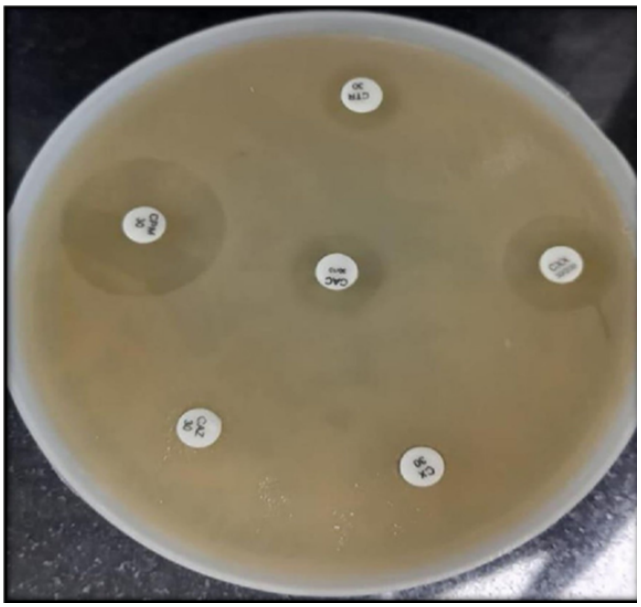
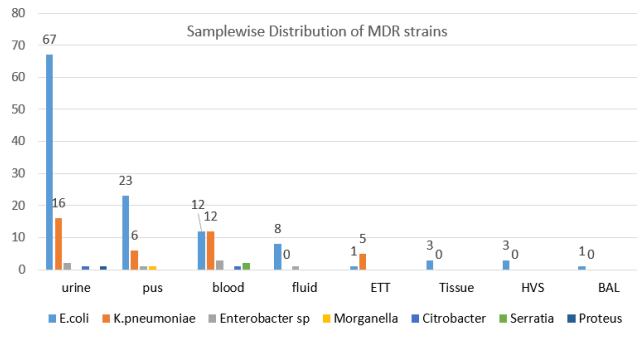
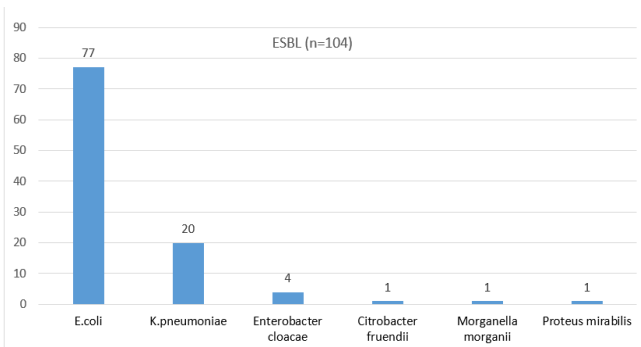


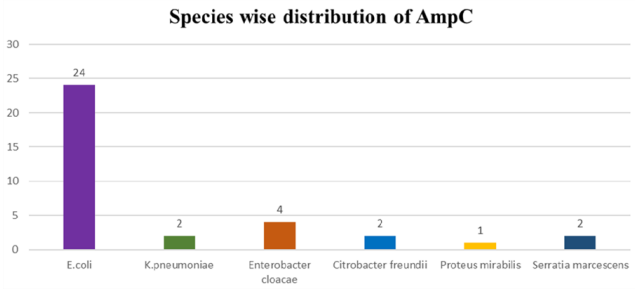
Figure 4: ESBL and AmpC co-producer, Increase in zone size of cefoxitin-cloxacillin, Ceftzidime-clavulanic acid and cefepime-suceptible



Graph 1: Sample wise distribution of MDR *Enterobacteriaceae* species



Graph 2: Species wise distribution of ESBL producing *Enterobacteriaceae*



Graph 3: Species wise distribution of Amp C producing *Enterobacteriaceae*

and meropenem (85.5%). Similar findings were given by Balaji et al (2019). *E.coli* isolates from their study, were susceptible to colistin (97-100%), followed by meropenem (67-89%), and amikacin (27-88%).[18]

In the present study, the prevalence of ESBL producing *Enterobacteriaceae* was 61%. John et al have al reported 62% prevalence of ESBL producing *Enterobacteriaceae* in Uganda (2015).[19] Marit et al in (2016) reported that, the prevalence of fecal carriage of ESBL producing organism was 34.3% in children suffering from diarrhea. The prevalence of fecal carriage of ESBL was more among hospitalized children (50.4%) than children from community (11.6%).[20]

In the present study, detection of ESBL enzyme was done by combination disk method as recommended by CLSI. In a study conducted by Dejenie et al, a total 265 isolates were positive for ESBL production by screening method. Out of these strains, 246 (92.8%) strains were confirmed by combination disk test and 224 (84.5%) were confirmed by double disk synergy test.[21]

ESBL producing *E.coli* strains were most susceptible to colistin (100%), followed by fosfomycin (98.7%), tigecycline (96%), meropenem (96%), and amikacin (94.8%). Mohamad et al (2021) have also found that, ESBL producing strains were mostly susceptible to imipenem (99.2%), meropenem (97.2%) and amikacin (97.9%).[22]

The prevalence of AmpC was 20% in the present study. Common organisms producing this enzyme were *E.coli* (68.5%) and *Enterobacter cloacae* (11.4%). Similar findings were recorded by Pandithage et al in Sri Lanka (2022).[23]

We preferred cefoxitin-cloxacillin double disk synergy test for determining the presence of Amp C enzyme. It is a cheaper method and easy to perform in hospital-based laboratory.[9] Microbiologists can confirm the presence of Amp C enzymes on the same day of AST reporting. All Amp C positive strains were confirmed by modified three-dimensional test (MTDT). But MTDT is cumbersome to perform and requires technical skill. Maraskhole et al (2014) have detected Amp C enzymes in 135 strains (out of 2933) by cefoxitin screen method. The isolates were further confirmed by cefoxitin- cloxacillin double disk synergy test. A total 91.85% strains gave positive results with 500μg cloxacillin disk and 90.37% strains gave positive results with 300 μg cloxacillin disk. All these strains were also confirmed by Modified three-dimensional test (MTDT).[24]

In this study, Amp C producing *E.coli* strains were most susceptible to colistin (100%), followed by meropenem (95.8%), fosfomycin (95.8%), amikacin (91.6%) and tigecycline (87%). Reza et al have also reported similar finding in 2018.[25]

In our study, 26 (15%) strains produced both the enzymes. In a study conducted by Uma et al (2021) in Haryana, 27% of *E.coli* isolates were co-producers.[26]

ESBL producing strains are inhibited by clavulanic acid or tazobactam when combined with beta lactam drugs.[4] Organisms producing Amp C enzymes respond well to cefepime and carbapenems.[5] Out of 26 Amp C producing *E.coli* strains, 20 (76.9%) strains were co-producers. So, susceptibility to cefepime was poor (25%) among Amp C positive strains in our study. Presence of multiple resistance mechanisms in gram negative bacilli can result in therapeutic failure. Such strains can be treated only with higher antimicrobial drugs like carbapenem or colistin.[1] To avoid overuse of higher antimicrobials like carbapenem, colistin, ceftazidime avibactam-aztreonam etc, it is important to detect enzymes like ESBL, AmpC and carbapenemases and report it to clinician along with automated AST reports. This will help clinician to choose the right drug for treatment and avoid overuse of higher antimicrobials.

5. Conclusion

Organisms producing ESBL and Amp C enzymes are difficult to treat with cephalosporins due to their resistance mechanisms. Hence, they are treated with higher level of antibiotics like carbapenems, aminoglycosides, tigecycline, fosfomycin and beta-lactam beta- lactamase inhibitor drugs.

Certain antimicrobial agents like third generation cephalosporins, clavulanic acid, imipenem etc. are capable of inducing Amp C enzyme production. So, these antimicrobials will produce more harm than benefit if the organism is ESBL and AmpC Co-producer. Therefore, detection of these phenotypes will help the clinician in choosing the right antimicrobial treatment for the patient. Routine reporting of presence of Amp C and ESBL enzymes by phenotypic methods can be easily implemented in clinical microbiology laboratory. Cefoxitin-cloxacillin disk test is a useful, rapid, and easy method for detection of Amp C beta lactamase enzyme by phenotypic method in clinical microbiology laboratory.

6. Limitations of study

Co-production of ESBL and/or amp C enzymes along with carbapenemase was not detected. Confirmation of ESBL and/or amp C enzymes was not done by molecular method.

7. Conflict of Interest

Authors declare there is no conflict of interest.

8. Source of Funding

None.

9. Acknowledgement

None.

References

1. Salvia T, Dolma KG, Dhakal OP, Khandelwal B, Singh LS. Phenotypic Detection of ESBL, AmpC, MBL, and Their Co-occurrence among MDR Enterobacteriaceae Isolates. *J Lab Physicians*. 2022;14(3):329–35.
2. Kaup S, Roopashree S. Prevalence of various Beta-lactamases in Enterobacteriaceae in a tertiary care hospital in South India: A Cross-sectional study. *IP Int J Med Microbiol Trop Dis*. 2021;7(3):186–91.
3. Giddi S, Dasari S, Suryakumari C. Prevalence of Extended Spectrum Beta- Lactamase Producers among Various Clinical Samples in a Tertiary Care Hospital: Kurnool District, India. *Int J Curr Microbiol Appl Sci*. 2017;6(8):2857–63.
4. Bush K, Jacoby GA. Updated functional classification of β-lactamases. *Antimicrob Agents Chemother*. 2010;54(3):969–76.
5. Mandell D, B. 2015.
6. James-II L. M100 Performance Standards for Antimicrobial. Clinical and Laboratory Standards Institute; 2022.
7. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β- lactamase production in Enterobacteriaceae: Review and bench guide. *Clin Microbiol Infect*. 2008;14(Suppl 1):90–103.
8. Naseer F, Iqbal R, Ikram N, Shoaib M, Javaid M, Mehmood RT, et al. Phenotypic cofirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended spectrum beta lactamase (ES*B*L) producing Uropathogens. *J Appl Biotechnol Bioeng*. 2017;3(3):344–9.
9. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Böttger EC, Hombach M, et al. Practical Approach for Reliable Detection of AmpC Beta-Lactamase-Producing Enterobacteriaceae. *J Clin Microbiol*. 2011;49(8):2798–803.
10. Khari FIM, Karunakaran R, Rosli R, Tay ST. Genotypic and phenotypic detection of AmpC β- lactamases in Enterobacter spp. Isolated from a teaching hospital in Malaysia. *PLoS One*. 2016;11(3):e0150643. doi:10.1371/journal.pone.0150643.
11. Shahid M, Malik A, Agrawal M, Singhal S. Phenotypic detection of extended-spectrum and AmpC β-lactamases by a new spot-inoculation method and modified three-dimensional extract test: Comparison with the conventional three-dimensional extract test. *J Antimicrob Chemother*. 2004;54(3):684–7.
12. Bronson JJ, Barrett JF, et al. Quinolone, everninomycin, glycylcycline, carbapenem, lipopeptide and cephem antibacterials in clinical development. *Curr Med Chem*. 2001;8(14):1775–93.
13. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. *Indian J Med Microbiol*. 2006;24(3):208–11.
14. Jacoby GA, Medeiros AA. More extended-spectrum beta-lactamases. *Antimicrob Agents Chemother*. 1991;35(9):1697–704.
15. Van Dulm E, Tholen ATR, Pettersson A, Van Rooijen MS, Willemsen I, Molenaar P, et al. High prevalence of multidrug resistant Enterobacteriaceae among residents of long term care facilities in Amsterdam, the Netherlands. *PLoS One*. 2019;14(9):222200. doi:10.1371/journal.pone.0222200.
16. Alajmi RZ, Alfouzan WA, Mustafa AS. The Prevalence of Multidrug-Resistant Enterobacteriaceae among Neonates in Kuwait. *Diagnostics (Basel)*. 2023;13(8):1505. doi:10.3390/diagnostics13081505.
17. Eshetie S, Unakal C, Gelaw A, Ayelign B, Endris M, Moges F, et al. Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. *Antimicrob Resist Infect Control*. 2015;4:12. doi:10.1186/s13756-015-0054-7.
18. Veeraraghavan B, Walia K. Antimicrobial susceptibility profile & resistance mechanisms of Global Antimicrobial Resistance Surveillance System (GLASS) priority pathogens from India. *Indian J Med Res*. 2019;149(2):87–96.
19. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC Pharmacol Toxicol*. 20152;16:14. doi:10.1186/s40360-015-0013-1.
20. Tellevik MG, Blomberg B, Kommedal Ø, Maselle SY, Langeland N, Moyo SJ, et al. High Prevalence of Faecal Carriage of ESBL-Producing Enterobacteriaceae among Children in Dar es Salaam, Tanzania. *PLoS One*. 2016;11(2):e0168024. doi:10.1371/journal.pone.0168024.
21. Teklu DS, Legese MH, Eshetu K, Negeri AA, Fentaw S, Addis T, et al. omparison of Double Disk Synergy Test and Combination Disk Test Methods for the Detection of Extended-Spectrum Beta-Lactamase Production among Enterobacteriaceae. *EC Microbiology*. 2019;15(6):411–20.
22. Gharavi MJ, Zarei J, Roshani-Asl P, Yazdanyar Z, Sharif M, Rashidi N, et al. Comprehensive study of antimicrobial susceptibility pattern and extended spectrum beta-lactamase (ESBL) prevalence in bacteria isolated from urine samples. *Sci Rep*. 2021;11(1):578. doi:10.1038/s41598-020-79791-0.
23. Perera P, Gamage S, De-Silva HSM, Jayatilleke S, Silva N, Aydin A, et al. Phenotypic and genotypic distribution of ESBL, AmpC β-lactamase and carbapenemase-producing Enterobacteriaceae in community-acquired and hospital-acquired urinary tract infections in Sri Lanka. *J Glob Antimicrob Resist*. 2022;30:115–22. doi:10.1016/j.jgar.2022.05.024.
24. Maraskolhe DL, Deotale VS, Mendiratta DK, Narang P. Comparision of three laboratory tests for detection of Ampc β lactamases in klebsiella species and E. Coli. *J Clin Diagn Res*. 2014;8(6):5–8.
25. Ghotaslou R, Sadeghi MR, Akhi MT, Hasani A, Asgharzadeh M. Prevalence and Antimicrobial Susceptibility Patterns of ESBL, AmpC and Carbapenemase-producing Enterobactericeae Isolated from Hospitalized Patients in Azerbaijan. *Iran J Pharm Res*. 2018;17:79–88.
26. Chaudhary U, Agarwal S, Raghuraman K. Identification of extended spectrum beta lactamases, AmpC and carbapenemase production among isolates of Escherichia coli in North Indian tertiary care centre. *Avicenna J Med*. 2018;8(2):46–50.

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