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# BIOLOGICAL SCIENCES

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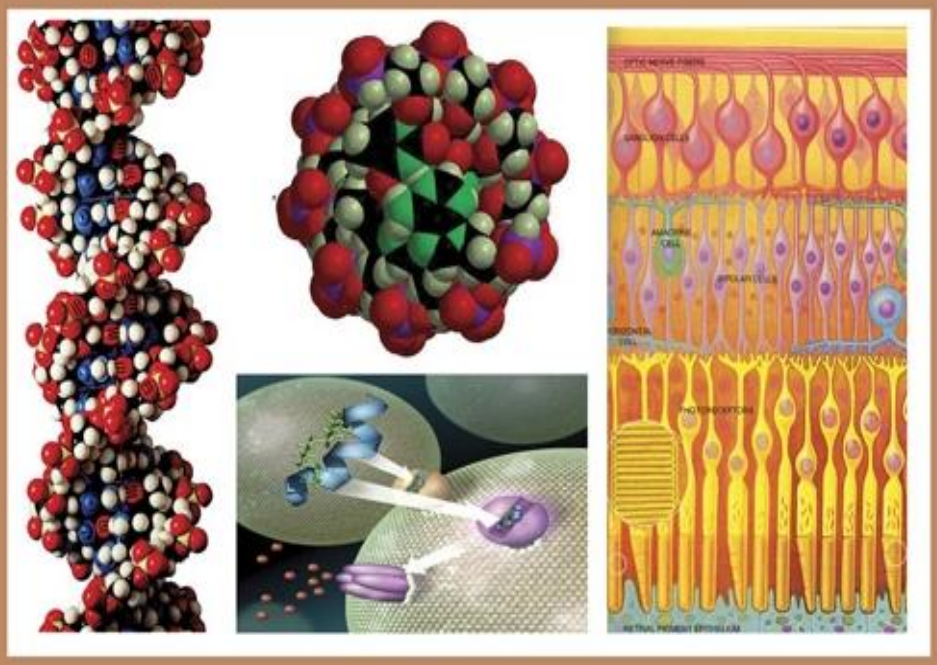

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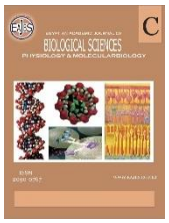

# Increase AmpC co-producer ESBL phenotypic detection by using Tazobactam with Cefepime Increase AmpC co-producer ESBL Phenotypic Detection

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**ABSTRACT**

**Introduction:** Isolates that co-produce an inhibitor-resistant beta-lactamase like AmpC are unsuitable for clavulanate and third-generation cephalosporin phenotypic techniques to detect ESBL.**Aim:** Cefepime and tazobactam improve AmpC co-producer ESBL phenotypic identification.**Materials and Methods:** In total, one hundred isolates of *Escherichia coli* (40), *Klebsiella spp*. (30), *Proteus spp* (20), and *Enterobacter spp* (10) were analysed. These isolates were obtained from urine and pus samples taken from Lady Reading Hospital Peshawar, Pakistan over the course of three months, beginning in January 2022 and continuing through March 2022. Both ESBL and AmpC production in the isolated bacteria was investigated at the same time. The synthesis of AmpC was demonstrated through a modified three-dimensional test (MTDT). The findings of the classic double disc synergy test, the phenotypic disc confirmatory test (PDCT), and the modified double disc synergy test (MDDST) were compared to determine whether ESBL production was present.**Results:** The improved three-dimensional test verified AmpC synthesis in 38 (38.1%) of 100 positive isolates. ESBL production was verified in 100 of the 82 ESBL-positive isolates. 20 (20%) DDST identified ESBLs. MDDST ESBL-positive isolates were discovered by cefotaxime and ceftazidime + clavulanate, which detected 60 (66.6%) and 10 isolates, respectively. 20 (10.5%) MDDST-detected ESBL producers were AmpC co-producers. DDST found no ESBL in AmpC-positive isolates, however, MDDST found 15 with co-production. Cefepime detected ESBL with AmpC production best in MDDST. In 10 (10.3%) isolates with piperacillin-tazobactam, amoxicillin-clavulanate synergized. In AmpC co-producers, third-generation cephalosporins cefotaxime, ceftazidime, and cefpodoxime did not detect ESBL**Conclusion:** ESBL detection is improved by piperacillin-tazobactum-cefepime double disc synergy testing.

## INTRODUCTION

Extended-spectrum beta-lactamases, often known as ESBLs, and AmpC beta-lactamases are becoming an increasing source of worry for both microbiologists and clinicians. ESBLs and AmpC beta-lactamases transmit resistance to many antibiotics, which can result in unsuccessful therapy. *Klebsiella species* are the most common suspects in their production. *Escherichia coli*, although it might also happen in other types of gram-negative bacteria. Extended-spectrum beta-lactamases, or ESBLs, are generally plasmid-mediated enzymes that hydrolyze penicillins, third-generation cephalosporins, and aztreonam (Pfaller, *et al.*, 2006).

They are vulnerable to beta-lactamase inhibitors, even though they are inactive against cephamycins (cephoxitin and cefotetan) and (clavulanic acid). AmpC co-production complicates ESBL phenotypes. Cephalosporinases known as AmpC -lactamases are distinguished from extended-spectrum beta-lactamases (ESBLs) by their capacity to hydrolyze cephamycins. Clavulanic acid has only a moderate effect on AmpC -lactamases, making this kind of enzyme resistant to its inhibition (Fortineau,, *et al.,* 2001). Whereas the genes that code for ESBLs are found on plasmids, the genes that code for AmpC -lactamases can be encoded either on plasmids or in the chromosomes. *Proteus mirabilis spp., Enterococcus spp., Corynebacterium freundii, Micrococcus morganii,* and *Streptomyces* were all examples of bacteria that include the chromosomally encoded AmpC gene. There is a possibility that chromosomal AmpC expression might be either constitutive or inducible (Chaudhary *et al.,* 2004). There is evidence that *K. pneumoniae, E. coli, Salmonella species,* and *Klebsiella species* all have plasmid-mediated AmpC. At first, it was believed that plasmid-mediated AmpC genes were not inducible; nevertheless, it has since been discovered that there are inducible Amp C genes on plasmids to change a cephalosporin report from susceptible or intermediate to resistant. If the isolate is shown to produce ESBLs and the test is performed (Thomson *et al.,* 2001). However, the identification of ESBLs in AmpC co-producing bacteria has proven troublesome for both the microbiologists and the clinicians who are trying to assist direct patients to the right antibiotic medication (Mohanty *et al.,* 2010). A pressing need arises, then, for the creation of laboratory testing procedures that can reliably identify the presence of these enzymes The majority of laboratories, especially in poor countries, lack the resources to perform the molecular procedures necessary for reliable ESBL detection. Different phenotypic methods have been suggested for routine use to find out if gram-negative bacteria make ESBLs. These utilise a -lactamase inhibitor, often clavulanate, in conjunction with third-generation cephalosporins (3GC) such as ceftriaxone, ceftazidime, and ceftaxime (Mohanty *et al.,* 2010). But the confirmatory test based on inhibitors works best for isolates that don't make a beta-lactamase like AmpC that is resistant to inhibitors.ESBL detection may be obscured by large levels of AmpC generation. In addition, clavulanate may operate as an inducer of high-level AmpC, resulting in false-negative ESBL identification due to an increase in resistance to screening agents. As a cure for this issue, tazobactam and sulbactam, which are considerably less likely to produce AmpC -lactamases, are chosen as inhibitors for ESBL detection (Mohanty *et al.,* 2010). Whereas cefepime, which is of the fourth generation of cephalosporins, is a superior choice as an indicator medication (Modi *et al.,* 2012). In this study, the original double disc synergy test and the phenotypic disc confirmatory test were compared with the modified double disc synergy test (MDDST) using cefepime as an indicator drug and piperacillin-tazobactam as an inhibitor for ESBL detection. Cefepime is a more reliable detection agent for ESBLs in the presence of AmpC -lactamases because it is minimally affected by high-level (Modi *et al.,* 2012).

## MATERIALS AND METHODS

The research was carried out at the Lady Reading hospital in Peshawar, Pakistan, under the department of microbiology. The institution's ethics committee gave its stamp of approval to the research project. A total of one hundred isolates of *Escherichia coli* (40), *Klebsiella spp* (30), *Proteus spp* (20), and *Enterobacter spp* (10) were analyzed. These isolates were obtained from urine and pus samples taken from hospitalised patients over the course of three months, beginning

in January 2022 and continuing through March 2022.

**Antimicrobial Susceptibility Testing:**

The antimicrobial susceptibility of the isolates was evaluated using the disc diffusion technique in accordance with CLSI recommendations. Cefotaxime (40 µg), cefpodoxime (30 µg), ceftriaxone (20 µg), cephoxitin (40 µg), gentamycin (15 µg), amikacin (30 µg), ciprofloxacin (15 µg), norfloxacin (20µg), nitrofurantoin (80µg), cotrimoxazole (25µg), piperacillin/tazobactam (120/10 µg). All antibiotics were tested using Mueller–Hinton agar.

**Test for ESBL Production:**

To test for ESBL generation, we chose all the strains that had a diameter of less than 30 mm for cefotaxime and less than 20 mm for ceftriaxone.

Phenotypic confirmatory disc diffusion test (PCDDT). This test was carried out in accordance with the recommendations provided by CLSI with the utilisation of control strains of *Escherichia coli* ATCC26913 beta-lactamase negative) and *Klebsiella pneumoniae* ATCC801190 (ESBL positive). Ceftazidime or cefotaxime tested in combination with clavulanic acid showed an increase in zone diameter greater than or equal to A >=5mm. when compared to the zone diameter measured when each antibiotic was tested alone (Manchanda *et al.*, 2012).

**Modified Double Disk Synergy Test:**

The original DDST was adapted to identify ESBLs in AmpC co-producers by separating the discs of cefepime and piperacillin-tazobactam by 20-40mm. Discs of cefpodoxime, ceftazidime, cefotaxime, and cefepime were inserted at distances varying from 17 mm to 25 mm from MHA, along with a disc of augmentin. Positive results from any of the three confirmatory tests (PDCT, DDST) for ESBL generation were considered conclusive evidence that the isolates in question produced ESBLs (Kateregga, *et al.,* 2012).

**Detection of AmpC β-lactamases Modified three –dimensional test (MTDT):**

Following an initial screening with cephoxitin (20 g) disc, the presence of AmpC -lactamases was determined by MTDT. Screen positive for AmpC -lactamase synthesis was determined for isolates with a cefoxitin zone diameter <18 mm. The MHA test organism was cultured overnight in a clean microcentrifuge tube. The bacterial mass was pelleted by centrifugation at 3000 rpm for 15 minutes after peptone water was added. A crude enzyme extract was made by rapidly freezing and thawing the bacterial pellet for around 10 cycles. After preparing a grass culture of E. coli ATCC26913 on MHA plates, we added a cefoxitin disc (20 µg) to the top of the media. A sterile surgical blade was used to make linear incisions 3 cm long, stopping 3 mm from the edge of the cefoxitin disc. Using a sterile Pasteur pipette, we drilled 8-millimeter-diameter wells into the slits, starting 5 millimetres in from the slit's outermost edge. Enzyme extract was added to the wells in 10 mL intervals until they were completely stocked. It is estimated that 30-40µl of the extract was deposited in the well. Overnight, the plates were kept in a 37°C incubator. There were three distinct types of findings. AmpC-producing isolates were selected among those that exhibited pronounced changes in the cefoxitin inhibition zone. AmpC non-producer isolates had no distortion, whereas AmpC producer isolates had minor distortion. We utilised a *Klebsiella pneumoniae* strain as a standard since we know it to be AmpC-positive s (Kateregga, *et al.*, 2012).

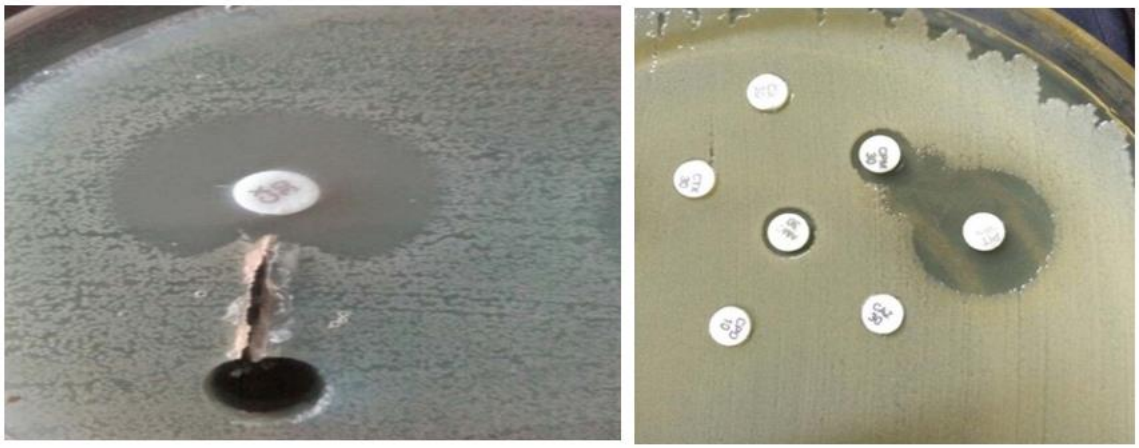
## RESULTS

A total of 100 isolates obtained from urine and pus cultures included *Escherichia coli (40), Klebsiella pneumoniae* (30), and *Proteus spp.* (20) *Enterobacter spp.* (10). All the isolates were screened for both ESBL and AmpC β-lactamase production. Among these, 43 (43.4%) isolates *Escherichia coli* (20), *Klebsiella pneumoniae* (13*), Proteus spp.*

(6) *Enterobacter spp.* (4), were screened positive for ESBL and 47 isolates (47.4%) *Escherichia coli (20), Klebsiella pneumoniae (17), Proteus spp. (4) Enterobacter spp.* (6) were screened positive for AmpC. Of these 43 isolates screened for ESBL, (43.4%) *Escherichia coli* (20/100,20.2%), *Klebsiella pneumoniae* (13/100,13.7%), *Proteus spp.* (6/100, 6%) *Enterobacter spp.* (4/100, 4.4%), were confirmed positive by either of the confirmatory tests used i.e., PDCT, DDST, or MDDST [Table/Fig 1]. ESBL-positive isolates with cefotaxime and cefotaxime+clavulanate detecting 58 and ceftazidime and ceftazidime+ clavulanate detecting additional 6 isolates. AmpC production was detected in 47 (47.1%) isolates by modified three-dimensional test *Escherichia coli (40/100 41.2%), Klebsiella pneumoniae (30/100, 32.8%), Proteus spp. (10/100, 10%) Enterobacter spp. (9/100, 9.1%)* [Table/Fig-1, 2]. All the 20 isolates which were additionally detected ESBL producers by MDDST showed positive three-dimensional tests.

**Table/Fig-1:** Distribution of ESBLs and AmpC in the isolates

| Organisms | Total isolated | ESBL +ve | AmpC +ve |
|---|---|---|---|
| Escherichia coli | 40 | 20 (40.2%) | 20(41.2%) |
| Klebsiella | 30 | 13(13.7%) | 17(17.8%) |
| Proteus | 20 | 6 (6%) | 4 (4%) |
| Enterobacter | 10 | 4 (4.4%) | 6 (6.1%) |
| Total | 100 | 162(66.1%) | 113(46.1%) |



Table/Fig-1]: Positive Modified three-dimensional test (Enhanced growth of surface organism at the point where slit inserted the zone of inhibition of cefoxitin). [Table/Fig-2]: MDDST showing synergism of only cefepime and piperacillin.

## DISCUSSION

Since its initial identification more than a decade ago, ESBL-producing microbes have been the subject of ever-increasing levels of alarm. Various studies conducted in Asia have indicated a prevalence incidence ranging from 55–85 percent. Research that was conducted in India found that ESBL production was present in 72% of the isolates. There is a possibility that up to 42% of Escherichia coli and up to 50% of *Klebsiella pneumoniae* isolates in Latin America are positive for ESBL (Kateregg *et al.,* 2012). Systemic infections brought on by ESBL-producing *Enterobacteriaceae* were linked to extremely detrimental clinical outcomes. ESBLs were first described in a restricted number of bacteria, such as *Escherichia coli* and *Klebsiella spp* but they have now expanded to additional genera, primarily Enterobacter and Proteus spp. More than 200 ESBLs have

been identified based on the physical qualities they possess, and it has been discovered that clavulanic acid, sulbactam, and tazobactam are able to suppress the activity of these ESBLs. This characteristic is one of the factors that contribute to their identification in the laboratory. However, due to the development of numerous enzymes, such as inhibitor-resistant ESBL variants, plasmid-borne AmpC, and the creation of ESBLs in AmpC-producing bacteria, ESBL phenotypes have recently gotten more complicated. These phenotypes typically display multidrug resistance, which may or may not be picked up by standard susceptibility testing. The inability of clinical laboratories to detect such complex resistance phenotypes is a serious challenge that they are currently facing. It is possible that this inability was a major factor in the uncontrolled spread of ESBL-producing organisms and the treatment failures that have been associated with this phenomenon. It is possible that organisms that produce AmpC serve as covert repositories for extended-spectrum beta-lactamases. Therefore, regular detection of ESBL synthesis in these organisms by clinical microbiology laboratories is significant for epidemiological and therapeutic reasons. The increase in zone width in DDST caused by ESBL producers can be entirely hidden by AmpC -lactamases since they are resistant to -lactamase inhibitors like clavulanic acid. Modifications of DDST, such as the use of cefepime and piperacillin-tazobactam together, or the use of AmpC enzyme inhibitors such as boronic acid compounds, cloxacillin, and new inhibitors such as Syn2190, have been suggested for detecting ESBLs in isolates that co-produce AmpC-lactamase. (Kaur *et al.,* 2012). have emphasised the use of cefepime discs in DDST for the detection of ESBLs in Enterobacter species. This novel version of the double-disk test (MDDT) utilising a combination of cefepime (FEP) and piperacillin-tazobactam (TZP) was assessed to identify ESBLs and was reported to be the most sensitive test (detected 56.1%) for ESBL detection in AmpC co-producers. A successful three-dimensional test with cefoxitin indicates cefoxitin hydrolysis and distinguishes between AmpC generation and decreased outer membrane permeability. In our investigation, 68.2% of isolates were resistant to cefoxitin, whereas 42% of three-dimensional tests were positive. Intriguingly, MDDST was positive in all 18 isolates with a positive three-dimensional test, i.e., co-producers of AmpC and ESBL. Cefepime was the most effective cephalosporin for identifying ESBL in the presence of AmpC because it is less susceptible to AmpC -lactamases. Cefotaxime and ceftazidime were unable to demonstrate any zone enhancement in the presence of a lactamase inhibitor in AmpC co-producers because the AmpC -lactamase produced a zone of resistance to the cephalosporin. Different Indian investigations have reported prevalence rates of AmpC-producing strains (10-80%) depending on geographic area, antimicrobial susceptibility pattern, and detection method (phenotypic or genotypic). India and worldwide are reporting more enterobacteriaceae co-producing AmpC and ESBLs.Turkey found ESBL in 46%, AmpC in 3%, and both in 38%.

**Conclusion**

Since these organisms frequently generate numerous lactamases, the prevalence of ESBLs in clinical isolates can easily be overestimated with the current CLSI-approved procedures i.e. DDST and PDCT. Since AmpC -lactamases can inhibit clavulanate synergy, it may be possible to improve ESBL detection by adapting double disc synergy tests that include piperacillin-tazobactam and cefepime.

**REFERENCES**

Chaudhary, U., and Aggarwal, R. (2004). Extended spectrum β-lactamases (ESBL)–an emerging threat to clinical therapeutics. *Indian journal of medical microbiology, 22*(2): 75-80.

Fortineau, N., Poirel, L., and Nordmann, P. (2001). Plasmid-mediated and inducible cephalosporinase DHA-2

from Klebsiella pneumoniae, *Journal of Antimicrobial Chemotherapy*, *47*(2): 207-210.

Kateregga, J. N., Kantume, R., Atuhaire, C., Lubowa, M. N., and Ndukui, J. G. (2015). Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC pharmacology and Toxicology*, *16*(1): 1-6.

Kaur, J., Mahajan, G., and Chand, K. (2016). Enhancing phenotypic detection of ESBL in AmpC co-producers by using cefepime and tazobactam. *Journal of clinical and diagnostic research: JCDR*, *10*(1): DC05.

Manchanda, V., and Singh, N. P. (2003). Occurrence and detection of AmpC β-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. *Journal of Antimicrobial Chemotherapy*, 51(2): 415-418.

Modi, D., Patel, D., Patel, S., Jain, M., Bhatt, S., and Vegad, M. M. (2012). Comparison of various methods for the detection of extended spectrum beta lactamase in Klebsiella pneumoniae isolated from neonatal Intensive Care Unit, Ahmedabad. *National journal of medical research*, *2*(03): 348-353.

Mohanty, S., Gaind, R., Ranjan, R., and Deb, M. (2010). Use of the cefepime-clavulanate ESBL Etest for detection of extended-spectrum beta-lactamases in AmpC co-producing bacteria. *The Journal of Infection in Developing Countries*, *4*(01): 024-029.

Mohanty, S., Gaind, R., Ranjan, R., and Deb, M. (2010). Use of the cefepime-clavulanate ESBL Etest for detection of extended-spectrum beta-lactamases in AmpC co-producing bacteria. *The Journal of Infection in Developing Countries*, 4(01): 024-029.

Pfaller, M. A., and Segreti, J. (2006). Overview of the epidemiological profileand laboratory detection of extended-spectrum β-Lactamases. *Clinical Infectious Diseases*, 42(Supplement_4): S153-S163.

Swenson, J. M., Patel, J. B., and Jorgensen, J. H. (2011). Special phenotypic methods for detecting antibacterial resistance. *Manual of clinical microbiology*, 1155-1179.

Thomson, K. S., Sanders, C. C., and Moland, E. S. (1999). Use of microdilution panels with and without β-lactamase inhibitors as a phenotypic test for β-lactamase production among Escherichia coli, Klebsiella spp., Enterobacter spp., Citrobacter freundii, and Serratia marcescens. *Antimicrobial agents and chemotherapy*, *43*(6): 1393-1400.