Section: Microbiology

Original Research Article

AmpC PRODUCING KLEBSIELLA: THE SILENT RISE OF A DIFFICULT-TO-TREAT PATHOGEN

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ABSTRACT

Background: The emergence of AmpC β-lactamase-producing Klebsiella spp. is an increasing concern due to its role in antimicrobial resistance and therapeutic failure.

Materials and Methods: A cross-sectional study was conducted in the Department of Microbiology at M.G.M.M.C., Indore, from July 2024 to September 2024, to detect AmpC β-lactamase production in Klebsiella isolates from pus samples. A total of 100 Klebsiella isolates were included. Antimicrobial susceptibility testing was performed according to CLSI guidelines, and screening for AmpC production was done using cefoxitin resistance. Confirmatory phenotypic methods included Double Disc Synergy Test (DDST) and Disc Approximation (DA) test. Out of 100 isolates, 38% were cefoxitin-resistant and screened positive for AmpC production. Among these, 45% were confirmed by DDST and 32% by DA test, indicating higher sensitivity of DDST. The study also demonstrated significant multidrug resistance, with the highest resistance observed against ceftazidime (91%) and comparatively lower resistance to levofloxacin (38%).

Results: A total of 100 klebsiella isolates were obtained from 797 pus samples processed, which yielded 312 Gram negative bacterial isolates.

Conclusion: AmpC β-lactamase production is prevalent among Klebsiella isolates and contributes to increased resistance to β-lactam antibiotics. Simple, cost-effective phenotypic methods like DDST and DA test can be effectively used for routine detection, aiding in appropriate antimicrobial therapy and infection control.

Keywords: Klebsiella isolates, AmpC β-lactamase, β-lactam antibiotics, Phenotypic methods.

INTRODUCTION

Klebsiella species is an important member of the family Enterobacterales and has emerged as one of the major causes of healthcare-associated infections worldwide. In recent years, Klebsiella species has gained significant clinical importance because of the rapid emergence and dissemination of multidrug-resistant (MDR) strains.[1]

Antimicrobial resistance among Klebsiella species is a major global health concern. Increasing resistance to β-lactam antibiotics has limited treatment options and increased morbidity, mortality, and healthcare burden. Therefore, accurate detection and surveillance of AmpC β-lactamase-producing isolates are essential for effective therapy and infection control.[2]

AmpC β-lactamase-producing organisms are difficult to detect because they may appear susceptible on routine phenotypic tests. This can lead to false susceptibility reports and treatment failure. Due to the increasing global spread and geographic variation of AmpC enzymes, early and accurate detection is essential for proper antimicrobial therapy, epidemiological surveillance, laboratory diagnosis, and infection-control measures.[3] Considering the increasing prevalence

of AMR and the limited data regarding AmpC β-lactamase-producing isolates, continuous surveillance and accurate detection of these resistant pathogens are essential for guiding appropriate antimicrobial therapy, implementing effective infection-control measures, and preventing further dissemination of resistance in healthcare settings.[4]
Phenotypic confirmatory test for AmpC β-lactamases production is Phenyl boronic acid method, Cefoxitin Cloxacillin- Double disc synergy test, AmpC TRIS EDTA disc test, Disc approximation test (Induction based method). Pseudo-susceptibility seen in AmpC producers leads to resistance to Extended-spectrum cephalosporins resulting in inappropriate antimicrobial regimens and therapeutic failure.[5] We are comparing the Phenotypic methods like the Double Disc Synergy Test (DDST) and Disc Approximation Test using Cefoxitin and Cefoxitin/Cloxacillin because, these tests are effective for routine detection in clinical microbiology laboratories.[3]

MATERIALS AND METHODS

This Cross-sectional study was conducted in the Department of Microbiology, M.G.M.M.C., Indore. The study carried out over a period of three months from July 2024 to September 2024. A total of 100 klebsiella isolates obtained from pus sample received in the laboratory were included in the study.

Study aim: To detect AmpC β-lactamases producing klebsiella species.

Inclusion criteria:

Only Klebsiella isolates in pus samples.

Exclusion criteria

Repeat sample from same patient.

Procedure: All Pus samples received were processed as per standard guidelines and Antimicrobial Susceptibility Test for all Klebsiella isolates was performed as per CLSI M-100-ED34. These isolates were screened for Cefoxitin resistant (zone diameter<18mm).7 All screen positive Cefoxitin resistant isolates were subjected to DDST & Disc approximation test.

Disc approximation test (Induction based method): A 0.5 McFarland bacterial suspension was prepared, lawn culture was done for Klebsiella isolates on the Muller-Hinton agar plate. A Ceftazidime (30µg) disk was placed at the centre of the plate then Imipenem (10 µg), Cefoxitin(30µg) and Amoxicillin/Clavulanate (20/10 µg) disk were placed at a distance of 20mm from the Ceftazidime disk. The plate was inverted and incubated overnight at 370 C. After overnight incubation, if there is any blunting or flattening of zones of inhibition between the Ceftazidime disk and the inducing substrates (Imipenem, Cefoxitin and Amoxicillin/Clavulanate disk) it was considered as a positive result for AmpC production.[6]

Cefoxitin Cloxacillin-Double disc synergy test (CC-DDST): This test was based on inhibitory effect of Cloxacillin on AmpC production. Lawn culture was done for Klebsiella isolates on the Muller-Hinton agar plate. Cefoxitin/Cloxacillin disc (30 µg /200 µg) and Cefoxitin disc (30 µg) were place d on Muller Hinton agar. A difference of >4mm zone between the two discs was an indication of AmpC production.6

RESULTS

A total of 100 klebsiella isolates were obtained from 797 pus samples processed, which yielded 312 Gram negative bacterial isolates.

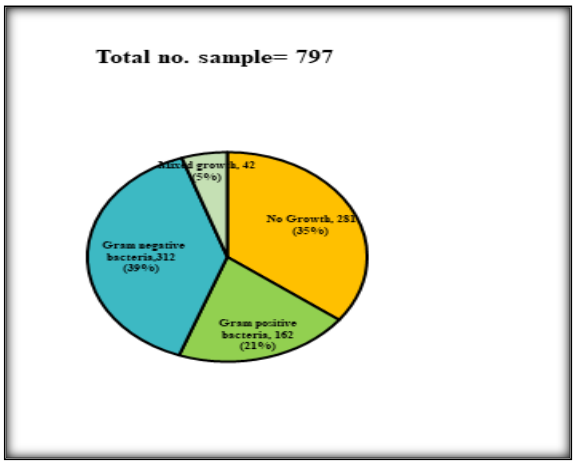

Figure 1: Diagrammatic Representation-1

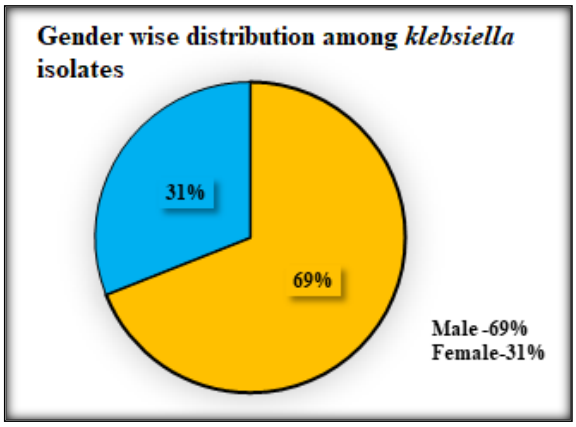

Figure 2: Diagramatic Representation-2

- Screening test (Total Klebsiella isolates tested:100)
- Screening test for AmpC-38 (38%)

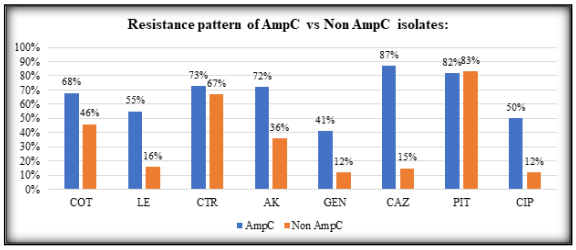

Figure 3: Antibiotic resistance pattern of klebsiella isolates

Abbreviation: COT-Co-trimoxazole (Trimethoprim + Sulfamethoxazole), LE-Levofloxacin, CTR-Ceftriaxone, AK-Amikacin, GEN-Gentamicin, CAZ-Ceftazidime, PIT-Piperacillin-Tazobactam, CIP- Ciprofloxacin.

Table 1: Comparison between two phenotypic methods

Phenotypic tests	Positive	Percentage
Only DDST	10	27%
Only DA	05	14%
Both (DDST &DA)	07	19%

DISCUSSION

This study aimed to assess the Antimicrobial resistance patterns and the prevalence of AmpC β-lactamase production in Klebsiella spp. isolates from pus samples in a tertiary care setting. Of the 797 samples analyzed, 516 (64.8%) showed bacterial growth, with 100 isolates identified as Klebsiella spp., underscoring the significance of Klebsiella as a common pathogen in pyogenic infections. Regarding AmpC β-lactamase detection, in this study, Cefoxitin screening demonstrated 38% positivity and 62% negativity. These findings are comparable to those reported by Ronni Mol et al., who observed a similar positivity rate of 38% and 61% negativity. In contrast, higher rates of cefoxitin positivity were reported by B. Madhumati et al. (67%)8 and Dhanashree P. Inamdar et al. (57%).[5]

Confirmatory testing with Double Disk Synergy Test (DDST) and Disc Approximation Test demonstrated that 17 isolates (45%) were AmpC-positive via DDST, while 12 isolates (32%) were confirmed using the Disc Approximation.

In this study, AmpC detection by disc approximation demonstrated 32% positivity, which was slightly lower than the findings reported by Dhanashree Inamdar et al. (37.5%)5, Ronni Mol et al. (34.7%)10, and Khatereh et al. (36.8%).[9]

Similarly, AmpC detection by disc synergy in the present study showed 45% positivity, which was lower than that observed by Ronni Mol et al. (56.5%)10 but higher than the study conducted by Dhanashree Inamdar et al. (31.5%).[5] Data regarding disc synergy were not reported by Khatereh et al. The comparatively higher detection rate observed with the disc synergy method may be attributed to its better sensitivity for AmpC β-lactamase detection, as inhibitor-based methods more effectively demonstrate enzyme inhibition. The higher sensitivity of DDST corroborates findings from other studies, which emphasize the importance of choosing appropriate diagnostic methods for accurate detection.

Above studies highlighting the increasing prevalence of AmpC enzymes in Klebsiella spp., which complicates treatment due to resistance to Cephalosporins and Beta-lactamase inhibitors. The observed variations may be attributed to differences in study population, sample size, geographical distribution, and local antimicrobial resistance patterns.

- Our findings demonstrated substantial resistance to multiple antibiotics, with Ceftazidime showing the highest resistance rate (91%) which is a growing concern, as third-generation Cephalosporins are commonly used in treating Gram-negative infections.
- In contrast, Levofloxacin exhibited the lowest resistance rate (38%) among the antibiotics tested.
- This resistance rate is indicative of Extended-spectrum β-lactamase (ESBL) and Amp C-producing strains, aligning with similar findings in previous research.

Limitations

- No molecular tests were conducted to confirm the presence of AmpC beta lactamase.
- The sample size in this study is relatively small.

CONCLUSION

The DDST using Cefoxitin and Cefoxitin/Cloxacillin and Disc approximation are effective, cost-efficient Phenotypic methods for AmpC detection in Klebsiella isolates. Their implementation can guide appropriate antibiotic therapy and aid in infection control in healthcare settings.

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