Ampicillinase C Beta-lactamase Producers among Isolates of Enterobacteriaceae in a Tertiary Care Centre: A Descriptive Cross-sectional Study

Shusila Khadka,1 Achut Barakoti,1 Ram Prasad Adhikari,1 Laxmi Kant Khanal,1 Jyotshna Sapkota1

1Department of Microbiology, Nepal Medical College and Teaching Hospital, Jorpati, Kathmandu Nepal.

ABSTRACT

Introduction: Ampicillinase C beta-lactamase-producing organisms are often resistant to multiple antimicrobial agents, and therapeutic options against these pathogens are limited. Limited information is available regarding Ampicillinase C beta-lactamase producers. The aim of this study was to find out the prevalence of Ampicillinase C beta-lactamase producers among isolates of Enterobacteriaceae in a tertiary care centre.

Methods: A descriptive cross-sectional study was carried out in the Clinical Microbiology Laboratory of a tertiary care centre from May 2021 to October 2021. Ethical approval was received from the Institutional Review Committee (Reference number: 044-077/078). Isolates of Enterobacteriaceae from various clinical samples were collected by convenience sampling. Ampicillinase C screening for beta-lactamase producers among the Enterobacteriaceae isolates was done using cefoxitin (30 μg) disc. Detection of Ampicillinase C beta-lactamase producers among the screen-positive isolates was done by cefoxitin-cloxacillin double-disc synergy test. An increase in the zone size of ≥4 mm was considered as Ampicillinase C beta-lactamase producers. Point estimate and 95% Confidence Interval were calculated.

Results: Among the total 481 isolates of Enterobacteriaceae, 49 (10.19%) (7.50-12.90, 95 % Confidence Interval) were detected as Ampicillinase C beta-lactamase producers among isolates of Enterobacteriaceae.

Conclusions: The prevalence of Ampicillinase C beta-lactamase producers was lower than in other studies done in similar settings. Meropenem could be a drug of choice for the treatment of infections due to Ampicillinase C beta-lactamase-producing gram-negative bacteria.

Keywords: antibiotic; beta-lactamase; Enterobacteriaceae; gram-negative bacteria.
Limited information is present in AmpC β-lactamase-producing bacteria.

The objective of this study was to find out the prevalence of AmpC β-lactamase producers among Enterobacteriaceae isolates in a tertiary care centre.

METHODS

This was a descriptive cross-sectional study carried out among isolates of Enterobacteriaceae in the Clinical Microbiology Laboratory of Nepal Medical College and Teaching Hospital from May 2021 to October 2021. Ethical approval was received from the Institutional Review Committee (Reference number: 044-077/078). All the clinical isolates of Enterobacteriaceae were included whereas duplicated samples/isolates were excluded from the study. Convenience sampling was done and the sample size was calculated using the formula:

\[
n = \left(\frac{Z^2 \times p \times q}{e^2}\right)
\]

\[
= 1.96^2 \times \frac{0.407 \times 0.593}{0.05^2}
\]

\[
= 371
\]

Where,

\(n\) = minimum required sample size

\(Z\) = 1.96 at 95% Confidence Interval (CI)

\(p\) = prevalence of AmpC β-lactamase producers, 40.7%6

\(q\) = 1 - p

\(e\) = margin of error, 5%

A total of 481 isolates of Enterobacteriaceae were taken. All clinical samples (pus, blood, urine, stool, sputum, pleural fluid, CSF) received for culture and sensitivity in the Clinical Microbiology laboratory were processed following standard protocol.7 In brief, the specimens were inoculated in culture plates (urine in cysteine lactose electrolyte deficient (CLED) media), pus in blood agar and Mac-Conkey agar, sputum and body fluids in blood agar, Mac-Conkey agar and chocolate agar. All inoculated plates were incubated at 37°C for 24 hours aerobically. All received blood culture bottles were incubated at 37°C and after 24 hours, sub-cultured in blood agar and Mac Conkey agar every alternate day for seven days. Bacterial isolates of the family Enterobacteriaceae were then identified further by studying colony characters, gram stain and biochemical tests. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method following standard clinical laboratory and standard institute (CLSI) guidelines.8 Screening of AmpC β-lactamase producers was done by using a cefoxitin disc (30 µg). Isolates were considered potential AmpC β-lactamase producers (screen positive) if the zone of inhibition for cefoxitin was ≤18 mm (CLSI susceptible breakpoint).5,11 Isolates of Enterobacteriaceae with screening test positive were subjected to cefoxitin-clavulanic acid (30-200 µg) double-disk synergy test. A difference in the cefoxitin-clavulanic acid inhibition zones minus the cefoxitin-alone zones of 4 mm or more was considered indicative of AmpC β-lactamase producers.5,8

Data were entered and analysed in Microsoft Excel 2013. Point estimate and 95% Confidence Interval were calculated.

RESULT

Among a total of 481 isolates of Enterobacteriaceae, 49 (10.19%) (7.50-12.90, 95% CI) were AmpC β-lactamase producers (Table 1).

**Table 1. Distribution of the isolates of Enterobacteriaceae among the AmpC β-lactamase producers (n= 49).**

| Producers Isolate | AmpC β-lactamase producers | n (%)  |
|--------------------|---------------------------|--------|
| Escherichia coli   | 38                         | 77.55  |
| Klebsiella pneumonia | 11                        | 22.45  |

AmpC β-lactamase producers among Enterobacteriaceae isolates, is found to be higher in urine specimens than lower respiratory specimen (Table 2).

**Table 2. Proportion of AmpC β-lactamase among Enterobacteriaceae isolates in different clinical specimens (n= 49).**

| Specimen          | Positive AmpC β-lactamase |
|-------------------|----------------------------|
|                   | n (%)                      |
| Urine             | 43                         | 87.75  |
| Lower respiratory | 6                          | 12.24  |

Antibiotic susceptibility testing showed that almost all of the AmpC β-lactamase-producing bacteria were sensitive to carbapenems (meropenem) and tigecycline. On the other hand, they showed marked resistance to fluoroquinolones and cotrimoxazole (Table 3).

**Table 3. Resistance pattern of AmpC β-lactamase-producing bacteria (n= 49).**

| Antibiotic              | Resistant isolates (E.coli n= 38) | Resistant isolates of K. pneumoniae (n= 11) |
|-------------------------|----------------------------------|------------------------------------------|
| Piperacillin tazobactam | 2 (5.26)                         | 4 (36.36)                                |
| Ceftriaxone             | 38 (100)                         | 11 (100)                                 |
| Ciprofloxacin           | 26 (68.42)                       | 6 (54.54)                                |
| Cotrimoxazole           | 11 (28.94)                       | 7 (63.63)                                |
Amikacin 2 (5.26) 4 (36.36)  
Tigecycline 4 (10.52) 2 (18.18)  
Meropenem - -

DISCUSSION

In this study, AmpC β-lactamase detection among Enterobacteriaceae isolates was done by a phenotypic method of cefoxitin- cloxacillin double-disc synergy following the screening using cefoxitin disc. Among the 481 isolates of Enterobacteriaceae, 16.83% were positive for AmpC β-lactamase screening test out of which 49 (10.2%) isolates were AmpC β-lactamase producers. Enterobacteriaceae are responsible for a large proportion of serious, life-threatening infections and resistance to multiple antibiotics in these organisms is an increasing global public health problem. The most common resistance of these organisms is to the third generation of cephalosporins.

The possible cause of the high rate of detection of AmpC β-lactamase producers in this study is lower as compared to ours because variations in the prevalence rate can occur according to time and place. As in our study, this study also showed E. coli 71.4% as the predominant AmpC β-lactamase producers. In our study, E. coli accounted for 77.6% (38 out of 49) and K. pneumonia accounted for 22.4% (11 out of 49) of the AmpC β-lactamase producers. A study done in India also showed E.coli as the predominant AmpC β-lactamase producers.

The AmpC β-lactamase producers were susceptible to carbapenems but showed a higher rate of resistance to fluoroquinolones and cotrimoxazole. A study done in Kathmandu, Nepal also showed that AmpC β-lactamase producers exhibited a high rate of resistance to fluoroquinolones and aminoglycosides and susceptibility to carbapenems. Few other studies also have shown that AmpC β-lactamase producers are susceptible to carbapenems. A study done in Pakistan showed that AmpC β-lactamase-producing E. coli were multidrug-resistant and resistant to cotrimoxazole, ciprofloxacin, and gentamycin. So, AmpC β-lactamase producers are usually resistant to commonly used antibiotics and carbapenems could be the drug of choice.

In this study, molecular techniques could not be used due to the lack of resources. The study was conducted in a single tertiary care centre.

CONCLUSIONS

Our study showed that the prevalence of AmpC β-lactamase producers was lower as compared to other studies done in similar settings. In our study, AmpC β-lactamase-producing bacteria were resistant to most of the commonly used antibiotics. Meropenem could be a drug of choice for the treatment of serious infections due to AmpC β-lactamase producing gram-negative bacteria. Identification of AmpC β-lactamase producers may aid in hospital infection control and help the physician to prescribe the most appropriate antibiotic.

Conflict of Interest: None.
5. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Bottger EC, Hombach M. Practical approach for reliable detection of AmpC beta-lactamase-producing Enterobacteriaceae. J Clin Microbiol. 2011 Aug;49(8):2798-803. [PubMed | Full Text | DOI]

6. Aryal SC, Upreti MK, Sah AK, Ansari M, Nepal K, Dhungel B, et al. Plasmid-mediated AmpC β-lactamase CITM and DHAM genes among gram-negative clinical isolates. Infect Drug Resist. 2020 Nov 24;13:4249-61. [PubMed | Full Text | DOI]

7. Henry D. Clinical Microbiology Procedures Handbook. 2nd ed. Washington D.C.: ASM press; 2004. [Full Text]

8. Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial susceptibility testing, (30th edition). Approved Standard. Wayne, PA: Clinical and Laboratory Standards Institute, 2020. [Full Text]

9. Inamdar DP, Anuradha B. Phenotypic methods for detection of Amp C β lactamases in gram negative clinical isolates of a tertiary care hospital. Indian J Microbiol Res. 2020;7(2):125–9. [PubMed | Full Text | DOI]

10. Helmy MM, Wasfi R. Phenotypic and molecular characterization of plasmid mediated AmpC β-lactamases among Enterobacteriaceae. J Med Microbiol Infec Dis. 2014;2(4):143-6. [PubMed | Full Text | DOI]

11. Saad N, Munir T, Ansari M, Gilani M, Latif M, Haroon A. Phenotypic identification and antibiotic susceptibility pattern of AmpC beta-lactamase producing clinical isolates of Enterobacteriaceae. Biomed Res Int. 2014,2014:171548. [PubMed | Full Text | DOI]

12. Tan TY, Ng LS, Koh TH, Hsu LY. Evaluation of screening methods to detect plasmid-mediated AmpC in Enterobacteriacea. Antimicrob Agents Chemother. 2009 Apr;53(4):1469-71. [PubMed | Full Text | DOI]

13. Adhikari RP, Shrestha S, Rai JR, Amatya R. Antimicrobial resistance patterns in clinical isolates of Enterobacteriaceae. Nepal Med J. 2018;1(2):p74-8. [PubMed | Full Text | DOI]