Prevalence, multidrug-resistance and risk factors for AmpC β-lactamases producing Escherichia coli from hospitalized patients

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Abstract
Introduction: Multi-drug resistance among AmpC β-lactamases producing Escherichia coli isolates is alarming. The study aimed to know the prevalence and presumptive antibiogram of AmpC producing Escherichia coli isolates and to determine the associated risk factors.

Methodology: Escherichia coli isolated from various clinical specimens from hospitalized patients during the study period (January 2018-December 2018) were taken for the study. Standard biochemical reactions were used for organism identification. Antibiotic susceptibility testing was done using Kirby-Bauer method as per CLSI guidelines. Cefoxitin resistance was taken as screening tool to detect AmpC producing strains. The phenotypic confirmation was done using modified three-dimensional test. Multiplex PCR was used to detect pAmpC.

Results: A total non-duplicate consecutive 470 Escherichia coli were isolated from various clinical specimens of hospitalized patients during the study period. Cefoxitin resistance was observed in 51.9% (244/470). Modified three dimensional test was positive in 115/244 (47.1%) strains. Genotypic characterization of phenotypic positive AmpC strains showed presence of CIT and DHA genes among 33/115 and 19/115 isolates respectively. The overall prevalence of pAmpC producing E. coli was found to be 52/470 (11.1%). Multidrug resistance (MDR) was observed in 42/52 (80.7%) pAmpC strains. Antimicrobial use, prolonged hospitalization and interventions were associated risk factors for AmpC producing isolates.

Conclusions: A high prevalence of multidrug resistance among AmpC producing strains suggests plasmid mediated spread of drug resistance in E. coli. Every hospital should formulate and implement infection control policies at-least for the risk group patients to control the dissemination of such microbes as infection prevention is better than infection control.

Key words: E. coli; AmpC β- lactamase; multiplex PCR; multidrug resistance; MDR.

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Introduction

Beta-lactam antibiotics are one of the most commonly prescribed antibiotics. Alarming rise in resistance to beta-lactams is a public health challenge. A variety of β-lactamases: ESBLs, AmpC β -lactamases and Metallo β -lactamases have emerged as the mechanism of resistance towards beta lactam antibiotics among the Gram-negative bacteria [1]. AmpC β-lactamases are clinically important cephalosporinases which confer resistance to a wide variety of both narrow and broad spectrum cephalosporins, beta-lactam/beta-lactamase inhibitor combinations and aztreonam [2]. Initially these enzymes were chromosomally mediated but had disseminated to plasmids. Thus, the plasmid mediated AmpC genes have been derived from inducible chromosomal genes that have become mobilized to other genera or species of different organisms. The commonly reported genotypes of AmpC are CIT, DHA, ACC, FOX, MOX and EBC [3].

Escherichia coli (E. coli), a universal commensal bacterium is most frequently recovered in the clinical laboratories and has been incriminated in the infectious diseases involving virtually every human organ system. It causes a wide variety of intestinal and extra-intestinal infections, such as diarrhea, urinary tract infections, septicemia, wound infections and meningitis [4,5].

In the last few years, emergence and wide dissemination of E. coli strains showing resistance to broad-spectrum antimicrobial agents due to AmpC β-lactamases production has been reported world-wide. Major risk factors for colonization or infection with
these resistant organisms are prolonged exposure to antibiotics, prolonged Intensive Care Units (ICU) stay, nursing home residency, severe illness, catheterization, instrumental intervention and residence in an institution with high rate of use of third generation cephalosporins [6,7].

Plasmid mediated AmpC (pAmpC) mediated resistance in E. coli poses a therapeutic challenge due to multidrug resistance. This study was conducted in MMIMSR, Mullana to know the prevalence and risk factors for AmpCβ-lactamase producing Escherichia coli isolated from various clinical specimens of hospitalized patients. The antibiotic sensitivity pattern of these strains was also studied to guide the clinicians about the choice of class of antibiotics as treatment options.

**Methodology**

The present study was conducted in the Microbiology department on all Escherichia coli isolated from urine, pus, body fluids, blood and other clinical specimens from hospitalized patients of MMIMSR, Mullana, Ambala over a period of one year (January, 2018 to December, 2018).

Bacterial growth obtained on culture plates after overnight incubation was identified by colony characteristics, gram staining, motility and various biochemical reactions [8].

All the E. coli isolates identified and confirmed were subjected to antibiotic sensitivity testing using various antibiotics such as ampicillin (2μg), ceftazidime (30μg), cefotaxime (30μg), ceftriaxone (30μg), cefoxitin (30μg), aztreonam (30 μg), cefepime (30μg), amikacin (30μg), gentamycin (10 μg), ciprofloxacin (5μg), imipenem (10μg), meropenem (10 μg), amoxicillin-clavulanic acid (20 + 10μg), cefoperazone-sulbactam (75/30 μg) and cotrimoxazole (1.25/23.75 μg) by the Kirby-Bauer method as per CLSI guidelines [9]. E. coli ATCC 25922 was used as a quality control strain.

The isolates which were resistant to one or more third generation cephalosporins were screened for AmpC β-lactamase production. The isolates which yielded <18 mm zone diameter around cefoxitin disc were taken as putative AmpC producers and were subjected to modified three-dimensional tests (M3DT) for phenotypic confirmation. In the modified three-dimensional test; the isolates which showed clear distortion of zone of inhibition of cefoxitin disc by enhanced growth of surface organism were taken as AmpC producers [10].

For molecular characterization, all the phenotypic confirmed AmpC harboring E. coli strains were subjected to multiplex PCR to identify pAmpC genotype. Plasmid DNA extraction was done using plasmid DNA extraction kit by Macherey-Nagel (Düren, Germany). The extracted DNA was amplified using the primers for the six family specific AmpC genes namely: FOX, MOX, CIT, DHA, EBC and ACC. The amplified products were analyzed by gel electrophoresis in 2% agarose gel stained with ethidium bromide [3].

The antibiotic sensitivity pattern of genotypic confirmed AmpC strains (pAmpC) was studied to know the prevalence of multi drug resistance among pAmpC producers. The isolated that were resistant more than one antimicrobial agent from three or more antimicrobial classes were taken as MDR [11].

**Results**

In the present study a total of 470 clinically significant Escherichia coli were isolated during one-year study duration (January- December, 2018) from urine, pus, body fluids, blood and other clinical specimens received in the Microbiology department of MMIMSR, Mullana, Ambala. Out of 470 E. coli isolates 224 (51.9%) were cefoxitin resistant. A total of 115 strains (47.1%) were found to be AmpC producers by phenotypic test (modified three-dimensional test) (Figure 1). pAmpC genes were detected by multiplex PCR in 52 (45.2%) among 115 phenotypic confirmed strains. The genes detected in these isolates were CIT (33/115) and DHA (19/115). Multiplex PCR showing CIT gene among the isolates is shown in Figure 2. Other four genotypes were not found in any isolate.
Predominately pAmpC isolates were from urine samples 26 (50%) followed by pus 10 (19.1%), peritoneal fluid 8 (15.4%), blood 2 (3.8%), respiratory specimens 2 (3.8%), HVS 2 (3.8%), CSF 1 (1.9%) and others 1 (1.9%). The presence of risk factors or any intervention in patients infected with pAmpC producing E. coli is depicted in Table1.

The antibiotic sensitivity pattern of pAmpC E. coli is depicted in Table 2. Almost all pAmpC producing strains were resistant to ampicillin, 3rd generation cephalosporins and aztreonam. High level of resistance was observed against ciprofloxacin 46 (88.5%), amoxyclav41 (78.8%), cotrimoxazole 43 (82.7%), amikacin 41 (78.8%), gentamycin 39 (75%), cefepime 27 (52%), cefoperazone-sulbactam 25 (48%). However AmpC producing strains showed good sensitivity to carbapenem group of antibiotics. pAmpC strains were found to more resistant than non pAmpC strains. Multidrug resistance (MDR) was observed in 42 (80.7%) pAmpC strains.

**Discussion**

In our study the prevalence of AmpC β-lactamase phenotype was found in 47.1% E. coli isolates. The prevalence from other studies ranged from 31.1 to 89.7% [12-14].

However the genotype was confirmed (pAmpC) among (52/115) 45% phenotypic confirmed isolates with predominance of CIT gene (32 strains) followed by DHA (20 strains). The prevalence of pAmpC genes in other studies ranged from 4.6% to 38.1% [12,14]. The predominance of CIT gene among the E. coli isolates in our study as well as from Gram-negative bacilli in other studies suggests a rapid dissemination of the plasmid mediated CIT gene posing a substantial threat with the emergence of multidrug resistance.

| Risk factor/intervention     | No. of AmpC producing E. coli isolated (%) |
|------------------------------|-------------------------------------------|
| Age (>60 years)              | 24 (46.1%)                                |
| Urinary catheter             | 27 (52%)                                  |
| Endotracheal tube            | 5 (9.6%)                                  |
| Peritoneal dDrain            | 3 (5.8%)                                  |
| Central line                 | 1 (1.9%)                                  |
| Post-surgery state (>7 days) | 34 (65.4%)                                |
| Prolonged hospital stay (>7 days) | 28 (53.8%) |
| Previous antibiotics use     | 47 (90%)                                  |
| Immune-compromised patients  | 9 (17.3%)                                 |
| Other                        | 5 (9.6%)                                  |

Plasmids that encode AmpC genes often carry many other resistance genes. We found that 42/52 (80.7%) of the pAmpC producing E. coli showed resistance to most important alternative drug choices for treating infections: aminoglycosides and fluoroquinolones. This makes it difficult to treat multidrug resistant pAmpC β-lactamase producing E. coli. High frequency of MDR pAmpC β-lactamase producing E. coli has been reported world-wide and it is continuously increasing. In such cases we are left with handful of antibiotics only like carbapenems. The emerging resistance even to the higher end antibiotics like carbapenems is making the condition even more worrisome.

In our study various risk factors were associated with infections by pAmpC producing E. coli. The risk factors associated with pAmpC producing isolates have

| Antibiotic                       | Resistance no. (%) |
|----------------------------------|--------------------|
| Ampicillin                       | 52 (100%)          |
| Ceftazidime                      | 51 (98%)           |
| Cefotaxime                       | 52 (100%)          |
| Ceftriaxone                      | 51 (98%)           |
| Cefoxitin                        | 52 (100%)          |
| Aztreonam                        | 52 (100%)          |
| Cefepime                         | 27 (52%)           |
| Amoxicillin+ Clavulanic acid     | 41 (78.8%)         |
| Cefoperazone-sulbactam           | 25 (48%)           |
| Imipenem                         | 2 (3.8%)           |
| Meropenem                        | 3 (5.8%)           |
| Amikacin                         | 41 (78.8%)         |
| Gentamycin                       | 39 (75%)           |
| Ciprofloxicin                    | 46 (88.5%)         |
| Cotrimoxazole                    | 47 (90.4%)         |
been well documented in various studies [7,15]. Noor-ul-Ain Jameel et al. found infection caused by AmpC β-lactamase producing *E. coli* among (76.5%) intravenous line, (22.4%) endotracheal tube, 12.9% surgery and 7.1% urinary catheters use [16]. The burden of pAmpC producing *E. coli* strains can be reduced by minimizing the use of invasive devices and preventing the misuse of antibiotics. Proper infection-control practices and antibiotic stewardship program are essential to prevent spreading of pAmpC producing bacteria.

**Conclusions**

Antibiotic resistance is a global threat but India is the epicenter. In India there is irrational of antibiotics because of over the counter sale of antibiotics without any prescription and diagnosis. Also antibiotic stewardship program are also not well established in most hospitals in countries like India, targeting the group of patients with risk factors for MDR strains infections might help in reducing the spread of MDR strains. In patients without a clear indication of infection, antimicrobial therapy should be stopped. Each hospital should have its antibiotic policy so that in case of start of empirical therapy narrow spectrum rather than broad spectrum antibiotics could be used.

**References**

1. Bajaj P, Singh NS, Virdi JS (2016) *Escherichia coli* β-Lactamases: What really matters. Front Microbiol 7:417.
2. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39:1211-1233.
3. Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153-2162.
4. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18:657-686.
5. Koneman (2006) The *Enterobacteriaceae*. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Textbook of Diagnostic Microbiology. 6th Edition. Philadelphia: Wolters Kluwer Health. 213-293.
6. Mathur P, Kapil A, Das B, Dhawan B (2002) Prevalence of extended spectrum beta lactamase producing Gram-negative bacteria in a tertiary care hospital. Indian J Med Res 115:153-157.
7. Chaudhary U, Aggarwal R (2004) Extended spectrum -lactamases (ESBL) - an emerging threat to clinical therapeutics. Indian J Med Microbial 22: 75–80.
8. Koneman (2017) The *Enterobacteriaceae*. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Textbook of Diagnostic Microbiology. 7th Edition. Philadelphia: Wolters Kluwer Health. 214-302.
9. Clinical and Laboratory Standard Institute (CLSI) (2015) Performance Standard for Antimicrobial Susceptibility Testing, 25th informational supplement. CLSI document M100-S25. (ISBN 1-56238-989-0)
10. Manchanda V, Singh NP (2003) Occurrence and detection of AmpC beta-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. J Antimicrob Chemother 51: 415-418.
11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giakoumetti G, Giske GJ, Honorez L, Judson CA, Morens DM, Pessoa-Leite M, Pollock JR, Simonetti G, Vardakas KZ, Warnock DW (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-281.
12. Mohamudha PR, Harish BN, Parjia SC (2012) Molecular description of plasmid-mediated AmpC β-lactamases among nosocomial isolates of *Escherichia coli* and *Klebsiella pneumoniae* from six different hospitals in India. Indian J Med Res 135:114-119.
13. Yilmaz NO, Agus N, Bozcal E, Oner O, Uzel A (2013) Detection of plasmid-mediated AmpC β-lactamase in *Escherichia coli* and *Klebsiella pneumoniae*. Indian J Med Microbiol 31: 53-59.
14. Bala R, Bansal R, Jindal R, Jindal N (2017) Occurrence of plasmid mediated AmpC β-lactamase genes and their types among the clinical isolates of *Escherichia coli* from a tertiary care hospital of Punjab (North India). Scholars Academic J Biosciences 5:790-793.
15. Pai H, Kang CI, Byeon JH, Lee KD, Park WB, Kim HB, Kim EC, Oh MD, Choe KW (2004) Epidemiology and clinical features of bloodstream infections caused by AmpC-type-beta-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48: 3720-3728.
16. Jameel NU, Ejaz H, Zafar A, Amin H (2014) Multidrug resistant AmpC β-lactamase producing *Escherichia coli* isolated from a paediatric hospital. Pak J Med Sci 30: 181-184.

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