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Brief Original Article

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.

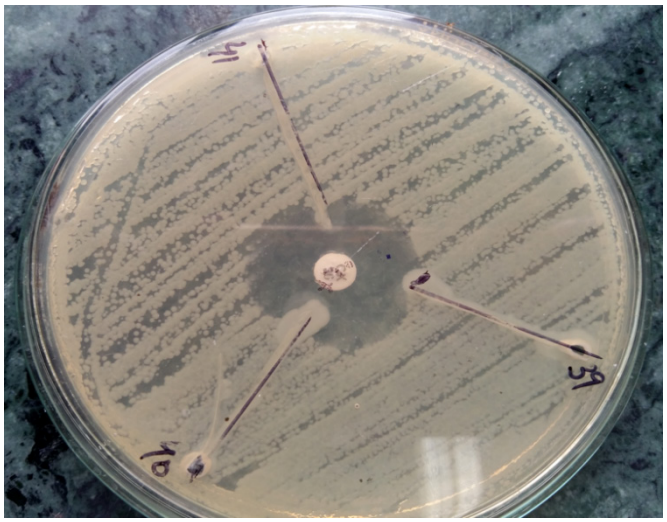
Methodology

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.

Figure 1. Modified three-dimensional test (showing indentation of zone around cefoxitin disc).



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

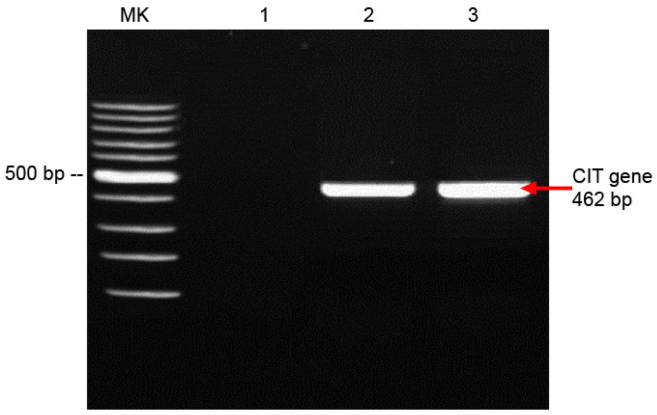
In our study the prevalence of AmpC β lactamase phenotype was found in 47.1% *E. coli* isolates. The prevalence from other studies ranged from 31.1 to 89.7%) [12-14].

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.

Table 1. Various risk factors/interventions among patients infected with pAmpC producing *E. coli* (n = 52), more than one risk factor was also present among patients.

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	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%)

Figure 2. Multiplex PCR showing plasmid-encoded AmpC β-lactamase gene (CIT) in plasmids extracted from *E. coli* isolates. Lane MK: (100 bp DNA Ladder by Thermo Scientific).



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

Table 2. Antibiotic resistance pattern of pAmpC β-lactamase producing *E. coli.*

Antibiotic	Resistance no. (%), n = 52
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%)
Ciprofloxacin	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.

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