Original Research Article

Prevalence of extended spectrum β-lactamase, AmpC β-lactamase and metallo β-lactamase mediated resistance in *Escherichia coli* from diagnostic and tertiary healthcare centers in south Bangalore, India

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ABSTRACT

Background: The increasing reports on multidrug resistant *Escherichia coli* has become a potential threat to global health. Here, we present a cross-sectional study to characterize extended spectrum β-lactamase, AmpC β-lactamase and metallo β-lactamase producing *E. coli* isolated from different human clinical samples.
Methods: A total of 300 clinical Gram negative bacterial isolates were collected and re-characterized for the identification of *E. coli* following standard microbiological techniques. The antimicrobial susceptibility of *E. coli* isolates was initially screened by Kirby-Bauer disk diffusion and MIC methods. The resistant isolates were confirmed to be ESBL, AmpC and MBL producers by their respective phenotypic confirmatory tests of combined disc method.
Results: We identified 203 (68%) *E. coli* and 97 (32%) Non-*E. coli* isolates. The highest recovery of *E. coli* was from urine samples 72 (35%). Combined disc method using ceftazidime/ceftazidime+clavulanic acid and cefotaxime/cefotaxime+clavulanic acid confirmed 156 (79%) and 144 (73%) *E. coli* as ESBL producers, respectively. Thirty-four (34%) and 16 (27%) resistant *E. coli* isolates were confirmed to be AmpC and MBL producers, likewise.
Conclusions: Increased prevalence of ESBL, AmpC and MBL producing *E. coli* were observed. Beta-lactamase mediated resistance appears to be prime mechanism in the multidrug resistant *E. coli*. Thus, early detection of beta lactamase producing *E. coli is* necessary to avoid treatment failure and prevent the spread of MDR.

Keywords: AmpC β-Lactamase, *Escherichia coli*, Extended Spectrum β-Lactamase, Healthcare centers, Metallo β-Lactamase

INTRODUCTION

The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria is widely accepted as a major problem that has been observed over the last decade.[1] Countries where infection prevention and control (IPC) protocols are lacking have become the main foci for developing multidrug resistance.[2]

Enterobacteriaceae included the largest number of Gram-negative and facultative anaerobic organisms which can be found in the clinical samples. *Escherichia coli*, member of *Enterobacteriaceae* family is one of the most important cause of nosocomial and community acquired infection.[3] *E. coli* serovars ranges from highly pathogenic to nonpathogenic strains, and cause several clinical manifestations, including bacteremia, sepsis, meningitis, gastroenteritis and Urinary Tract Infections (UTIs).[3]

In the recent past, there are alarming reports about the emergence and spread of antimicrobial resistant *E. coli* strains from all around the world. These strains are associated with high morbidity, mortality, increased length of hospitalization and cost of health care.[4] Resistance to third-generation cephalosporin's, poses a great challenge in a developing country like India.[5]

Among tropical countries, India has emerged as the focal point of antimicrobial resistance. Various strategies are used by bacteria to remain immune against the deleterious effects of antibiotics. The most important mechanism of resistance is antibiotic hydrolysis mediated by the bacterial enzyme β-lactamase. Beta-lactamases are bacterial enzymes which make the β-lactam antibiotics inactive by hydrolyzing the β-lactam ring.[6]

The rapid global dissemination of *E. coli* harboring plasmid-borne extended-spectrum β-lactamases (ESBLs), plasmid mediated AmpC β-lactamases (AmpC) and Metallo β-lactamases (MBL) represents a significant clinical threat.[6,7] ESBLs belong to Group 2be of Bush's functional classification.[8] ESBLs have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group (third generation cephalosporins and aztreonam) and are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam.[9] ESBL producing isolates, in addition to being resistant to β-lactam antibiotics, often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones.[9] AmpC beta-lactamases are well defined enzymes with broad substrate specificity and classified as class C according to Ambler and group 1 by Bush-Jacoby-Medeiros.[10] These enzymes, both chromosomal and plasmid mediated show an action spectrum similar to ESBLs. Plasmid-mediated AmpC β-lactamases have a broad substrate profile that includes penicillin, cephalosporins, and monobactams.[7,9]

Carbapenems represented a great advance for the treatment of serious bacterial infections caused by β-lactam resistant bacteria. Due to their broad spectrum of activity and stability to hydrolysis by most β-lactamases, the carbapenems have been the drugs of choice for treatment of infections caused by penicillin or cephalosporin resistant Gram negative bacilli.[11]

However, extensive and imprudent use of the carbapenems, poor sanitation and large population has facilitated the emergence of carbapenem resistant bacteria.[12] Resistance to carbapenem is predominantly mediated by metallo-β-lactamases, a class B type of β-lactamases that recognize bivalent metal ions.[11,12]

The multidrug resistant *E. coli* isolates that are present in the hospital environment and in the community pose not only therapeutic problems but also serious concerns for infection control management. The lack of screening for these multidrug resistant pathogens coupled with poor antibiotic stewardship and surveillance systems increases their burden in the community. Thus, early detection of ESBL, AmpC and MBL producing *E. coli* is crucial to establish appropriate antimicrobial therapy and to prevent their inter-hospital and intra-hospital dissemination.[13] The present study was undertaken to isolate and characterize ESBL, AmpC and MBL producing *E. coli* in different clinical samples from diagnostic and tertiary healthcare centers.

METHODS

Collection of isolates and laboratory processing

This cross-sectional study was performed from June 2015 to December 2016 at the Microbial Pathogenesis and Pathogen Diversity Laboratory, ICAR-NIVEDI, Bangalore. A total of 300 consecutive, non-repetitive, clinical Gram negative bacterial isolates were collected from Microbiology division of two diagnostic laboratories and a tertiary care referral hospital located in South Bangalore.

The isolates were derived from samples of pus, urine, sputum, blood, wound and other body fluids. The demographic information and the history of each patient was obtained from his/her records. The isolates were re-characterized for the identification of *E. coli* following standard microbiological techniques as described by American Society of Microbiology.[14]

Tests for ESBL-production

Screening test

All the *E. coli* isolates were screened for ESBLs by disc diffusion method.[15] In the presumptive test to detect potential ESBL producers, all the isolates were screened for susceptibility to ceftazidime (30μg) and cefotaxime (30μg) antibiotic discs (Himedia, Mumbai). Results were interpreted based on the CLSI guidelines as follows: zones of inhibition of ≤22mm for ceftazidime and ≤27mm for cefotaxime indicated ESBL production. The less susceptible or resistant isolates were subjected to confirmatory test.

Confirmatory test

The ESBL producing *E. coli* isolates were confirmed by CLSI phenotypic confirmatory test of combined disc assay method.[15] One disc each of ceftazidime (30μg) and cefotaxime (30μg) alone and one in combination with clavulanic acid (10μg) were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The ESBL-producing strains showed ≥5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone. *E. coli* ATCC 25922 were used as quality control strain.

Tests for AmpC-production

Screening test

The isolates were screened for presumptive AmpC production by testing their susceptibility to cefoxitin (30µg) and cefotetan (30µg) antibiotic discs (Himedia, Mumbai) using Kirby Bauer disk diffusion method. All the isolates with an inhibition zone diameter of ≤14mm for cefoxitin and ≤12mm for cefotetan, were labelled as AmpC positive and were subjected to confirmatory test.[15]

Confirmatory test

AmpC producers were confirmed by phenotypic confirmatory test of combined disc assay method.[16] One disc of cefoxitin (30µg) alone and one in combination with cloxacillin (200µg) were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The *E. coli* strains demonstrating a zone diameter around the cefoxitin+cloxacillin disc ≥5mm than the zone diameter around the cefoxitin disc alone were considered as AmpC producers. *E. coli* ATCC 25922 were used as quality control strain.

Tests for MBL-production

Screening test

All the 203 *E. coli* isolates were screened for MBL production by testing their susceptibility to imipenem (10µg) and meropenem (10µg) antibiotic discs (Himedia, Mumbai) using Kirby Bauer disk diffusion method. All the isolates with an inhibition zone diameter of ≤19mm were considered as screen positive for MBL and were subjected to confirmatory test.[15]

Confirmatory test

All screen positive *E. coli* isolates were confirmed for metallo- β -lactamase production as described by.[17] One disc of imipenem (10µg) alone and one in combination with EDTA (750µg/mL) were placed at a distance of 20 mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The *E. coli* strain demonstrating a zone diameter ≥7mm around the imipenem/EDTA disc compared to that of imipenem disc alone was considered to be positive for the presence of MBLs. *E. coli* ATCC 25922 were used as quality control strain.

*Antimicrobial susceptibility testing by MIC method*

All the isolates which were positive for ESBL, AmpC and MBL production by initial screening test were tested by MIC method utilizing broth microdilution procedure (Vitek 2 compact system, bioMerieux, India) following

the CLSI guidelines.[15] Same respective antibiotics were tested for ESBLs, AmpC and MBL production as used in initial screening tests by disc diffusion method.

RESULTS

Beta lactamase enzymes are of increasing clinical concern. ESBLs are most commonly produced by *Escherichia coli* and *Klebsiella spp*. However, they may also be present in other gram negative bacteria.[3] Many MDR bacteria produce multiple β-lactamases including combinations of these different enzymes.

The infections which are caused by MDR Gram negative bacteria that produce ESBL/AmpC/MBL enzymes have been reported with an increasing frequency and are associated with a significant morbidity and mortality.[18] Prolonged antibiotic exposure, overstay in hospitals, severe illness, unprecedented use of third generation cephalosporin, and increased use of intravenous devices or catheters are important risk factors for infection with MDR *E. coli*.[19]
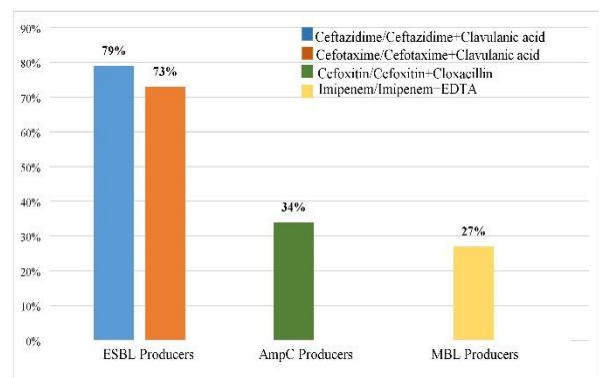
Figure 1: Phenotypic confirmatory test for ESBL/AmpC/MBL production by combined disc assay method.

The present study identified 203 (68%) *E. coli* isolates and 97 (32%) Non-*E. coli* out of 300 Gram negative bacteria collected from diagnostic and tertiary health care centers. The current study demonstrated that 72 (35%) of *E. coli* were isolated from urine samples followed by 54 (27%) and 35 (17%) from pus and respiratory specimens, respectively (Table 1). With regard to urinary tract infection among hospitalized patients, many researchers indicated its incidence as 31-47%.[20,21]

The use of invasive device i.e. urinary catheters has a significant association with hospital acquired urinary tract infections, for it provides either a portal of entry for microorganism or a place for colonization of microorganisms.

Similarly, respiratory infections in hospital setting are of particular concern due to the risk of transmission to patients who are ill and/or immunocompromised.[22]

Table 1: Isolation of *E. coli* and non-*E. coli* isolates from various clinical samples.

Clinical samples	Total no. of *E. coli* isolates n (%)	Total no. of non-*E. coli* isolates n (%)
Urine	72 (35)	14 (14)
Pus	54 (27)	18 (18.5)
Sputum	35 (17)	37 (38)
Blood	20 (10)	14 (14)
Wound	10 (5)	6 (6)
Other body fluids	12 (6)	8 (8)
Total	203 (68)	97 (32)

Correct identification of ESBL positive *E. coli* in due time is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms.[23] Early detection will definitely help in controlling hospital infections which are caused by this group of organisms. In the present study, initial screening test by disk diffusion method identified possible ESBL producing *E. coli* between 76% (Cefotaxime) and 97% (Ceftazidime) (Table 2). MIC testing of disk diffusion resistant isolates identified complete resistance in 38% and 35% of isolates to cefotaxime and ceftazidime, respectively. Intermediate resistance was observed in 40% and 45% of isolates for the same antibiotics, correspondingly (Table 2). Among the 197 (97%) cephalosporin resistant *E. coli* isolates, 156 (79%) were found to be ESBL positive by ceftazidime/ceftazidime+clavulanic acid and 144 (73%) of isolates were found ESBL positive by cefotaxime/cefotaxime+clavulanic acid combined disc method (Figure 1). Others have also reported 50-70% prevalence of ESBL producing *E. coli*.[24,25] ESBL production varies from hospital to hospital because of variation in selection of type of antibiotics. The selective pressures which are generated by the indiscriminate use of the beta-lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamases.[26]

Table 2: Initial screening of ESBL/AmpC/MBL producing *E. coli* by Kirby-Bauer disc diffusion and MIC methods.

Antimicrobial Agent	Kirby-Bauer disc diffusion method		Minimum inhibitory concentration method	
	Intermediate	Resistance	Intermediate	Resistance
Ceftazidime	6 (3%)	197 (97%)	36 (45%)	28 (35%)
Cefoxitime	21(10%)	154 (76%)	32 (40%)	30 (38%)
Cefotetan	36 (18%)	140 (69%)	18 (23%)	28 (35%)
Cefoxitin	30 (15%)	120 (59%)	28 (35%)	29 (36%)
Meropenem	53 (26%)	63 (31%)	11 (14%)	13 (16%)
Imipenem	81 (40%)	59 (29%)	9 (11%)	11 (14%)

DISCUSSION

Isolates producing AmpC β-lactamases raise special concerns as these isolates have been responsible for several nosocomial outbreaks and high rate of clinical failure among infected patients.[27] Till date, several phenotypic tests for the identification of AmpC producing isolates have been developed. However, there are presently no CLSI approved tests for identification of AmpC β-lactamase producing bacterial pathogens. AmpC β-lactamase producing *E. coli* is being increasingly reported from many parts of the world.[28,29]

In our study, initial screening test identified probable AmpC producing *E. coli* between 59% (Cefoxitin) and 69% (Cefotetan) (Table 2). MIC testing of resistant *E. coli* isolates identified complete resistance and intermediate resistance to cefoxitin in 36% and 35% of isolates, respectively (Table 2). Similarly, complete resistance and intermediate resistance to cefotetan was observed in 35% and 23% of isolates. Confirmatory test of 120 (59%) resistant *E. coli* revealed 34 (34%) isolates as AmpC producing *E. coli* by testing with cefoxitin alone and cefoxitin+cloxacillin (Figure 1).

The present study showed much higher prevalence rates of AmpC producing *E. coli*, than the ones ranging from 2% to 10% reported from various parts of the world.[30,31] However, several other studies have reported much higher incidence ranging from 14-49% of AmpC producing isolates of *E. coli*.[32,33] The increasing prevalence of AmpC β-lactamase resistance among *E. coli* is becoming a serious problem worldwide. High-level AmpC production is typically associated with in-vitro resistance to third-generation cephalosporins and cephamycins. In connection with this, high clinical treatment failures with broad-spectrum cephalosporins have been documented.[31]

Carbapenems are considered to be one of the antibiotics of last resort for treatment of infections caused by multi drug resistant bacteria such as *E. coli*.[11] Alarms have been raised over and over on the dangers of spreading of carbapenem resistant bacteria in hospitals and

communities, where they can be acquired if IPC programs are not in place.[12] In this study, initial screening test identified prospective MBL resistant *E. coli* isolates between 29% (Imipenem) and 31% (Meropenem) (Table 2). MIC testing of Imipenem and Meropenem detected complete resistance in 16% and 14% of isolates, however, intermediate resistance was observed in 14% and 11% of isolates respectively (Table 2). Out of 59 (29%) Imipenem resistant *E. coli* isolates, 16 (27%) were confirmed as MBL producers by the combined disk test utilizing Imipenem alone and Imipenem-EDTA (Figure 1). Studies have reported 3-25% of MBL producing *E. coli* strains from hospitalized Patients.[13,34] We observed higher prevalence of MBL resistant *E. coli* than reported by Bora *et al,* 2014 (19%) and Bandekar *et al,* 2011 (16%).[35,36] Antibiotic overuse is an important contributor for the emergence and spread of resistance; association between carbapenem consumption and resistance has been previously documented. However, since last 15years, acquired resistance which is mainly mediated by MBLs to these life-saving antimicrobials has been increasingly reported worldwide including India not only in *E. coli*, but also among members of *Enterobacteriaceae*.

CONCLUSION

The early detection of beta lactamase producing *E. coli* would be important for the reduction of morbidity and mortality and also to avoid the dissemination of such strains within the community. The present study observed increased prevalence of ESBL, AmpC and MBL producing *E. coli*. This study underlines a real threat from the emergence of pan drug-resistant bacteria in near future. The spread of ESBL/AmpC/MBL producing *E. coli* has been noticeably rapid worldwide including India, indicating that continuous monitoring systems and effective infection control measures area absolutely required.

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