Phenotypic detection of extended spectrum β-lactamase and AmpC β-lactamase in urinary isolates of *Escherichia coli* at a tertiary care referral hospital in Northeast India

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ABSTRACT

Objective

Urinary tract infections (UTIs) are the most prevalent infections worldwide, mostly caused by *Escherichia coli*. Emerging antibiotic resistance due to extended spectrum β-lactamase (ESBL) and AmpC β-lactamase production limit the use of β-lactam antibiotics against the infections caused by *E. coli.* We detected the production of ESBL and AmpC β-lactamase in urinary isolates of *E. coli,* recovered from a tertiary care referral hospital in Northeast India.

Materials and Methods

A total of 140 *E. coli* urinary isolates were recovered during October 2008 to January 2009. Antibiotic susceptibility testing and ESBL detection were carried out according to Clinical Laboratory and Standards Institute (CLSI) guidelines. Phenotypic detection of AmpC β-lactamase was carried out by AmpC disc method.

Results

Among the 140 urinary isolates, 112 isolates (80%) were multi-drug resistance (MDR). ESBL was detected in 67.14% (94/140) of *E. coli* isolates. AmpC β-lactamase was detected in 22.34% of ESBL producing *E. coli* isolates.

Conclusions

Routine testing for ESBL and AmpC β-lactamase in *E. coli* urinary isolates with conventional antibiogram would be useful for strict antibiotic policy implementation in hospitals, to estimate the impact of increased drug resistance and to take steps for reducing their resistance.

Key words: AmpC β-lactamase, ESBL, *E. coli*, MDR, UTI.

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Introduction

Despite of the widespread availability of antibiotics, urinary tract infections (UTIs) remain the second most common infectious presentation in humans, both in the community and the hospital settings. It affects approximately 150 million people worldwide annually which results in more than 6 billion US dollars loss to the global economy.[1] As many as 80% of UTIs are caused by *Escherichia coli.* Antimicrobial resistance in uropathogenic *E. coli* is of major concern worldwide due to its increasing resistance to several commonly prescribed antibiotics.[2] Presently, production of extended spectrum β-lactamase (ESBL) and AmpC β-lactamase limits the use of β-lactam antibiotics against the infections caused by *E. coli*.[3]

ESBLs are enzymes that have the ability to hydrolyze and cause resistance to various types of newer β-lactam antibiotics, including the expanded-spectrum cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime) and monobactams (e.g., aztreonam), but not the cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., imipenem, meropenem, and ertapenem). Conversely, ESBLs are susceptible to inhibition by the β-lactamase inhibitors i.e., clavulanic acid, tazobactam, or sulbactam.[4] On the other hand, AmpC β-lactamase confers resistance to a wide variety of â-lactam drugs, including α-methoxy-β-lactams, narrow, expanded and broad-spectrum cephalosporins, monobactams and most significantly β- lactam plus β-lactamase inhibitor combinations (*viz.*, ampicillin-clavulanic acid, piperacillin/tazobactam, *etc.*), which are generally stable against ESBL.[5] Many clinical laboratories have problems detecting ESBL and AmpC β-lactamase. Confusion exists about the importance of these resistance mechanisms, optimal test methods, and appropriate reporting conventions. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures.[6] Therefore, we performed this study to detect the ESBL and AmpC β-lactamase phenotypes among the urinary isolates of *E. coli* at a tertiary care referral hospital in Northeast for effective management of UTIs.

Materials and Methods

A total of 140 non-duplicated *E. coli* isolates were recovered from the urine samples at Department of Microbiology, Guwahati Medical College and Hospital in Northeast India. Samples were obtained from both hospitalized and non-hospitalized patients between October 2008 to January 2009. Standard microbiological techniques were used for isolation and identification of the isolates.[7] Prior to testing, all isolates were stored in 15% glycerol-supplemented Luria-Bertani medium at 80^0C. The study was carried out with consent from the institutional ethics committee.

All the isolates were subjected to antimicrobial susceptibility testing to 16 different β-lactam and non β-lactam antibiotics by standard disc-diffusion method using commercially available discs as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[8] The antibiotics tested were: ampicillin(10µg), cephalexin(30µg), cefpodoxime (10µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefepime (30µg) , aztreonam (30µg), cefoxitin (30µg), piperacillin/tazobactam (100/10µg) and imipenem (10µg), co-trimoxazole (25µg), nalidixic acid (30µg) ciprofloxacin (5µg), gentamicin (10µg) and amikacin (30µg). All the antibiotic discs and media were procured from Hi-media, Mumbai, India.

The initial screening and phenotypic confirmatory tests recommended by the CLSI for ESBL detection were carried out to evaluate the incidence of ESBL.[8] All *E. coli* isolates were screened for ESBL production by disc diffusion method using ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefpodoxime (10µg) and aztreonam (30µg) antibiotic discs. Phenotypic detection of ESBL was carried out by CLSI confirmatory method using cefotaxime (30µg) and ceftazidime (30µg) and a disc of cefotaxime plus clavulanic acid (30/10µg) and ceftazidime plus clavulanic acid (30/10µg). A positive phenotypic confirmatory test was defined as a ≥5 mm increases in inhibition zone diameter between at least one of the combination discs with clavulanic acid versus the inhibition zone tested alone.

Phenotypic detection of AmpC β-lactamase was carried out by AmpC disc method.[9] A lawn culture of *E. coli* ATCC 29522 was prepared onto a Mueller Hinton agar (MHA) plate. The sterile discs (6 mm in diameter) were moistened with the sterile saline (20 µL) and inoculated with several colonies of the test bacteria. The inoculated disc was then placed besides a cefoxitin disc (almost touching) onto the inoculated plate. After overnight incubation at 37°C, the plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result).

E. coli ATCC 25922 was used as the negative control and *Klebsiella pneumoniae* ATCC 700603 was used as the ESBL positive control.

Results

A total of 140 isolates of *E. coli* were recovered from urine samples submitted for routine microbiological analysis from both indoor and outdoor patients. The isolates were subjected to antimicrobial susceptibility testing to 16 different antimicrobial agents. Out of 140 urinary isolates of *E. coli*, all the isolates (100%) were resistant to ampicillin, 85.71% (120/140) to cephalexin, 71.43% (100/140) to cefpodoxime, 70.0% (98/140) to cefotaxime, 67.14% (94/140) to ceftazidime, 57.86% (81/140) to ceftriaxone, 53.57% (75/140) to cefepime, 27.14% (38/140) to cefoxitin, 63.57% (89/140) to aztreonam and 29.28% (41/140) to piperacillin/tazobactam, 57.85% (81/140) to co-trimoxazole, 72.14% (101/140) to nalidixic acid, 62.14% (87/140) to ciprofloxacin, 21.43% (30/140) to gentamicin and 12.86% (18/140) to amikacin. Among the 140 isolates, 112 isolates (80.0%) were MDR (resistant to ≥3 antibiotics). However, all the 140 isolates (100%) were found to be sensitive to imipenem.

The CLSI screening test for ESBL detection was positive for 71.43% (100/140) *E. coli* isolates. 67.14% (94/140) of *E. coli* isolates were found to be ESBL producers by CLSI confirmatory test. The predictive value for screening of ESBL producers was highest with cefpodoxime (94.0%) and least with aztreonam (81.0%). Among the 94 ESBL producers, AmpC β-lactamase was detected in 22.34% (21/94) isolates by AmpC disc method. All the isolates co-producing ESBL and AmpC β-lactamase were found to be resistant to cefoxitin.

Table1: Percentage susceptibility of *E. coli* urinary isolates (n=140) to the selected antibiotics:

Antimicrobial Agents	Sensitive(S)%	Intermediate(I)%	Resistant(R)%
Ampicillin	0.0	0.0	100
Cephalexin	12.86	1.43	85.71
Cefpodoxime	18.57	10.0	71.43
Cefotaxime	27.86	2.14	70.0
Ceftazidime	30.0	2.86	67.14
Ceftriazone	40.71	1.43	57.86
Cefepime	42.86	3.57	53.57
Aztreonam	32.14	4.28	63.58
Cefoxitin	69.29	3.57	27.14
Co-Trimoxazole	36.43	5.72	57.85
Nalidixic acid	25.72	2.14	72.14
Ciprofloxacin	35.0	2.86	62.14
Gentamycin	73.57	5.0	21.43
Amikacin	81.44	4.28	14.88
Piperacillin/tazobactam	67.86	2.86	29.28
Imipenem	100	0.0	0.0

Discussion

UTIs are usually treated with broad-spectrum cephalosporins, fluoroquinolones and aminoglycosides.[1] This study reveals that larger number of *E. coli* (80%) recovered from UTI in this region are MDR. Similar to the findings of present study, previous reports from India also indicated the high prevalence of MDR phenotypes in *E. coli*.[10-11] The high level of resistance to β-lactam antibiotics exhibited by majority of *E. coli* isolates in the present study may result from the production of ESBL as well as AmpC β-lactamase. The existence of resistance to piperacillin/tazobactam in 29.28 % of *E. coli* urinary isolates is a cause of concern. However, none of the *E. coli* urinary isolates was found to be resistant to the carbapenem antibiotic used in the study. Among the non β-lactam antibiotics, the higher resistance rate was observed for nalidixic acid, ciprofloxacin and co-trimoxazole. Comparatively, lower rate of resistance to aminoglycosides was observed. Most UTIs in developing countries are treated on an empirical basis; thus treatment should be based on available local data regarding the susceptibility of common pathogens to antibiotics. Unfortunately, most patients who can afford drugs are prone to self-treatment in the absence of any laboratory investigation. Therefore, an increase in antibiotic resistance is to be expected and it is important to determine the distribution of pathogens responsible for

UTIs and their patterns of resistance to the main available antibiotics.[12]

Over the past few years, the prevalence of ESBL producing strains among clinical isolates has been steadily increasing resulting in limitations of therapeutic options. The prevalence of ESBL and AmpC β-lactamase in clinical isolates vary greatly with different geographic areas and rapidly changing over time. In our study, the occurrence of ESBL producers in urinary isolates of *E. coli* was found to be 67.14% by CLSI phenotypic confirmatory test. Previous study from India have reported ESBL production in urinary isolates of *E. coli* vary from 18.5 to 60.7%.[13-18] In a study from North-East India, 33.82% of *E. coli* strains were tested positive for ESBL production.[19] The results of the present study indicate an elevated incidence of ESBL producing *E. coli* in this geographical area. Very recently, data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program in the Asia/Pacific region indicated the prevalence of ESBL producing *E. coli* as 42.2%. ESBL-positive *E. coli* rates were also relatively high in China (55.0%) and Thailand (50.8%).[20] A study from Turkish hospitals reported 74.6% of *E. coli* as ESBL producers.[21] An estimated 30,000 cases of human infection with ESBL-producing *E. coli* occur each year in the United Kingdom, and studies have found epidemic strains of ESBL-producing *E. coli* in the United Kingdom and throughout the world.[22]

Recently, isolates that produce both and ESBL and AmpC β-lactamase becoming more common in clinical isolates and it represents an emerging epidemiologic threat. This may be due to dissemination of plasmid mediated AmpC β-lactamase among the members of *Enterobacteriaceae*, sometimes in combination with ESBL. CLSI recommendations exist for detecting ESBL-producing isolates of *E. coli*, *Klebsiella* spp. and *Proteus mirabilis*, but there are no recommendations for detecting AmpC β-lactamase in any organisms. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures. Plasmid-mediated AmpC β-lactamase have been found in *E coli*, although this species can also increase the production of its normally weakly expressed chromosomal AmpC enzyme.[23]

Methods for detecting *E. coli* AmpC β-lactamase are technically demanding for clinical laboratories. Although non-susceptibility to the cephamycins suggests increased production of AmpC β-lactamase, organisms that produce these types of enzymes often go undetected and have been responsible for several nosocomial outbreaks.[24] Various phenotypic methods have been described by different researchers to detect AmpC β-lactamase. Among these, the Three Dimensional Enzyme Test (3DET) considered as a gold standard for AmpC detection, but it is labour intensive and not feasible in routine laboratory investigations.[25] The AmpC disc test is an easier, reliable and rapid method for detection AmpC β-lactamase and reported as sensitive as 3DET.[26] Therefore, we employed AmpC disc test to detect the AmpC β-lactamase production in ESBL positive isolates. It is important to note that phenotypic tests are unable to differentiate between plasmid-mediated AmpC activity and chromosomally encoded AmpC activity.[27]

In the present study, we found the co-existence of AmpC β-lactamase in 22.34% of ESBL producing *E. coli* urinary isolates. Rajini *et al.*[28] reported an incidence

of 44.4 % *E. coli* isolates as co-producer of both ESBL and AmpC β-lactamase. Another study from India also reported high prevalence (64.80 %) of ESBL and AmpC β-lactamase producing strains of *E. coli* isolates .[29] Singhal *et al.*[26] had reported low incidence (1.25%) of such co-existence among *E. coli* isolates. Our study emphasizes the importance of AmpC β-lactamase detection in urinary isolates as we detected higher co-existence of ESBL and Amp Cβ-lactamase among *E. coli* urinary isolates. However, all the urinary isolates of *E. coli* (100%), including the ESBL and AmpC β-lactamase producers were found to be sensitive to imipenem, making it most effective antibiotic in this study.

Conclusion

E. coli isolates producing ESBL are increasingly causing UTIs both in hospitalized and outpatients. Besides, co-production of ESBL and AmpC β-lactamase in clinical isolates of *E.coli* making therapy of UTI difficult and promoting greater use of expensive broad spectrum antibiotics, such as carbapenems. However, overuse of carbapenem may lead to emergence of carbapenem resistant strains, which is presently a major concern throughout the world. Therefore, early detection of ESBL as well AmpC β-lactamase is of paramount importance for surveillance and control of antibiotic resistance as well as to guide appropriate and judicious antibiotic usage.

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