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High occurrence of *bla*$_{CMY-1}$ AmpC lactamase producing *Escherichia coli* in cases of complicated urinary tract infection (UTI) from a tertiary health care centre in north India

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AmpC beta lactamase producing Gram-negative bacteria have emerged worldwide. It is important to distinguish plasmid mediated AmpC β lactamases from chromosomally mediated enzymes for surveillance, epidemiology and hospital infection control as plasmid mediated genes can spread to other organisms. Occurrence of *bla* $_{CMY-1}$ AmpC β-lactamase, a plasmid mediated cephamycinase was studied in 100 consecutive isolates of *Escherichia coli* from cases of complicated urinary tract infection (UTI). Screening for AmpC production was done by modified Hodge test, three dimensional test and AmpC disk test. All isolates showing a positive result by 2 out of 3 tests were then tested for *bla*$_{CMY-1}$ gene by PCR. Fifty nine isolates were positive for AmpC β lactamase production, 56.6 per cent were positive by PCR. Eight out of 13 isolates which were negative by EDTA disk method were positive by PCR, whereas none of the isolates negative by 3D and modified Hodge test was positive by PCR. Among admitted patients urinary catheterisation was the major risk factor followed by obstructive uropathy, three patients developed urosepsis. High occurrence of *bla*$_{CMY-1}$ AmpC β-lactamase warrants health care workers to endorse good hospital practices.

Key words AmpC β lactamases - *bla*$_{CMY-1}$ - *E. coli* - UTI

AmpC β-lactamases are clinically important cephalosporinases encoded on the chromosome of many *Enterobacteriaceae* and a few other organisms where they mediate resistance to cefazolin, cephalothin, cefoxitin, other penicillins, and β-lactamase inhibitor/ β-lactam combinations. Transmissible plasmids have acquired genes for AmpC enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal *bla*AmpC gene, such as *Escherichia coli*, *Klebsiella pneumoniae,* and *Proteus mirabilis*[1]. Therapeutic options for infections caused by Gram-negative organisms expressing plasmid mediated AmpC β-lactamases are limited because these organisms are usually resistant to all β-lactams except

cefepime, cefpirome and carbapenems. It is important to distinguish plasmid mediated AmpC β-lactamases from chromosomally mediated enzymes for surveillance; epidemiology and hospital infection control because plasmid mediated genes can spread to other organisms within the hospital. There are currently no guidelines to detect these though many phenotypic assays like modified Hodge test, three dimensional test (3D test), Tris EDTA disk tests and disk potentiation test using boronic acid, *etc.* are available[1]. Six families of plasmid mediated AmpC β-lactamases have been identified. These include MOX, CIT, DHA, ACC, FOX and EBC[2]. In a previous study we found high prevalence of AmpC β-lactamases in *E. coli* and *Klebsiella* spp. causing complicated UTI[3]. CMY-1 is a plasmid mediated cephamycinase first identified in 1989 from Korea and contributes to a significant proportion of cefoxitin resistant isolates of *E. coli* in Korea[4,5] and blood stream isolates of *K. pneumonia* (14 of 61 isolates producing AmpC β-lactamase)[6]. We report here high occurrence of bla_{CMY-1} AmpC β lactamase producing *E. coli* in cases of complicated UTI.

A prospective study was undertaken at the Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India, to know the occurrence of bla_{CMY-1} AmpC production in isolates of *E. coli* obtained from patients with complicated urinary tract infection (UTI). Over a period of one month (January 2008), 100 consecutive *E.coli* isolates grown in significant counts from urine sample of patients with complicated UTI were included in the study. Repeat isolates from the same patient were excluded. Complicated UTI was defined as infection developing in a patient with anatomically, physiologically or functionally compromised urinary tract. All demographic details of the patients were noted. The study protocol was approved by the ethics committee of the Institute. Antibiotic susceptibility was determined by using the Kirby Bauer's disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[7] using the Muller Hinton agar (Difco, USA) and antimicrobial disks (Oxoid, UK and Hi-Media, Mumbai). The following drugs were tested: ceftriaxone (30 μg), cefotaxime (30 μg), gentamicin (10 μg), amikacin (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), nitrofurantoin (30 μg), and co-trimoxazole (25 μg). If an organism showed resistance to all the above mentioned drugs, then it was tested against second line agents like cefoperazone-sulbactam (75 μg + 30 μg), piperacillin-tazobactam (75 μg + 10 μg) and imipenem (10 μg). *E. coli* ATCC 25922 was used as control for antibiotic susceptibility tests and preparing lawn culture for the phenotypic tests for AmpC β-lactamase production.

Isolates were initially screened for AmpC β-lactamase production by using a 30 μg disk of cefoxitin to detect AmpC production. All cefoxitin-resistant and cefoxitin-intermediate susceptible isolates were considered for further testing. For phenotypic confirmation, three tests, *viz.* modified Hodge test using cefoxitin disk[4], AmpC disk test using TRIS EDTA[8] and the modified three dimensional test[9], were used. Distortion of inhibitory zone >3 mm was taken as positive for scoring the 3D and modified Hodge test. A clearly visible indentation or flattening of zone of cefoxitin inhibition was scored as positive for the AmpC disk test. All isolates showing a positive result by two out of the three tests were tested for the presence of the bla_{CMY-1} gene by PCR as described earlier[10]. Briefly, PCR was done using the oligonucleotide primers P1 (5'-GTGGAT TCA TCC GAG AAG ATG-3') and P2 (5'-GATGGG CTT GTC CTC TTT CG-3'). The reaction conditions were 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, in a 20 μl volume mix, containing 1 U of Taq DNA polymerase, 0.2 pmol each of primer, 250 μM each of 4 dNTPs and 1 μl of the heat extracted template DNA. The presence of 258 bp band of the PCR products was detected by 1.5 per cent agarose gel electrophoresis.

Of the 100 isolates tested, 59 were positive for AmpC production. A total of 40 isolates were positive by all tests. The mean values of distortion zone for modified Hodge test and 3D test were 5.3 and 5.64 mm, respectively. The occurrence of antimicrobial resistance in these isolates was as follows; ceftriaxone-97 per cent (57/59), cefotaxime-95 per cent (56/59), gentamicin-92 per cent (54/59), amikacin-24 per cent (14/59), nalidixic acid-100 per cent (59/59), ciprofloxacin-97 per cent (57/59), norfloxacin-98.5 per cent (58/59), nitrofurantoin-19 per cent (11/59), co-trimoxazole-88 per cent (52/59). Eighteen of the 59 isolates were resistant to all first line drugs for which second line agents were tested. Resistance to cefoperazone plus sulbactam and piperacillin plus tazobactam was 72 per cent (13/18) each. All the 18 isolates were sensitive to imipenem. PCR established the presence of bla_{CMY-1} gene in 56.6 per cent (34/59) of the AmpC producing *E. coli* (Table). Eight of the 13 isolates which were negative by EDTA disk method were positive by

Table. Phenotypic tests and PCR for CMY-1 β-lactamase

Test	Phenotype positive	Phenotype negative
AmpC disk test	46	13
CMY-1 PCR positive	26	8
Modified Hodge test	50	9
CMY-1 PCR positive	29	0
Modified 3D test	56	3
CMY-1 PCR positive	32	0

PCR, whereas none of the isolates negative by 3D and modified Hodge test was positive by PCR.

Plasmid mediated cephamycinases represent clinically relevant new members of Class C β-lactamases which may spread both by translocation of the strains harbouring bla_{CMY} genes and by transfer of the genes among members of the family *Enterobacteriaceae* because of their location on R factors. The CMY-1 genes are found adjacent to an insertion sequence common region involved in gene mobilization into class-1 integrons[1]. Plasmid-mediated AmpC β-lactamases have been found worldwide but are less common than extended-spectrum β-lactamases (ESBLs), and in *E. coli*, these appear to be less often a cause of cefoxitin resistance than an increased production of chromosomal AmpC β-lactamase[1]. Currently 43 CMY alleles are known; of which CMY-2 are the commonest CMY type reported having the broadest geographic spread. CMY2 is an important cause of β-lactam resistance in non typhoid *Salmonella* strains in many countries, there have been only a few reports regarding CMY-1[6]. Of the 59 patients, 29 were males and 30 females, age ranged from 3 to 75 yr, with mean of 36 yr. There were 11 children <14 yr. Twenty six isolates were from outpatients. Among admitted patients urinary catheterisation was the major risk factor (20/33 cases) followed by obstructive uropathy (5 cases), malignancy (2 cases) and chronic renal failure (3 cases). Three patients developed urosepsis. In 48 patients, infection was of nosocomial origin denoting endemicity in our setting and calls for better infection control practices and surveillance for AmpC β-lactamase producing Gram-negative organisms.

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