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Correspondence

Multi-drug resistance in clinical isolates of Gram-negative bacilli in a tertiary care hospital of Assam

Sir,

The widespread use of β-lactam antibiotics has lead to the development of resistance to this group of antibiotics in bacterial pathogens due to various types of β lactamase production. Amongst the mechanisms of resistance to third generation cephalosporins, production of extended spectrum β-lactamases (ESBLs) and AmpC β lactamases is the most common[1]. Since both ESBL and AmpC β-lactamase are encoded on plasmids and confer a selective advantage to strains harbouring these in a hospital setting, it is important to know the occurrence of ESBL and AmpC producing strains as well as their antibiotic susceptibilities to newer agents to guide empirical therapy. This laboratory based study was carried out over a period of one year from May 2011 to April 2012 at the department of Microbiology, Assam Medical College and Hospital, Dibrugarh, Assam, India to determine the occurrence of multi-drug resistant Gram-negative bacilli producing AmpC β-lactamases and ESBL causing infection in hospitalized patients.

A total of 150 non repeat, non enteric consecutive clinical isolates of different Gram-negative bacilli obtained from various clinical specimens like blood, wound, swab, pus, CSF, urine, sputum suction tube aspirate, tracheal aspirate peritoneal fluid of hospitalized patients were identified biochemically by the standard methods[2].Isolates from mixed culture were excluded from the study. The antibiogram of the isolates was determined by the standard Kirby Bauer's disc diffusion method[3] and Clinical Laboratory Standards Institute (CLSI) recommendations[4].

Isolates that yielded a cefoxitin zone diameter of less than 18 mm and resistant to 3rd generation cephalosporins (screen positive) were tested for AmpC enzyme production by AmpC disc test[1] and modified three-dimensional test as described by Manchanda and Singh[5]. In modified three-dimensional test, fresh overnight growth of test organism from Mueller-Hinton agar (MHA) plate was taken in a micro centrifuge tube. Peptone water was added and centrifuged at 900*g* for 15 min. Crude enzyme extract was prepared by repeated freeze thawing at -80°C seven times. A lawn culture of *Escherichia coli* ATCC 25922 (Hi-media microbiogics) was prepared on MHA plates and cefoxitin (30 µg) discs were placed on the plate. Linear slits were cut using a sterile surgical blade 3 mm away from the cefoxitin disc; 30 to 40 µl of the enzyme extract was added to a well made at the outer edge of the slit, without overflowing. The plates were kept upright for 5 to 10 min until the liquid dried and were incubated at 37°C for overnight. Clear distortion of zone of inhibition of cefoxitin was taken as AmpC β lactamase producer.

Isolates that fall in the sensitivity range to cefotaxime, ceftazidime or ceftriaxone (3GC) and cefoxitin as per the CLSI[4] were subjected to disk antagonism test[6,7] for inducible AmpC detection. Dicks of inducing agent cefoxitin (CX) and cephalosporins [cefpirome (CPM), ceftazidime(CZ), ceftriaxone(CTR) and cefotaxime (CTX)] were placed on the surface of the test bacterial lawn on MHA plates on a lawn of bacterial culture of the suspected inducible AmpC β-lactamase producers separated by 15 mm. The plates were examined after overnight incubation at 37 ^{0}C. If blunting of the cephalosporin discs adjacent to the cefoxitin discs occurred, the organisms were considered to produce inducible AmpC β lactamase.

Screen positive isolates for possible ESBL production were confirmed by combined disc method (CLSI)[4] .

With modified disc diffusion method 108 isolates were identified as possible ESBLs producers and 113 isolates as possible AmpC producers resistant to

cefoxitin. Multi-drug resistance (resistance to 3 or more antibiotics) was detected with commonest phenotype being ampicillin, co-trimoxazole and ceftazidime resistance phenotype (50.6%).

AmpC β-lactamase (both plasmid mediated and inducible chromosomal) was detected in 48 (32%) Gram-negative isolates which was significantly higher than studies elsewhere[8-10]. Maximal occurrence of AmpC β-lactamase was found among non fermenting Gram-negative bacilli as shown in the Table. Modified three Dimensional test was found to be more sensitive in detection of plasmid mediated AmpC as it could detect three more isolates to be positive which were negative by AmpC disc test. Thirty eight (95%) of these isolates were cefoxitin resistant whereas the remaining two isolates were cefoxitin sensitive. This finding reiterates the limited usefulness of using cefoxitin as a screening disc to detect plasmid mediated AmpC beta lactamase producing isolates[5,10].

Inducible expression of chromosomal AmpC β-lactamases, although rare in *E. coli* and *Klebsiella pneumoniae*, is associated with a significant risk of therapeutic failure with all β-lactam drugs except carbapenems[11]. Disc antagonism test for chromosomal inducible AmpC β-lactamase was positive in eight isolates including four *K. pneumoniae* isolates and four non fermenting Gram-negative bacilli. Since earlier studies have not reported presence of this type of resistance in *Klebsiella* isolates[12], confirmation of this finding by molecular methods will be needed.

ESBL production was detected in 27.33 per cent isolates (41/150). However, in a previous study from this institute detected ESBL in 33 per cent isolates with highest incidence in *K. pneumoniae* (42.5%)[13]. Their study included both in-and out-patients' samples, whereas we included samples from hospitalized patients only. Increased occurrence AmpC β-lactamase in hospitalized patients may be a reason for relative decrease of ESBL in these isolates in our study. Co-existence of AmpC β-lactamase may give false negative result in detection of ESBL[8].

Co-existence of AmpC and ESBL was demonstrated in two *Klebsiella* isolates; which could be because of dissemination of plasmid mediated AmpC enzyme among *Entrerobacteriaceae* sometimes in combination with ESBL[14].

In the present study strong association was noticed among AmpC β-lactamase production and duration of hospitalization for more than 72 h ($P<0.01$) using chi square test. This shows that at present AmpC harbouring isolates are largely restricted to hospitalized patients only. However, no such association was observed in ESBL producers. This is in accordance with studies by other authors from India[5,15].

All the AmpC and ESBL positive isolates were multi-drug resistant. Concurrent resistance to amikacin and gentamicin was also significantly associated with production of AmpC β-lactamase and ESBL ($P < 0.05$) using Chi square test. These isolates also showed concurrent resistance to cefoxitin (96%), ampicillin (92%), ceftriaxone and cefotaxime (83%), ceftazidime (77%), co-trimoxazole (71%) and ciprofloxacin (69%), respectively. However, all the ESBL and AmpC producing isolates were sensitive to imipenem, thereby reiterating the continued efficacy of carbapenems as the first line agents for treatment of health care

Table. β lactamase production in various Gram-negative bacteria

Organisms	Total isolates N (%)	AmpC positive (N)	ESBL positive (N)	AmpC + ESBL positive (N)
Klebsiella spp.	55 (36.67)	16	15	2
Escherichia coli	49 (32.67)	14	15	0
Non-fermenting Gram-negative bacilli (NFGNB)	36 (24.00)	13	9	0
Pseudomonas aeruginosa	1(0.67)	0	0	0
Proteus vulgaris	3 (2.00)	1	0	0
Proteus mirabilis	2 (1.33)	1	0	0
Providencia spp.	1 (0.67)	1	1	0
Enterobacter spp.	1 (0.67)	0	1	0
Citrobacter spp.	2 (1.33)	2	0	0
Total	150 (100.00)	48	41	2

associated infections caused by the members of *Enterobacteriaceae* producing ESBL and AmpC β-lactamases.

In conclusion, we detected AmpC and ESBLs in the Gram-negative multi-drug resistant clinical isolates obtained from hospitalized patients. Strict antibiotic policies and measures to limit indiscriminate use of cephalosporins should be undertaken to minimize the emergence of such resistance.

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