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Extended-spectrum β-lactamase & carbapenemase-producing Gram-negative bacilli in neonates from a tertiary care centre in Dibrugarh, Assam, India

Sir,

The choice of first-line antibiotic for the initiation of treatment in cases of neonatal sepsis/meningitis is a challenge to a clinician. It is further complicated when it is suspected to be caused by drug-resistant bacteria. β-lactam antibiotics are used widely worldwide against infections caused by Gram-negative bacteria. Resistance is known to be due to various mechanisms among which production of extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs) is reported[1-12].

Presence of ESBLs and MBLs has been reported among bacterial isolates from the neonates[3-7]. New Delhi metallo-β-lactamase (NDM-1)-producing *Enterobacteriaceae* has also been isolated from neonates with sepsis in India[3,4,7]. The present study reports the presence of bla_{SHV}, bla_{TEM}, bla_{CTX-MU}, $bla_{OXA23-like}$, $bla_{OXA51-like}$, $bla_{OXA58-like}$ and bla_{NDM1} among the *Enterobacteriaceae* (*Klebsiella* and *Escherichia coli*) as well as non-fermenters (*Acinetobacter baumannii* and *Pseudomonas* spp.) obtained from the cerebrospinal fluid (CSF) of neonates from a tertiary care centre in Dibrugarh, Assam, India.

Consecutive admissions in the neonatal ward of Paediatrics department, Assam Medical College and Hospital, Dibrugarh, with symptoms of suspected meningitis were screened for bacterial aetiology from January 2013 to January 2015. All microbiological procedures were carried out in the Bacteriology division of Microbiology Laboratory, Regional Medical Research Centre, Dibrugarh. Lumbar puncture for CSF collection (volume up to 1 ml) was done at the neonatology unit. CSF analysis was done when there was clinical or proven sepsis and in whom blood culture grew microorganism. Ethical clearance for the study was obtained from the ethics committees of both the institutions (AMC/EC/8094 and RMRC/Dib/IEC(human)/2012-13/329). Written informed consent was obtained from parents/ guardians of all neonates.

A total of 67 (22.1%) of the 303 CSF samples tested were positive for pathogens. Gram-negative organisms were predominant (n=32, 48%). The most frequent Gram-negative bacteria isolated were *Acinetobacter baumannii* (n=12), *Klebsiella* spp. (n=8) and *Pseudomonas* spp. (n=6). Less frequently isolated were *Neisseria meningitidis* (n=2), *Escherichia coli* (n=1), *Sneathia* (n=1), *Cronobacter sakazakii* (n=1) and *Roseomonas cervicalis* (n=1). In the present study, isolates of *A. baumannii* (n=12), *Klebsiella* (n=8), *Pseudomonas* spp. (n=6) and *E. coli* (n=1) were characterized. Three isolates of *A. baumannii*, two of *Klebsiella* and one isolate of *Pseudomonas* detected by the direct CSF polymerase chain reaction (PCR)[13] were included for genotypic characterization. Isolates resistant to at least one of the following: cefotaxime, ceftriaxone, ceftazidime, or cefepime were screened for ESBL as per the Clinical Laboratory Standard Institute (CLSI) guidelines[14].

Screening for carbapenemase production was done by disc diffusion using ertapenem (10 µg) disc as per the CLSI[14]. A zone size of 19-21 mm was considered positive for carbapenemase production. Those showing carbapenemase production by screening test were tested by modified Hodge test (MHT)[14]. For quality control, *Klebsiella pneumoniae* ATCC BAA 1705 and BAA 1706 were taken as positive and negative controls. Carbapenem-resistant isolates were also tested for MBL production by phenotypic disc confirmatory test (PDCT)[15].

Genotypic analysis for bla_{NDM-1}, bla_{SHV}, bla_{TEM}, $bla_{OXA23-like}$, $bla_{OXA51-like}$, $bla_{OXA58-like}$ and bla_{CTX-MU} was performed for isolates found to be positive in ESBL and

MBL. Established primers were used on the genomic DNA for amplification of the different genes including bla_{SHV}, bla_{TEM}[16], $bla_{CTX\text{-}MU}$[17], $bla_{OXA23\text{-}like}$, $bla_{OXA51\text{-}like}$, $bla_{OXA58\text{-}like}$[18] and bla_{NDM1}[19]. Bacterial extraction was done with the Wizard Genomic DNA Purification Kit (Promega, USA). PCR on each isolate was carried out using Veriti 96-Well Thermal Cycler (Applied Biosystems, USA). Clinical isolates which were confirmed for the presence of different genes, namely bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}MU}$, $bla_{OXA23\text{-}like}$, $bla_{OXA51\text{-}like}$ and $bla_{NDM\text{-}1}$, by sequencing were taken as positive control.

ESBL was detected in eight (88.9%) isolates of *Acinetobacter*, one (20%) of *Pseudomonas*, six (100%) of the *Klebsiella* and the lone isolate of *E. coli*, by initial phenotypic screen test. Of these, only three (33%) of the *Acinetobacter* were confirmed as ESBL producers by PDCT. None of the isolates of *E. coli*, *Klebsiella* and *Pseudomonas* were positive for the confirmatory test. This may be because the PDCT does not detect all ESBLs. Some organisms with ESBLs contain other beta-lactamases resulting in a false-negative test. These β-lactamases include AmpC and inhibitor-resistant TEM. Hyperproduction of TEM and/or SHV β-lactamases in the organism with ESBL may also cause false-negative PDCT[20].

Carbapenemase production was detected in seven (77.8%) isolates of *Acinetobacter*, one (20%) of *Pseudomonas*, four (66.7%) of *Klebsiella* and the lone isolate of *E. coli* by initial screen and confirmed in the *E. coli*, six (66.7%) of *Acinetobacter* and three (50%) of the *Klebsiella* isolates by the MHT. The remaining isolates could be resistant to the carbapenem by a different mechanism other than carbapenemase production-like expression of an extended-spectrum cephalosporin-like Amp C. MBL was detected among seven (77.8%) *Acinetobacter*, the lone *E. coli* and four (66.7%) per cent *Klebsiella* isolates by PDCT.

Genotypic characterization (Table I) revealed that most common resistant genes harboured by *Acinetobacter* were bla_{NDM1} (41.7%) and $bla_{OXA51\text{-}like}$ (41.7%) followed by $bla_{OXA23\text{-}like}$ (25%), $bla_{OXA58\text{-}like}$ (16.7%), bla_{SHV} (16.7%), bla_{TEM} (8.3%) and $bla_{CTX\text{-}MU}$ (8.3%). *Klebsiella* harboured all the genes included except for $bla_{OXA58\text{-}like}$. The *E. coli* isolate harboured the ESBL gene bla_{SHV} and MBL gene bla_{NDM1} only. None of the six isolates of *Pseudomonas* harboured the β-lactamase genes tested. Table II shows the multiplicity of genes in the different isolates. The presence of single β-lactamase encoding gene was seen among 33 per cent of the *A. baumannii* (4/12) isolates and 12.5 per cent of *Klebsiella* (1/8) isolates. The presence of two genes in same host was seen in 23 per cent of *A. baumannii* (3/12) and 12.5 per cent of *Klebsiella* (1/8) isolates and in the only *E. coli* isolate. Three genes were detected in 25 per cent of *Klebsiella* (2/8) and 8.3 per cent of the *A. baumannii* (1/12) isolates. One isolate each of *Acinetobacter* (8.3%) and *Klebsiella* (12.5%) showed the presence of six and five genes, respectively. Co-existence of ESBL and carbapenemase-encoding genes was seen in eight isolates.

β-lactamase-producing bacteria are a major problem worldwide, and their prevalence varies in different geographical regions. *Enterobacteriaceae* is the common ESBL and MBL producers known in India[1-7,21,22]. Non-fermenters such as *Acinetobacter* and *Pseudomonas* are also known producers[15,21].

An Indian study among neonates found about 60 per cent of the *Klebsiella* and 75 per cent of the *E. coli* isolates to be ESBL producers[5]. In another study from West India, ESBL-producing *Klebsiella* and *E. coli* were 94.87 and 92 per cent, respectively[22]. *Klebsiella* (60%) was the most common ESBL producer followed by *E. coli* (30%) in a study by Vijayakanthi *et al*[6]. In a study carried out among hospitalized patients in a tertiary care centre in Assam, ESBL production was detected in 27.33 per cent isolates (41/150) and AmpC β-lactamase (both plasmid-mediated and inducible chromosomal) was detected in 32 per cent Gram-negative isolates[8]. In another study from a tertiary referral hospital in North-East India, the presence of bla_{OXA2} and other ESBL genes ($bla_{SHV\text{-}148}$, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$) in different Gram-negative bacilli has been reported[9]. ESBL genes namely SHV, TEM and CTX-M have also been described among *E. coli* isolates from Assam[10]. There also exists report on the detection of OXA-48 β-lactamase gene in *E. coli* and *P. aeruginosa* in Assam[11]. NDM and ESBL producing urinary *E. coli* and *K. pneumonia* isolates have also been reported from this region[12]. NDM-1 was seen among the *Klebsiella* (50%), *A. baumannii* (41.7%) and the lone *E. coli* isolate tested in the present study. Even though the existence of NDM-1 among Gram-negative isolates has been reported from North-East region, its demonstration in isolates from neonates is extremely limited[4].

Use of β-lactam antibiotics has led to the emergence of β-lactamases producers and is a great concern

Table I. Phenotypic and genotypic detection of resistance among Gram-negative isolates from cerebrospinal fluid (CSF) of neonates

ESBL detection among the Gram-negative isolates from CSF of neonates							
Isolates	Phenotypic characterization			Genotypic characterization			
	Total tested	Initial screen positive, number of isolates (%)	Positive by PDCT, number of isolates (%)	Total tested	bla_{SHV} positive, number of isolates (%)	bla_{TEM} positive, number of isolates (%)	$bla_{CTX\text{-}MU}$ positive, number of isolates (%)
Acinetobacter	9	8 (88.9)	3 (33.3)	12	2 (16.7)	1 (8.3)	1 (8.3)
Klebsiella	6	6 (100)	0	8	2 (25)	1 (12.5)	3 (37.5)
Pseudomonas	5	1 (20)	0	6	0	0	0
Escherichia coli	1	1 (100)	0	1	1 (100)	0	0
Carbapenemase detection among the Gram-negative isolates from CSF of neonates							
Isolates	Phenotypic characterization				Genotypic characterization			
	Total tested	Initial screen, number of isolates (%)	Positive for MHT, number of isolates (%)	Positive for MBL by PDCT, number of isolates (%)	Total tested	$bla_{OXA23\text{-}like'}$ number of isolates (%)	$bla_{OXA51\text{-}like'}$ number of isolates (%)	$+bla_{OXA58\text{-}like'}$ number of isolates (%)	$bla_{NDM\text{-}1'}$ number of isolates (%)
Acinetobacter	9	7 (77.8)	6 (66.7)	7 (77.8)	12	3 (25)	5 (41.7)	2 (16.7)	5 (41.7)
Klebsiella	6	4 (66.7)	3 (50)	4 (66.7)	8	2 (25)	2 (25)	0	4 (50)
Pseudomonas	5	1 (20)	0	0	6	0	0	0	0
E. coli	1	1 (100)	1 (100)	1 (100)	1	0	0	0	1 (100)

PDCT, phenotypic disc confirmatory test; MBL, metallo-β-lactamases; MHT, modified Hodge test; ESBL, extended-spectrum β-lactamases

Table II. Multiplicity of β-lactamase genes among the Gram-negative isolates in the study

Genes	*Acinetobacter baumannii* (n=12)	*Klebsiella* (n=8)	*Escherichia coli* (n=1)
6 genes			
$bla_{TEM} + bla_{SHV} + bla_{CTX\text{-}MU} + bla_{OXA23\text{-}like} + bla_{OXA51\text{-}like} + bla_{NDM\text{-}1}$	1	None	None
5 genes			
$bla_{SHV} + bla_{CTX\text{-}MU} + bla_{OXA23\text{-}like} + bla_{OXA51\text{-}like} + bla_{NDM\text{-}1}$	None	1	None
3 genes			
$bla_{TEM} + bla_{CTX\text{-}MU} + bla_{NDM\text{-}1}$	None	1	None
$bla_{OXA51\text{-}like} + bla_{OXA58\text{-}like} + bla_{NDM\text{-}1}$	1	None	None
$bla_{SHV} + bla_{CTX\text{-}MU} + bla_{NDM\text{-}1}$	None	1	None
2 genes			
$bla_{OXA58\text{-}like} + bla_{NDM\text{-}1}$	1	None	None
$bla_{OXA51\text{-}like} + bla_{NDM\text{-}1}$	1	None	None
$bla_{SHV} + bla_{NDM\text{-}1}$	None	None	1
$bla_{OXA23\text{-}like} + bla_{OXA51\text{-}like}$	1	1	None
Single gene			
bla_{SHV}	1	None	None
$bla_{OXA23\text{-}like}$	1	None	None
$bla_{OXA51\text{-}like}$	1	None	None
$bla_{NDM\text{-}1}$	1	1	None

for the clinicians since these are multidrug-resistant causing therapeutic failure. Carbapenems are the drug of choice for life-threatening infections with such ESBL-producing organisms. This has probably led to the emergence of carbapenem resistance among the organisms creating another great challenge for treatment. The present study also found the existence carbapenem resistance among the Gram-negative isolates. Carbapenemase production was phenotypically confirmed in the lone isolate of *E. coli*, 77.8 per cent of *A. baumannii* and 66.7 per cent of the *Klebsiella* isolates. Emerging reports of carbapenem resistance among *A. baumannii* are also available[3,7,11,18].

Though the present study was limited due to its small sample size, it still highlighted the emergence of carbapenem resistance among common bacterial isolates from neonatal infection and the presence of carbapenemase encoding genes for OXA and NDM-1. Thus, screening for these and taking appropriate measures for control of their spread are of major importance.

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