RESEARCH ARTICLE

AmpC beta-lactamases in Klebsiella pneumoniae: An emerging threat to the paediatric patients

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Abstract

Objective: To determine the burden of AmpC beta-lactamase producing Klebsiella pneumoniae and its antimicrobial profile among paediatric patients.

Methods: This cross-sectional study was conducted at the Microbiology Department of The Children's Hospital and the Institute of Child Health in Lahore, Pakistan, from May 2014 to April 2015, in which isolates of Klebsiella pneumoniae were screened by using the cefoxitin disc. Confirmation was done by inhibitor-based method using 400 micro grams of boronic acid dispensed on the cefoxitin discs. The zone sizes of cefoxitin with and without the boronic acid were compared. The antimicrobial susceptibility testing was performed using Kirby Bauer disc diffusion method.

Results: Positive cultures yielded 585 Klebsiella pneumoniae out of which 220(37.6%) strains were AmpC beta-lactamase-positive on the basis of cefoxitin screening and 126(21.53%) were positive on the basis of inhibitor-based confirmatory method. Most of the infected patients 73(57.9%) were neonates. All AmpC beta-lactamase-producing strains were resistant to cephalosporins. They also exhibited resistance to ciprofloxacin 109(86.5%), amikacin 98(77.8%), levofloxacin 8(77.8%), cefoperazone-sulbactam 81(64.3%), piperacillin-tazobactam 82(65.1%), meropenem, 56(44.4%) and imipenem 32(25.4%).

Conclusion: Prompt identification of AmpC beta-lactamases using inhibitor-based confirmatory test can help reduce the burden of these pathogens.

Keywords: AmpC β-lactamase, Multidrug resistant Klebsiella pneumoniae, Inhibitor-based method. (JPMA 68: 893; 2018)

Introduction

AmpC beta (β)-lactamases are clinically substantial enzymes and are associated with the resistance to a large variety of β-lactam drugs except carbapenems and cefepime.[1] AmpC β-lactamases are clinically significant as they can hydrolyse penicillins, cephalosporins and cephamycins. They resist to a wide variety of β-lactamase inhibitor including α-methoxy-β-lactam such as cefoxitin. They are distinct from extended-spectrum beta-lactamases (ESBLs) by their ability to hydrolyse cephamycins and they are not affected by β-lactamase inhibitors.[2] In the Ambler structural classification of β-lactamases, AmpC enzymes belong to class C, while in the functional classification scheme of Bush these are assigned to group 3.[3]

Genes for AmpC β-lactamases are encoded on the chromosomes of several members of the family enterobacteriaceae. Plasmid mediated AmpC β-lactamases are thought to have originated from chromosomes of several enterobacteriaceae species and are infrequently inducible.[4] Plasmids carrying genes for AmpC β-lactamases often carry multiple resistant genes, including genes for resistance to aminoglycosides, quinolones, chloramphenicol, sulfonamide, tetracycline, and trimethoprim as well as genes for other β-lactamases such as CTX-M-3.[5] AmpC enzymes are located in periplasm, typically having molecular mass of 34 to 40 kDa and isoelectric points of >8.0.[4] Plasmids with these genes can spread among members of the family Enterobacteriaceae and have been documented in many countries.[6]

Plasmid mediated AmpC β-lactamase producing Klebsiella (K.) pneumoniae and Escherichia (E.) coli have been responsible for nosocomial outbreaks of infections and colonisation.[7] AmpC β-lactamases are associated with erroneous antimicrobial susceptibility in routine testing.[8] AmpC β-lactamases are not inhibited by β-lactamase inhibitors such as clavulanic acid.[3] Strains with AmpC genes are often resistant to multiple antibiotic. Cefepime is a poor inducer of AmpC β-lactamases, rapidly perforates through the outer cell membrane and is not thoroughly hydrolysed by the enzyme.[9] Temocillin, a 6-alpha-methoxy derivative of ticarcillin is active in vitro against many AmpC-

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producing Enterobacteriaceae.[10] Colistin sulphate and tigecycline are another option for the treatment of AmpC producing bacteria which are resistant to carbapenems.[11]

The current study was planned to observe the AmpC β-lactamases which limits the therapeutic options for infections caused by gram-negative organisms and are usually resistant to all the β-lactam antibiotics. The ultimate objectives was to determine the burden of AmpC β-lactamases in K. pneumoniae, efficacy of cefoxitin and inhibitor-based method for the detection of AmpC β-lactamases in K. pneumoniae and to determine the antimicrobial resistance profile of AmpC producing K. pneumoniae among paediatric patients.

Subjects and Methods

This cross-sectional study was conducted at the Microbiology Department of The Children's Hospital and the Institute of Child Health in Lahore, Pakistan, from May 2014 to April 2015 for which the ethical approval was granted by the institutional review board. Clinical samples of blood, urine, cerebrospinal fluid (CSF), pus, ear swabs and various tips were processed to detect K. pneumoniae harbouring AmpC β-lactamase. The collected samples were of both genders of up to 15 years of age. Only those samples were processed which showed resistance to cefoxitin 30µg disc. The isolates susceptible to cefoxitin were excluded. The samples were processed to isolate K. pneumoniae to identify AmpC producing strains and to report antimicrobial profile.

The K. pneumoniae isolates were tested for AmpC β-lactamase production using the cefoxitin disc (30 µg). Isolates that yielded zone diameters of less than 18mm were considered positive for AmpC β-lactamase production in the screening test. All the isolates were tested for the confirmation of AmpC β-lactamase production utilising a disc of cefoxitin (30µg) with and without boronic acid (400µg), placed on the Muller Hinton aga r plate and the plates were incubated overnight at 37 degree Celsius (Figure-1). Boronic acid was prepared by dissolving 120mg of phenylboronic acid in 3mm of dimethyl sulfoxide (DMSO). Three millilitres of sterile distilled water was added to this solution, and freshly prepared 20µl stock solution of boronic acid was dispensed on cefoxitin. A zone size of ≥5mm around the disc of cefoxitin-containing boronic acid in comparison to cefoxitin alone was reported as an AmpC β-lactamase producer.

Antimicrobial susceptibility testing was performed by using Kirby Bauer disc diffusion method.[12] Bacterial suspensions were prepared in accordance with the 0.5 McFarland's turbidity standard. The suspension was streaked on the Mueller Hinton agar plate. The antibiotic discs of amikacin, gentamicin, co-amoxiclav, cefotaxime, ceftriaxone, ceftazidime, cefuroxime, cefixime, cefoxitin, ciprofloxacin, moxifloxacin, levofloxacin, piperacillin-tazobactam, cefoperazone-sulbactam, meropenem and imipenem were used for antimicrobial susceptibility testing. After overnight incubation at 35-37°C, the diameter of each zone of inhibition was measured. Result of each isolate were reported as sensitive, intermediate or resistant to the antimicrobial disc predicated on the interpretation chart of zone sizes recommended by Clinical and Laboratory Standard Institute (CLSI) manual 2013.[13]

Results

Of the 26,602 clinical samples processed, 585(2.2%) isolates were K. pneumoniae. And of them, 220(37.6%) isolates were AmpC β-lactamase producer on screening, while 126(21.53%) isolates were AmpC β-lactamase producer on inhibitor-based confirmatory method (Table-1).

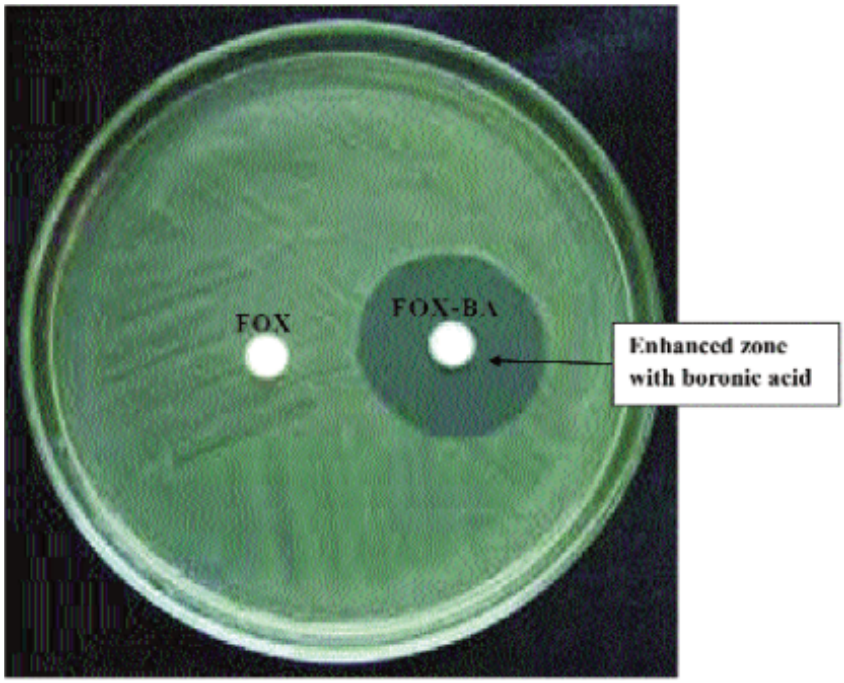

Figure-1: Cefoxitin alone and in combination with boronic acid.

Table-1: Frequency of AmpC β-lactamase producing Klebsiella pneumoniae (n=585).

Klebsiella pneumoniae	AmpC Screening	Inhibitor Based Confirmation
AmpC β-lactamase	220	126
Non-AmpC	365	459

Table-2: Demographic distribution of patients infected with AmpC β-lactamase producing K. pneumoniae (n=126).

	Frequency	Percentage
Gender		
Male	84	66.7%
Female	42	33.3%
Age Groups		
Neonates	73	58%
Infants	19	15.1%
1 year-5 year	15	11.9%
5 year-10 year	09	7.1%
10 year-15 year	10	7.9%

(Neonates=less than 28 days, infants=29 days-1 year).

Table-3: Source distribution of AmpC β-lactamase producing Klebsiella pneumoniae (n=126).

Clinical Specimens	Frequency	Percentage
Blood	58	46.0%
Urine	34	27.0%
Pus	10	7.8%
CSF	7	5.6%
Endotracheal tube	7	5.6%
Pleural Fluid	4	3.2%
Tracheal Secretions	4	3.2%
Wound Swab	1	0.8%
Sputum	1	0.8%

CSF: Cerebrospinal fluid.

AmpC-producing K. pneumoniae were isolated from 84(66.7%) male and 42(33.3%) female patients. There were 73(58%) neonates and 19(15.1%) infants (Table-2).Out of the 126(21.53%) AmpC β-lactamase-producing K. pneumoniae isolates, 58(46.0%) were obtained from blood, 34(27.0%) from urine, 10(7.8%) from pus and 7(5.6%) from CSF (Table-3). The overall outcome showed that 110(87.3%) patients were discharged after successful treatment. The outcome of the 5(4.0%) patients who left against medical advice (LAMA) remained unknown. There were 11(8.7%) cases of mortality (Table-4). All of the 126(100%) isolates were resistant to co-amoxiclav, ceftazidime, cefotaxime, cefuroxime, cefixime, ceftriaxone and cefoxitin. There were 56(44.4%) isolates which showed resistance against meropenem (Table-5). The relationship of outcome and in vitro susceptibility pattern of pathogens against meropenem and imipenem showed that majority of the mortality cases were infected with

Table-4: Outcome of the infected patients (n=126).

Outcome	n (%)
Discharge	110 (87.3)
Death	11 (8.7)
LAMA	5 (4.0)

LAMA: Leave against medical advice.

Table-5: Antimicrobial resistance profile of AmpC producing Klebsiella pneumoniae (n=126).

Antibiotics	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Co-amoxiclav	126 (100)	0 (0)	0 (0)
Ceftazidime	126 (100)	0 (0)	0 (0)
Ceftriaxone	126 (100)	0 (0)	0 (0)
Cefotaxime	126 (100)	0 (0)	0 (0)
Cefixime	126 (100)	0 (0)	0 (0)
Cefuroxime	126 (100)	0 (0)	0 (0)
Cefoxitin	126 (100)	0 (0)	0 (0)
Moxifloxacin	114 (90.5)	0 (0)	12 (9.5)
Gentamicin	114 (90.5)	0 (0)	12 (9.5
Ciprofloxacin	109 (86.5)	7 (7.9)	10 (5.6)
Levofloxacin	98 (77.8)	1 (0.8)	27 (21.4)
Amikacin	98 (77.8)	5 (4)	23 (18.3)
Piperacillin-Tazobactam	82 (65.1)	15 (11.9	29 (23.0)
Cefoperazone-Sulbactam	81 (64.3)	10 (7.9)	35 (27.8)
Meropenem	56 (44.4)	22 (17.5)	48 (38.1)
Imipenem	32 (25.4)	23 (18.3)	71 (56.3)

Table-6: Outcome vs. in vitro susceptibility with meropenem and imipenem (n=126).

Outcome	Meropenem			Imipenem		
	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate
Discharge	46	46	18	25	68	17
Death	7	1	3	4	2	5
LAMA	3	1	1	3	1	1

LAMA: Leave against medical advice.

meropenem and imipenem-resistant bacteria (Table-6).

Discussion

AmpC β-lactamases, in contrast to extended-spectrum beta-lactamases (ESBLs), not only hydrolyse broad and extended-spectrum cephalosporins but are resistant to inhibition by β-lactamase inhibitors such as clavulanic acid.[14] In our study 21.53% K. pneumoniae were AmpC β-lactamase-positive. A study conducted on 100 isolates of K. pneumoniae reported 32 (32%) isolates of AmpC β-lactamase producers.[15] A frequency of 33% AmpC β-lactamase producers was reported in135 isolates of K. pneumoniae.[2] Another review indicated 19.6% frequency of AmpC β-lactamase producing K. pneumoniae at Mansoura University Hospitals, Egypt.[16] There were 66.7% of AmpC β-lactamase producers found in male patients in our study. A study from a tertiary healthcare centre in Kano, Northwest Nigeria, showed 60% prevalence of AmpC β-lactamases producers in males and 40% in females.[17] The prevalence of 66.3% AmpC β-lactamases in males reported in another study.[18] Highest occurrence rate of 57.9% AmpC β-lactamase-producing K. pneumoniae were found in neonates. A study reported 42.86% AmpC β-lactamase-producing K. pneumoniae among patients <10 years of age and 36.84% in 11-20 years of age.[19] Occurrence of AmpC β-lactamase-producing strains of K. pneumoniae was different in various specimens. High occurrence of 58(46.0%) AmpC β-lactamase-producing K. pneumoniae was found in blood in the present study. A study described the occurrence of AmpC β-lactamases in different specimen such as urine (86.8%), blood cultures (7.7%) and other body sites (5.5%).[20] In the present study, all AmpC β-lactamase-producing K. pneumoniae were isolated from different wards. The highest numbers of the strains were isolated from Neonatal Nursery Unit (37.3%). Another study reported the prevalence rate of 45% AmpC β-lactamase-producing isolates from intensive care unit (ICU) and 22% from burn unit.[2] Another study showed the prevalence of AmpC β-lactamases in different wards (60%) from outpatients, (28%) from hospitalised patients and (12%) from ICU.[20]

The isolated strains of AmpC β-lactamase-producing K. pneumoniae showed resistance to multiple antibiotics. All of the AmpC β-lactamase-producing K. pneumoniae were resistant to co-amoxiclav and cephalosporins while variable resistance pattern was seen with other antibiotics. There were 32(25.4%) isolates resistant to imipenem. One study noted that the resistant pattern of AmpC β-lactamases to piperacillin-tazobactam (36.73%), amikacin (73.46%), ciprofloxacin (53.06%) and gentamicin (69.38%). All of the organisms were susceptible to imipenem.[21] In another study AmpC β-lactamase-producing isolates showed 40.5% resistance to azithromycin, 18.2% to co-amoxiclav, 27.0% ciprofloxacin, 67.5% to cefuroxime, 81.0% cefotaxime and 63.0% ceftazidime.[22] A study reported over AmpC β-lactamase from five Indian tertiary medical centres in 2012 showed 13.6% resistance pattern to amikacin, 27.8% to piperacillin-tazobactam and 81.8% to levofloxacin.[23] The antimicrobial profile of AmpC producing isolates vary in different studies. AmpC harbouring P.aeruginosa were multidrug resistant, the maximum sensitivity (89.1%) was seen with imipenem, followed by moderate activity with piperacillin-tazobactam (51.5%), amikacin (47.5%), carbenicillin (43.5%) and co-trimoxazole (43.5%), and poor susceptibility patterns with some of the other drugs.[24] Most of the studies showed that imipenem is a drug of choice in most of the AmpC β-lactamases producers.[11]

A study in a tertiary care hospital of Islamabad, Pakistan, reported high number of plasmid mediated AmpC β-lactamase-producing E. coli and K. pneumoniae.[25] A study conducted in Rawalpindi, Pakistan, recommended the use of disc approximation test to detect the AmpC beta-lactamase-producing E. coli and K. pneumoniae.[26]

The present study was a single-centre study. The detection of AmpC β-lactamase genes could not be done due to limited resources.

Conclusions

The outcome of the patients showed that the infections with AmpC β-lactamase-producing K. pneumoniae lead to significantly high rate of mortality among paediatric patients. Effective infection control practices can reduce the number of such cases. Imipenem was the drug of choice for the treatment of infections caused by AmpC-producing K. pneumoniae. Inhibitor-based confirmatory test is a simple and the best method for the detection of AmpC β-lactamases in routine clinical microbiology laboratories.

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Conflict of Interest: None.

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