

## ORIGINAL ARTICLE

# HAEMATOGENOUS INFECTIONS CAUSED BY EXTENDED SPECTRUM BETA LACTAMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* IN PEDIATRIC PATIENTS

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### ABSTRACT:

**Background:** Present study was aimed to determine the proportion of extended spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* strains from the pediatric intensive care unit (PICU), and to identify risk factors for these infections. **Materials and Methods:** A retrospective study was conducted from January 2014 to December 2015. Data pertaining to demographics, length of stay, outcome, and relevant risk factors previously defined in the literature was collected. **Results:** A total of 226 non-duplicate strains and fifty isolates of *Klebsiella pneumoniae* were isolated from the blood of pediatric patients. The frequency of isolation of *Klebsiella pneumoniae* was 30/226 (13.27%) isolates. ESBL-producing *K. pneumoniae* was most commonly isolated in the pediatric intensive care unit. Patients with infections due to ESBL-producing *Klebsiella* had a longer duration of hospital stay as well as PICU stay. **Conclusion:** *K. pneumoniae* is the most common gram-negative bacterial isolate responsible for bloodstream infections in pediatric patients. Further prospective studies with phenotypic characterization of the ESBL-producing organisms, as well as analysis of initial therapy, treatment failure incidence, are needed to determine the burden of ESBL-producing organisms in the pediatric population.

**Key words:** Antimicrobial resistance, extended spectrum beta lactamase (ESBL), haematogenous infection, *Klebsiella pneumoniae*, pediatric patients.

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## INTRODUCTION

Symptomatic bacteremia is a major problem among children and requires urgent rational antibiotics therapy. Gram-negative organisms are responsible for a significant proportion of nosocomial infections in adults and children. It is also a significant cause of high morbidity and mortality.<sup>1</sup> The antibiotic susceptibility pattern of Gram-negative organisms has been changing from last decade, with a

gradual shift towards more resistant organisms to antibiotics.<sup>2</sup>

*Klebsiella pneumoniae*, a gram-negative bacterium, was the most common causative agents in pediatric bloodstream infection.<sup>3</sup> It is a successful opportunistic pathogen and has been associated with various ailments such as urinary tract infections, septicaemia, respiratory tract infections and diarrhea.<sup>4</sup>

*Escherichia coli* and *Klebsiella* species, that produced  $\beta$ -lactamases with an extended spectrum of activity. The extended-spectrum  $\beta$ -lactamases (ESBLs) produced by these organisms are capable of hydrolyzing the  $\beta$ -lactam ring of several antibiotics. This delivers a wide variety of antibiotics, including third-generation cephalosporins, monobactams, and penicillins, ineffective in the treatment of infections caused by these organisms. Organisms that produce ESBLs typically retain in vitro susceptibility to cefoxitin, cefotetan, and carbapenems, although treatment failures have been seen.<sup>5-7</sup>

Pediatric risk factors have not been clearly elicited as of yet. Zaoutis et al identified female sex, corticosteroid use within 60 days of acquiring the infection, and infection with *Klebsiella* species as being independently predictive of bloodstream infections due to ESBL-producing organisms in children.<sup>8</sup> Recent reports have highlighted the emergence of ESBL producing strains endowed with an extremely wide spectrum of antibiotic resistance, including resistance to trimethoprim, amikacin, streptomycin and gentamicin. Kim et al identified prior hospitalization, ICU admission within the preceding 30 days, mechanical ventilation, presence of a central venous catheter, development of breakthrough bacteremia during antibiotic therapy, and exposure to extended-spectrum cephalosporins within 30 days of infection as risk factors for acquiring infections caused by ESBL-producing *E. coli* or *Klebsiella pneumoniae* in a cohort of neonates with bacteremia.<sup>9</sup> Due to the irrational use of antibiotics, *K. pneumoniae* isolated from blood culture is mostly multi- drug resistant (resistant to three or more antibiotic classes), often producing beta-lactamases.<sup>10</sup>

Present study was carried out to determine the prevalence of ESBL-producing *K. pneumoniae* strains isolated from blood in children and to identify risk factors for these infections.

## MATERIALS AND METHODS

Ethical permission from Institutional ethical board has been taken before the commencement of this study. A retrospective study was conducted from January 2014 to December 2015. Blood culture samples from hospitalized children younger than 18 years of age were collected. Data pertaining to demographics, length of stay, outcome, and relevant risk factors previously defined in the literature was collected.

All blood samples were routinely cultured in pediatric blood culture bottles. *Klebsiella* isolates that were obtained as a pure and predominant growth from the clinical specimens were only considered for the present study. The organisms were identified based on colony morphology and biochemical reactions

### Antimicrobial susceptibility testing

Disc diffusion method was used to determine the susceptibility of micro-organisms to different antimicrobial agents such as ampicillin, cefalotin, ceftriaxone, cefazidime, amoxicillin/clavulanic acid, piperacillin/tazobactam, meropenem, imipenem, ertapenem, gentamicin, amikacin, co-trimoxazole and ciprofloxacin. The results were interpreted as per National Committee for Clinical Laboratory Standards (NCCLS) recommendations. *Escherichia coli* ATCC 25922 strain was used for quality control

### ESBL detection by Double Disc Diffusion Synergy Test (DDST):

In the DDST, synergy was determined between a disc of augmentin (20 mg amoxicillin and 10 mg clavulanic acid) and a cefotaxime, cefazidime and ceftriaxone discs were placed at a distance of 30 mm apart on a lawn culture of the resistant isolate under test on Mueller-Hinton Agar (MHA, Hi-Media). The microorganism was labeled as ESBL-positive if the zone of inhibition around one or more cephalosporin discs was extended on the side nearest to the amoxicillin/clavulanic acid. This increase occurs because the clavulanic acid present in the augmentin disc inactivates the ESBL produced by the test organism. Results obtained were statistically analyzed using SPSS (Statistical Package for the Social Sciences) 9.0.

## RESULTS

A total of 226 non-duplicate strains and thirty isolates of *Klebsiella pneumoniae* were isolated from the blood of pediatric patients. The frequency of isolation of *Klebsiella pneumoniae* was 30/226 (13.27%) in 2015. *K. pneumoniae* was the second leading isolated microorganism, and the first among Gram-negative isolates out of the total 226. (Table 1)

There was a high frequency of ESBL-producing *K. pneumoniae*: 70% (21 out of 30)

All the isolates were found to be resistant to a minimum of 3 antibiotics to which they were tested. Hence all the isolates were considered to be multidrug resistant.

**Table 1:** In vitro resistance to antimicrobials of ESBL-positive and ESBL-negative *K. pneumoniae* strains isolated from blood of pediatric patient.

Antibiotic	ESBL positive strains (%age)		ESBL negative strains (%age)	
	Sensitive	Resistant	Sensitive	Resistant
Cefazolin	0	100	42.5	57.5
Cetriaxone	0	100	50	50
Cetazidime	0	100	50	50
Piperacillin	65.5	35.5	76	24
Amoxicillin/clavulanic acid	24.5	65.5	50	50
Imipenem	100	0	100	0
Meropenem	100	0	100	0
Ertapenem	100	0	100	0
Gentamicin	0	100	50	50
Amikacin	48.5	51.5	76	24
Ciprofloxacin	86.8	13.2	50	50

**Table 2:** ESBL-positive and ESBL-negative *K. pneumoniae* isolates in various pediatric wards

Wards	ESBL positive (%age)	ESBL-negative (%age)
Pediatric Surgery	12	9
PICU	82	64
Others	6	27

**Table 3:** Median Length of Pediatric Intensive Care Unit (PICU) and Hospital Stay in Days, and Interquartile Ranges (IQRs)

Organism	PICU LOS (IQR)	Hospital LOS (IQR)
ESBL-	16 (6-26)	28 (8-48)
ESBL+	42 (9-75)	58.5 (19-96)
P value	0.025	0.286

LOS: Length of stay

100% of the isolates showed resistance to all the three 3GC antibiotics (Cefazolin, Cetriaxone, Cetazidime) and this resistance was observed to coexist with resistance to other antibiotics. (Table 1)

ESBL-producing *K. pneumoniae* was most commonly isolated in the pediatric intensive care unit (PICU). (Table 2)

Patients with infections due to ESBL-producing *Klebsiella* had a longer duration of hospital stay as well as PICU stay. (Table 3)

**DISCUSSION**

There has been an increasing prevalence of ESBL-producing organisms during the past decade. Recent estimates for ESBL-producing organisms are reported to be around 12% in ICUs in the United States.<sup>8</sup> In one series of 728 neonates with Gram-negative sepsis,

ESBLs were detected in 86.6% of *Klebsiella* isolates and 63.65% of *E coli* isolates.<sup>11</sup>

Results of present study has shown that *K. pneumoniae* is the second most common etiological agent of haematogenous infections in pediatric patients, and the first among gram-negative bacteria.

The frequency of isolation of *Klebsiella pneumoniae* was 30/226 (13.27%) isolates. The prevalence of the pathogen observed was in accordance with other authors. In a study conducted by Mamishi, *K. pneumoniae* was the most frequently isolated from blood cultures in children. The author found that the frequency of *K. pneumoniae* was 8.5% .<sup>12</sup>

The incidence of ESbL -producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past years and accounts for 6 to 17% of all nosocomial urinary tract infections.<sup>13</sup>

Recent studies have revealed that patients with septicemia caused by ESBL-producing organisms had significantly a higher fatality rate than those with non-ESBL isolates.<sup>14</sup> Our study shows an increase of ESBL- producers.

Resistance to other antimicrobial drugs among ESBL-producing *K. pneumoniae* was commonly found. Malkan Rad reported that Gentamicin has low susceptibility among these isolates.<sup>14</sup> Similar findings has also been obtained in present study. Resistance to amikacin and this antibiotic should be used with caution when treating empirically.

In addition to 100% resistance to 3GC and gentamicin, 51.5% of the isolates showed resistance to amikacin, 65.5% to and 65% to Amoxicillin/clavulanic acid. In present study resistance to 3GC was found to coexist with resistance to other antibiotics. Since all the isolates showed multidrug resistance, the therapeutic strategies to control infections due to *Klebsiella* spp. has to be carefully formulated. The therapeutic use of all 3GC should be avoided against *Klebsiella* spp. that appear resistant to any such compound.<sup>15</sup>

Since all the isolates were sensitive Imipenem, Meropenem and Ertapenem, they might serve as the drug of choice for the treatment of infections due to ESBL producing *K. pneumoniae* strains.

Ciprofloxacin showed around 86.80% effectiveness towards ESBL+ *K.pneumoniae* tested in this study. Similar results have been also found in the Nwadioha study from Kano.<sup>16</sup>

ESBL production is coded by genes that are prevalently located on large conjugative plasmids of 80-160 Kb in size. Since these plasmids are easily transmitted among different members of the enterobacteriaceae, accumulation of resistance genes results in strains that contain multiresistant plasmids. For this reason, ESBL-producing isolates are resistant to a variety of classes of antibiotics. Moreover, the emergence of these multiple resistant *Klebsiella* strains is unfortunately accompanied by a relatively high stability of the plasmids encoding ESBLs. Conjugative dissemination of ESBL coding plasmids might facilitate the spread of antibiotic resistance among different members of enterobacteria.<sup>17</sup>

A 2007 review of the Gram-negative “health care crisis” recognized a general consensus that resistance is associated with negative clinical outcomes.<sup>18</sup>

Ramphal et al<sup>19</sup> examined numerous studies in an attempt to determine the effect of infections with ESBL-producing organisms on clinical outcomes.

The investigators discussed several comparative studies where ESBL status had no effect on outcomes, but also some studies that did find an association with poor outcome. They concluded that many of the studies were underpowered. Several other studies have demonstrated that patients with infections due to ESBL-producing bacteria had worse clinical outcomes than patients with infections caused by non-ESBL-producing organisms.<sup>13-17</sup>

Infections due to these multidrug-resistant Gram-negative organisms result in increased costs of medical care, primarily driven by increased length of stay in this population.<sup>15,17</sup> In this study, lengths of hospital and PICU stay, as well as survival to discharge, were used as markers of outcome. (Table 2 and 3) Patients with infections due to ESBL-producing *Klebsiella* had a longer duration of hospital stay as well as PICU stay. Present study hypothesize that patients with infections caused by ESBL-producing organisms had higher hospital-related costs directly related to prolonged duration of resource use in the ICU.

There were some limitations to this study, including its retrospective design, small sample size, lack of genotypic analysis, and use of multiple isolates from some patients; these limit the widespread extrapolation of these data

#### CONCLUSION:

*K. pneumoniae* is the most common gram-negative bacterial isolate responsible for haematogenous infections in pediatric patients. A rational use of antibiotics, especially in this tender age group, in order to achieve a relatively high level antibiotic activity against offending bacterial organisms, is recommended and more importantly, avoiding misuse and overuse of antibiotics may reverse the undesired effects of multidrug resistant and ESBL producing *Klebsiella*.

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