

## FREQUENCY OF EXTENDED SPECTRUM $\beta$ -LACTAMASE PRODUCING KLEBSIELLA PNEUMONIAE AND ESCHERICHIA COLI ISOLATES

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*Extended spectrum beta lactamases (ESBLs) that mediate resistance to 3rd generation cephalosporins are now observed worldwide in all species of Enterobacteriaceae especially Klebsiella pneumoniae and Escherichia coli. ESBL producing organisms are responsible for nosocomial infections and may result in hospital outbreaks. Present study is a descriptive study. It was planned to determine the frequency of extended-spectrum  $\beta$ -lactamase (ESBL) production among Klebsiella pneumoniae and Escherichia coli isolated from different clinical specimens from patients admitted to different teaching hospitals of Lahore (Services Hospital, Mayo Hospital and Sir Ganga Ram Hospital). It was carried out in Department of Pathology, Services Institute of Medical Sciences (SIMS), Lahore from October 2005 to August 2006. A total of 324 clinical isolates of Klebsiella pneumoniae and Escherichia coli recovered from different clinical specimens like blood, urine, sputum, pus / wound swabs were included in this study. All isolates were screened and confirmed for extended spectrum beta lactamase (ESBL) production by Kirby-Bauer disk diffusion method on Mueller Hinton agar using NCCLS guidelines. Out of a total of 324 isolates, 184 were Klebsiella pneumoniae and 140 were Escherichia coli. Among these, 47.82% were Klebsiella pneumoniae and 38.57% Escherichia coli isolates were ESBL producer. ESBL production was the highest among isolates from blood (50%) isolates from Mayo Hospital, Lahore (56.25%). It is concluded that there is a high frequency of ESBL production among nosocomial isolates of Klebsiella pneumoniae and Escherichia coli. These isolates being resistant to 3rd generation cephalosporins and many other classes of antibiotics pose a special therapeutic challenge. Therefore, these isolates should be routinely tested for ESBL production.*

Beta lactam antimicrobial agents are the most common drugs used for the treatment of bacterial infections<sup>1</sup>. Production of  $\beta$ -lactamases that hydrolyze beta lactam ring is the main strategy used by the bacteria to inactivate these drugs and thus is the most common mechanism of bacterial resistance against these agents. There are many types of beta lactamases which vary both in their ability to inactivate a given beta lactam drug as well as their susceptibility to inhibitors such as Clavulanic acid, Sulbactam and Tazobactam. Extended spectrum beta lactamases (ESBLs) that mediate resistance to 3rd generation cephalosporins are now observed worldwide in all species of Enterobacteriaceae especially *Klebsiella pneumoniae* and *Escherichia coli*.<sup>2,3</sup> ESBL producing organisms are responsible for nosocomial infections and may result in hospital outbreaks<sup>4</sup>. These organisms are frequently resistant to other groups of antibiotics including amino glycosides, quinolones and cotrimoxazole<sup>5</sup>.

Although ESBL production was first reported in 1983<sup>6</sup>, many clinical laboratories and medical community are not fully aware of the importance of ESBLs and methods to detect these.<sup>7,8</sup> Reasonably reliable methods are now available by which ESBL production can be detected by clinical microbiology laboratories. These tests rely on initial screening followed by confirmatory tests as prescribed by NCCLS<sup>9</sup>.

In Pakistan, a few studies have been performed to assess the frequency of ESBL production among Gram negative isolates in Rawalpindi/ Islamabad and Karachi.<sup>10-12</sup> However, data about the extent of this problem in Lahore could not be retrieved. Present study was planned to determine frequency of ESBL production among nosocomial *Klebsiella pneumoniae* and *Escherichia coli* isolates from different teaching hospitals of Lahore. This would create a better understanding of this problem and help in formulating proper antibiotic policy.

## MATERIALS AND METHODS

Present study comprise of 324 non duplicate isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) recovered between October 2005 to August 2006. These were isolated from different clinical specimens including blood (n = 94), urine (n = 118), pus / wound swabs (n = 72) and other specimens (n=40) of patients admitted to different teaching hospitals of Lahore (Services Hospital, Sir Ganga Ram Hospital and Mayo Hospital). The isolates were identified with the help of colony morphology on blood agar, MacConkey agar plates, Gram stain character and standard biochemical tests<sup>13</sup>. The isolates were tested for sensitivity against different antibiotics by Kirby-Bauer disk diffusion method on Mueller Hinton agar using NCCLS guidelines<sup>9</sup>. During the present study sensitivity disks of Aztreonam (30 ug), Cefotaxime (30 ug), Ceftazidime (30 ug) and Ceftriaxone (30 ug) were used for screening of ESBL production. Diameter of zone of inhibition of growth was measured and interpreted according to NCCLS criteria<sup>9</sup>. The isolates with reduced sensitivity against two of four drugs mentioned above were interpreted as suspected ESBL producers.

Phenotypic confirmation of these provisional ESBL producers was done by testing with sensitivity disks of Ceftazidime and Cefotaxime alone and in combination with Clavulanic acid (10 ug) as recommended by NCCLS<sup>9</sup>. For this purpose, discs of Ceftazidime + Clavulanic acid (30/10 ug) and Cefotaxime + Clavulanic acid (30/10ug) were used. All the isolates showing increase in the zone of inhibition of 5 mm or more for either antimicrobial agent in combination with Clavulanic acid versus its zone when tested alone were labeled as confirmed ESBL producers. Chi-square test was used for evaluation of statistical significance of the results.

**Table 1:** Results of *Klebsiella pneumoniae* and *Escherichia coli* isolates for ESBL production.

Isolates	Suspected ESBL producers		Confirmed ESBL producers	
	Number	Percentage	Number	Percentage
<i>Klebsiella pneumoniae</i> (n = 184)	96	52.17	88	47.82*
<i>Escherichia coli</i> (n = 140)	67	47.8	54	38.57
Total (n = 324)	163	50.31	142	43.82

\*  $p > 0.05$  (no significant difference between *K. pneumoniae* and *E. coli*)

## RESULTS

A total of 324 isolates of *K. pneumoniae* (n = 184) and *E. coli* (n = 140) were studied. On initial screening 50.31% isolates were observed to provisional ESBL producers, whereas on further testing 43.82% isolates were found to confirm ESBL producers. ESBL production was observed to be higher among *K. pneumoniae* isolates (47.82%) as compared to *E. coli* (38.57%). However, there was no significant difference ( $p > 0.05$ ) as shown in Table 1. ESBL production was the highest among isolates from blood (50%) and isolates from Mayo Hospital, Lahore (56.25%) as shown in Tables 2, 3. However, the difference did not reach statistical significance. Detailed results are shown in the respective tables.

## DISCUSSION

ESBLs are plasmid mediated TEM and SHV derived enzymes. These are capable of hydrolyzing broad spectrum cephalosporins and Monobactam. However, these are inactive against Cephamecins and Carbapenems<sup>14</sup>. ESBL producing organisms also exhibit co resistance to many other classes of antibiotics resulting in limitation of therapeutic options. The prevalence of antibiotic resistance to other groups of antibiotics among ESBL producing *E. coli* and *K. pneumoniae* has increased markedly in recent years and increases the possibility that traditional, empiric antimicrobial regimens may also become ineffective<sup>15</sup>. These enzymes are most commonly produced by *Klebsiella* species and *E. coli*. But may also occur in other Gram negative bacteria including species of *Enterobacter*, *Salmonella*, *Proteus*, *Citrobacter*, *Pseudomonas* etc<sup>8</sup>.

In the present study, 47.82 percent out of 184 *K. pneumoniae* and 38.57 percent out of 140 *E. coli* isolates were ESBL producer. Different studies carried out by other workers in various parts of the world on ESBL production show quite variable results. In a study carried out in Singapore<sup>15</sup>, frequency of ESBL production among *Klebsiella* species was similar to that in the present study (44%), whereas frequency among *E. coli* isolates was much lower (16.1%).

Generally ESBL production has been observed to be higher among *Klebsiella* isolates than in *E. coli* isolates.<sup>16</sup> Frequency of production among *E. coli* has also been found to be low i.e. 5% in Korea and 23.3% in Indone-

sia<sup>1</sup>. In a nationwide study in Spanish hospitals, ESBL producing strains of *Klebsiella* and *E. coli* were isolated from majority of the hospitals (90%). Prevalence of ESBL producing strains was 0-24% and 0-16.7% for *E. coli* and *K. pneumoniae* respectively. It was also observed that ESBL production was higher among *Klebsiella* isolates of hospital origin<sup>17</sup>.

Frequency of ESBL production in the present study is slightly higher than that observed in our neighbouring country, India. In a study carried out in Chandigarh, 38.5% *Klebsiella* and 24.7% *E. coli* isolates were ESBL producer.<sup>18</sup> In another study carried out in a tertiary care hospital in India, ESBL was detected in 30.18% *K. pneumoniae* isolates<sup>19</sup>.

Studies on ESBL production among Gram negative isolates belonging to enterobacteriaceae family carried out in Pakistan also give variable results. In a study carried out in Rawalpindi, frequency of ESBL production among Gram negative bacilli was observed to 45 percent<sup>10</sup>. While in a study carried out at Islamabad, prevalence of ESBL in enterobacteriaceae was found to be 37.5%, in nosocomial and 6% in out-patient isolates. ESBL production was very high in *K. pneumoniae*, followed by 33.3% in enterobacter and 28.5% in *E. coli* isolates<sup>11</sup>. In another study carried out in Rawalpindi, it was observed that 35% of enterobacteriaceae isolates were ESBL producers with the highest prevalence among *K. pneumoniae* isolates<sup>20</sup>. The results of the study done in Karachi show that 30% isolates belonging to enterobacteriaceae family were ESBL producers. It was also observed that there was no difference between the results of combined disc and double disc methods for detection of ESBL production<sup>12</sup>. From the above discussion it is clear that ESBL production is a significant problem all over the world and also that figures in Pakistan are much high.

In the present study, ESBL production was higher among isolates from blood (50%), followed by urine (43.2%), pus / wound swabs (40.27%) and other samples (37.5%). Various risk factors have been implicated in the selection and spread of ESBL producing strains. ESBL producing Enterobacteriaceae strains are often found in areas of hospital or community where antibiotic use is heavy and patient's condition is critical<sup>21</sup>. In a

**Table 2:** Distribution of ESBL producing isolates of *K. pneumoniae* and *E. coli* according to clinical source of specimen.

Clinical specimen	ESBL producers	
	Number	Percentage
Blood (n = 94)	47	50*
Urine (n = 118)	51	43.22
Pus/wound swabs (n = 72)	29	40.27
Others (n = 40)	15	37.5

\* p>0.05 (no significant difference among different specimens)

**Table3:** Distribution of ESBL producing isolates of *K. pneumoniae* and *E. coli* according to hospital.

Hospital	Number of isolates	ESBL producers	
		Number	Percentage
Services Hospital	184	74	40.22
Sir Ganga Ram Hospital	76	32	42.1
Mayo Hospital	64	36	56.25*

\* p>0.05 (no significant difference among different hospitals)

prospective observational study based on findings in different hospitals all around the world, it was observed that overall 30.8% episodes of nosocomial bacteraemia and 43.5% episodes acquired in intensive care units were due to ESBL producing organisms. Previous administration of antibiotics containing oxyimino group (cefuroxime, Cefotaxime, Ceftriaxone, Ceftazidime or Aztreonam) was associated with bacteraemia due to ESBL producing organisms<sup>22</sup>. All these observations indicate the real threat of this problem, particularly when we know that only treatment option for ESBL infections especially blood stream infections, are highly costly drugs like Carbapenems. Although flouroquinolones are also among the therapeutic options. yet this option is limited because of high percentage of resistance against these drugs (13.7-65.5%) in ESBL producing strains.<sup>3,23,24</sup>

Comparison of frequency of ESBL production among isolates from different teaching hospitals of Lahore show that isolates from Mayo Hospital had the maximum percentage (56.25%). The figures were similar (around 40%), among isolates from Services Hospital and Sir Ganga Ram Hospital.

Keeping in view the facts that ESBL producing

Gram negative bacteria are quite common in our hospital environment and that these isolates tend to be multi drug resistant, it is imperative that all microbiology laboratories should regularly test and report these isolates. Use of more than one type of oxymino cephalosporins as suggested in NCCLS for screening will improve the sensitivity of detection. This should be followed by confirmatory test for ESBL production. This method works well for *Klebsiella* and *E. coli*. For other members of enterobacteriaceae, efforts to standardize this test are still going on. It may be pointed out that some organisms with ESBLs contain other lactamases like AmpCs and inhibitor resistant TEMs. These can mask ESBL production in the phenotypic test; thus resulting in false negative results<sup>1</sup>. As for control of evolution and spread of ESBL producing organisms in hospital setting is concerned, strict control on antibiotic abuse along with adoption of infection control measures is the only answer at the moment.

In **conclusion** there is a high frequency of ESBL production among nosocomial isolates of *Klebsiella pneumoniae* and *Escherichia coli*. These isolates being resistant to 3rd generation cephalosporins and many other classes of antibiotics pose a special therapeutic challenge. Therefore, these isolates should be routinely tested for ESBL production.

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