

## Extended Spectrum- $\beta$ -Lactamase producing *Klebsiella pneumoniae* at a tertiary care setup in Kashmir, India: Comparative phenotypic detection and antimicrobial susceptibility pattern

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### Abstract

**Background & objectives:** Betalactams are the most widely used group of antimicrobials, however growing resistance to these invaluable drugs mediated by *extended spectrum β-lactamases (ESBLs)* and AmpC enzymes is a major concern. The present study was undertaken to determine the prevalence of these enzymes, their effect on antimicrobial susceptibility pattern and compare different phenotypic detection tests, in clinical isolates of *Klebsiella pneumoniae* in a tertiary care hospital.

**Methods:** 103 clinical isolates of *Klebsiella pneumoniae* were isolated and identified by standard microbiological procedures. Antimicrobial susceptibility testing was carried out by Kirby-Bauer method. Ceftazidime and Cefotaxime were used for screening potential ESBL producers. Confirmation was done by a combination of double disk synergy test (DDST), phenotypic confirmatory disk diffusion tests (PCDDTs) and Etest.

AmpC detection was carried out using Cefoxitin 30 µg disk..

**Results:** Overall prevalence of ESBL producers was 71.8% and 27.1% were AmpC betalactamase producers (derepressed mutants). While DDST was able to detect 34.8%, PCDDTs detected 78.3% of ESBL producers. ESBL producers mediated very high resistance to both betalactams and non-betalactams. Prolonged hospital stay and prior use of 3rd generation cephalosporins were identified as important risk factors for ESBL acquisition.

**Interpretation & Conclusion:** ceftazidime and cefotaxime proved to be reasonably good screening agents. PCDDTs are more efficient ESBL detectors than DDST. ESBLs not only pose a great threat to future of betalactams but they also endanger the utility of many non-betalactams. In order to ensure rationale treatment, ESBL detection and reporting might assume a priority in near future.

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**Keywords:** AmpC, ESBL, Etest, *Kebseilla pneumoniae*, multidrug resistance

### INTRODUCTION

The specter of drug resistance in general and multidrug resistance in particular looms large ahead as never before. The war waged between microorganisms and antimicrobials continues to flare

up unabated with each partner developing new weaponry and seeking novel ways of combat. Betalactam group of antibiotics are the workhorse antimicrobial agents but the rising *ESBL* mediated

**Table 1.** Distribution pattern of ESBL producers & non-producers in outpatients and inpatients

Distribution	ESBL producers		Non-producers		Total	
	n	%	n	%	n	%
Inpatient	71	95.9	7	63.6	78	91.7
Outpatient	3	4.1	4	36.3	7	8.2
Total	74	100	11	100	85	100

P value < 0.004

resistance is giving clinicians a tough time as the therapeutic options are getting bewildered.

Betalactamases continue to be the leading cause of resistance to betalactam antibiotics in gram negative bacteria. In recent years there has been an increased incidence and prevalence of *ESBLs*, enzymes that hydrolyze and cause resistance to oxyimino-Cephalosporins and aztreonam<sup>1, 2</sup>. *ESBLs* are more prevalent in *Klebsiella pneumoniae* than in any other enterobacterial species and outbreaks of infections caused by *ESBL* producing strains have been reported widely<sup>3</sup>.

*Klebsiella* is a very frequent isolate in our hospital setting and a significant proportion being MDR, poses a formidable challenge. Moreover *Klebsiella* being the commonest organism besides *Escherichia coli* to elaborate *ESBLs*, complicates the problem as their detection has a direct bearing on the use of 3<sup>rd</sup> generation cephalosporins and their in time reporting ensures rationale in treatment.

The present study, the first of its kind from Kashmir was primarily designed to detect extended spectrum β-lactamases (ESBLs) and AmpC enzymes (AmpC β-lactamases in case of *Klebsiella* are plasmid mediated enzymes that are derepressed mutants, constitutively elaborated in heavy amounts with out the need for an inducing agent<sup>4,5</sup> in clinical isolates of *Klebsiella pneumoniae*, to compare different phenotypic detection tests, to study the antimicrobial susceptibility patterns and the possible risk factors involved.

## MATERIAL AND METHODS

The study group comprised of a total of 103 *Klebsiella pneumoniae* isolates obtained from different clinical specimens such as blood, urine, sputum, pus and other body fluids that were received in the bacteriology section of department of microbiology Sher-i-Kashmir Institute of Medical Sciences, Kashmir-India for a period of six months. Both in and outpatients were included in the study and one sample from each patient was analyzed. Relevant information, such as in- or out-patient status, hospital stay at the time of sample collection and history of 3<sup>rd</sup> generation cephalosporin use in the preceding two weeks was obtained. All the samples



**Figure 1.** Enhancement of zone of inhibition towards the central clavulanate containing disk indicative of *ESBL* production in a *Klebsiella pneumoniae* strain (positive DDST)

were processed and identified as per the standard microbiological protocols and procedures<sup>6</sup>. Isolates confirmed as *Klebsiella pneumoniae* were studied for their antimicrobial susceptibility pattern, extended spectrum β-lactamase, AmpC β-lactamase and combined *ESBL* + AmpC production.

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method with the following set of antibiotics: ceftazidime (30μg), cefotaxime (30μg), ceftriaxone (30μg), cefpirome (30μg), ciprofloxacin (5μg), ofloxacin (5μg), gentamicin (10μg), ampicillin (10μg), imipenem (30μg), aztreonam (30μg), nitrofurantoin (300μg) and Cotrimoxazole (trimethoprim 1.25μg + sulphamethoxazole 23.75μg).

## Screening

Ceftazidime and cefotaxime were included in the primary panel for screening potential *ESBL* producers. Isolates with inhibition zone diameter of ≤ 22mm for ceftazidime and ≤ 27mm for cefotaxime

**Table 2.** Hospital stay of patients infected with ESBL producing and non-producing Klebsiella

Hospital stay (no. of days)	ESBL producers n	ESBL producers %	Non –producers n	Non –producers %	Total n	Total %
0-7 days	22	29.7	8	72.7	30	35.3
>7 days	52	70.3	3	27.3	55	64.7
Total	74	100	11	100	85	100

P value < 0.007

were considered as potential ESBL producers as per the NCCLS guidelines and put to confirmatory testing by a double disk synergy test (DDST) and two phenotypic confirmatory disk diffusion tests (PCDDT A and PCDDT B)<sup>7,8</sup>.

#### Double Disk Synergy Test:

In the DDST ceftazidime ,cefotaxime and ceftriaxone 30 µg each were placed at a distance of 15mm edge to edge from a centrally placed augmentin disk containing 20 µg of amoxicillin + 10 µg of clavulanic acid. Result was inferred if the inhibition zone around the these 3<sup>rd</sup> generation antibiotic disks increased towards the disk containing clavulanate i.e. augmentin<sup>9,10</sup>(Figure 1).

#### Phenotypic Confirmatory Disk Diffusion Tests:

Ceftazidime and cefotaxime 30 µg each were used alone and in combination with 10 µg of clavulanic acid in the phenotypic confirmatory disk diffusion tests A (PCDDT A) and B (PCDDT B) respectively. Individual discs were placed at least 3cm centre to centre apart. An increase in zone diameter of either ceftazidime or cefotaxime by ≥ 5mm with clavulanic acid versus its diameter when tested alone was considered as ESBL positive<sup>7,9</sup> (figure 2).

Susceptibility or resistance to Cefoxitin 30µg disk was used to differentiate ESBLs from AmpC betalactamases. Isolates were considered as ESBL producers when they showed synergy with clavulanate in any one or more of the confirmatory tests and tested sensitive to both cefoxitin and Imipenem<sup>4,11</sup> (figure 3). Isolates were considered as AmpC betalactamase producers when they failed to show synergy with clavulanate in any of the confirmatory tests and tested sensitive to imipenem but resistant to cefoxitin<sup>4,5,11</sup> (figure 4). If the test isolate showed synergy with clavulanate in any one or more of the confirmatory tests but tested resistant to cefoxitin it was considered as producing both ESBLs and AmpC betalactamases simultaneously<sup>4</sup>.



**Figure 2.** Ceftazidime and cefotaxime showing an increase in zone diameter of > 5mm with the addition of clavulanic acid, indicative of ESBL production in a *Klebsiella pneumoniae* isolate (positive PCDDT)

#### Etest

Etest ESBL strips of ceftazidime TZ (0.5-32µg/ml) and TZL ceftazidime (0.064-4µg/ml) plus 4µg/ml clavulanic acid were used to confirm 15 representative isolates positive for ESBL production by disk diffusion tests. The strips have a cephalosporin gradient at one end and a cephalosporin plus clavulanate gradient at the other. ESBL production was inferred if the MIC ratio for cephalosporin alone: cephalosporin + clavulanate ≥ 8<sup>12</sup> (figure 5). The strips were strictly used as per the guidelines of the manual provided by the manufacturer.

All the antibiotic disks used were purchased from Hi-Media laboratories, Mumbai except cefotaxime and cefotaxime + clavulanic acid that were acquired from Becton, Dickinson and company Maryland USA. Etest ESBL strips of ceftazidime and

**Table 3.** History of prior 3<sup>rd</sup> generation cephalosporin consumption in patients infected with ESBL producing and non-producing *Klebsiella* pneumonia

H/O prior 3 <sup>rd</sup> GC consumption	ESBL producers n	ESBL producers %	Non -producers n	Non -producers %	Total n	Total %
3 <sup>rd</sup> GC consumed	67	90.5	0	0	67	78.8
3 <sup>rd</sup> GC not consumed	7	9.5	11	100	18	21.2
Total	74	100	11	100	85	100

P value < 0.000

ceftazidime + clavulanic acid were purchased from AB BIODISK solna, Sweden. *E.coli* ATCC 25922 was used as a negative ESBL control. Statistical analysis was done using Fisher exact test. P ≤ 0.05 was considered significant.

## RESULTS

The overall prevalence of ESBL producing *Klebsiella pneumoniae* was 71.84 %( n=74/103) with 95.9% obtained from inpatients, 70.3% having a hospital stay of more than a week and 90.5% having a history of prior 3<sup>rd</sup> generation cephalosporin use in the preceding two weeks. The differences were statistically significant from non-producers as depicted in tables' 1, 2 and 3 respectively. However 13.5% (10/74) of these isolates were producing AmpC β-lactamases simultaneously. Majority of ESBL producing *Klebsiella* were recovered from blood (44.59%) followed by surgical wound site and urine, (16.21%) from each. Maximum numbers of ESBL producing *Klebsiella* were obtained from Neonatology (20.2%) followed by (16.2%) from post operative ward (POW) and (13.5%) from SICU. Patients with sepsis yielded highest number of ESBL producers (20.2%) followed by patients with malignancy (10.8%) and subdural & extradural haematoma (SDH/EDH) (9.4 %)Table 4.

ESBL producing *Klebsiella* mediated 100% resistance to aztreonam and greater than 90% to 3<sup>rd</sup> generation cephalosporins & cefpirome. Among the non-beta lactams highest resistance was against gentamicin (91.9%) and the lowest against ciprofloxacin (44.6%). Differences were statistically significant from non-producers as shown in table 5. All isolates irrespective of the enzyme status were resistant to ampicillin and sensitive to imipenem.

Among a total of 103 isolates screened 89.3 %( n=92) were screen positive using either ceftazidime or cefotaxime. In the screen positives 69.6 %( n=64) were ESBL producers, 19.5 %( n=18) AmpC producers and 10.8 %( n=10) produced both enzymes simultaneously. Thus the overall prevalence of AmpC producers was 27.1% (28/103) of which 35.7 %( 10/28) were concomitantly ESBL producers. While DDST was able to detect only 34.8% (32/92),



**Figure 3.** A phenotypically confirmed ESBL producing strain of *Klebsiella pneumoniae* showing resistance to 3<sup>rd</sup> generation cephalosporins, cefpirome and aztreonam while maintaining susceptibility to cefoxitin and imipenem.

PCDDT A using ceftazidime/ceftazidime + clavulanate and PCDDT B using cefotaxime/ cefotaxime + clavulanate detected 69.6 % (64/92) and 68.5% (63/92) ESBL producers respectively. Both phenotypic confirmatory disk diffusion tests together (A+B) increased the detection to 78.3% (72/92). The detection rate was 80.4% (74/92) considering the results of all the three tests.

All the 15 representative isolates ESBL positive by disk diffusion, tested positive by Etest. Respective MICs of ceftazidime & ceftazidime + clavulanate and their ratios are shown in table 6.

## Discussion

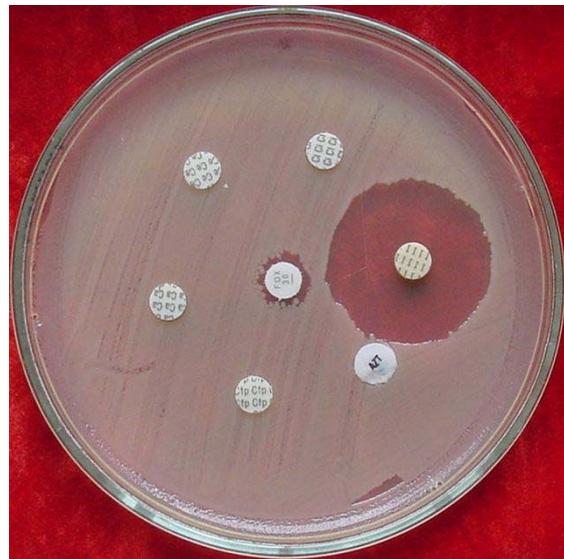
The overall prevalence of ESBLs in *Klebsiella pneumoniae* in the current study was 71.8% (74/103). Wide variations in the prevalence of ESBL producing *Klebsiella* have been reported in various studies. SENTRY antimicrobial surveillance program in 1997-99 from all over the world showed that ESBL

**Table 4.** Distribution pattern of ESBL producing *Klebsiella* isolated from various sites

Specimen	Total ESBLS	
	n	%
Blood	33	44.9
Surgical wound site	12	16.2
Urine	12	16.2
Tracheal aspirate	7	10.9
Burn wounds	5	5.9
Endotracheal tip	5	5.9
Total	74	100

producing *Klebsiella pneumoniae* may account for about 45% in Latin America, 25% in western pacific, 23% in Europe and 8% in USA<sup>13</sup>. The prevalence of ESBL producing *Klebsiella species* in Japan is 5%, Taiwan 21.7%, Philippines 31.3%, Malaysia/Singapore 38% and Indonesia 33.3%<sup>9</sup>. Duttaroy et al from Gujarat India in 2005 reported 58% prevalence of ESBL producing *Klebsiella pneumoniae* isolated from different clinical specimens using the DDST<sup>14</sup>. Padmini et al from Coimbatore India in 2004 reported 40% prevalence of ESBL producing *Klebsiella Pneumoniae* in urinary isolates<sup>15</sup>. The higher prevalence of 71.8% in our study could be probably due to the use of three confirmatory tests compared to a single test used by different studies from other parts of the country. Secondly 93.2 % (n=96/103) isolates in our study had come from hospitalized patients where the prevalence of ESBL producers runs very high. The higher prevalence compared to western countries can be explained by the fact that western countries have strict infection control policies and practices, efficient and effective antibiotic audit systems, shorter average hospital stays, better nursing barriers, and other important health care measures that are known to substantially decrease the chances of acquisition and spread of ESBL strains. The uncontrolled use of 3rd generation cephalosporins at our hospital could be a leading contributory factor to the high ESBL prevalence observed in this study.

The difference in the in- and out-patient distribution between ESBL producers and non-producers in the present study was statistically significant. These results point to the fact that ESBLs are largely a problem of hospitalized patients who share numerous risk factors. ESBL positive *Klebsiella Pneumoniae* has been primarily incriminated in hospital acquired infections. Paterson et al reported that 84% of ESBL *Klebsiella Pneumoniae* were hospital acquired of which 44% were acquired in ICU, in a multicentre prospective observational study<sup>9</sup>. There was a statistically significant difference in the



**Figure 4.** *Klebsiella pneumoniae* isolate showing resistance to 3<sup>rd</sup> generation cephalosporins, aztreonam and cefoxitin but maintaining susceptibility to imipenem indicative of production of an AmpC enzyme (derepressed mutant)

hospital stays of patients infected with ESBL producing and non-producing *Klebsiella spp.* Similar findings have been reported in several other studies<sup>16, 17</sup>. Prolonged hospital stay as such is a very important risk factor for acquisition and transmission of an ESBL producing strain. The present study also revealed a statistically significant difference between the prior use of 3rd generation cephalosporins and subsequent infection with an ESBL producing or non-producing *Klebsiella pneumoniae*. Extended spectrum beta-lactams are commonly included in the empirical treatment of gram negative sepsis. The increasing use of broad spectrum cephalosporins have became one of the major factors responsible for high rate of selection of ESBL producing microorganisms<sup>18</sup>.

The ESBL producers showed very high resistance to betalactam antimicrobials compared to non-producers that appeared uniformly susceptible. The differences were statistically significant. Resistance conferred by ESBL producing *Klebsiella Pneumoniae* to ceftazidime, cefotaxime and ceftriaxone was 89.2%, 90.5% and 94.6% respectively. One of the important observations in our study was the very high level of resistance to 4th generation cephalosporin cefpirome (91.9%) which was not expected, as many ESBL producers remain susceptible to the 4th generation cephalosporins. However our results are consistent with many studies reported in literature<sup>19</sup>. The associated co-resistance to non-betalactams,

gentamycin cotrimoxazole, nitrofurantoin, ofloxacin and ciprofloxacin by *ESBL* producers was 91.9%, 77.0%, 58.3%, 58.1% and 44.6% respectively. Non-producers were 100% susceptible to all these drugs except cotrimoxazole against which resistance of 18.2% was observed. The differences were statistically significant in our study. Similar resistance patterns have been reported from other parts of India<sup>15,20</sup>. Our study results well support the fact that *ESBL* producers not only confer high levels of resistance to 3rd generation cephalosporins but also to non-beta-lactams like aminoglycosides, quinolones and cotrimoxazole Table 7.

In the present study 10.8%, 9.5% and 5.4% of *ESBL* producers appeared falsely susceptible to the 3rd generation cephalosporins ceftazidime, cefotaxime and ceftriaxone respectively, using the routine inhibition zone diameters for interpretation. The finding highlights the importance of CLSI/NCCLS *ESBL* break points and underscores the need for *ESBL* confirmatory testing. Several studies have reported such false susceptibility to 3rd generation cephalosporins by *ESBL* producers<sup>21</sup>. Screen positivity in this study was 89.3% (92/103) using either ceftazidime or cefotaxime. All the screen positive isolates, in the phenotypic confirmatory testing proved to possess a beta-lactamase belonging to a major class with a maximum probability of 80.4% (74/92) for isolation of an *ESBL* producer. Rest 19.5% (18/92) of screen positive isolates proved to have an AmpC enzyme. As such screening with ceftazidime & or cefotaxime can be reasonably helpful in predicting the likelihood of presence of an *ESBL/AmpC* producing isolate. The sensitivity of screening *ESBLs* in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one antimicrobial agent among ceftazidime, cefotaxime, ceftriaxone, aztreonam and cefpodoxime will improve the sensitivity of detection. Cefpodoxime seems to be the logical indicator as most *ESBLs* show obvious resistance to it; however, putting up both ceftazidime and cefotaxime is a good alternative as advised by NCCLS/CLSI<sup>7,22,34</sup>.

Using the DDST, PCDDTs and cefoxitin for *ESBL/AmpC* detection in the screen positive *Klebsiella* isolates, 69.5% (64/92) produced *ESBLs* only, 19.5% (18/92) AmpC beta-lactamase only and 10.8% (10/92) produced both. Guleri A et al reported in a pilot study from Glasgow, using cefoxitin disk for detecting AmpC production, Etest *ESBL* and *ESBL* combination disks for *ESBL* detection in 12 screen positive *Klebsiella* isolates that 83.3% (10/12) of isolates produced *ESBLs* only, none AmpC beta-lactamase only and 16.6% (2/12) produced both<sup>23</sup>. The higher percentage (83.3%) of *ESBL* producers in the study compared to 69.5% in our

study could be because of the fact that the authors used both Etest *ESBL* strips as well as *ESBL* combination disks of ceftazidime, cefotaxime, cefpodoxime, cefepime and cefpirome with and without clavulanate. The use of more Etest *ESBL* strips and combination disks increases the overall sensitivity of *ESBL* detection and using cefepime/cefpirome combination disks can be particularly very helpful in *ESBL* detection in isolates simultaneously producing AmpC beta-lactamases. *ESBL* detection is sometimes difficult in strains producing AmpC enzymes simultaneously but the use of a more stable AmpC cephalosporin (cefepime/cefpirome) can overcome this problem<sup>24</sup>. Because we did not use cefepime/cefpirome combination disks, probably we might have failed to detect *ESBLs* in some AmpC producing strains. The reason for the difference between the percentages of AmpC enzymes in the two studies could be probably because of the relatively small number (12) of *Klebsiella* isolates tested in the study compared to 92 in the present study.

Among the screen positive isolates, confirmed by different detection tests, DDST alone detected 34.8% (32/92), PCDDT A detected 69.6% (64/92) and PCDDT B detected 68.5% (63/92) of *ESBL* producers. Both phenotypic confirmatory disk diffusion tests (A+B) detected 78.3% (72/92) and all the three tests (DDST + PCDDT A + PCDDT B) put together were able to detect 80.4% (74/92) of *ESBL* producers. Although the specificity of DDST has been well documented, its sensitivity has been variably reported as 76.5%, 93.3%, 87% and 79% in various studies<sup>14</sup>. The current study where in DDST detected 34.8% of *ESBL* producers is closer to the reported sensitivity of 27.3%<sup>21</sup>. Wide variations in sensitivities of DDST reported in different studies result because of the very meticulous and intricate standards required for double disk synergy testing. DDST needs precise placement of disks, clavulanate may not inhibit all *ESBLs* and the test may be unable to detect *ESBLs* in strains producing chromosomal cephalosporinases. The different sensitivities of DDST could also be attributed to different disc spacings used in different studies. Some studies report using two different disk spacings simultaneously in an attempt to increase the sensitivity<sup>14</sup>. Phenotypic confirmatory disk diffusion test A and PCDDT B detected 69.6% and 68.5% *ESBL* producers respectively. The detection is increased to 78.3% if the results obtained using both tests are taken into consideration. Fatima H.M'Zali et al from leads UK reported a study of comparative *ESBL* detection by different phenotypic detection tests in the family enterobacteriaceae<sup>25</sup>. The sensitivity of *ESBL* detection with MDD test using Ceftazidime ± Clavul-

**Table 5.** Comparison of antimicrobial resistance patterns of ESBL producing (n=74) and non-producing (n=11) *Klebsiella pneumoniae*

Antimicrobials	ESBL producers(n=74)	Non- producers(n=11)	P value
Ampicillin	74	11	Not significant
Ceftazidime	66	0	0.001
Cefotaxime	67	0	0.001
Ceftriaxone	70	0	0.000
Cefpirome	68	0	0.001
Aztreonam	74	0	0.000
Ciprofloxacin	33	0	0.022
Ofloxacin	43	0	0.008
Cotrimoxazole	57	2	0.034
Gentamicin	68	0	0.001
Imipenem	0	0	Not significant

inic acid (PCDDT A) in the study was 86% and 65.5% using Cefotaxime ± clavulanic acid (PCDDT B). The sensitivity increased to 93% when results obtained using both agents. The higher detection of 93% in the study compared to 78.3% in the present study could be explained by the fact that the authors used epidemiologically distinct enterobacteriaceae strains known to possess a beta-lactamase characterized by molecular methods, while as we in our study used screen positive *Klebsiella* isolates only likely to harbor a beta lactamase enzyme. The second reason for this variation could be because in the referred study only 7% (7/100) isolates carried an AmpC enzyme compared to 30.4% (28/92) in the present study. ESBL detection can be difficult in strains simultaneously producing an AmpC beta-lactamase that quite often can mask an ESBL<sup>26</sup>. The third reason could be probably that we used differences in the zone diameters instead of ratio of the zone diameters for interpretation.

The results obtained in our present study can be analyzed to infer that DDST is not a very sensitive method for ESBL detection 34.8% only; PCDDT A and PCDDT B are better detectors 69.6% and 68.5% respectively and almost equally sensitive. The detection can be increased further to 78.3% by using both ceftazidime and cefotaxime ESBL combination disks. Putting up a DDST along with PCDDT (A + B) does not increase the detection appreciably (from 78.3% to 80.4%). As such putting up a DDST at times can be an uneconomical, labour intensive and unyielding exercise.

The 15 representative isolates positive for ESBL production by disk tests, tested positive by Etest



**Figure 5.** MIC of ceftazidime/ceftazidime + clavulanic acid (TZ/TZL) = >32/0.75 = >42. Clear cut ESBL positive

strips of ceftazidime and ceftazidime + clavulanic acid. In all cases a ≥ 8 fold reduction in MIC of ceftazidime was observed when combined with clavulanate. Though Etest strips are highly precise and sensitive their high cost is a limiting factor for their routine use.

Once an *ESBL* producing strain is detected the laboratory should report it resistant to all penicillins, cephalosporins and aztreonam even if they test as susceptible<sup>27, 28</sup>. Treatment failures and death have occurred when cephalosporins were used against

**Table 6.** MICs of ceftazidime (TZ) & ceftazidime + clavulanate (TZL) in the 15 representative isolates tested by Etest ESBL strips

MIC $\mu\text{g/ml}$ TZ	MIC $\mu\text{g/ml}$ TZL	Ratio of TZ/TZL	Interpretation
24	0.50	24/0.50=48	ESBL positive
16	0.125	16/0.125=128	ESBL positive
>32	0.75	>32/0.75=>42	ESBL positive
>32	1.0	32/1.0=>32	ESBL positive
>32	<0.064	>32/<0.064=>500	ESBL positive
12	1.5	12/0.5=8	ESBL positive
8	0.25	8/0.25=32	ESBL positive
6	<0.064	6/0.064=>93	ESBL positive
2	0.125	2/0.125=16	ESBL positive
4	0.094	4/0.094=>42	ESBL positive
>32	<0.064	>32/<0.064=>500	ESBL positive
16	0.125	16/0.125=128	ESBL positive
16	<0.064	16/<0.064=>250	ESBL positive
>32	0.125	>32/0.125=>256	ESBL positive
>32	0.75	>32/0.75=>42	ESBL positive

*ESBL* producers that appeared susceptible in vitro<sup>28, 29</sup>. Although beta lactamase inhibitors have tremendous activity against *ESBLs* in vitro, their clinical effectiveness against serious infections due to *ESBL* producing organisms is somewhat controversial. Hyperproducing strains may produce enough beta-lactamase to overcome the effect of the betalactamase inhibitors. For these reasons betalactamase inhibitors may not be optimal therapy for serious infections due to *ESBL* producing organisms. However they are useful for less serious infections such as UTI<sup>30, 31</sup>. Non-betalactam antimicrobial agents such as fluoroquinolones, aminoglycosides and cotrimoxazole remain viable alternatives for the treatment of *ESBL* producing *Klebsiella* strains susceptible to these agents<sup>32</sup>. By far the carbapenems (meropenem, imipenem and ertapenem) remain the drug of choice for serious infections by *ESBL* producing *Klebsiella pneumoniae*<sup>7, 32</sup>.

Presently *Klebsiella pneumoniae* infections with *ESBL* are associated with severe outcomes such as 2.5 fold higher crude mortality, 5 fold higher mortality to infections and 3.4 fold higher delay in appropriate therapy. Also co selection with other resistances especially fluoroquinolones, aminoglycosides and sulfonamides further limit therapeutic options and especially bloodstream infections caused by *ESBL* producing organisms markedly increase the rates of treatment failure and death.<sup>33</sup>

Countering the threat posed by *ESBLs* should follow a multipronged approach. Not only is efficient detection, early reporting and rationale treatment modifications important but various other measures

**Table 7.** Antimicrobial susceptibility pattern of *ESBL* producing *Klebsiella pneumonia* as compared to 100% sensitivity in non *ESBL* producers

Drug	Sensitive/Resistance %ge
Gentamicin	8.1/91.9
Cotrimaxazole	23/77
Nitrofurantoin	41.7/58.3
Ofloxacin	41.9/58.1
Ciprofloxacin	55.4/44.6

also need to be emphasized equally. Efficient infection control policies and practices, restricted and judicious use of cephalosporins and simple measures like thorough hand washing are equally important.

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