DETECTION OF ESBL AND AMPC β-LACTAMASES IN KLEBSIELLA SPP. AND EVALUATION OF RISK FACTORS IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA

Pavithra K[1], Shrikala Baliga[2], Shalini Shenoy[3]

[1]Assistant Professor, Department of Microbiology, A. J. Institute of Medical Sciences and Research Centre, Mangalore.
[2]Professor, Department of Microbiology, Kasturba Medical College, Mangalore.
[3]Professor, Department of Microbiology, Kasturba Medical College, Mangalore.

ABSTRACT

BACKGROUND
Multi-drug resistant strains of Klebsiella as a result of the production of β lactamases like ESBL and AmpC β lactamases pose a big problem. The aim of the study is to detect ESBL and AmpC β lactamases among Klebsiella spp. and to evaluate the risk factors associated with acquiring these beta lactamases.

MATERIALS AND METHODS
ESBL detection was done by the method advocated by the CLSI and AmpC production by the modified three-dimensional test. A detailed history was taken; therapeutic details and outcome were assessed. Statistical analysis was done by χ^2 test.

Settings and Design- The isolates from samples of patients attending outpatient departments and admitted to wards and ICU of a tertiary care centre were included in study.

RESULTS
Among our isolates 104 (69.3%) were ESBL producers and 13 (8.6%) AmpC producers. Both ESBL and AmpC β lactamase production was seen in twelve isolates.

There was significant association between hospital stay of more than 5 days, treatment with broad-spectrum antibiotics, admission to ICU/NICU and ESBL production.

CONCLUSIONS
The present study revealed a high rate of ESBL production and the coexistence of ESBL and AmpC. There is a need to devise and standardise more simple and reliable methods to detect them.

KEYWORDS
ESBL, AmpC, Risk Factors.

BACKGROUND
Klebsiella is well known to clinicians as a cause of community-acquired pneumonia and as a nosocomial pathogen, which can be multidrug resistant.[1]

ESBLs are usually plasmid mediated and are inhibited by clavulanic acid.[2,3] AmpC β-lactamases are poorly inhibited by clavulanic acid and provide resistance to cephamycins and oxyimino β lactams.[4] Coexistence of ESBL and AmpC β-lactamases complicates the picture because high level expression of AmpC β-lactamase may mask the recognition of ESBL.[5] Also, the current methods of identification can result in underestimation of the prevalence of these organisms.[6]

This study will focus on the detection of ESBL and AmpC β-lactamases among the clinical isolates of Klebsiella spp. in our hospital and evaluate the risk factors associated with acquiring these beta lactamases.

MATERIALS AND METHODS
The study was conducted in a tertiary care centre. A total of 150 non-repeat Klebsiella isolates from various clinical sources from inpatients and outpatients of our hospital were included in the study.

A detailed history, which included the following points was taken;
1. Age.
2. Sex.
3. Presenting complaints, any underlying disease and probable risk factors like diabetes mellitus, haematological malignancies.
4. Clinical diagnosis.
5. Any previous antimicrobial therapy.

6. Performance of any invasive procedures and the duration of the presence of invasive devices.
7. History of any long-term hospitalisation.
8. Any previous steroid or immunosuppressant intake.
9. Outcome- whether there was improvement, recovery, condition unchanged or death.

Klebsiella was biochemically identified up to the species level.

ESBL Detection
Cephalosporin/Clavulanate Combination Disks Method
The method advocated by the CLSI was adopted.[7] Disk tests were performed using cefotaxime (30 μg) and cefotaxime/clavulanic acid (30 μg/10 μg) and ceftazidime (30 μg) and ceftazidime/clavulanic acid (30 μg/10 μg) from Becton Dickinson.

Quality Control
Klebsiella pneumoniae ATCC 700603 strain was used as positive control for ESBL production and Escherichia coli ATCC 25922 as negative control for ESBL production.

Modified Three-Dimensional Test for AmpC Detection[8]
The test was performed by inoculating the surface of the agar with Escherichia coli ATCC 25922, a susceptible strain. A cylindrical plug of agar of diameter 4 mm was then removed. Cefoxitin 30 μg [from Becton Dickinson disk] was placed 2.5 cm away from the well. A slit was made from the well reaching up to 2 mm from the antibiotic disks. This well was then filled with a milky suspension of the test organism. The test was interpreted as positive if the inhibition zone around the β-lactam antibiotic disk was distorted in such a way that growth of the test organism appeared within the zone, behind the cup and fully reaching the cup.

Quality Control
For AmpC production: A strain of Providencia alcalifaciens, which is a known producer of AmpC β-lactamase was used as a positive control and Escherichia coli ATCC 25922 as the negative control for AmpC production.

RESULTS
A total of 150 strains of Klebsiella were isolated from various clinical samples.

Out of this, 56 were isolated from blood (37.3%), 41 from urine (27.3%) and the rest from pus, respiratory tract specimens, catheter tips, sterile fluids and tissues as shown in Table 1.

The isolates were identified up to the species level by biochemical tests. Among the 150 isolates, 129 (86%) were identified as Klebsiella pneumoniae and 21 (14%) were identified as Klebsiella oxytoca.

Among the 150 isolates, 104 (69.3%) were ESBL producers. Thirty nine (37.5%) of ESBL producers were isolated from blood, 25 (24.03%) from urine and 23 (22.1%)

from pus. The rest were distributed among other clinical specimens (as shown in Table 2). Of the ESBL producers, 99 (95.2%) were K pneumoniae and five (4.8%) were K oxytoca.

AmpC detection test was positive in 13 of the total 150 isolates (8.6%) of which six were from pus. Eleven of the AmpC producers were K pneumoniae and two were K oxytoca. The distribution of AmpC positive isolates among various clinical samples is given in Table 3.

Twelve of the isolates were positive for both ESBL and AmpC β lactamases- 10 of K pneumoniae and two of K oxytoca produced both ESBL and AmpC β lactamases.

Evaluation of Risk Factors
Hospital stay of more than 5 days and treatment with broad-spectrum antibiotics was seen in 56 (61.5%) of the ESBL producers, whereas it was seen only in 10 (27.7%) of the ESBL negative isolates. Forty five (49.5%) of ESBL positive cases were admitted to ICU/NICU and had undergone therapeutic and/ or diagnostic invasive procedures/were on dialysis. Evaluation of the presence of other risk factors is shown in Table 4.

Among the 43 neonates, 36 (83.7%) were low birth weight babies, 1 (2.3%) had aspiration pneumonia and 1 (2.3%) had birth asphyxia.

Only 9 among the 13 AmpC positive isolates had history of hospital stay of average 15 days while 60 of the negative isolates had the history of hospital stay. Seven positive isolates had history of broad-spectrum antibiotic therapy and underwent invasive therapeutic/diagnostic procedures whereas 46 and 44 respectively of negative isolates had the same history.

By X^2 test, there was significant association (p value <0.05) between hospital stay of more than 5 days (p=0.005), treatment with broad-spectrum antibiotics (p=0.005), admission to ICU/NICU (p=0.012), invasive procedures (p=0.012) and ESBL production in Klebsiella isolates. There was no significant association (p value >0.05) between these risk factors and the production of AmpC β-lactamases by the clinical isolates.

Outcome
Out of the total 150 isolates, 65 patients showed improvement in their condition, 12 recovered completely, 39 expired while outcome was not known in 34 cases. Among the expired cases, 30 (76.92%) isolates were ESBL producers and one (2.5%) was AmpC producer.

DISCUSSION
Extended-spectrum β-Lactams are commonly included in the empirical antibiotic regimen for the treatment of infections by gram-negative bacteria.[9] The resistance of Klebsiella spp. to these third generation cephalosporins is a serious concern to treating doctors.

Detection of multiple resistance in gram-negative organisms is a challenge to clinical microbiologists. Although, CLSI recommendations exist, they are limited to ESBL-producing E. coli, Klebsiella and Proteus species. No

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recommendations exist for ESBL detection and reporting for other organisms and for detection of AmpC beta-lactamases.[3,4] Distinguishing between the two β-lactamases is of considerable therapeutic interest to a clinical microbiologist as the presence of AmpC can mask the presence of ESBL. In our study, we have aimed to detect these two beta lactamases.

Majority of our isolates were Klebsiella pneumoniae subsp. pneumoniae (86%) followed by Klebsiella oxytoca (14%). This is similar to the results elsewhere in the world.[10,11]

The rate of ESBL production ranges widely. The present study revealed that 69.3% of our Klebsiella isolates were ESBL producers. Prevalence of ESBL producing Klebsiella spp. as reported by other investigators in India - 25.6%, 24.6%, 30.18%, 58% and 80.0%.[12,13,,14,15] Worldwide too, the rate varies significantly. A study in Norway showed 84% of their Klebsiella isolates as ESBL producers.[16] A study by Winokur et al found percentage of ESBL production as 45% in Latin America, 25% in Western Pacific region, 23% in Europe, 8% in United States, 5% in Canada.[17]

ESBL production was observed both in K pneumoniae as well as K oxytoca isolates in many studies.[2,11] Of the ESBL producers in our study, 99 (95.2%) were K pneumoniae and 5 (4.8%) were K oxytoca. Study by Livermore and Yuan showed that ESBL production was more common in K pneumoniae (93.2% of ESBL producers) than in K oxytoca (6.8% of ESBL producers).[11] In case of K oxytoca, resistance to cephalosporins could be due to the hyperproduction of a chromosomal K1 β-lactamase due to mutation. But, hyperproduction confers resistance to cefotaxime, ceftriaxone and cefpodoxime, but not to ceftazidime.[18] Since, all the isolates of this study showed resistance to both cefotaxime and ceftazidime, it can be concluded that cephalosporin resistance in the K oxytoca is due to ESBL production and not due to hyperproduction.

In the present study, AmpC detection was done by modified three-dimensional test. It was found to be a very sensitive test by many authors.[4,8,19] The test was positive in 13 of the total 150 isolates, i.e. 8.6%. This is higher than the 1.1% obtained in a study by Coudron et al[19] and 0.9% in a European study by Livermore.[11] Indian studies have demonstrated varied results Singhal et al[20] found that 6.18% among their isolates were AmpC producers, which was similar to our results.[19] Slightly higher rates were observed by other authors- 33.3% by Manchanda and Singh,[8] 31.1% by Shahid et al[13] and 39.1% by Mohamudha Parveen.[21]

Simultaneous occurrence of ESBL and AmpC enzymes were noted in 6 isolates (9.8%) by Shahid et al[13] whereas 12 of our isolates showed production of both ESBL and AmpC.

In the present study, ESBL producing Klebsiella as well as multidrug-resistant Klebsiella were significantly associated (p value <0.001) with hospital stay of more than 5 days (61.5%) and admission to ICU/NICU (49.5%) when compared to the non-ESBL producers (27.7% for hospital stay and 25% admission to ICU/NICU). Though the

colonisation with resistant organisms was not studied here, the results indicate that patients get colonised and get infected with resistant organisms as the length of hospital stay increases and admission to areas like ICU/NICU.

Treatment with broad-spectrum antibiotics was significantly associated with ESBL positivity. 49.5% of ESBL positive strains among our isolates had history of treatment with broad-spectrum antibiotics while only 25% of ESBL negative had the same history. Thus, we can conclude that a high rate of ESBL production by Klebsiella strains could be due to the selective pressure imposed by the use of antimicrobials. These results are consistent with other studies from India as well as abroad.[22,23]

A Spanish study by Rodriguez et al demonstrated that diabetes mellitus and the elderly are independent risk factors for infections with ESBL producing organisms along with prior antibiotic therapy and hospital stay.[24] But, the present study did not show significant association with diabetes mellitus (10 of positive cases- 10.9%) and patients with malignancy and on chemotherapy (4 cases). This maybe because about one third of our isolates were from children and neonates.

Studies have shown that risk factors for infections with AmpC producers include long hospital stay, admission to ICU, indwelling devices and prior administration of broad-spectrum cephalosporins.[25,26] The present study did not reveal any association between these risk factors and AmpC production. In our study, AmpC production was seen only in 13 isolates, hence the sample size was inadequate to comment on the risk factors.

The outcome was assessed based on the improvement in clinical status and the physician's opinion as to treatment success or failure. Tests to assess bacteriologic cure, i.e. negative cultures after 72 hrs. of treatment were not done in any of the cases.

The patients in whom recovery and improvement was seen were treated mainly with ciprofloxacin, amikacin and piperacillin/tazobactam according to the antibiotic susceptibility test report. Paterson has recommended fluoroquinolones and carbapenems for treatment of infections with ESBL producing organisms,[3] while Gavin et al have seen improvement in their patients with piperacillin/tazobactam.[27]

CONCLUSION
The coexistence of ESBL and AmpC is quite alarming. The detection of ESBL by CLSI proposed method is an easy and reliable method. They are not labour intensive and the interpretation is not subjective.

Modified three-dimensional method of detecting AmpC beta-lactamase, which we have used is quite sensitive, but labour intensive. There is a need to devise and standardise more simple and reliable methods to detect these beta lactamases so that they can be used in any diagnostic laboratory.

Clinical Specimens	Number (%)
Blood	56 (37.3)
Urine	41 (27.3)
Pus	31 (20.7)
Respiratory tract	17 (11.3)
Others	5 (2)
Total	**150**

Table 1. Distribution of Isolates among Clinical Specimens

Samples	ESBL Positive (%)
Blood	39 (37.5)
Urine	25 (24.03)
Pus	23 (22.1)
Respiratory tract specimens	13 (12.5)
Catheter tips	2 (0.02)
Tissues	1 (0.01)
Fluids	1 (0.01)
Total	**104**

Table 2. ESBL Producers from the Clinical Samples

Samples	AmpC Positive (%)
Blood	2 (15.4)
Urine	3 (23.1)
Pus	6 (46.2)
Respiratory tract specimens	1 (7.7)
Catheter tips	1 (7.7)
Tissues	0
Fluids	0
Total	**13**

Table 3. Distribution of AmpC Positive Isolates among the Clinical Samples

Risk Factors	ESBL Positive (%)	ESBL Negative (%)
Hospital stay	56 (61.5)	10 (27.7)
ICU Admission	45 (49.5)	9 (25)
Invasive procedures/Dialysis	45 (49.5)	9 (25)
Diabetes mellitus	10 (10.9)	2 (5.5)
Malignancy	4 (4.3)	0 (0.0)

Table 4. Evaluation of Risk Factors ESBL Production

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