Prevalence of ESBL Producing Klebsiella Species Isolated from Respiratory Samples at Intensive Care Units.

R. Hymavathi1, S. Shanthi Kumar2, G. Swarnalatha3, A. Surekha4

1Assistant Professor, Department of Microbiology, Kurnool Medical College, Kurnool, Andhra Pradesh, India.
2Professor & HOD, Department of Microbiology, Kurnool Medical College, Kurnool, Andhra Pradesh, India.
3Assistant Professor, Department of Microbiology, Kurnool Medical College, Kurnool, Andhra Pradesh, India.

Received: May 2018
Accepted: May 2018

Copyright: © the author(s), publisher. Annals of International Medical and Dental Research (AIMDR) is an Official Publication of “Society for Health Care & Research Development”. It is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Extended-spectrum β-lactamase-producing K. pneumoniae (ESBL-Kp) has recently become an important nosocomial pathogen. Intensive care units infections are predominant hospital acquired infections especially in tertiary health care. It is essential to know about ESBL prevalence in tertiary health care to take necessary actions by curbing resistant microorganisms. We aimed to study ESBL prevalence in Klebsiella species among respiratory samples from ICU patients. Methods: Respiratory samples collected from Intensive care unit patients, were processed under aseptic precautions. All Klebsiella isolates were subjected to routine antibiotic susceptibility testing by modified Kirby Bauer’s disc diffusion method and also for ESBL testing along with control. The results were interpreted as per CLSI (Clinical Laboratory Standards Institute) guidelines. Results: 153 (47.2%) Klebsiella species were isolated from 324 culture positive respiratory samples. Out of the 153 isolates which were screened for ESBL production, 90 (58.8%) isolates were found to be ESBL positive by CLSI disc diffusion. On performing confirmatory tests on the 90 isolates which were ESBL positive by screening tests, 84 (54.9%) were found to be ESBL producers on CLSI phenotypic confirmatory test (PCT) and 88 (57.5%) were found to be ESBL producers on Double Disc Synergy Test (DDST). Conclusion: Discussing with clinical microbiologist about antibiotic therapy helps clinicians to tackle with these resistant pathogens; also prevents transmission of resistant bugs and indirectly aid to increase life expectancy of patients.

Keywords: Klebsiella, Extended spectrum β-lactamases.

INTRODUCTION

The genus Klebsiella is a member of Enterobacteriaceae family, ubiquitous in nature and found in water, soil and mucosal surfaces of mammals. Klebsiella colonizes respiratory tract, gastrointestinal tract, urinary tract, eye of humans. It is more problematic pathogen causing higher mortality and morbidity rates at Intensive care units. The incidence of infections caused by multidrug resistant Klebsiella strains has increased, became an important cause of hospital acquired infections. The most important mechanism of resistance to beta lactam antibiotics is due to the production of beta lactamases by Klebsiella. The enzymes are thought to have evolved from penicillin binding proteins with which they show some sequence homology. This development was likely due to the selective pressure by beta lactam producing soil organisms found in the environment. Because of their increased spectrum of activity against the oxyimino-

cephalosporins, these enzymes are called extended spectrum beta lactamases (ESBL) which are capable of hydrolyzing and inactivating a wide variety of beta lactam antibiotics like third generation cephalosporin’s and penicillin’s.[3-4] The ESBL enzymes are plasmid mediated enzymes capable of hydrolyzing and inactivating a wide variety of β lactams – third generation cephalosporins, penicillins and aztreonam. These enzymes are the result of mutations of TEM 1 and TEM 2 and SHV1. The resistant organisms are now a worldwide problem. The majority of ESBL producing strains are Klebsiella pneumoniae, K oxytoca, and Escherichia coli.[5] Extended-spectrum β-lactamase-producing K. pneumoniae (ESBL-Kp) has recently become an important nosocomial pathogen.[6] In the tertiary hospitals of China, approximate 50% nosocomial-acquired infections are caused by ESBL-Kp and blaCTX-M-15 and blaCTX-M-14 are the predominant genotypes.[7] Recently, the prevalence rate of ESBL-Kp in community-acquired infections is increasing even causing invasive infection.[8] Intensive care units infections are predominant hospital acquired infections especially in tertiary health care. Resistant microorganisms are quite common in ICU’s, dissemination also occurs easily.
when there is breakage in chain of Infection control practices. It is essential to know about ESBL prevalence in tertiary health care to take necessary actions by curbing resistant microorganisms. We aimed to study ESBL prevalence in Klebsiella species among respiratory samples from ICU patients.

**MATERIALS AND METHODS**

For this prospective study, a total of 748 respiratory samples of Intensive care unit patients sent for bacterial culture and sensitivity were included. Respiratory samples include sputum, throat swab, BAL, mini BAL. Study was undertaken at Department of Microbiology, Kurnool Medical College, Government General Hospital, Kurnool from Feb 2016 to Dec 2017. Respiratory samples collected from Intensive care unit patients, advised to collect in a sterile leak proof universal container along with proper labelling related to patient details. All respiratory samples were processed under aseptic precautions, direct gram stain observed for all samples; inoculated on Nutrient agar, Mac Conkey agar, Blood agar then incubated at 370C for overnight and colonies were processed.

Respiratory samples showing pure and predominant growth of Klebsiella species correlating well with clinical condition were included in this study. Klebsiella species were identified by gram stain as gram negative, non-motile, rod shape measures about 0.3 to 1.5µm and 0.5 to 5µm; colony characteristics such as round, regular, large, convex, mucoid, glistening and by biochemical reactions. All Klebsiella isolates were subjected to routine antibiotic susceptibility testing by modified Kirby Bauer’s disc diffusion method and also for ESBL testing along with control (Klebsiella pneumoniae ATCC 700603). The results were interpreted as per CLSI (Clinical Laboratory Standards Institute) guidelines. Antimicrobial discs used includes amoxyclav – 20/10 µg, piperacillin+tazobactum, cefotaxime – 30 µg, ceftazidime – 30 µg, ceftazidime+ clavulanic acid 30+10 g disc at a distance of 25 mm apart. An increase of ≥5 mm in zone of inhibition for ceftazidime + clavulanic acid compared to ceftazidime was confirmed as ESBL producers.

**RESULTS**

A total of 748 respiratory samples were collected from ICU’s in this study period. Out of 748 respiratory samples 153 (20.4%) were Klebsiella isolates. Culture positivity of respiratory samples from ICU’s was 43.3% (324 out of 748).

153 (47.2%) Klebsiella species were isolated from 324 culture positive respiratory samples. 80 (52.2%) out of 153 Klebsiella isolates were observed in > 50 years age group ICU patients predominantly. 35 Klebsiella species were isolated from respiratory samples of 0-10 years age group children. Klebsiella species were predominantly isolated from males (60.1%) when compared to females (39.8%).

**Table 1: Klebsiella isolates prevalence in various parameters**

<table>
<thead>
<tr>
<th>S.N o.</th>
<th>Parameters</th>
<th>No. of Klebsiella isolates</th>
<th>Percentage (%)</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age in years</td>
<td>0-10</td>
<td>35</td>
<td>22.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11-20</td>
<td>5</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21-30</td>
<td>8</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>31-40</td>
<td>8</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>41-50</td>
<td>17</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>51-60</td>
<td>58</td>
<td>37.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>61 and above</td>
<td>22</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Sex</td>
<td>Males</td>
<td>92</td>
<td>60.1</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>61</td>
<td>39.8</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Days in Hospital</td>
<td>≥7 days</td>
<td>104</td>
<td>67.9</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 7 days</td>
<td>49</td>
<td>32.1</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Association with comorbidities</td>
<td>Yes</td>
<td>107</td>
<td>69.9</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>46</td>
<td>30.1</td>
<td></td>
</tr>
</tbody>
</table>
Patients spent for ≥ 7 days in hospital (67.9%) were most commonly affected by Klebsiella respiratory tract infection. Parameters including sex, number of days stay in hospital, association with comorbidities were statistically significant [Table 1]. Out of 153 klebsiella pneumoniae isolates, 139 (90.8%) were sensitive to Meropenem, 134 (87.5%) were sensitive to amikacin, 126 (82.3%) were sensitive to gentamicin, 122 (79.7%) were sensitive to cefotaxime, 108 (70.5%) were sensitive to tetracycline, 78 (50.9%) were sensitive to ciprofloxacin, 68 (44.4%) were sensitive to ceftazidime/clavulanic acid, 65 (42.4%) were sensitive to piperacillin+tazobatum, 55 (35.9%) were sensitive to ceftazidime, 52 (33.9%) were sensitive to cefotaxime, 34 (22.2%) were sensitive to amoxyclav [Figure 1].

Out of the 153 isolates which were screened for ESBL production, 90 (58.8%) isolates were found to be ESBL positive by screening tests, 84 (54.9%) were found to be ESBL producers on CLSI phenotypic confirmatory test (PCT) and 88 (57.5%) were found to be ESBL producers on Double Disc Synergy Test (DDST). On comparison of PCT and DDST, the p value was 0.72, which is not significant statistically [Table 2]. Sensitivity of PCT at 95% CI was 85.7% to 97.4% (Ave - 93.1%) and specificity was 89.3 to 99.6 (Ave - 96.9). On assessment of DDST sensitivity at 95% (Ave - 93.1%) and specificity was 89.3 to 99.6 (Ave - 95.4).

Table 2: ESBL estimation among Klebsiella isolates by PCT and DDST confirmatory tests

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates tested</th>
<th>PCT</th>
<th>DDST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>species</td>
<td>153</td>
<td>84</td>
<td>54.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88</td>
<td>57.5</td>
</tr>
</tbody>
</table>

DISCUSSION

In the last 5 years, emerging of Klebsiella ESBL producers has become a significant problem and also identification of such isolates has proved to be a challenging task in the microbiology laboratories. In the last two decades, CTX-M replaces SHV as the major type of ESBLs disseminating worldwide.[12] Dramatically resistance is increasing to cephalosporins amongst Escherichia coli and Klebsiella spp., revealed by Asia, Latin America and European survey, largely contingent on the spread of CTX-M ESBLs.[13] It has been suggested that the frequent acquirement of plasmids harboring blaCTX-M is largely responsible for the increase of CTX-M-producing Enterobacteriaceae. Extended-spectrum β-lactamase-producing K. pneumoniae (ESBL-Kp) has recently become an important nosocomial pathogen.[14]

In the present study, 153 (47.2%) Klebsiella species were isolated from 324 culture positive respiratory samples. Jing Zhang et al.[15] reported Klebsiella pneumoniae is predominantly isolated from sputum samples (73.9%) followed by urine (11.2%), blood (5.5%), abscess (3.3%), throat swab (2.4%), and unknown specimen (3.6%). Infections are quite common in ICUs, especially respiratory tract infections. There is no much extensive studies ESBL prevalence in ICUs. So we selected this study to do in a tertiary care hospital.

Out of 153 klebsiella pneumoniae isolates, 139 (90.8%) were sensitive to Meropenem, 134 (87.5%) were sensitive to amikacin, 122 (79.7%) were sensitive to gentamicin, 122 (79.7%) were sensitive to cefotaxime, 108 (70.5%) were sensitive to tetracycline, 78 (50.9%) were sensitive to ciprofloxacin, 68 (44.4%) were sensitive to ceftazidime/clavulanic acid, 65 (42.4%) were sensitive to piperacillin+tazobatum, 55 (35.9%) were sensitive to ceftazidime, 52 (33.9%) were sensitive to cefotaxime, 34 (22.2%) were sensitive to amoxyclav in this study.

Jing Zhang et al.[16] reported Klebsiella pneumoniae isolates shown 65.4% susceptibility to cefotixin, 30.9% were susceptibility to gentamicin, 79.5% were susceptibility to amikacin, 51.3% were susceptibility to cefepime, 51.3% were sensitive to levofoxacin, 99.5% were sensitive to meropenem and imipenem. They did a study on 587 Klebsiella pneumoniae isolates collected from 31 county hospitals locating in 11 provinces representing seven major geographic regions of China.

Vemula Sarojamma et al.[16] reported out of 100 isolates tested for their antibiogram, 61% isolates have shown susceptibility to 3rd-generation cephalosporins and 39% were resistant. Amoxyclavin showed the highest percentage of resistance followed by tetracyclines and cefotimoxazole. Numerous virulence factors have been described in Klebsiella spp responsible to evade defence mechanisms and for developing resistance towards various antibiotics. Virulence factors includes Extracellular capsule with about 80 different capsular (K) antigens are known. In addition to the

Figure 1: Antibiotic sensitivity pattern of Klebsiella species
capsule, there are about five somatic or O antigens, fimbrial and nonfimbrial adhesins, Additional virulence determinants for Klebsiella spp include the ability of the organism to scavenge iron from the surrounding medium using secreted siderophores, that is, enterochelin and aerobactin.[17-19] Out of the 153 isolates which were screened for ESBL production, 90 (58.8%) isolates were found to be ESBL positive by CLSI disc diffusion. On performing confirmatory tests on the 90 isolates which were ESBL positive by screening tests, 84 (54.9%) were found to be ESBL producers on CLSI phenotypic confirmatory test (PCT) and 88 (57.5%) were found to be ESBL producers on Double Disc Synergy Test (DDST). Sensitivity and specificity of PCT and DDST are almost similar, PCT was found to be better test which is supported by other studies.[20, 21] In similar to our study Lakshmi mumari et al.[22] observed similar ESBL prevalence detection by PCT and DDST i.e., 26.6%. Vernula saroojamma et al.[16] documented PCDDT was found to be better than DDST in the detection of ESBLs. Many of the studies have done on Klebsiella isolates ESBL production from clinical isolates and documented prevalence of ESBL as 23.6% from Chennai,[23] 25.6% from Nagpur,[24] ESBL prevalence rate depends on various factors like infection control practices, carriage rate in community, antibiotic policy, colonization, prolonged length of stay in hospital, mechanical ventilation.[25-28] Prevalence of ESBL producing Klebsiella isolates reported from Turkey 78.6%,[29] China 51%,[30] Spain 20.8%,[31] USA 4.2-44%.[32] Management and treatment of ESBL-producing K. pneumoniae infections can be challenging and is evolving. Cephalosporins are therefore not recommended for the treatment of bloodstream and serious infections caused by these pathogens.[33] However, cephalosporins have been used successfully to treat less serious infections such as UTIs and pneumonia.[32] In contrast, the empiric use of β-lactam-β-lactamase inhibitors in intensive-care units appears to have a small, but significant protective effect and reduces infections caused by ESBL-producing K. pneumonia.[34] The use of other non-β-lactam antibiotics, such as quinolones, has been effective in the treatment of infections caused by ESBL-producing organisms in animal models.[34] Currently, the carbapenems, that is, imipenem and meropenem, are the only class of antimicrobials that have consistently been effective against ESBL-producing K. pneumonia.[32]

Conventional methods for detection of ESBL production by pathogenic organisms play an important role along with Automated MIC sensitivity testing. Conventional methods mainly used for epidemiological purposes and also helps when there is increased MICs may go unnoticed by laboratory personnel.

CONCLUSION

Klebsiella species are predominant pathogens among respiratory tract infections. DDST and PCT are conventional confirmatory diagnostic tests for ESBL detection with good sensitivity. ESBL production rate higher among Klebsiella species isolated from Intensive care units. Clinicians should aware about spread of resistant pathogens among critically ill patients. Discussing with clinical microbiologist about antibiotic therapy helps clinicians to tackle with these resistant pathogens; also prevents transmission of resistant bugs and indirectly aid to increase life expectancy of patients.

REFERENCES

11. Clinical Laboratory Standards Institute Performance Standards For Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement; Supplemental Table 2A-S1. Screening and Confirmatory Tests for ESBLs in Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, and Proteus mirabilis for Use With Table 2A, M100-S21;31(1):4849.