Dissemination of CTX-M-15 β-Lactamases Harbouring E. coli and Klebsiella Pneumonia Strains from Tertiary Care Hospital India

**ABSTRACT**

**Introduction** Extended spectrum β-lactamases (ESBL) producing gram negative bacteria (GNB) have spread ominously worldwide particularly Escherichia coli (E. coli) and Klebsiella pneumonia (K. pneumoniae). Hospital outbreaks of multidrug resistant K. pneumoniae and E. coli are often caused by a new type of strain, the ESBL producers. The present study was undertaken to detect the prevalence of E. coli and K. pneumoniae with reference to CTX-M-15 ESBL.

**Methods** Identification and confirmation of E. coli and K. pneumoniae were done by standard conventional methods. Antibiotic susceptibility testing was done by Kirby Bauer’s disc diffusion technique and interpretations of susceptibility were done according to the CLSI (Clinical Laboratory Standard Institute) 2008 guidelines. Genotypic characterization of CTX-M-15 were done by polymerase chain reaction.

**Results** Total of 91 E. coli and 79 K. pneumoniae were isolated. Amongst all E. coli isolates, 47 (51.64%) isolates were ESBL producers while 8 (8.79%) were MBL producers. Maximum ESBL producers E. coli were from patients admitted in the ICU. Amongst all K. pneumonia, 64.55% strains were ESBL producers while 10.12% strains were MBL producers. 61 strains were detected as CTX-M-15 β-lactamases producing ESBL. Of the total 61, 26 (42.62%) were E. coli and 35 (57.37%) were K. pneumonia.

**Conclusions** This study highlights the need for monitoring the spread of this multidrug resistant clonal complex throughout the nation and provides better understanding of the contribution of clonal dissemination among multidrug resistance GNB.

**KEYWORDS** ESBL, K. pneumoniae, E. coli, CTX-M-15, MBL

**INTRODUCTION**

Extended spectrum β-lactamases (ESBL) producing gram negative bacteria (GNB) have spread ominously worldwide particularly Escherichia coli (E. coli) and Klebsiella pneumonia (K. pneumoniae). Infections caused by GNB are now a significant concern for the development of therapies against it. The excessive use of cephalosporins in the treatment of GNB infections resulted in an increase bacterial resistance to these drugs. ESBL genes responsible for resistance are mainly found in mobile genetic elements that can readily spread through bacterial populations. ESBL producing K. pneumoniae usually expresses resistance to various antibiotics; therefore their antibiotic therapy is limited to a few expensive drugs which, in most cases, are not available in developing countries. In the 1990s, a novel type of ESBL, the CTX-M enzyme emerged worldwide, called The CTX-M pandemic. Specific CTX-M subgroups have been localized to different geographic areas. This phenomenon accelerated rapidly especially during the last 5 years. Currently, the most widely distributed CTX-M enzyme is CTX-M-15 which was first detected in E. coli from India in 2001. Multi drug resistant CTX-M-15-producing E. coli and K. pneumonia are emerging worldwide especially since 2003 as an important pathogen causing onset and hospital acquired infections. A single clone of CTX-M-15 producing E. coli named ST131 isolated from several countries including Spain, France, Switzerland, Lebanon, Kuwait, and Korea. This clone is associated with serogroup O25 belonging to CTX-M-15 ESBL.

**DISCLAIMER:** Any views expressed in this paper are those of the authors and do not reflect the official policy or position of the Department of Defense.
to highly virulent phylogenetic group B2 and harbours multi drug resistant IncF1 plasmids. Despite the high prevalence of these isolates in nosocomial infections, large studies to investigate the molecular epidemiology of these isolates in India are still lacking. Due to the universal emergence of clone ST131 isolates that produce CTX-M-5 β-lactamases we have designed a study to investigate the prevalence and characteristics CTX-M-15 ESBL producing E. coli and K. pneumonia isolated from various clinical infections.

In pediatric wards, nosocomial K. pneumoniae infections are especially troublesome particularly in premature infants and intensive care units (ICUs). K. pneumoniae are often the pathogens involved in neonatal sepsis in both early-manifestation and late-manifestation infections. Due to the extensive spread of antibiotic-resistant strains, especially ESBL producing strains, there has been renewed interest in K. pneumoniae infections.

Hospital outbreaks of multidrug-resistant K. pneumoniae is often caused by a new type of strain, the ESBL producers. The incidence of ESBL-producing strains among clinical K. pneumoniae isolates has been steadily increasing over the past several years. Frequencies of up to 40% have been reported in certain regions. Currently, the available data suggest a further increase in the incidence of ESBL-producing isolates. As a result, the therapeutic options are becoming limited, so that in the near future there will be an urgent need for hospital infection control measures that counter the spread of ESBL-producing bacteria.

The present study was undertaken; to isolate and confirm E. coli and K. pneumonia from various clinical samples by conventional methods, to study the antibiotic susceptibility pattern of above isolates with special reference to phenotypic detection of ESBL and to do genetic characterization of ESBL producing E. coli and K. pneumoniae in relation to CTX-M-15 pandemic clone.

**METHODS**

*Place of conducting research.* Department of Microbiology, Dr. D. Y. Patil Medical College Hospital And Research Centre (Dr. D.Y.Patil University Pune) Pimpri, Pune – 411018, India

*Duration.* June 2015 to December 2015

*Sample size.* E. coli and K. pneumonia isolated during the study period, in the Microbiology department Dr. D.Y. Patil Medical College Hospital And Research Centre Pimpri Pune – 411018.

*Ethical statement.* Study was approved by the Institutional Ethics Committee - Dr. D. Y. Patil Medical College. Reference no.: DYPMC/I.E.S.C./03/15.

*Data collection.* Data on sociodemographic variables such as age, sex and clinical data included immune status, underlying disease, a history of hospitalization; previous hospitalization and antimicrobial therapy, use of mechanical ventilator, presence of an indwelling catheter, the associated focal infection, and parameters for determination of disease severity were collected.

*Sample processing.* Samples were processed on Blood Agar and MacConkey agar and incubated at 37°C. Identification and confirmation was done by standard conventional methods. Antibiotic susceptibility testing was done by Kirby Bauer’s disc diffusion technique and interpretations of susceptibility will be done according to the CLSI (Clinical Laboratory Standard Institute) 2008 guidelines.

**Test for ESBL production**

CLSI confirmatory test. Test was performed on Muller-Hinton agar. Ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30/10 µg) were placed on Muller-Hinton agar and incubated at 37°C. The organism was considered as ESBL producer if there is ≥ 5 mm increase in zone diameter of ceftazidime/clavulanic acid disc than that of ceftazidime alone. E. coli ATCC25922 and K. pneumonia strain 48188 were used as negative and positive control respectively.

**PCR detection of CTX-M-15 on ESBL isolates**

The presence of genes will be determined by PCR using primer targeting newly described CTX-M-15 E. coli and K. pneumonia by using the following primers:

Gndbis f(5’ATACCGACGACGCCGATCTG-3’)

rbOB25b r (5’TGCTATTATGCGCGACG-3’).

Annealing temperature of 60°C was used to generate a PCR product of 300 bp with the conditions as described. The reaction mixture will be analyzed by
electrophoresis on 2% agarose gel, and the amplicons will be visualized by staining with ethidium bromide.

RESULTS

A total of 91 E. coli and 79 K. pneumoniae were isolated from various clinical samples received in the microbiology laboratory. Maximum E. coli were isolated from urine samples followed by pus and fluid while maximum K. pneumoniae were isolated from various body fluids followed by blood and sputum samples.

Amongst all E. coli isolates, 47 (51.64%) isolates were ESBL producers while 8 (8.79%) were MBL producers. Maximum ESBL producers E. coli were from patients admitted in the ICU. 89.01% E. coli strains were resistant to Amikacin, 74.72% E. coli were showed resistant to Amoxycillin while 48.35% E. coli showed resistant to Cotrimaxazole (Fig. 1).

Amongst all K. pneumonia, 64.55% strains were ESBL producers while 10.12% strains were MBL producers. Maximum ESBL and MBL producers were from Endotracheal secretion secretions and blood samples followed by pus samples. 84.81% K. pneumonia strains were resistant to Cefotaxime, 82.27% strains were resistant to Ceftriaxone. Tetracyclines, Amikacin, Amoxycillin resistant were found in 50.63%, 43.3%, 62.02% strains of K. pneumonia, respectively. Amongst all MBL producers, strains 6(75%) were from ICU patients.

Maximum numbers of MDR GNB were isolated from NICU followed by surgery and medicine wards.

Table 1 | Antimicrobial susceptibility of clinical isolates of E. coli.

<table>
<thead>
<tr>
<th>Name of antibiotics</th>
<th>Susceptibility pattern of GNBs (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptibility (%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>10 (10.98)</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>23 (25.27)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>31 (34.09)</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>29 (31.86)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>37 (40.65)</td>
</tr>
<tr>
<td>Cotrimaxazol</td>
<td>43 (47.25)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>22 (24.17)</td>
</tr>
<tr>
<td>*Cefazidime + Calvulanic acid (n = 258)</td>
<td>47 (51.64)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>64 (70.32)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>80 (87.91)</td>
</tr>
<tr>
<td>*Imipenem + EDTA (n = 100)</td>
<td>08 (10.12)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>47 (51.64)</td>
</tr>
</tbody>
</table>

*Cefazidime + Calvulanic acid disc susceptibility testing was done on only cefazidime resistance strains (Fig. 2). Imipenem + EDTA disc susceptibility testing were done on only Imipenem resistance strains (Fig. 3).

K. pneumonia was predominantly isolated from NICU. From OPD urine samples E. coli was isolated as most common urinary pathogen as community acquired urinary tract infections. From pulmonary medicine 13 (16.45%) K. pneumonia was isolated.

All ESBL producers’ strains i.e. 47 (51.64%) and 51 (64.55%) E. coli and K. pneumonia respectively were subjected for detection of CTX-M 15 β-lactamases production. Of the total 98 ESBL from E. coli and K. pneumonia, 61 strains were detected as CTX-M-15 β-lactamases producing ESBL. Of the total 61, 26 (42.62%) were E. coli and 35 (57.37%) were K. pneumonia.
with a higher mortality rate and early administration of carbapenem use may reduce the rate of mortality among patients with infections caused by ESBL-producing organisms\textsuperscript{22-25}. Theoklis et al. (2009), Dhumal et al. (2012) and Savita Jadhav et al. (2012) were reported growing trend of ESBL producing Enterobacteriaceae in PICU in Taiwan and India respectively\textsuperscript{18,19,24}. while Kyung Kim et al. (2002) reported high prevalence of ESBL-producing E. coli and K. pneumonia in PICU in Korea. E. Lebessi et al. (2002) have reported high prevalence of ESBL-producing E. coli and K. pneumonia in NICU from Athens, Greece\textsuperscript{23}. From India, Savita Jadhav et al. (2012) have reported increasing incidence of multidrug resistance K. pneumoiae infections in hospital and community and reported MBL producers from NICU and PICU\textsuperscript{26}. The studies described by Nichols Chanoin et al. (2020) that bacteremia caused by ESBL-producing strains is associated with a higher mortality rate and early administration of carbapenem use may reduce the rate of mortality among patients with infections caused by ESBL-producing organisms. The risk factors for bacteremia caused by ESBL-producing strains is associated with a higher mortality rate and early administration of carbapenem use may reduce the rate of mortality among patients with infections caused by ESBL-producing organisms. The risk factors for bacteremia caused by ESBL-producing strains of E. coli or K. pneumoniae included hospitalization, care in an ICUs, ventilator care, antibiotic use within the preceding 30 days, the presence of a central venous access catheter, and the development of breakthrough bacteremia during antibiotic therapy. Exposure to extended-spectrum cephalosporins was a risk factor for infection with ESBL-producing strains, while other antimicrobial agents did not increase the risk. Similar finding were reported by Yun-Kyung et al. from Korea (2002). Infection with ESBL-producing organisms was significantly more among the neonates for whom expressed breast milk was fed through external feeding.
tubes in comparison to the neonates fed at the breast. Previous study showed that the use of the external feeding tube was an independent risk factor for colonization by multiple antibiotic-resistant *K. pneumoniae* and *E. coli* but the subjects were not neonates. Further studies with a larger population need to be done to establish this association. Similar finding were reported from India by Subhasree Roy et al. (2013)²⁴.

All phenotypic ESBL producers’ strains were further subjected for molecular detection by PCR for the presence of CTX-M-15 β-lactamases production. 61 strains were detected as CTX-M-15 β-lactamases producing ESBL. Of the total 61, 26 (42.62%) were *E. coli* and 35 (57.37%) were *K. pneumoniae*. The clonal diversity and the possibility of dissemination of such plasmid mediated genes raise concern as experiments in the laboratory show that transfer of such genes can occur by conjugation. In addition, the presence of integron-1 in some isolates probably allows dissemination of CTX-M-15 ESBL gene. The rate of resistance to most antibiotics was alarming, suggesting that the WHO recommended ampicillin and gentamicin combination as first line treatment of neonatal sepsis may no longer be effective. Resistance to 3rd generation cephalosporins and monobactam was seen in these isolates, being higher amongst *E. coli* isolates. Ciprofloxacin resistance was more frequent in *E. coli* isolates, as reported previously. Carbapenems which are reserve drugs for treatment of neonatal sepsis, showed 100 per cent sensitivity²⁶. The increase in the number of ESBL- producing isolates by *Enterobacteriaceae* in PICUs outbreaks were reported by several authors worldwide. The dissemination of ESBL-producing *Enterobacteriaceae* is a consequence of the clonal expansion of a few epidemic strains and the spread of resistance plasmids among bacterial organisms which has been associated with community as well as hospital acquired. Since the resistance displayed by bacteria reflects the environment in which the organism thrives, immediate action, including reinforcement of infection control measures, should be taken to prevent further spread of the resistant bacteria²⁷. Though the study is limited and conducted over a period of two months by the small sample size, it highlights the presence of CTX-M-15 in clonally diverse *K. pneumoniae* and *E. coli* isolates indicating that CTX-M-15 ESBL is probably disseminated horizontally. The high occurrence of ESBL organisms and a transmissible resistance gene (*M₉CTX-M*) is of great concern in a country with high population density and infant mortality rate.

**CONCLUSION**

This study highlights the need for monitoring the spread of this multidrug–resistant clonal complex throughout the nation and provides better understanding of the contribution of clonal dissemination among multidrug resistance GNB. This study would definitely benefit the development of intervention and prevention strategies to control the spread of such multi drug resistant clonally related groups of strains in clinical settings as well as in the community and the environment.

**ACKNOWLEDGEMENTS**

We would like to extend our thanks to STS 2015, Indian council of medical research [ICMR] New Delhi. This study was partially funded by ICMR. We also thankful to chairmen and members of the Dr. D. Y. Patil University Pune for their help and support. We acknowledge assistance provided by Miss. Neelam Sing and Miss. Anuradha kadluk at different stages of this study.

**REFERENCES**