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Extended Spectrum β-Lactamases and Metallo β-Lactamases Production in *Klebsiella pneumoniae* Isolates Causing Pneumonia in Rural Population of Uttar Pradesh, India

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A B S T R A C T

The drug resistance among the gram negative bacteria is present as a serious global problem. ESBLs and MBLs are the important members of beta-lactamases produced mainly by gram negative bacteria and are responsible for mediating resistance to extended-spectrum cephalosporins and carbapenem. These enzymes are commonly detected in the members of the Enterobacteriaceae like Klebsiella pneumoniae and Escherichia coli. A cross-sectional study was conducted at Department of Microbiology, Santosh Medical College & Hospital Ghaziabad by using *K. pneumoniae* isolated from sputum samples. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique. ESBL and MBL production were detected by using phenotypic methods. Out of the 200 sputum samples, 72 samples were confirmed and identified as *K. pneumoniae.* Out of total 72 isolates, 48 (66.6%) were found to be resistant to Ceftazidime, Ceftriaxone, Cefotaxime and 24 (33%) isolates were found to be ESBL producers. On the other hand 40 (55.5%) isolates were found to be resistant to imipenem and 16 (22.2%) isolates were found to be MBL producers.

Introduction

The members of the Enterobacteriaceae family have an important role in nosocomial and community acquired infections. In recent years, *K. pneumoniae* have become important pathogens in various types of infections (Sunilkumar Biradar and Roopa, 2015). *Klebsiella* species is a Gram negative opportunistic nosocomial pathogen and is known to cause community acquired infections. *Klebsiella* is known to cause bacterial pneumonia, urinary tract infection, wound infections, blood infections and infections in the intensive care unit (Fish and Ohlinger, 2006). Antimicrobial resistance has become a serious public health problem worldwide. Infections caused by resistant bacteria are associated with increased morbidity and mortality than those caused by susceptible pathogens (Travers and Barza, 2002). Infections caused by resistant bacteria led to prolonged hospital stays, increased health care costs and in many cases to untreatable infections (Byarugaba, 2004). The incidence of infection due to extended-

spectrum β-lactamase (ESBL) producing organisms has increased sharply especially in *Escherichia coli* and *Klebsiella pneumoniae* and also by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The growing prevalence of ESBL producing organisms is responsible for increased use of carbapenems (Nordmann P *et al.,* 2009). But extensive and sometimes unnecessary use of carbapenems has facilitated the emergence of carbapenem resistant bacteria by production of carbapenem hydrolysing enzyme, metallo-β-lactamase (MBL). MBL producing *Pseudomonas* and *Acinetobacter* isolates are common cause of nosocomial infections but other MBL producing gram negative bacteria like E. coli and *Klebsiella* spp. are also emerging as a public health concern worldwide (Manoj Kumar *et al.,* 2015).

With this in mind, the present study was designed to increase awareness and demonstrate the need to detect and monitor the occurrence of novel-lactamases ESBLs and MBL. The goal was to investigate the occurrence (not prevalence) of these types of - lactamases in our tertiary care hospital and to use the findings to alert health professionals about the need for vigilance and preparedness. Although these enzymes may be produced by a variety of organisms, the study focused only on *Klebsiella pneumoniae* because this species is the most common ESBL-producing organism and MBL production has also increased in over the period.

Materials and Methods

This study was conducted at Department of Microbiology, Santosh Medical College & Hospital Ghaziabad. Fresh expectorated sputum samples were collected in a sterile wide mouth container. Patient was asked to rinse mouth thoroughly with water to reduce the amount of oral bacteria that are normally present that can contaminant the sputum.

Total of 200 sputum samples with clinical history of pneumonia were cultured on Blood agar and MacConkey agar and the isolates were identified by standard biochemical tests like Gram staining (Gram negative short and stout bacilli seen), Indole test (negative), Voges-Proskauer test (positive), Citrate utilization test (positive), Urease production test (positive), Triple sugar iron agar (A/A with gas), acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests (K.N. Ravichitra *et al.,* 2014).

Antimicrobial susceptibility testing

All *Klebsiella pneumoniae* isolates were screened for antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Hi-Media) and interpreted as per CLSI guidelines (K. N. Ravichitra *et al.,* 2014). A log phase broth culture inoculums of the isolate with a turbidity equivalent to McFarland 0.5 standard ($1.5x10^8$ CFU/ml) was prepared and lawn cultured on the Mueller-Hinton agar and allowed to dry. Antibiotic discs were applied to the Mueller Hinton agar surface with the help of sterile forceps (Joshua T Freeman *et al.,* 2014).

ESBL detection tests

Screening test

All *K. pneumoniae* isolates were screened for ESBLs by disc diffusion method. In the presumptive test to detect potential ESBL producers, all the isolates were screened for susceptibility to ceftazidime (30μg) and cefotaxime (30μg) antibiotic discs (Himedia, Mumbai). Results were interpreted based on the CLSI guidelines as follows: zones of inhibition of ≤22mm for ceftazidime and ≤27mm for cefotaxime indicated ESBL production. The less susceptible or resistant

isolates were subjected to confirmatory test (CLSI, 2015).

Confirmatory test

The ESBL producing *K. pneumoniae* isolates were confirmed by CLSI phenotypic confirmatory test of combined disc assay method.15 One disc each of ceftazidime (30µg), Ceftriaxone (30µg) and cefotaxime (30µg) alone and one in combination with clavulanic acid (10µg) were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The ESBL-producing strains showed ≥5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone. *E. coli* ATCC 25922 was used as quality control strain (CLSI, 2015 and Tewari *et al.,* 2018).

MBL Detection tests

Screening test

All *K. pneumoniae* isolates were screened for MBL production by testing their susceptibility to imipenem (10µg) antibiotic discs (Himedia, Mumbai) using Kirby Bauer disk diffusion method. All the isolates with an inhibition zone diameter of ≤19mm were considered as screen positive for MBL and were subjected to confirmatory test (CLSI, 2015).

Confirmatory test

All screen positive *K. pneumoniae* isolates were confirmed for metallo- β -lactamase production. One disc of imipenem (10µg) alone and one in combination with EDTA (750µg/mL) were placed at a distance of 20 mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight

at 37°C. The E. coli strain demonstrating a zone diameter ≥7mm around the imipenem/EDTA disc compared to that of imipenem disc alone was considered to be positive for the presence of MBLs. *E. coli* ATCC 25922 were used as quality control strain (CLSI, 2015 and Khosravi Y *et al.,* 2012).

Results and Discussion

Out of the 200 sputum samples, 72 samples were confirmed and identified as *K. pneumoniae.* Age wise distribution of *K. pneumoniae* among males & females is shown in Table 1.

Among the 72 isolates of *K. pneumoniae* 52 (72.2%) were from male patients and 20 (27.7%) were from females. Maximum isolation was found in the age group of above 50 years and least isolation was found in the age group of 10-20 years.

In the present study maximum numbers of isolates were found to be sensitive to Amikacin (80.5%), Gentamycin (80.5%), Co-Trimaxazole (69.4%) and Piperacillin (45%). Maximum number of isolates was found to be resistant to Ceftazidime (66.6%), Cefpodoxime (75%), Imipenem (55.5%) & Ofloxacin (61.1%).

Out of total 72 isolates, 48 (66.6%) were found to be resistant to Ceftazidime, Ceftriaxone, Cefotaxime and 24 (33%) strains were found to be ESBL producers.

Out of 72 isolates, 40 (55.5%) were found to be resistant to imipenem and 16 (22.2%) strains were found to be MBL producers.

Beta lactams are commonly included in the empirical antibiotic regimens for treatment of infections caused by gram negative bacteria. Indiscriminate use of broad spectrum

Cephalosporins has become one of the major reasons for the high production of extended spectrum beta lactamase by gram negative bacteria. Until recently, carbapenems were the choice for the therapeutic management of multidrug-resistant gram-negative bacterial infections. Currently, the spread of carbapenem-resistant bacteria has caused greater concern due to the limited choice in antibiotics for treating infection (Behera *et al.,* 2008 and Palucha, 1999).

In our study isolation rate of *K. pneumoniae* was 36% from sputum samples, however study done by Kholy and Amani (2011) showed 32.6%isolation rate of *Klebsiella pneumoniae*. According to the study of Vemula Sarojamma (2011) the isolation rate of *K. pneumoniae* was 31.2% which is also in contrast to our results. Research done by S Baby Padmini (2004) revealed 14.11% isolation rate which is lower comparative to our study (Table 2).

Table.1 Age and sex wise distribution of *K. pneumoniae*

Age	Male	Female
10-20 years	4	0
20-30 years	12	2
30-40 years	8	6
40-50 years	10	4
>50 years	18	8
Total	52	20

Table.2 Antibiotic sensitivity pattern of *K. pneumoniae*

Antibiotics	Sensitive
Amikacin	80%
Gentamycin	80%
Co-Trimaxazole	69%
Piperacillin	45%
Ceftazidime	33%
Ceftriaxone	33%
Cefotaxime	33%
Imipenem	55%
Ofloxacin	39%
Ciprofloxacin	55%
Tetracycline	40%

	Ceftazidime/Ceftriaxone/Cefotaxime Resistant	ESBL producers
Klebsiella pneumoniae	48 (67%)	24 (33%)

	Imipenem Resistant	MBL producers
Klebsiella pneumoniae	40(55%)	16 (22%)

The susceptibility pattern of *K. pneumoniae* observed in our study showed maximum sensitivity to Amikacin (80%) and Gentamycin (80%) followed by Co-trimaxazole (69%), Ciprofloxacin (55%), and Imipenem (55%). Kholy and Amani (2011) reported susceptibility to Amikacin (66.5%), Gentamycin (42%), Co-Trimaxazole (24.3%), and Ciprofloxacin (31%) whereas Asati Rakesh Kumar (2013) reported susceptibility to Amikacin (93%), Gentamycin (41%), Co-Trimaxazole (47%) and Ciprofloxacin (51%).

In the present study *K. pneumoniae* was found to be highly resistance to Ceftazidime (77%), Ceftriaxone (77%), Cefotaxime (77%) followed by Ofloxacin (61%) and Tetracycline (60%). VemulaSarojamma (2011) in their study also reported high resistant to Ceftazidime (61%), Amoxyclav (39), Imipenem (84%), Amikacin (56%), Gentamycin (40%) whereas Asati Rakesh Kumar (2013) reported higher resistance to Tetracycline (85%), Ceftizoxime (73%), Cefuroxime (66%). VemulaSarojamma (2011) also reported resistance to Ceftazidime (61%), Kholy and Amani (2011) reported lower resistance to ceftazidime (25%). I. Shukla (2004) reported higher ceftazidime resistance (88.3%) compared to our study.

It is important for clinical microbiology laboratories to be able to detect ESBLs and carbapenem-hydrolyzing beta - lactamases because these enzymes are either associated with problems of false susceptibility or have significant infection control implications, or both. Extended-spectrum β-lactamases (ESBLs) are frequently encountered among clinical Enterobacteriaceae, predominantly in *E. coli* and *Klebsiella pneumoniae*.

The present study showed much lower occurrence of ESBL and MBL producing *Klebsiella pneumoniae*, which was ranging from 30% to 75% reported from various parts of the world. Present study showed 33% ESBL production among *Klebsiella pneumoniae* isolates. Another study from U.S. (Ellen Smith Moland *et al.,* 2002) revealed 75% occurrence of ESBL producer in Klebsiella pneumoniae isolates from 24 U.S. Hospitals. Kholy and Amani (2011) have shown that 64% K. pneumoniae isolates were ESBL producers, Baby Padmini (2004) have reported 40% ESBL producers which were at higher side compared to present study Whereas I. Shukla (2004) have shown that 30% *K. pneumoniae* isolates were ESBL producers and Singh P (2018) reported only 6.7% *K. pneumoniae* isolates were positive for ESBL production in his research.

In our study 22% *Klebsiella pneumoniae* isolates were MBL producers Whereas S. Nagaraj (2012) reported 75% of MBL production While in a study done by Giske CG (2011) showed 69.4% of MBL production rate in *K. pneumoniae* isolates causing pneumonia.

Antibiotic overuse is an important contributor for the emergence and spread of resistance. Since last 15years, acquired resistance which is mainly mediated by ESBLs and MBLs has been increasingly reported worldwide including India not only in K. pneumoniae, but also among members of Enterobacteriaceae.

Detection of ESBL and carbapenemases in Enterobacteriaceae is still very important for infection control and epidemiological point of view, and may impact greatly on patient management.

Acknowledgment

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