Prevalence and antibiogram of extended spectrum β- lactamase producing *Klebsiella pneumoniae* and *Proteus mirabilis* in clinical isolates

Shireen Rana[1*], S.B. Siddesh[2], R. Vijayaraghavan[3]

[1]Tutor, Department of Pharmacology & PhD Research Scholar, Saveetha University
[2]Professor & HOD, KBN Institute of Medical Sciences [3]Director of Research, Saveetha University

***Corresponding Author:**
Email: shirinrana44@gmail.com

Abstract

Rapid emergence of ESBL production in bacteria like *Klebsiella* and *Proteus species* has put forward a major challenge in their detection as well as treatment options. This study was conducted from Oct 2012 to May 2015 to know the prevalence and antibiogram of ESBL producing *Klebsiella pneumonia & Proteus mirabilis*. Blood, sputum, pus, wound samples were collected and processed; identification of isolates was done as per standard methods. ESBL detection was done with double disk test and combination disk and confirmed with MIC reduction test. 208/578 (36.0%) &126/ 405 (31.1%) isolates of *Klebsiella pneumoniae & Proteus mirabilis* were ESBL producers respectively. The present study showed a high percentage of ESBL producers among clinical isolates of *Klebsiella pneumoniae and Proteus mirabilis* to frequently used antibiotics. Knowledge of antibiogram studies will help physicians in choosing an empirical treatment. To know the changing trends in resistance regular antibiotic susceptibility studies should be conducted.

Key words: Extended spectrum beta lactamases (ESBL), Klebsiella pneumoniae, Proteus mirabilis, antibiogram, Resistance

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Introduction

The occurrence of extended-spectrum beta lactamases (ESBLs) in gram-negative bacteria has increased in recent years, which has led to worldwide concern regarding the control of bacterial infections[1]. However the prevalence of ESBL producers varies with areas and time, a relatively high prevalence rate of ESBL producers in the Asia-Pacific area were reported by several surveillance studies.[2] Currently, antibiotic resistance to bacteria is being commonly reported worldwide. Due to indiscriminate use of antibiotics the situation is alarming all over the globe due to development of antibiotic resistance. In addition, bacteria have the ability to transmit gene and acquire resistance to antibiotics which are utilized as therapeutic agents and transferring the resistance from one bacteria to other[3].

Globally resistant Extended spectrum β lactamases (ESBL) bacteria are increasingly reported in both community and hospital settings[4]. They are mainly found in Escherichia coli, Klebsiella species and Proteus species but can occur in other members of Enterobacteriaceae family[5]. Failure in the treatment and clinical mortality are also more likely to occur in patients infected with ESBLs-producing Proteus mirabilis[6]. In recent years carbapenemase producing Enterobacteriaceae has emerged and spread globally, especially isolates carrying genes encoding *K. pneumoniae* carbapenemase and NDM (New Delhimetallo-β-lactamase) carbapenemases, that has compromised options available to cure the disease[7,8].

ESBL have the capacity to hydrolyze and produce resistance to various type of newer β-lactam antibiotics including the extended–spectrum cephalosporins, monobactams and carbapenems[9]. Reports of previous studies in India found ESBL production varying from 6% to 87%[10-12]. It is necessary to find the prevalence of ESBL strains and resistance to different antibiotics which are essential to formulate guidelines for the proper treatment of infections caused by them and to control the spread. Hence the present study was conducted.

Material and Methods

Different clinical samples like purulent material from wounds or abscesses, sputum, blood or pus collected from 1640 patients were cultured to isolate the organisms. Demographic data (such as age, sex) of the patients was recorded prior to sample collection. Samples were processed and isolates were identified as per standard methods[13].

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined by Kirby-Bauer disk diffusion method as per CLSI guidelines[14]. Antimicrobial disks used were Ampicillin (10µg), Amoxycillin-clavulanic acid (20/10µg), Piperacillin (100µg), Piperacillin-tazobactam (100/10µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg),Ofloxacin

(5µg), Cefuroxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Gentamicin (10µg), Amikacin (30µg), Tobramycin (30µg, Co-trimoxazole (1.25/23.75 µg), Aztreonam (30µg) and Imipenem (10µg). [Hi Media, Mumbai]

Screening test for ESBLs

Screening of ESBLs was done as per CLSI guidelines, isolates showing inhibition zone size of ≤22 mm with Ceftazidime (30 µg), ≤25 mm with Ceftriaxone (30 µg), and ≤27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production

Confirmatory tests for ESBLs

1. **Double disc synergy test:** Organisms to be tested were inoculated onto Muller-Hinton agar plate by lawn culture. A disc containing amoxyclav (Amoxycillin + clavulanic acid) was placed at center of the plate. Ceftazidime, Ceftriaxone andCefotaxime were placed with the interdisc distance (edge to edge) of 15 mm from the amoxyclav disc. The plates were incubated at 37°C for overnight. Enhancement of zone of inhibition towards amoxyclav by any one of these drugs such as ceftazidime, cefotaxime, ceftriaxone was considered as positive result.

2. **Phenotypic confirmatory test with combination disc:** Disk of Ceftazidime (30µg) and a disk of Ceftazidime +Clavulanic acid (30 µg/10 µg), cefotaxime (30mcg) and a disk of cefotaxime + clavulanic acid (30mcg/10mcg) were used. Both the disks were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Muller Hinton Agar plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured. When there was an increase of >5 mm in inhibition zone diameter around combination disk with clavulanic acid versus the inhibition zone diameter in disk alone was confirmed positive for ESBL production. K. pneumoniae ATCC 700603 (an ESBL producer) and E. coli ATCC25922 were used as positive and negative controls respectively.

3. **MIC reduction test (E test)**: The isolates positive with combination disk test were further confirmed for ESBL production by this test. Minimum inhibitory concentration of the isolates was determined by E test method.

Concerntration range of antibiotic used are as follows
Ceftazidime: 0.25-16 µg/ml
Ceftazidime + Clavulanic acid: 0.016-1 µg/ml
When the ratio of the value obtained for Ceftazidime (CAZ): The value of Ceftazidime in combination with Clavulanic acid (CAZ+) is more than 8 or no zone is obtained for CAZ and Zone obtained in CAZ+ indicates that the strain is an ESBL producer[15]. *K. pneumoniae* ATCC 700603 (an ESBL producer) was used as control strain. Analysis of data obtained was done using Microsoft excel (2010 version). The results are explained in frequency and percentage.

Results

Out of 1640 samples collected, 578 *K. pneumoniae* and 405 *P. mirabilis* were isolated. The age and sex distribution of *K. pneumoniae* cases is shown in Table 1.

Table 1: Age and sex distribution of K. pneumoniae cases (n=578)

Age group (years)	Male	Female	Total
0-10	7	4	11
11-20	41	44	85
21-30	46	50	96
31-40	61	64	125
41-50	40	44	84
51-60	49	51	100
61-70	36	41	77
Total	**280**	**298**	**578**

Maximum numbers of cases were from females in the age group of 31-40 years.

Out of 578 *K. pneumoniae*, 208 (36.0%) were ESBL producers. The antibiotic resistance pattern of ESBL and non-ESBL producers is shown in Table 2.

Table 2: Antibiotic resistance of *Klebsiella pneumoniae* (Resistance pattern)

Antibiotic	ESBL producers (n=208) n (%)	Non ESBL producers (n=370) n (%)
Ampicillin	208 (100)	101 (27.2)
Amoxycillin-clavulanic acid	135 (65.0)	57 (15.4)
Piperacillin	208 (100)	122 (33)
Piperacillin-tazobactam	54 (26.0)	11 (3.0)
Nitrofurantoin	38 (18.2)	70 (19)
Ciprofloxacin	95 (45.6)	145 (39.1)
Ofloxacin	91 (43.7)	112 (30.2)

Cefuroxime	85 (40.8)	49 (13.2)
Ceftriaxone	63 (30.2)	39 (10.5)
Ceftazidime	71(34.1)	36 (9.7)
Gentamicin	100 (48.0)	88 (23.7)
Amikacin	95 (45.6)	75 (20.2)
Tobramycin	93 (44.7)	73 (19.7)
Co-trimoxazole	81 (39.0)	57 (15.4)
Aztreonam	0	0
Imipenem	0	0

Highest resistance was seen with Piperacillin and Ampicillin and least with Imipenam and Aztreonam. The age and sex distribution of *P. mirabilis* cases is shown in Table 3.

Table 3: Age and sex distribution of *Proteus mirabilis* cases (n=405)

Age group (years)	Male	Female	Total
0-10	3	2	5
11-20	15	12	27
21-30	34	42	76
31-40	43	46	89
41-50	35	34	69
51-60	42	54	96
61-70	20	23	43
Total	**192**	**213**	**405**

Maximum number of cases were from females in the age group of 51-60 years.

Out of 405, *P. mirabilis* 126(31.1%) were ESBL producers. The antibiogram of ESBL and non-ESBL producers is shown in Table 4.

Table 4: Antibiogram of P. mirabilis (Resistance pattern)

Antibiotic	ESBL producers (n=126) n (%)	Non ESBL producers (n=279) n (%)
Ampicillin	126 (100)	279 (100)
Amoxycillin-clavulanic acid	105 (83.3)	228 (81.7)
Piperacillin	115 (91.2)	210 (75.2)
Piperacillin-tazobactam	23 (18.2)	5 (1.7)
Nitrofurantoin	15(12.0)	24 (8.6)
Ciprofloxacin	51 (40.4)	74 (26.5)
Ofloxacin	41 (32.5)	50(18.0)
Cefuroxime	28 (22.2)	24 (8.6)
Ceftriaxone	24 (19.0)	19 (6.8)
Ceftazidime	24 (19.0)	26 (9.3)
Gentamicin	26 (20.6)	26 (9.3)
Amikacin	15 (12.0)	13 (4.6)
Tobramycin	15 (12.0)	19(6.8)
Co-trimoxazole	13 (10.3)	21 (7.5)
Aztreonam	0	0
Imipenem	0	0

Highest resistance was seen with Ampicillin, Amoxycillin-clavulanic acid and Piperacillin, least with Aztreonam and Imipenem

Discussion

In the present study, prevalence of ESBL producing *K. pneumoniae* was 36.0%. Other studies conducted in India have reported prevalence of ESBL producing *K. pneumoniae* varying from 6% to 87%[10-12]. In recent years, reports from USA, Canada, China, and Italy found a significant increase in ESBL producers[16,17]. *Klebsiella pneumoniae* was found to be highly resistant to Ampicillin and Piperacillin and least to Aztreonam and Imipenem (Table 2). In other studies also Imipenem was reported as the most effective drug[18].

After Imipenem and Aztreonam, the most sensitive drugs for *Klebsiella pneumoniae* were Piperacillin + Tazobactam and Nitrofurantoin. Other studies support these findings[19].

In the present study, out of 405 *Proteus mirabilis*, 126 (31.1%) were ESBL producers. Infection was more in females belonging to the age group of 51-60 years, may be because of predisposing factors like surgery, sepsis, catheterization and diabetes mellitus. There is lack of data regarding prevalence of ESBL producing *Proteus mirabilis*; studies conducted outside India have reported prevalence of 3% to 20%[20-22]. Highest resistance was seen with Ampicillin, Amoxycillin-&Clavulanic acid and Piperacillin, least with Aztreonam and Imipenem, same as with ESBL producing *K. pneumoniae* (Table 4). After Imipenem and Aztreonam, the most sensitive drugs for P. mirabilis were Aminoglycosides and Nitrofurantoin. Similar finding were reported by Levy et al[23].

Antibiotic resistance pattern among *K. pneumoniae* and *P. mirabilis* vary widely. This might be because of disease pattern, geographical variation and local antibiotic use. There is a lack of data from India on antibiotic consumption, the available data suggest that it is higher than other developing nations of the world. Rates are further lower in developed nations. In past 10 years there is a significant increase in the use of carbapenem and Piperacillin - tazobactam[24]. The other causes for development of antibiotic resistance in *K. pneumoniae* might be due to production of Amp C beta lactamases.[25]

Limitations of the study

We did not test for Amp C and metallo beta lactamse (MBL) Future studies should include testing for Amp C and metallo beta lactamse (MBL).

Conclusion

The present study showed a high percentage of ESBL producers among clinical isolates of *K. pneumoniae* and *P. mirabilis* to commonly used antibiotics. Knowledge of antibiogram studies will help physicians choosing an empirical treatment. To know the changing trends in the resistance regular antibiotic susceptibility studies should be conducted. In addition to monitoring of Imipenem sensitivity and routine testing of newer carbapenemes like Meropenem and Ertapenem should be carried out further to detect early resistance and save these powerful antibiotics for life threatening infections.

Conflict of interest: None

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