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Prevalence of ESBL Producing Klebsiella Species and Their in-Vitro Antimicrobial Susceptibility Pattern in A Tertiary Care Hospital

Akila.K[1], Nithyalakshmi.J[2], Mohanakrishnan.K[3], Sumathi.G[4]
Department Of Microbiology, Sri Muthukumaran Medical College Hospital And Research Institute, Chikkarayapuram, Chennai, India.

Abstract: *Infections caused by extended spectrum beta-lactamase (ESBL) producing Klebsiella spp. are associated with higher morbidity and mortality with limited treatment options. As there is no centralized national data in India, the prevalence of ESBL producing Klebsiella spp. is obtained from various scattered publications across the country. This varies widely from 10.10% to 87.00%. So it is important to do periodic surveillance at each institutional level to monitor the prevalence ESBL producers and take measures to contain their spread. This retrospective study was undertaken to identify the prevalence of ESBL producing Klebsiella spp. and their susceptibility pattern in a tertiary care hospital. Total of 209 Klebsiella spp. isolates were identified from various clinical specimens. Antimicrobial susceptibility pattern and detection of ESBL production was done as per standard protocols. Out of 209 Klebsiella spp., 80 (38.28%) were found to be ESBL producers. All ESBL producing Klebsiella pneumoniae strains were susceptible to imipenem. The highest degree of resistance was observed with cefotaxime. The resistance level to aminoglycosides was low. The knowledge about the local prevalence of resistant bacteria helps to treat patients better by judicious use antibiotics. Strict implementation of infection control measures is essential to reduce the prevalence of resistant bacteria.*

Keywords: *extended spectrum beta lactamase (ESBL), Klebsiella species, and susceptibility pattern*

I. Introduction

Genus Klebsiella under Enterobacteriaceae family has some medically very important species like Klebsiella pneumoniae and Klebsiella oxytoca. They cause variety of sporadic to frequent infections in vulnerable groups in community. In health care settings they cause endemic infections and epidemic outbreaks. They are one of the frequent extended spectrum beta-lactamase (ESBL) producers among gram-negative bacteria. [1]

Beta-lactam antibiotics are one of the earliest to come into clinical practice and still the most routinely and widely prescribed antibiotics as a first line choice against common community and hospital acquired infections. It accounts for about 55-60% of total global antibiotic consumption. [2] The use of various groups of antibiotics in the last 60 years had saved countless lives and reduced the burden of infectious diseases on humanity but also exerted considerable selection pressure on bacteria. The inevitable evolutionary survival response was the emergence of resistant strains through genetic mutation and the rapid spread of resistance mechanism through mobile genetic elements across intra and inter-species. [3, 4] The frequent modes of resistance include enzymatic hydrolysis, target site modification, reduced uptake and enhanced efflux of antibiotics. [5]

Enzymatic hydrolysis by beta-lactamases is the leading cause of resistance to beta-lactam antibiotics especially in gram-negative bacteria. [4] In fact, the penicillinase, the first beta-lactamase was identified prior to the release of penicillin into clinical practice. [6, 7] Everytime a new beta lactam antibiotic is introduced, the mutation and dissemination of beta lactamase encoding gene swiftly followed. [7] Currently, the number of unique gene alleles for beta lactamases exceeds 1500. (Catalogued/Curated by G. Jacoby and K. Bush, http://www.lahey.org/Studies/ [8]

The newer beta lactamase enzymes exhibit expanded substrate specificity and variable beta lactamase inhibitor susceptibility. The enzymes with hydrolytic ability against penicillins, second and third generation cephalosporins and monobctams but not against cephamycins with beta lactamse inhibitor susceptibility are called as "extended spectrum beta lactamases" (ESBL). [9, 10] They are structurally serine beta lactamases belonging to Ambler class A, C and D. In functional Bush-Jacoby-Medeiros classification they are placed under 2be, 2d groups. [11]

Klebsiella spp are ubiquitous in nature. This coupled with their ability to survive in medical equipment and in the hands of hospital personnel had caused many outbreaks of infection in hospitals across the world. [1,

12] Pneumonia, UTI and primary pyogenic liver abscess are some of the community-acquired infections caused by Klebsiella spp. worldwide. [13] This study was done to identify the prevalence of ESBL producing Klebsiella spp. and their sensitivity pattern in a 750-bedded tertiary care hospital.

II. Materials And Methods

2.1. Clinical Isolates

A retrospective record based study to look at the prevalence of ESBL producing Klebsiella spp. was done in a tertiary care teaching hospital. A total of 209 Klebsiella spp. organisms were identified from culture and sensitivity reports. They were isolated from various clinical samples like urine, sputum, pus and blood obtained from both inpatients and outpatients of all age groups and both sexes over a period of one year (Jan 2015 to Dec 2015). Isolates were identified to species level based on colony morphology and biochemical reactions as per standard procedures. [14]

2.2. Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests of all the isolates were performed using Kirby-Bauer disc diffusion method on Mueller Hinton agar as per Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). [15] The antimicrobial discs used were Cefotaxime (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Nitrofurantoin (300units), Amikacin (30µg), Gentamicin (10µg), Co-trimoxazole (25µg), Imipenem (10 µg) and Nalidixic acid (30 µg).

2.3. Screening & Confirmatory Test For ESBL

The screening for ESBL production was based on the size of specific zone of inhibition in millimeters to indicator cephalosporins, Cefotaxime and Ceftazidime. The zone of inhibition less than 27mm for Cefotaxime and 22 mm for Ceftazidime were taken as indicators for possible ESBL production. Subsequent phenotypic confirmatory test for ESBL production was done using combined disc diffusion method. The discs of Ceftazidime (30mcg) and Ceftazidime plus Clavulanic acid (30/10mcg) were placed on the surface of Mueller Hinton agar with 20 mm distance between two discs. Overnight incubation was done at 37 degree Celsius. An increase of > 5 mm in zone diameter of Ceftazidime plus clavulanic acid in comparison to the zone diameter of Ceftazidime alone was taken as the confirmatory test for ESBL production. [15]

III. Results

A total of 209 Klebsiella spp. isolates were identified from various clinical samples collected over a period of one year (Jan 2015-Dec 2015). Most frequent isolates of Klebsiella spp were Klebsiella pneumoniae 185 (88.51%) followed by Klebsiella oxytoca 24 (11.49%). Out of 209 Klebsiella isolates, 80 (38.76%) were found to be ESBL producers and 129 were non-ESBL producers. There were no ESBL producing Klebsiella oxytoca isolates found. The ESBL producing Klebsiella pneumoniae were more often isolated from sputum (50%), followed by pus (47.22%) and urine (29.36%).

Among the third generation cephalosporins, high degree of resistance was observed with cefotaxime followed by ceftazidime. All ESBL producing Klebsiella pneumoniae strains were susceptible to imipenem. The resistance level to aminoglycosides was low and it was moderate with co-trimoxazole, fluoroquinolones and nitrofurantoin.

IV. Discussion

In the present study, the prevalence of ESBL producing Klebsiella spp. is 38.28% (80 ESBL producers out of total 209 Klebsiella spp isolates). The studies which are listed in Table.5 from other geographical regions of India, done over the last 12 years reported a widely variable prevalence ranging from 10.10% to 87%. [16-29]

The factors responsible for such wide variability could be sample size & type, demographic factors of patient cohort such as age, gender, medical illness, community versus hospital patients and prior cephalosporin use. [17, 30] The type of the test used to detect ESBL production, the indicator cephalosporin used and the innoculum effect can also influence the results. [29, 31] AmpC hyper production can confound the results. Few authors advocate that the initial screen then confirmatory test approach for ESBL detection is not ideal and less sensitive than confirmatory testing in the routine susceptibility testing itself. [32]

India ranked first in the world by consuming 12.9×10^9 Standard units (SU) of antibiotics in total, in the year 2010. It is equivalent to 10.7 standard units per person and second only to United States of America (USA) in per capita antibiotic consumption in the same year. [2] The increase in cephalosporin consumption from around 1.0×10^9 standard units in 2000 to nearly 4.0×10^9 standard units in 2010 is particularly noteworthy in India. [33, 34] It is well known that antibiotic use selects for resistant bacteria and the

indiscriminate and excessive use of beta-lactam antibiotics is in itself a driving force for clinically significant increase in the incidence of ESBL producing bacteria. [4]

This study observed no ESBL producers in Klebsiella oxytoca that comprised about 11.49% of total Klebsiella isolates (24 out of total 209 isolates).[Table 2] The SMART study India group reported 10% Klebsiella oxytoca among Klebsiella spp. causing intra-abdominal infections, out of which 60% were ESBL producers. [25] Another study reported 11% Klebsiella oxytoca among Klebsiella spp isolated from various specimens. [36]

In the present study, urine samples were the major source of ESBL producing strains followed by sputum samples. About 40% of ESBL producing strains were from urine and 38.75% from sputum which is consistent with size of the samples. The female patients and urine samples were the largest in the present study. The gender & sample wise distribution of Klebsiella isolates are presented in Table 1.The sample wise distribution of ESBL producing Klebsiella spp is presented in Table 3. The other studies which tested different types of specimens had reported a variable order depending on patient cohort and number of samples in each type they included. [Table 5]

Antimicrobial susceptibility testing of ESBL producing & non-ESBL Klebsiella isolates exhibited 100% resistance to ampicillin and is similar to a study done by Varaiya et al [19] which reported 0 % sensitivity to ampicillin among ESBL producing Klebsiella pneumoniae isolates. Another study done by Agrawal et al [17] had reported similar results. In this study about 45% of isolates were resistant to co-trimoxazole. Amikacin had excellent activity against 88.75% isolates of ESBL producing Klebsiella pneumoniae. All ESBL producing Klebsiella isolates were susceptible to imipenem in this study and is similar to other comparative studies mentioned in Table 7.[17-19,21,22, 26-29] Hawser et al (SMART study India group) reported 6-7% of isolates from intra abdominal infections were resistant to imipenem. [25]

The antibiotic resistant property in ESBL producers is not only restricted to beta-lactam group but also includes other classes of antibiotics. The proportion of resistant isolates among ESBL producing isolates in each sample types is presented in Table 4.The proportion of resistant isolates against various classes of antibiotics is higher in ESBL producers than non-ESBL producers and is shown in Figure 1. Usually, the organisms acquire multiple resistant mechanisms along with ESBL encoding genes through horizontal gene transfer rendering multiple antibiotics ineffective. The similar results are found in a study conducted by Vijayakanthi et al in neonatal intensive care. [37]

Infections caused by resistant strains have very few treatment options and are associated with higher morbidity and mortality. As antibiotic stock is dwindling, resistance to available antibiotics become the most pressing public health threat in the world. A joint meeting of representatives from various medical societies of India laid out a five year targeted, achievable plan to tackle the challenge of antimicrobial resistance called as 'Chennai Declaration.' It is an important national initiative. The international community hailed the initiative. It is up to the numerous stakeholders in the health care community of India to implement it effectively to increase the shelf life of available antibiotics in the face of dwindling number of new antibiotics in the pipeline.[38]

V. Tables & Figures

Table 1: Gender (columns) & Sample-wise (rows) Distribution of Klebsiella spp isolates

Sample	Male(n)	Female (n)	Total (%)
Urine	26	83	**109 (52.15)**
Sputum	33	29	**62 (29.67)**
Pus	21	15	**36 (17.22)**
Blood	1	1	**2 (0.96)**
Total(%)	**81 (38.76)**	**128 (61.24)**	**209 (100)**

Table 2: Distribution of Klebsiella spp.

Species	No of isolates	Percentage of total
Klebsiella pneumoniae	185	88.51%
Klebsiella oxytoca	24	11.49%

Table 3: Sample-wise Distribution of ESBL producing Klebsiella. spp

Sample	ESBL (n=80) (%)
Urine	32 (40.00 %)
Sputum	31 (38.75 %)
Pus	17 (21.25 %)
Blood	0

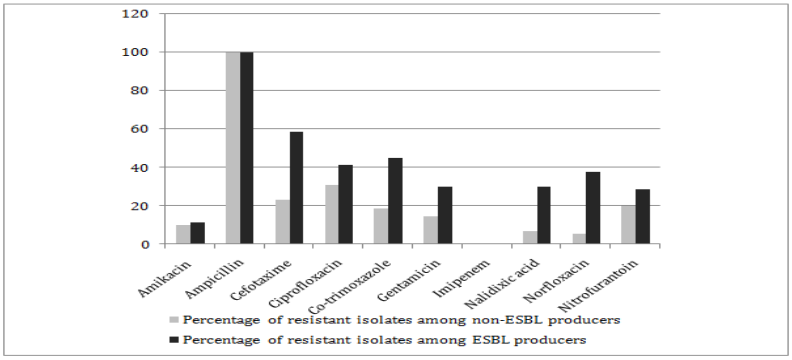
Table 4: The proportion of resistant isolates among ESBL producers in each sample types

Antibiotic discs used	Urine n=32 (%)	Sputum n=31 (%)	Pus n=17 (%)	Total n=80 (%)
Ampicillin	32 (100)	31 (100)	17 (100)	80 (100)
Cefotaxime	22 (68.75)	16 (51.61)	9 (52.94)	47 (58.75)
Amikacin	4 (12.50)	2 (6.45)	3 (17.65)	9 (11.25)
Gentamicin	8 (25.00)	7 (22.58)	9 (52.94)	24 (30.00)
Ciprofloxacin	13 (40.62)	13 (41.94)	7 (41.18)	33 (41.25)
Co-trimoxazole	10 (31.25)	15 (48.39)	11 (64.71)	36 (45.00)
Imipenem	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Nalidixic acid	7 (21.86)	12 (38.71)	5 (29.41)	24 (30.00)
Norfloxacin	15 (46.88)	7 (22.58)	8 (47.06)	30 (37.50)
Nitrofurantoin	9 (31.25)	8 (25.81)	6 (35.29)	23 (28.75)

Table 5: The prevalence of ESBL producing Klebsiella spp. in comparative studies

Data source	Place & Year of study	Patient cohort	Type of samples	Number of Klebsiella isolates	ESBL producers	Carbepenem resistance
Kumar et al [16]	Hyderabad, 2006	In & outpatients	All types	464	47 (**10.10%**)	Not done
Agrawal et al [17]	Pune, 2008	In & outpatients	All types	176	28 (**16.00%**)	0%
Vemula et al [18]	Kadappa, 2011	In & outpatients	All types	100	17 (**17.00%**)	0%
Variaya et al [19]	Mumbai, 2008	inpatients	Diabetic foot ulcer (pus, wound sawbs)	80	16 (**20.00%**)	0%
Tankhiwale et al [20]	Nagpur, 2004	In & outpatients	Urine	82	21 (**25.60%**)	Not done
Shukla et al [21]	Aligarh, 2004	Hospital isolates	All types	106	32 (**30.18%**)	0%
Babypadmini et al [22]	Coimbatore, 2004	Hospital isolates	Urine	58	23 (**40.00%**)	0%
Metri et al [23]	Bijapur, 2012	In & outpatients	Urine	58	26 (**44.90%**)	Not mentioned
Taneja et al [24]	Chandigarh, 2008	In & outpatients	Urine	39	20 (**51.20%**)	-
Hawser et al SMART study [25]	7 hospitals India, 2010	Inpatients	Intra-abdominal samples	100	55 (**55.00%**)	6-7%
Rao et al [26]	Davangere, 2008	Hospital isolates	All types	30	17 (**62.20%**)	0%
Rudresh et al [27]	Bangalore, 2011	Hospital isolates	All types	79	50 (**63.30%**)	0%
Sharma et al [28]	Jaipur, 2013	In & outpatients	All types	179	120 (**67.04%**)	0%
Manchanda et al [29]	Delhi, 2005	In & outpatients	All types	100	87 (**87.00%**)	0%

Fig 1: Comparison of antimicrobial resistance patterns of non-ESBL & ESBL producers



VI. Conclusion

The high prevalence of ESBL producing Klebsiella spp. in India is a cause for concern. The surveillance studies are essential in every institute to monitor the prevalent resistant organisms and their susceptibility pattern. This will help in formulating guidelines for judicious use of antibiotics and call for strict adherence to infection control measures.

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