

Prevalence of ESBL-Producing *Klebsiella pneumoniae* Isolated from Pneumonia Cases, in a Tertiary Care Hospital

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Abstract: *Klebsiella* is an important human pathogen that has the potential to cause severe infections. *K. pneumoniae* is gaining renewed interest because of emergence of multidrug resistance due to ESBL production. Total of 100 sputum samples from pneumonia cases were studied, 27 out of 53 that yielded growth, were culture positive for *Klebsiella pneumoniae*. Males were most commonly affected. 37% of all *Klebsiella* isolates were ESBL producers exhibiting multidrug resistance to commonly used antibiotics like cephalosporins. All isolates were sensitive to carbapenems. This study aims to determine the prevalence of ESBL producing *Klebsiella Pneumoniae* and antimicrobial sensitivity profile to plan a proper hospital infection control program to prevent the spread of resistant strains.

Keywords: pneumonia, sputum culture, Blood agar, kelbsiella, ESBL.

INTRODUCTION

Klebsiella is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family [1]. It is the most common causative agent of community acquired and nosocomial infections. It causes pneumonia, urinary tract infection, other pyogenic infections, septicemia and rarely diarrhea [2].

They are nearly always naturally resistant to ampicillin [3]. Emergence of antimicrobial resistance among *Klebsiella* species to broad spectrum beta-lactam antibiotics via extended-spectrum beta-lactamase (ESBL) production is becoming an increasing problem worldwide [4].

Relentless use of β -lactam antibiotics in the clinical practice has resulted in the appearance of newer β -lactamases such as extended spectrum β -lactamases (ESBLs), are typically plasmid mediated and seen mainly in *Klebsiella pneumoniae* and *Escherichia coli*. ESBLs are rapidly evolving group of beta-lactamase which share the ability to hydrolyse third generation cephalosporins and aztreonam, yet are inhibited by clavulanic acid. They are derived from genes for TEM-1, TEM-2 or SHV-1 by mutations that alter the amino acid sequence around the active site of beta-lactamases [5].

Prevalence of ESBLs varies from an institute to another. Previous studies from India and abroad have reported ESBL production varying from 8 to 80%. However, there is paucity scientific information available on antibiotic profile with rate of ESBL production in *Klebsiella pneumoniae* isolates. Keeping in view the above facts, the present study was

undertaken to find the prevalence of ESBL producers among *Klebsiella pneumoniae* isolates at our institute.

MATERIALS AND METHODS

This study was undertaken in a 750 bedded multi-specialty referral hospital in Hyderabad catering to both urban and semi-urban populations. This prospective study was carried out after obtaining ethical clearance in the Department Of Microbiology, Osmania general hospital, Hyderabad, Telangana.

Source of Data

The study was conducted in 2017 over 1 year time period. 100 patients having fever with respiratory symptoms suggestive of pneumonia, attending medical out-patient and admitted in Osmania General Hospital, Hyderabad were included in the study after taking informed consent.

Sputum collection

Sputum (deeply coughed) from the patients is collected in sterile wide mouthed leak proof container.

In patients who could not expectorate sputum spontaneously, sputum induction was done using 3% hyper-tonic saline nebulization.

Sputum processing

Macroscopic appearance: the nature of the sputum was observed-purulent, muco-purulent, mucoid, or blood stained. Microscopic examination: Gram's stain – Bartlett's grading system was used for assessing the quality of sputum samples.

Culture – Sputum samples were inoculated on 5% sheep Blood agar, Chocolate agar and Mac Conkey agar and incubated overnight at 37°C.

Klebsiella pneumoniae strains were identified by their morphology and biochemical characteristics. Morphology of *Klebsiella pneumoniae* identified were large, dome-shaped, mucoid colonies on blood agar and lactose fermenting colonies on Mac Conkey agar. On Gram-staining, Gram-negative, short, plump, straight rods were seen. The biochemical characters identified were negative indole test, negative methyl red test, positive Voges-Proskauer test, and positive citrate utilization test, and positive urease test, acid and abundant gas production from glucose, lactose, sucrose, and maltose and mannitol sugar fermentation tests [1].

Antibiotic sensitivity – Kirby Bauer disc diffusion method

Antibiotic sensitivity of clinical *Klebsiella pneumoniae* isolates was done by Bauer's and Kirby's

disc diffusion method according to the CLSI guidelines. Commercially available Imipenem (10 µg), Piperacillin (100 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Ceftazidime (30 µg), Amoxicillin Clavulanate (20 / 10µg), Ceftazidime Clavulanate (30 / 10 µg) and Cefotaxime (30 µg) antibiotic discs were used.

Detection of E.S.B.L Producing Organism – *Klebsiella* isolates showing resistance to 3rd Generation Cephalosporins (Ceftazidime- <17mm, cefotaxime <27mm) were selected for ESBL confirmatory test as per CLSI guidelines 2015 [6]. The ESBL phenotypic confirmatory test was done by Disc Potentiation Test.

Disc Potentiation Test – Inoculum was prepared and standardized using 0.5 McFarland and swabbed on to Mueller Hinton Agar plate and discs containing Ceftazidime 30 µg and Ceftazidime+ Clavulanate (30 / 10 µg) are placed with 25 mm distance and incubated at 37°C for 16-18 hours. An increase of ≥5 mm in zone of inhibition for ceftazidime + clavulanic acid compared to ceftazidime was confirmed as ESBL producers.

RESULTS AND DISCUSSION

A total of 53 samples, out of 100 samples that were collected, yielded growth, out of which 27 *Klebsiella pneumoniae* were isolated. Males were more commonly affected and maximum number of isolates, were found in age group 56-75 years (Table-1).

Table-1: Distribution of *Klebsiella* isolates according to age and sex

Age group	No of Pts	Males	Females	<i>K.pneumoniae</i>
15-25	8	2	1	3
26-35	13	1	1	2
36-45	12	1	2	3
46-55	13	2	-	2
56-65	26	5	3	8
66-75	21	6	2	8
76-85	5	-	-	-
86-95	2	1	-	1
Total	100	18	9	27

Majority (60%) of the patients from whom *Klebsiella pneumoniae* was isolated (n=27) were over 45 years of age, majority were habituated to smoking, or had COPD. Old age, smoking and underlying respiratory diseases such as COPD impair pulmonary defences and predispose to pneumonia caused by gram

negative bacteria [7]. Our hospital being a tertiary referral hospital we receive patients with wide range of severity, many of them carrying multiple co morbidities. These patients might have been exposed to antibiotics for treatment of respiratory or non-respiratory tract infections.

Table-2: Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Antibiotic disc	Susceptibility	Percentage %	Resistance	Percentage %
Piperacillin	14	51.8	13	48.2
Amikacin	20	74	7	26
Ceftazidime	14	51.8	13	48.2
Cefotaxime	15	55.5	12	44.5
Ciprofloxacin	19	70.3	8	29.7
Amoxicillin + clavulanate	16	59.2	11	40.8

Ceftazidime + clavulanate	26	96.2	1	3.8
Imipenem	27	100	0	0

Klebsiella pneumoniae were susceptible to Ceftazidime clavulanate (96%), Imipenem (100%), Amikacin (74%) and ciprofloxacin (70.3%). Resistance to Piperacillin, Ceftazidime and Cefotaxime was 48%, 48% and 45% respectively (Table-2).

In the present study, *K. pneumoniae* isolates showed 74% susceptibility towards Amikacin, whereas 85.6% sensitivity was reported by K. V. Ramana *et al.* 2015 [8] and 83% by Sunil Vijay *et al.* [9]. In another study from Jaipur by Preeti Srivastav [10], 40% susceptibility was reported.

Klebsiella pneumoniae isolates in the present study showed 70.3% susceptibility against Ciprofloxacin. O Okesola *et al.* [11] found 61.1% sensitivity to ciprofloxacin, whereas K.V. Ramana *et al.* [8], found that 75% isolates were resistant to ciprofloxacin.

Table-3: Prevalence of Extended Spectrum Beta Lactamases in *Klebsiella pneumoniae*

Organism	No of isolates	ESBL positive	%
<i>K.pneumoniae</i>	27	10	37

37% of *Klebsiella pneumoniae* were found to be ESBL producers, which were confirmed by phenotypic disc potentiation method. Out of 10 ESBL isolates, 7 of 10 were obtained from in-patients and 3 of 10 were from out-patient.

The prevalence rate of ESBLs was 37% in the present study; Shukla *et al.* [12] detected ESBL in 30.18%. Sunil Vijay [9] found prevalence rate of 18.3%. P. N. Sridhar Rao *et al.* [14] detected ESBL from clinical isolates in Davangere and found that 61.7% of isolates were ESBL. In the study by Shashidhar Vishwanath [15] from KMC Manipal in 2013, 65% of ESBL producing *K.pneumoniae* were recovered.

Prevalence of ESBL producers in any hospital depends upon various factors like antibiotic policy, the carriage rate among the hospital personal, and the type of disinfection used especially in ICU [16].

The emergence of multidrug resistant strains can be explained by the inappropriate use of antibiotics. The increasing trend of antimicrobial resistance is most worrisome for Gram-negative bacteria because there has been little successful development of new antibiotic agents targeting this class of pathogen [17].

CONCLUSION

The present study was undertaken to know the prevalence ESBL producing *Klebsiella pneumoniae*, so that specific treatment can be advocated.

Klebsiella pneumoniae in the present study showed resistance of 49% towards Ceftazidime and 45% to cefotaxime. Sunil vijay [9] found 43% resistance to ceftazidime and 47% to cefotaxime. In another study conducted by Shukla [12] in N. India, *K. pneumoniae* isolates showed 67.5% and 78.3% resistance to Ceftazidime and Cefotaxime respectively. It was observed that Gram negative bacteria were resistant to commonly used antibiotics, which is alarming and this could be the reason for considerable resistance [13].

Klebsiella pneumoniae in our study showed 96% susceptibility to Ceftazidime Clavulanate whereas Sunil Vijay [9] reported only 71% sensitivity to Ceftazidime Clavulanate. 100% sensitivity was showed towards Imipenem in present study whereas Sunil Vijay [9] and K.V Ramana [8] reported 88% and 78.6% sensitivity towards Imipenem.

37% of the *Klebsiella pneumoniae* were ESBL producers. As this constitutes a considerable proportion, it is necessary and useful to perform screening and confirmatory tests for phenotypic detection of these organisms in routine work.

Overtreatment with antimicrobials of acute uncomplicated bronchitis, which is largely due to viruses, has led to emergence of multidrug resistance among pathogens. Therefore, cautious and judicious use of antimicrobial agents will reduce the burden of multidrug resistance and thereby enabling better patient management and limiting the resultant morbidity and mortality arising from Pneumonia.

Considering the fact that prevalence of ESBL producers in any hospital varies between different geographical regions, there is need to regularly update our knowledge. This will help in formulating antibiotic policy and thus helps the clinicians in initiating suitable antimicrobial treatment.

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