

# Prevalence of ESBL-mediated Resistance among Hospital and Community isolates of *Klebsiella pneumoniae* in Warangal

Usha Vidya Rani S<sup>1</sup>, Ramanamma MV<sup>2</sup>, Mukherjee AL<sup>3</sup>, Suneesh Jacob A<sup>4</sup>

<sup>1</sup>Dr. Shodavaram Usha Vidya Rani, Assistant Professor, Department of Microbiology, S.V. Medical College, Tirupati, Andhra Pradesh, India, <sup>2</sup>Dr. Mallajosyula Venkata Ramanamma, Director of Medical Education (Retd.), Government of Andhra Pradesh, Hyderabad, Andhra Pradesh, India, <sup>3</sup>Dr. Arava Lazarus Mukherjee, Assistant Professor, Department of Orthopedics, ACSR Govt. Medical College, SPSR Nellore, Andhra Pradesh, India. <sup>4</sup>Dr. Akkarapakam Suneesh Jacob, Research Scholar, Indian Institute of Technology, Kanpur, Uttar Pradesh, India.

**Address for correspondence:** Dr. Shodavaram Usha Vidya Rani, E-mail: ushavidyarani@yahoo.co.in

## Abstract

**Introduction:** Extended Spectrum  $\beta$ -Lactamase (ESBL) producing *Klebsiella pneumoniae* were prevalent in the hospital environment and were responsible for many hospital associated infections (HAIs). The present study was aimed at identifying these strains in the hospital environment so as to guide the clinician in planning antibiotic policy. **Material and Methods:** 200 clinical isolates of *Klebsiella pneumoniae* constituted the study group. 100 isolates were from the community and 100 from the hospital. Based on their susceptibility or resistance to 3<sup>rd</sup> generation cephalosporins (3GCs), the isolates were characterized into two groups. Group I consisted of sensitive strains (128 numbers) which showed a zone diameter of more than 17mm and group II consisted of resistant strains (72 numbers) which showed a zone diameter of less than 17mm to ceftazidime (30 $\mu$ g), cefotaxime (30 $\mu$ g) and ceftriaxone (30 $\mu$ g). Isolates of group II were tested for ESBL production using two methods namely double disc synergy test (DDST) and phenotypic confirmatory disc diffusion test (PCDDT). **Results:** Among the 72 resistant strains, 36 (50%) were ESBL producers and 36 (50%) were non-ESBL producers. Among the hospital isolates, the percentage of ESBL producers was more (30%) compared to the community isolates (6%). **Conclusion:** About one third of the hospital isolates of *Klebsiella pneumoniae* showed ESBL production whereas ESBL mediated resistance was low in community isolates. This study substantiated the need for planning proper antibiotic policy to reduce mortality due to Gram-negative sepsis.

**Keywords:** Extended spectrum beta lactamases (ESBLs), Double disc synergy test (DDST), Hospital associated infections (HAIs), Phenotypic confirmatory disc diffusion test (PCDDT) and Third generation cephalosporins (3GCs).

## Introduction

*Klebsiella pneumoniae* is one of the commonest organisms causing sepsis in the hospital as well as in the community. Colonization in the oropharynx especially of hospitalized patients may be the source of lung infections in debilitated patients [1]. Nosocomial outbreaks due to multi-drug resistant *Klebsiella pneumoniae* are also reported [2]. ESBLs are more prevalent in *Klebsiella pneumoniae* than any other Enterobacteriaceae species and are often undetected by routine susceptibility testing methods where only one disc of any one of the 3GCs is used. Occurrence of ESBL producing *Klebsiella pneumoniae* has been

reported in India [3]. As no documented information is available with regard to the extent of spread of the ESBL producing strains of *Klebsiella pneumoniae* in the hospital and community in Warangal region an attempt was made to identify ESBL producing *Klebsiella pneumoniae* among hospital and community isolates using two different techniques namely double disc synergy test (DDST) [4] and phenotypic confirmatory disc diffusion test (PCDDT) [5, 6].

## Material and Methods

Two hundred clinical isolates of *Klebsiella pneumoniae* isolated from various clinical samples of both out-patients and in-patients constituted the study material.

Manuscript received 27<sup>th</sup> April 2016  
Reviewed: 14<sup>th</sup> May 2016  
Author Corrected: 24<sup>th</sup> May 2016  
Accepted for Publication 11<sup>th</sup> June 2016

One hundred isolates were from the out-patients and a hundred from in-patients. Institution ethical committee approval has been obtained. Identification of the strains was done as per the standard guidelines [1] (Figures 1 and 2). Antibiogram of the isolates was studied with a set of eight antibiotic discs consisting of amoxicillin (30µg), gentamycin (10µg), amikacin (30µg), ciprofloxacin (30µg), cefuroxime (30µg), ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg). The

isolates were categorized into two groups based on their susceptibility or resistance to 3GCs. Group I consisted of sensitive strains which had shown a zone diameter of more than 17 mm for all the 3GCs. Group II were resistant strains, which had shown a zone diameter of less than 17 mm for any one of the 3GCs [7]. Strains belonging to the second group were tested for ESBL production by 2 methods namely DDST and PCDDT.

Double disc synergy test (DDST) was done using amoxicillin 20µg and clavulanic acid 10µg (augmentin), and discs of 3GCs (ceftazidime, ceftriaxone and cefotaxime). The test inoculum (0.5 McFarland's turbidity) was spread onto Mueller-Hinton agar (MHA) by using a sterile cotton swab. A disc of augmentin (20 µg amoxicillin + 10 µg clavulanate) was placed on the surface of the MHA, then discs of cefotaxime (30µg), ceftazidime (30 µg) and ceftriaxone (30 µg) were kept 16-20 mm apart from augmentin disc (center to center). The plates were incubated at 37°C overnight. The enhancement of the zone of inhibition of the cephalosporin disc towards the clavulanic acid disc was inferred as synergy and the strain was considered as an ESBL producer [4] (Figure 3).



**Fig. 1:** Lactose fermenting mucoid colonies of *Klebsiella pneumoniae* on MacConkey's agar



**Fig 2:** Biochemical reactions of *Klebsiella pneumoniae*



**Fig 3:** ESBL production detected by DDST



**Fig 4:** ESBL detected by DDST & PCDDT on same plate

Phenotypic confirmatory disc diffusion test (PCDDT) was performed as a disc diffusion test as recommended by the CLSI. The test inoculum (0.5 McFarland's turbidity) was spread onto the MHA by using a sterile cotton swab and then a ceftazidime (Ca) disc containing 30 µg of the antibiotic and ceftazidime + clavulanic acid (CaC) disc containing 20

$\mu\text{g}+10 \mu\text{g}$  of the antibiotics respectively were placed at a distance of 30 mm from each other. The plates were incubated overnight at  $37^{\circ}\text{C}$  and the results were read. A  $> 5$  mm increase in the zone diameter for CaC versus its zone diameter when it was tested alone by Ca confirmed an ESBL producing organism. All the discs were obtained from Hi-Media, India. In PCDDT, an increase of zone diameter more than 5 mm around a ceftazidime+clavulanic acid disc when compared to ceftazidime disc alone was considered as ESBL producer [5, 6] (Figure 4). The control strains used in the present study were *Klebsiella pneumoniae* ATCC 700603 (as positive control), *Escherichia coli* ATCC 25922 (as negative control) and in-house control.

## Results

The overall prevalence rate of 3GC-resistant *Klebsiella pneumoniae* was 36% (Hospital and Community isolates together). Among the two hundred isolates of the test group, 72 were resistant to the 3GCs (zone diameter of less than 17mm). (TABLE I). The prevalence of 3GC resistance was more in the hospital (60%) compared to the community isolates (12%). Among the 3GC resistant hospital isolates 50% were ESBL producers. (TABLE II). The maximum percentage (26%) of ESBL producing strains was from pus samples. (TABLE III).

**Table I: Sensitivity and resistance patterns of *Klebsiella pneumoniae* isolates to 3GCs.**

Total No. of isolates	Sensitive to 3GCs		Resistant to 3GCs	
	No.	Percentage	No.	Percentage
200	128	64%	72	36%

**Table II: Distribution of *Klebsiella pneumoniae* isolates based on ESBL production.**

Group	Total no. of isolates	Sensitive to 3GCs		Resistant to 3GCs			
		No.	Percentage	ESBL producers		Non ESBL producers	
				No.	Percentage	No.	Percentage
Hospital	100	40	40%	30	30%	30	30%
Community	100	88	88%	6	6%	6	6%
<b>Total</b>	<b>200</b>	<b>128</b>	<b>64%</b>	<b>36</b>	<b>18%</b>	<b>36</b>	<b>18%</b>

**Table III: Sample-wise distribution of ESBL producing *Klebsiella pneumoniae*.**

Samples	No. of <i>Klebsiella pneumoniae</i> isolates	ESBL producing <i>Klebsiella pneumoniae</i> isolates	
		No.	Percentage
Pus	46	12	26.08%
Sputum	106	12	11.32%
Urine	42	8	19.04%
Stool	4	2	50.00%
Blood	2	2	100.00%

**Table IV: Percentage of ESBL in different study groups in India**

S. No.	Place	Author	No. studied	No. of ESBL producers	Percentage
1	Delhi	Revathi [9]	100	53	53.00%
2	Chennai	Subha and Ananthan [10]	120	8	6.66%
3	Aligarh, U. P.	Shukla et al [11]	120	32 (by PCDDT) 29 (by DDST)	26.66% 24.16%
4	Haryana	Singhal et al [12]	272	173	63.60%
5	Chennai	Menon et al [13]	70	14	20.00%
6	Warangal, A. P.	Usha et al	200	72	36.00%

## Discussion

The predilection of ESBLs for *Klebsiella pneumoniae* in the hospital environment is probably due to their longer survival in the hospital thus facilitating cross infection [6]. In the hospital, the environmental sources of ESBL producing organisms are ultrasonography coupling gel, bronchoscopes, blood pressure cuffs, glass thermometers to name a few. Sink basins and babies' baths have also been discovered to have been contaminated [8]. The present study was the first attempt to know the percentage of 3GC resistant *Klebsiella pneumoniae* isolates in Warangal.

Revathi from Delhi tested 100 clinical isolates of Gram-negative bacilli for production of ESBLs with the E-test ESBL strip. 53 isolates were ESBL positive [9]. Subha and Ananthan from Chennai studied a total of 120 isolates of *Klebsiella pneumoniae*. 87% of the isolates showed resistance to all the 3GC antibiotics. ESBL production was detected in 8 strains. The ESBL activity could experimentally be transferred to recipient *Escherichia coli* K12 J62-2 [10]. Shukla et al from Aligarh, Uttar Pradesh, India tested 120 multi-drug resistant strains of *Klebsiella pneumoniae*. The strains were studied for ESBL production by PCDDT and DDST. 72% of the isolates were resistant to all the 3GCs. 32 strains showed ESBL production by PCDDT and 29 by DDST. Resistance was transferred to recipient *Escherichia coli* K12 J-62-1 [11]. Singhal et al from Haryana screened 272 isolates for ESBL and AmpC  $\beta$ -lactamase by modified double disc approximation method. 173 of the isolates (64%) were found to be ESBL positive [12].

Menon et al studied ESBL production among members of Enterobacteriaceae in a tertiary care center in Chennai. The methods used were DDST and three dimensional tests. Among 70 strains, 20% were ESBL producers. Three dimensional test was found to be better than DDST. In DDST method a distance of 15 mm was maintained between 3GC and Augmentin [13].

In our present study of 200 isolates 72 (36%) were found to be ESBL producers. The range of percentages was from 6 to 64%. (TABLEIV). This is understandable as the prevalence of ESBL producers of any hospital depends upon various factors like the hospital antibiotic policy, the carriage rate of the organism in the hospital environment and the type of disinfectants practiced in the hospitals especially in the intensive care units. These strains are often undetected because of the usage

of only one antibiotic disc belonging to 3GCs. Strains showing significant zones of inhibition often pass off as sensitive while in reality they are resistant often with ESBL production. Thus it is mandatory to include a minimum of any three of the antibiotics of 3GCs. Strains resistant to any one of the three 3GCs are to be taken as resistant and should be tested for ESBL production. Gastrointestinal colonization among hospitalized patients is a significant risk factor for spreading ESBL resistance in the hospital environment [14].

The observations in the present study indicate the possible methods of clinical approach as detailed below to reduce mortality and morbidity due to ESBL producing hospital strains of *Klebsiella pneumoniae*: avoid injudicious use of 3GCs without assessing the sensitivity pattern of the clinical isolate, formulate a policy of empirical therapy in high risk units [15] and devise protocols to reduce the prevalence of drug resistant bacteria in the hospital environment. In short, the prevention and management of infections caused by ESBL producing organisms requires a well coordinating activity of the microbiologist, the clinician, the hospital paramedics and the hospital infection control team.

## Conclusions

Among 200 clinical isolates studied, 72 (36%) strains were resistant to 3GCs. Out of them 60 were from hospital environment and 12 were community isolates. ESBL producers were more in the hospital isolates (30%) compared to the community isolates (6%). Most of the ESBL producing *Klebsiella pneumoniae* isolates were from pus samples. To get an accurate picture of resistance to 3GCs, the isolate has to be tested for susceptibility to at least three of the 3GCs.

**Acknowledgements:** Authors are grateful to Dr. B. Appala Raju, Professor and Head of the Department of Microbiology, PSG Institute of Medical Science and Research, Coimbatore for extending help to procure material for the present study and thankful to Dr. I. L. Ramesh, our well-wisher for all his moral support and encouragement towards the study and last but not the least, Dr. Hari Krishna, Asst. Professor of Physiology for extending help in computer and online techniques.

**Funding:** Nil, **Conflict of interest:** None initiated.

**Permission from IRB:** Yes

## References

1. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Eds. Enterobacteriaceae, Antimicrobial Susceptibility Testing. In: Color Atlas and Text Book of Diagnostic Microbiology. 5<sup>th</sup> Edition, 1997. J. B. Lippincott Company, Philadelphia, New York. 207, 779-798, 831.
2. Mahon CR and Manuselis, Jr. Enterobacteriaceae In: Text book of Diagnostic Microbiology, 1995. W. B. Saunders Company, a Division of Harcourt Brace & company. Philadelphia London Toronto Montreal Sydney Tokyo. 456-457.
3. Subha A, Ananthan S. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. Indian Journal of Medical Microbiology, (2002) 20(2):92-95.
4. Jarlier V, Nicolas M, Fournier G, Philippon A. Extended spectrum  $\beta$ -lactamases conferring transferable resistance to newer  $\beta$ -lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev Infect Dis 1988; 8:67-78.
5. Clinical Laboratory Standards: Performance standards for antimicrobial susceptibility testing; Eighth informational supplement. M100-S8. NCCLS, Wayne, PA: 1998; 7767 Clinical and Laboratory Standards Institute. 2005 guidelines by CSLI/NCCLS–CSLI informational supplement. Approved standard M100-S15 Wayne, PA; 2005; 565.
6. Paterson DL and Bonomo RA. Extended-spectrum  $\beta$ -lactamases: a Clinical Update. Clinical, Microbiology Reviews. Oct. 2005. p. 657-686.
7. National Committee for Clinical Laboratory standards: performance Standards for antimicrobial susceptibility test, 5<sup>th</sup> ed. (Villanova, PA: NCCLS) 1993: DOCUMENT M2 – A5.
8. Eisen, D., E. G. Russell, M. Tymms, E., J. Rper, M. L., Grayson, and J. Turnidge, 1995, Random amplified polymorphic DNA.
9. Revathi G, Singh S. Detection of Expanded spectrum cephalosporin Resistance due to inducible  $\beta$ -lactamases in hospital isolates. Indian J Med Microbiol 1997; 15 (3):113-115.
10. Subha A, Ananthan S. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. Indian Journal of Medical Microbiology, (2002) 20(2):92-95.
11. Shukla I, Tiwari R, Agarwal M. Prevalence of Extended Spectrum  $\beta$ -lactamase Producing Klebsiella pneumoniae in a Tertiary Care Hospital, Indian Journal of Medical Microbiology. (2004)(2):87-91.
12. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chug S, Giand R, Rattan A. Evaluation of methods for AmpC  $\beta$ -lactamase in Gram-negative clinical isolates from tertiary care hospitals. Indian Journal of Medical Microbiology, (2005) 23 (2): 120-124.
13. Menon T, Bindu D, Kumar CPG, Nalini S, Thirumarayan MA. Comparison of Double Disc and Three Dimensional Methods to screen for ESBL producers in a Tertiary Care Hospital. Indian Journal of Medical Microbiology. (2006) 24 (2): 117-120.
14. Lucet JC, Decre D, Fichelle A, Joly-Guillou ML, Pernet M, Deblangye, et al. Control of a prolonged outbreak of Extended spectrum  $\beta$ -lactamase producing Enterobacteriaceae in a University Hospital. Clin Infect Dis 1999; 29:1411-8.
15. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of ESBL Producing Gram-negative bacteria in a tertiary care hospital. Indian J Medical Res 2002; 115:153-157.

## How to cite this article?

Usha Vidya Rani S, Ramanamma MV, Mukherjee AL, Suneesh Jacob A. Prevalence of ESBL-mediated Resistance among Hospital and Community isolates of Klebsiella pneumoniae in Warangal. *Int J Med Res Rev* 2016;4 (6):1005-1009. doi: 10.17511/ijmrr.2016.i06.24.