



Prevalence of Extended-Spectrum β -Lactamases producing *Klebsiella pneumoniae* isolates in a tertiary care hospital in South India

Authors

Rangnekar Aseem¹, Mallaya Shrikara, Shenoy Shalini, Gupta Shefali*

*Corresponding Author

Dr Shefali Gupta

Senior Research Associate, Department of Virology, PGI Chandigarh

Email: shefali.g2109@gmail.com

Abstract

Background: Growing antimicrobial resistance is of great concern in modern antibiotic era. Infections caused by multidrug resistant organisms like *Klebsiella pneumoniae* are among the leading causes of sepsis and infection related deaths.

Aim: This study was done to determine the prevalence of ESBL producing *Klebsiella* in the various clinical specimens by phenotypic methods.

Material and Methods: A prospective laboratory based study was done to determine phenotypically detect the presence of Extended Spectrum β -lactamases (ESBL's) in samples received over a period of two years. A total of 7644 gram negative bacterial isolates were recovered from a total of 16526 clinical specimens received in the microbiology laboratory, over the two year period. Among the 2497 isolates of *Klebsiella*, 1157 isolates were ESBL positive (46%).

Conclusion: Drug resistant *Klebsiella* producing ESBL are hard to treat, and infections caused by them can lead to fatal outcome. Effective antibiotic policy and rational use must be strongly and promptly implemented in all healthcare setups.

Keywords: ESBL, Antibigram, Drug resistant *Klebsiella pneumoniae*.

Introduction

Klebsiella are important bacterial pathogen isolated from hospitalised patients. The importance of *Klebsiella*, in the ever increasing number of gram negative aerobic bacillary nosocomial infections in the United States and India, has been well documented.⁽¹⁾ Epidemic and endemic nosocomial infections caused by *Klebsiella* are leading causes of morbidity and mortality.⁽²⁾

Multidrug resistant *Klebsiella* are emerging worldwide posing a big challenge to healthcare.⁽³⁾

Also, extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase (ESBL). Infection due to multi drug resistant *Klebsiella* leading to septicemia and meningitis may be fatal. Since more and more of these outbreaks have been caused by multidrug resistant strains, *Klebsiella* neonatal infections are becoming a major concern for the pediatrician.⁽⁴⁾⁽⁵⁾

Klebsiella pneumoniae are inherently resistant to penicillin, including the semisynthetic penicillin like the ampicillin. *Klebsiella* infections were treated with cephalosporin, fluoroquinolones and aminoglycosides. Extended spectrum beta-lactamase (ESBL) producers resistant to 3rd generation Cephalosporins' and Monobactams were first identified from Germany in 1983. Since then, their incidence has sharply increased. An increased prevalence of fluoroquinolone, aminoglycoside resistance overall and in ESBL producing strains has been noted.⁽⁶⁾

The most widely used classification of β -lactamases is the Ambler classification. It separates beta-lactamases into four major classes A to D based on amino acid sequence homology. Classes A, C and D are beta-lactamases with serine at their active site, while class B(also known as metallo-beta-lactamases) have zinc at their active site.⁽⁷⁾

Noticeable increase in the colonization rates with *Klebsiella* has been reported from patients receiving broad-spectrum or multiple antibiotics. Exhaustive use of antibiotics has led to the occurrence of multiply resistant *Klebsiella* strains in hospitals.⁽⁵⁾⁽⁶⁾⁽⁷⁾

This study focuses on antibiotic resistance patterns in clinical isolates of *Klebsiella* and the prevalence of ESBL among the various *Klebsiella* isolates. Due to constrained antibiotic options and growing resistance to the newer generations, it is imperative to study the prevalence of resistant organisms and document its increasing trend.

Materials and Methods

We identified clinical isolates of *Klebsiella pneumoniae* from various clinical specimens submitted to the microbiology laboratory, at a tertiary care hospital in Mangalore over a period of two years (Jan 2013-Jan 2015).

All isolates were identified morphologically and biochemically by standard procedures. The diagnosis of *K. pneumoniae* infection is confirmed by culture of clinically relevant sample; blood, sputum, urine, or aspirated body fluid, including

pleural effusion, pericardial effusion, synovial fluid, cerebrospinal fluid, and abscess material.⁽⁵⁾⁽⁸⁾⁽⁹⁾

In the setting of bacterial pneumonia, sputum Gram stain is done as a presumptive identification for an etiologic agent of implicated bacterial infection. The morphology, culture morphology, motility, and metabolic activities were used to identify *K. pneumoniae*.⁽⁸⁾⁽⁹⁾

Biochemical properties⁽⁵⁾⁽⁸⁾⁽⁹⁾⁽¹⁰⁾

Klebsiella pneumoniae ferments glucose, sucrose, lactose and mannitol, with production of acid and abundant gas. They are Indole test and Methyl Red test negative and Voges Proskauer (VP) test positive. *Klebsiella* are urease producing and utilizes citrate.⁽⁹⁾

Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed using Kirby-Bauer disk-diffusion method and interpretation was done according to CLSI 2014 guidelines.⁽¹²⁾

Detection of ESBL

ESBL production was detected by using the combination disc test for phenotypic confirmation. ESBLs are inhibited by β -lactamase inhibitors like clavulanic acid. Hence when tested by disk diffusion method using cefotaxime(30 μ g), cefotaxime-clavulanic acid(30/10 μ g) and ceftazidime(30 μ g) ceftazidime-clavulanic acid(30/10 μ g) there is an increase of ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone.⁽¹²⁾

Results

A total of 7644 gram negative bacterial isolates were recovered from a total of 16526 clinical specimens over the two year period. Among these, there were 2497 *Klebsiella* isolates, out of which 1157 isolates were ESBL positive (46%).

Table 1 Total number of different specimens and number (percentage) of *Klebsiella* and number (percentage) of ESBL-producing *Klebsiella pneumoniae*

Specimen	Total number received	Gram negative bacilli isolated	Number of <i>Klebsiella pneumoniae</i> isolates	Number of ESBL positive <i>Klebsiella pneumoniae</i>	ESBL positive <i>Klebsiella</i> (%)
Blood	3216	1223	563	114	20%
Urine	3120	2135	1300	481	37%
Sputum	1886	878	400	176	44%
Pus swabs	3782	1946	484	290	60%
Genital swabs(ECS)	1766	298	42	8	19%
CSF	522	199	21	0	0%
Eye	62	21	7	0	0%
Catheter tip	394	201	66	22	33%
Umbilical tips	116	66	16	4	25%
ET suction tips	256	159	36	18	50%
Placental membranes	436	99	10	10	100%
Ascitic fluid	352	123	34	2	6%
Pleural fluid	264	110	54	18	33%
Bile	24	13	8	8	100%
Gastric aspirate	6	3	4	0	0%
Knee aspirate	10	4	1	0	0%
Others(drain,BAL, peritoneal and pericardial fluids,breast milk)	314	166	14	6	43%
TOTAL	16526	7644	2497	1157	46%

Picture 1: Detection of ESBL: Increase of ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone. Disk CTX is cefotaxime (30 μ g), CTX-CLA cefotaxime-clavulanic acid(30/10 μ g) and CA is ceftazidime (30 μ g) CA-CL is ceftazidime-clavulanic acid(30/10 μ g)



Discussion

Klebsiella are known to cause severe community acquired infections, which include pneumonia, hepato biliary tract infections, osteomyelitis. Likewise they are also implicated in causing hospital acquired infections viz. ventilator associated infections, urinary tract infections post-operative wound infections and nosocomial sepsis, in patients admitted in Intensive Care Units.⁽¹³⁾⁽¹⁵⁾

In recent years increased incidence ESBL and AmpC enzymes have occurred due to wide spread use of broad-spectrum cephalosporins.⁽¹⁶⁾ β -Lactamases are the primary cause of bacterial resistance to β -lactam antibiotics. Infections due to multidrug resistant *Klebsiella pneumoniae* are on the rise and are an important cause of morbidity and mortality in the present times.

Our study is an attempt to document the alarming trends in antimicrobial resistance of *Klebsiella pneumoniae*. We studied the antimicrobial susceptibility pattern of the *Klebsiella* isolates and detected the presence of ESBL phenotypically.

The incidence of ESBL-producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past several years. Frequencies of up to 40% have been reported in several countries in Europe and Middle East.⁽¹³⁾

The maximum number of isolates of *Klebsiella* (2135) in our study were from the urine samples, followed by blood (1223) and sputum (878). However, 44% of sputum isolates were ESBL, 37% of the urinary isolates were ESBL positive while only 20% of the isolates from blood were ESBL positive. Among the total 7644 gram negative bacterial isolates that were recovered from a total of 16526 clinical specimens the number of *Klebsiella* isolates were 2497. Out of these, as much as 1157 isolates were ESBL positive (46%). Similar reports from across India are available in literature. Chakraborty et al in their study on extra intestinal *E.coli* reported that 71% of the isolates in a tertiary care hospital in South India were ESBL producers⁽¹⁴⁾ Similar findings was observed by Berrazeg et. al. in their study spanning several cities in two different

countries namely France and Algeria. They found that the number of ESBL producing isolates in Algeria were 88.6% and the average ESBL producers in both the countries combined was 57.7%⁽¹⁵⁾

Mathur et al. from a study done in All India Institute, New Delhi reported that 68% of isolates i.e. 458 out of 678 Enterobacteriaceae were ESBL producers. Among the isolated bacterial species, they found that ESBL production was most common in *Klebsiella* spp. (80%)⁽¹⁶⁾. Grover et al. of the Armed Forces Medical College (AFMC) pune, found that the prevalence of ESBL producers was 32%.⁽¹⁷⁾

Conclusion

The therapeutic options are becoming limited, so that in the near future there will be an urgent need for hospital infection control measures that counter the spread of ESBL-producing bacteria.

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