

Antibiogram and hypermucoviscosity pattern among *Klebsiella pneumoniae* isolates from respiratory samples: A tertiary care hospital study in South India

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Abstract

Klebsiella pneumoniae (*K. pneumoniae*) is frequently found as normal microbial flora of gastrointestinal tract, nasopharynx and skin, has emerged as a major nosocomial pathogen causing infections of the lower respiratory tract, wounds and urinary tract more commonly opportunistic infections in immune-compromised hosts. Respiratory tract infections are one among the most common nosocomial infections caused by *K. pneumoniae*. Multi-drug resistant *K. pneumoniae* (MDR-KP), along with extended-spectrum beta-lactamase (ESBL) producing strains are the cause of concern making it a difficult target for antibiotic chemotherapy. This study was conducted to know the antibiogram pattern and the occurrence of hypermucoviscous in clinical isolates of *K. pneumoniae* from respiratory samples in a tertiary care medical college hospital in South India. *K. pneumoniae* isolates were characterized from respiratory tract samples by using standard microbiological procedures showing hypermucoviscosity which were screened for antibiotic sensitivity test by using Kirby Bauer disc diffusion and also by double disk synergy test (DDST) for presumptive ESBL production. In our study we found that, among 87 respiratory samples of *K. pneumoniae* isolates, 79 (90.8%) were hypermucoviscous and 29 (33.3%) isolates were found to be ESBL producers. This shows that, ESBL producing *K. pneumoniae* isolates had a greater capacity to produce hypermucoviscosity (100%) than non-ESBL producing *K. pneumoniae* isolates (86.2%).

Keywords: *Klebsiella pneumoniae*, Antibiogram, Respiratory samples, Hypermucoviscosity, Hypervirulent.

Introduction

K. pneumoniae is the most common gram negative bacterium found in a wide range of nosocomial and community acquired infections.¹ These gram negative bacilli causes nosocomial infections such as pneumonia, urinary tract infections, bacteremia, wound infections, intra abdominal infections and neonatal septicemia.²⁻⁴ *K. pneumoniae* is a prominent nosocomial pathogen associated with respiratory tract infections including ventilator-associated pneumonia (VAP).

K. pneumoniae possesses an arsenal of both cell-associated and secreted virulence factors. Some of the important cell associated virulence factors that enable its survival in diverse environmental conditions and help in establishing infections are capsular polysaccharide, fimbrial adhesins, biofilm and lipopolysaccharide. The prominent extracellular or secreted virulence factors are hemolysins, proteases,

cytotoxin, siderophores, exotoxins, etc. *K. pneumoniae* is a pathogen which has the ability to colonize various tissues including upper and lower respiratory tract, urinary tract and skin wounds.⁵ *K. pneumoniae* pathogenicity is attributed to several virulence factors like fimbrial adhesins, lipopolysaccharides, capsule and siderophores. The exopolysaccharide capsule is associated with hyperviscous or hypervirulent phenotype of *K. pneumoniae*.⁶ This hypercapsule is responsible for the emergence of hypermucoviscous *K. pneumoniae* strains and aids bacteria to develop resistance to both antibiotics and host defense mechanisms. Hypermucoviscosity of *K. pneumoniae* strains can be assessed by a positive string test based on their ability to form mucoviscous strings using colonies grown on 5% sheep blood agar culture plates.⁷

Respiratory infections including pneumonia are often associated Hypermucoviscous strains of *K.*

pneumoniae and are known to produce ESBL among members of family *Enterobacteriaceae*.⁸ Beta lactam ring containing antibiotics such as penicillins and broad-spectrum cephalosporins can be made ineffective by ESBLs that shows resistance to antibiotics including carbapenemase producing strains isolated from respiratory samples. A reliable, simple and economic test is Double Disk Synergy Test (DDST) to detect production of ESBL strains *K.pneumoniae*. Studies have shown association between antibiotic drug resistance and hypermucoviscous nature among clinical isolates of *K. pneumoniae*.⁹

This study was conducted to know the local antibiogram pattern, ESBL production and hypermucoviscosity among respiratory strains of *K. pneumoniae* in a tertiary care hospital, Mangalore, Dakshina Kannada District, Karnataka, South India.

Materials and Methods

Phenotypic isolation and identification of respiratory *K. pneumoniae* strains:

A prospective study was conducted in the Department of Microbiology, Yenepoya Medical College and Hospital, Mangalore, Karnataka. Respiratory (sputum) samples were collected from suspected patients using standard specimen collection guidelines. 87 *K. pneumoniae* strains from respiratory samples were included in this study. Clinical samples were inoculated on Mac Conkey's agar and 5% Sheep Blood agar and incubated overnight at 37°C. Colonies of bacteria grown on the agar plates were identified by its morphology and biochemical reactions utilizing standard microbiological tests including pure and predominant growth from urine samples containing *K. pneumoniae* isolates.⁽¹⁰⁾

Antibiotic susceptibility testing

Conventional Kirby Bauer's disc diffusion method using Mueller- Hinton agar (MHA) plates were used to test bacterial susceptibility to antimicrobial agents as described by Clinical Laboratory Institute (CLSI) guidelines.⁽¹¹⁾ By adjusting to 0.5 McFarland turbidity standards, (1×10^8 cfu/ml), MHA plates were inoculated with a suspension of *K. pneumoniae*. The drugs used to test sensitivity were Netilmicin (30µg),

Piperacillin (100 µg), Piperacillin-Tazobactam (100/10µg), Amoxicillin-Clavulanic acid (20/10µg), Cefotaxime (30µg), Ampicillin ((10µg), Amikacin (30µg) Cefpodoxime (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg) and imipenem (10 µg). The culture plates were incubated 37°C overnight followed by measurement of zones of inhibition by comparing with the standard measurement chart.

Detection of ESBL production by double disk synergy test

K. pneumoniae isolates from respiratory samples showing resistance to III generation cephalosporins were tested for ESBL production by using Double Disk Synergy Test (DDST) as per CLSI guidelines.¹²

During testing procedure Amoxicillin – Clavulanic acid was placed in the centre of the lawn culture made on Muller Hinton Agar (MHA) plate and were inoculated with each of the test *K. pneumoniae* isolates that are found resistance towards any one or all the antibiotic disks of Ceftazidime, Cefotaxime and Cefpodoxime. The discs containing Ceftazidime, Cefotaxime and Cefpodoxime with each having a disc concentration of 30µg which are placed around the central amoxicillin – clavulanic acid disc with a centre to centre distance of 30 mm followed by incubation of plates at 37°C for 24 hrs. Any increase in zone of inhibition between any one of the cephalosporin disks with the central disk the isolate were considered to be an ESBL producer.

Detection of hypermucoviscous phenotype

The extracapsular polysaccharide is often associated with hypermucoviscosity or hypervirulence among strains of *K. pneumoniae*.⁶ This exopolysaccharide or hypercapsule is responsible for the emergence of hypermucoviscous *K. pneumoniae* strains that confer resistance to antibiotics. String test described by Fang *et al.*, was used to assess the hypermucoviscous phenotypic expression among respiratory strains of *K. pneumoniae*.¹ Isolates of *K. pneumoniae* were subcultured overnight on 5% sheep blood agar at 37°C. They were considered positive for the hypermucoviscous or hypervirulent phenotype if an inoculation loop touched to the surface of the colony

generated viscous strings of 5mm in length when pulled away from the colony.

Results and Discussion

This study showed higher resistance to ciprofloxacin, whereas the bacterial isolates were sensitive to amoxicillin-clavulanic acid, piperacillin-tazobactam and imipenem by Kirby Bauer disc diffusion method with antibiogram pattern of *K. pneumoniae* isolates (Table 1). Among the *K. pneumoniae* clinical isolates, 36 and 41 strains were resistant to Ceftazidime and Cefotaxime respectively. This study also revealed ESBL production among 29 (33.3%) isolates as confirmed by DDST (Fig. 1).

Antibiogram study pattern of ESBL producing and non ESBL producing respiratory *K. pneumoniae* isolates are shown in Table 2. Hypermucoviscous phenotype were seen in 79 (90.8%) of the *K. pneumoniae* isolates. Among the total samples, about 50 (57.5%) were positive for hypermucoviscosity by non-ESBL producing *K. pneumoniae*. All ESBL producing strains were positive for string test. Positive string test for hypermucoviscosity by clinical strains of respiratory isolates of *K. pneumoniae* is shown in Fig. 2.

K. pneumoniae are one of the common opportunistic pathogen which are associated with both community and hospital-acquired infections including

lower respiratory infections such as pneumonia.¹³ The major virulence factor of *K. pneumoniae* is mucoviscous extracapsular polysaccharide or hypercapsule and its role in several infections are well documented.

Antibiotic resistance is rapidly emerging among hypermucoviscous bacterial strains when compared to bacteria that lack hypercapsule.¹⁴ Factors such as multi-drug resistance are due to restricted penetration of antibiotics via exopolysaccharide capsule, development of drug resistance genes and reduction of bacterial growth.¹⁵

Among the total respiratory samples, about 90.8% of *K. pneumoniae* isolates showed hypermucoviscosity with has close relationship with an earlier study done by Zhang *et al.*, that has shown clear association with hypervirulent strains (74.7%) of *K. pneumoniae*.^{16,17} Our study also correlated with an earlier study conducted by Aljanaby *et al* which indicated 62.50% of hypermucoviscosity among ESBL producing isolates of *K. pneumoniae*.⁽¹⁸⁾ Higher prevalence of hypermucoviscosity and positive association with ESBL production were reported by Khaertynov *et al*, Hennequin *et al* and Lee *et al* among clinical isolates of hypermucoviscous or hypervirulent strains of respiratory *K. pneumoniae* clinical isolates.¹⁹⁻²¹

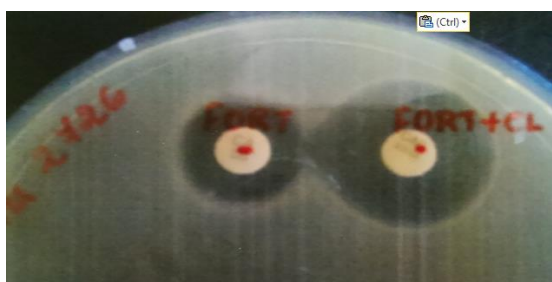
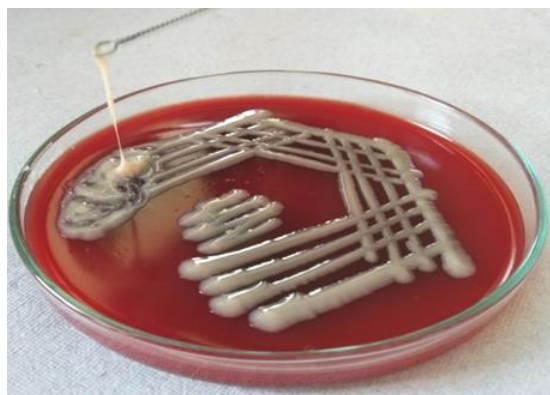
Table 1: Antibiogram pattern of *K. pneumoniae* isolates from respiratory samples (N=87):

Antibiotics	Sensitive (S) n (%)	Intermediate (I) n (%)	Resistant (R) n (%)
Ampicillin	0	0	87(100)
Amikacin	63(72.5)	3(3.4)	21(24.1)
Ceftazidime	49(56.3)	2(2.3)	36(41.4)
Cefotaxime	44(50.6)	2(2.3)	41(47.1)
Cefpodoxime	50(57.5)	2(2.3)	35(40.2)
Ciprofloxacin	32(36.8)	2(2.3)	53(60.9)
Netilmicin	63(72.5)	1(1.1)	23(26.4)
Piperacillin	58(66.7)	1(1.1)	28(32.2)
Pip-tazobactum	71(81.7)	1(1.1)	15(17.2)
Amoxi-clav	70(80.5)	1(1.1)	16(18.4)
Imipenem	73(83.9)	0	14(16.1)

Table 2: Antibiogram pattern of ESBL and non-ESBL producing *K. pneumoniae* isolates from respiratory samples (N=87):

Antibiotic tested (disc concentration)	Susceptibility of non-ESBL producing <i>K. pneumoniae</i> (n=58)			Susceptibility of ESBL producing <i>K. pneumoniae</i> (n=29)		
	S n (%)	I n (%)	R n (%)	S n (%)	I n (%)	R n (%)
Ceftazidime (30 µg)	49(56.3)	2(2.4)	7(8.0)	0(0)	0(0)	29(33.3)
Cefotaxime (30 µg)	44(50.6)	2(2.4)	12(13.7)	0(0)	0(0)	29(33.3)
Cefpodoxime (10 µg)	50(57.4)	2(2.4)	6(6.9)	0(0)	0(0)	29(33.3)
Amikacin (30 µg)	42(48.3)	2(2.4)	14(16.1)	21(24.1)	1(1.1)	7(8.0)
Netilmicin (30 µg)	42(48.3)	1(1.1)	15(17.2)	21(24.1)	0(0)	8(9.3)
Ciprofloxacin (5 µg)	25(28.7)	1(1.1)	32(37.0)	7(8.0)	1(1.1)	21(24.1)
Ampicillin (10 µg)	0 (0)	0 (0)	58(66.7)	0(0)	0(0)	29(33.3)
Piperacillin (100 µg)	39(44.8)	1(1.1)	18(20.7)	19(21.9)	0(0)	10(11.5)
Piperacillin-tazobactam (100/10 µg)	48(55.2)	0 (0)	10(11.5)	23(26.4)	1(1.1)	5(5.8)
Amoxicillin-clavulanic acid (20/10 µg)	47(54.0)	1(1.1)	10(11.5)	23(26.4)	0(0)	6(7.0)
Imipenem (10 µg)	51(58.6)	0(0)	7(8.0)	22(25.4)	0(0)	7(8.0)

S- Sensitive, I- Intermediate, R- Resistant.

**Fig. 1:** Double disk synergy test showing ESBL production among *K. pneumoniae***Fig. 2:** Demonstration of hypermucoviscous phenotype of *K. pneumoniae* by a positive string test

Conclusion

This study showed that, many strains of *K. pneumoniae* isolated from respiratory samples were more resistant to III generation Cephalosporins. Our study highlights a positive correlation and association

between antibiotic drug resistance and hypermucoviscosity by ESBL producing clinical strains of *K. pneumoniae* isolated from respiratory samples.

Source of Funding

None.

Conflict of Interest

None.

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