Hypermucoviscous uropathogenic strains of *Klebsiella pneumoniae* producing extended spectrum beta-lactamase: An experience in South Indian tertiary care hospital

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Abstract
Klebsiela pneumoniae (*K. pneumoniae*), belongs to *Enterobacteriaceae* family and is the most pathogenic species that is prominently reasons for infections such as septicaemia, pneumonia and also urinary tract infections (UTIs). The UTIs is due to Gram negative bacteria and could be both nosocomial and also community acquired which are very difficult to treat as a consequence of antibiotics resistance. The increased incidence of antibiotic resistance among these healthcare associated uropathogenic strains of *K. pneumoniae* leads to increase in mortality, morbidity rates makes the treatment of UTIs less cost effective. Increase in multidrug-resistance and production of extended spectrum Beta- lactamases (ESBLs) by uropathogenic *K. pneumoniae* strains which are of a greater public health concern. This study was conducted to know the occurrence of hypermucoviscosity and ESBL production in *K. pneumoniae* isolates from urine samples in a tertiary care medical college hospital in Dakshina Kannada, Mangalore, Karnataka, India. *K. pneumoniae* isolates were characterized from urinary tract samples by using standard microbiological procedures showing hypermucoviscosity which were screened to test antibiotic sensitivity by using Kirby Bauer disc diffusion and also by double disk synergy test (DDST) for presumptive ESBL production. In our study we found, among 80 samples of uropathogenic *K. pneumoniae* isolates, 78(97.5%) were hypermucoviscous or hypervirulent, 19(23.7%) 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 (96.7%).

Keywords: *Klebsiella pneumoniae*, Hypermucoviscous, Uropathogenic, Hypervirulent, ESBL production.

Introduction
Genitourinary system is often colonized by normal microbial flora including bacteria many of which may act as opportunistic infectious agents.[1] Key opportunistic pathogen for both hospital-acquired and community-acquired infections such as pneumonia, UTI and pyogenic liver abscess is found to be *K. pneumoniae* and group of disorders named urethritis, cystitis and acute and chronic pyelonephritis comes under Urinary Tract Infections (UTIs).[2,3]

The presence of indwelling urinary catheters is closely associated with UTIs caused by uropathogenic *K. pneumoniae* isolates.[4] *K. pneumoniae* UTI symptoms include increased frequency, dysuria, urgency of voiding, and hematuria. *K. pneumoniae* pathogenicity is attributed to several virulence factors like fimbrial adhesins, lipopoysaccharides, capsule and siderophores. The exopolysaccharide capsule is associated with mucoviscosity and an increase in its production confers the hypermucoviscous or hypervirulent phenotype of *K. pneumoniae*.[5] The extracapsular polycaccharide or 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.[6]

UTIs are often associated Hypermucoviscous strains of *K. pneumoniae* and are known to produce ESBL among members of family *Enterobacteriaceae*.[7] 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 urinary tract samples. A reliable, simple and economic test is Double Disk Synergy Test (DDST) to detect production of ESBL strains *K.pneumoniae* which is cost effective too. Many studies have shown link between antibiotic drug resistance hypermucoviscosity from clinical isolates of *K.pneumoniae*.[8]

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

Materials and Methods
Phenotypic Isolation and identification of uropathogenic K. pneumoniae strains:
A prospective study was conducted in the Department of Microbiology, Yenepoya Medical College and Hospital, Mangalore, Karnataka. Urine samples were collected from suspected UTI patients using standard specimen collection guidelines. 80 *K. pneumoniae* uropathogenic strains were included in this study. Clinical samples were inoculated on Mac Conkey's agar and 5% Sheep Blood agar and incubated overnight at 37ºC. 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.[9]

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.[10] By adjusting to 0.5 McFarland turbidity standards, ($1x10^8$ cfu/ml), MHA plates were inoculated with a suspension of *K. pneumoniae*. The drugs used to test sensitivity were Netilmicin (30µg), Piperacillin (100µg), Piperacillin-Tazobactam (100/10µg), Amoxicillin-Clavulanic acid (20/10µg), Cefotaxime (30µg), Ampicillin (10µg), Amikacin (30µg) Cefpodoxime (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg) and imipenem (10µg). The culture plates were incubated 37ºC 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. pneumonaie isolates showing resistance to III generation cephalosporins were tested for ESBL production by using Double Disk Synergy Test (DDST) as per CLSI guidelines.[11]

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*.[5] 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 uses to assess the hypermucoviscous phenotypic expression among uropathogenic strains of *K. pneumoniae*.[12] Isolates of *K. pneumoniae* were subcultured overnight on 5% sheep blood agar at 37^0C. They were considered positive for the hypermucoviscosity 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

Our study showed increased resistance to ciprofloxacin, whereas the bacterial isolates were sensitive to amoxicillin-clavulanic acid, piperacillin-tazobactam and imepenem by Kirby Bauer disc diffusion method with antibiogram pattern of *K. pneumoniae* isolates (Table 1). In the samples, 26 and 31 strains were resistant to Ceftazidime and Cefotaxime respectively. This study also revealed ESBL production among 19 (23.7%) isolates as confirmed by DDST (Fig. 1).

Antibiogram study pattern of ESBL producing and non ESBL producing uropathogenic *K. pneumoniae* isolates are shown in Table 2. Hypermucoviscous phenotype were seen in 78(97.5%) of the *K. pneumoniae* isolates. Among the total 61 strains, 59(96.7%) were positive for hypermucoviscosity by non-ESBL producing *K. pneumoniae*. All the 19 ESBL producing *K. pneumoniae* strains were positive for string test. Positive string test for hypermucoviscosity by clinical strains of uropathogenic isolates of *K. pneumoniae* is shown in Fig. 2.

The most common opportunistic pathogen which are associated with both community and hospital-acquired infections including UTIs is *K. pneumoniae*.[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 are seen more with hypermucoviscous bacterial strains when compared to bacteria that lack hypercapsule.[(14)] Various factor that offer resistance to antibiotics are due to restricted penetration of antibiotics via exoplolysaccharide capsule, development of drug resistance genes and reduction of bacterial growth.[15]

Among the total samples, about 97.5% of the uropathgenic *K. pneumoniae* isolates showed hypermucoviscosity with has correlated with an earlier study done by Zhang *et al.*, which has shown clear association with hypervirulent strains (74.7%) of *K. pneumoniae*.[16] 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*.[17] High prevalence of hypermucoviscosity and positive correlation between ESBL production and hypermucoviscosity among hypervirulent strains of uropathogenic isolates *K. pneumoniae* were seen in study reported by Khaertynov *et al* and Hennequin *et al* respectively.[18,19]

Table 1: Antibiogram pattern of *K. pneumoniae* isolates from urine samples (N=80):

Antibiotics	Sensitive (S) n (%)	Intermediate (I) n (%)	Resistant (R) n (%)
Ampicillin	0	0	80 (100)
Amikacin	56 (70)	2 (2.5)	22 (27.5)
Ceftazidime	53 (66.25)	1 (1.25)	26 (32.5)
Cefotaxime	47 (58.75)	2 (2.5)	31 (38.75)
Cefpodoxime	52 (65)	1 (1.25)	27 (33.75)
Ciprofloxacin	23 (28.75)	1 (1.25)	56 (70)

Netilmicin	56 (70)	2 (2.5)	22 (27.5)
Piperacillin	60 (75)	1 (1.25)	19 23.76)
Pip-tazobactum	67 (83.75)	1 (1.25)	12 (15)
Amoxi-clav	68 (85)	0	12 (15)
Imipenem	71 (88.76)	0	9 (11.25)

Table 2: Antibiogram pattern of ESBL and non-ESBL producing uropathogenic *K. pneumoniae* isolates (N=80)

Antibiotic tested (disc concentration)	Susceptibility of non-ESBL producing *K. pneumoniae* (n=61)			Susceptibility of ESBL producing *K. pneumoniae* (n=19)		
	S n (%)	I n (%)	R n (%)	S n (%)	I n (%)	R n (%)
Ceftazidime (30 µg)	50 (82.0)	3 (4.9)	8 (13.1)	0 (0)	0(0)	19(100)
Cefotaxime (30 µg)	45 (73.7)	2 (3.3)	14 (23.0)	0 (0)	0(0)	19(100)
Cefpodoxime (10 µg)	52 (85.2)	2 (3.3)	7 (11.5)	0 (0)	0(0)	19(100)
Amikacin (30 µg)	41 (67.2)	2 (3.3)	18 (29.5)	11 (57.9)	1(5.3)	7(36.8)
Netilmicin (30 µg)	41 (67.2)	1 (1.7)	19 (31.1)	10 (52.6)	1(5.3)	8(42.1)
Ciprofloxacin (5 µg)	17 (36.15)	2 (3.3)	42 (61.44)	5 (26.3)	0(0)	14(73.7)
Ampicillin (10 µg)	0 (0)	0 (0)	13 (100)	0 (0)	0(0)	12(100)
Piperacillin (100 µg)	41 (67.2)	0 (0)	20 (32.8)	10 (52.6)	1(5.3)	8(42.1)
Piperacillin-tazobactum (100/10 µg)	48 (78.6)	1 (1.7)	12 (19.7)	16 (84.2)	0(0)	3(15.8)
Amoxicillin-clavulanic acid (20/10 µg)	47 (77.0)	1 (1.7)	13 (21.3)	14 (73.7)	1(5.3)	4(21.0)
Imipenem (10 µg)	55 (90.2)	0 (0)	6 (9.8)	16 (84.2)	0(0)	3(15.8)
S- Sensitive, I- Intermediate, R- Resistant.						

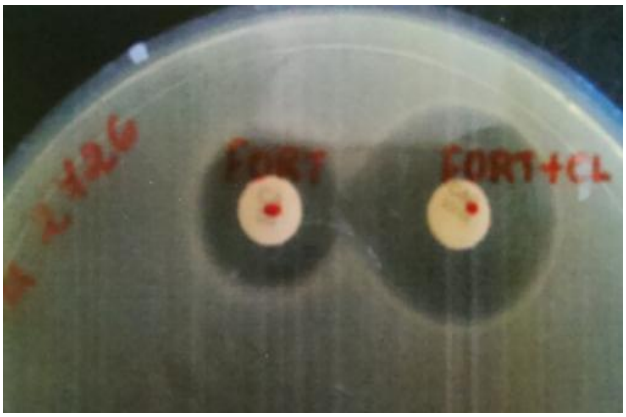

Fig. 1: Double disk synergy test showing ESBL production among *K. pneumoniae*

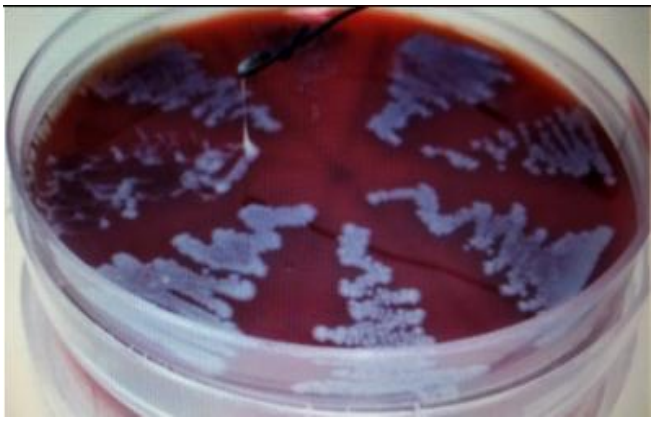

Fig. 2: Positive string test showing hypermucoviscosity among strains of *K. pneumonia*

Conclusion

Our study showed that many strains of *K. pneumoniae* isolated from samples of urine were strongly resistant to III generation Cephalosporins. Our study highlights a positive correlation and association between antibiotic drug resistance and hypermucoviscosity by ESBL producing isolates of *K. pneumoniae*.

Conflict of Interest: None.

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