Prevalence and Antibiotic susceptibility pattern among Klebsiella isolates from patients attending a tertiary care hospital

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Abstract

Introduction: *Klebsiella* is an important opportunistic pathogen that is found to be the causative of hospital acquired pneumonia, urinary tract infections, skin and soft tissue infections and bacteremia. It has developed resistance to most of the currently used antibiotics and even resistance to higher antibiotics like carbapenems is on the rise. Hospital acquired infections caused by multidrug resistant strains of *Klebsiella* species are associated with high rates of morbidity and mortality.

Aim: To assess the diversity and state of resistance of *K. pneumoniae* to antibiotics in a tertiary care centre**.**

Materials and Methods: *Klebsiella* strains were isolated from clinical samples received in microbiology laboratory in a tertiary care centre and were identified by standard biochemical techniques. 368 Klebsiella strains were included in the study. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method following CLSI guidelines. Quality control was performed by using standard strain *E. coli* ATCC 25922.

Results: 13.16% of strains isolated from clinical samples were found to be Klebsiella species. Out of 368 *Klebsiella* strains 42% were resistant to Ceftriaxone, 46% to Cotrimoxazole, 52% to Amoxycillin-clavulanicacid. Resistance to Meropenem & Piperacillin-Tazobactum was 10% & 12% respectively.

Conclusion: Detection of drug resistance mechanisms, formulation of a good antibiotic policy and a proper antibiotic stewardship program should be incorporated to combat emerging multidrug resistance which is a global threat.

Keywords: Multidrug resistance, *Klebsiella*, Kirby-Bauer disk diffusion method.

Introduction

Klebsiella is an important opportunistic pathogen that is found to be the causative of hospital acquired pneumonia, urinary tract infections, skin and soft tissue infections and bacteremia.[1] *Klebsiella* is a Gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the family *Enterobacteriaceae*. It has various virulence factors such as polysaccharide capsule, endotoxin, cell wall receptors and iron-scavenging systems.[2] *Klebsiella pneumoniae* is a superbug, which complicates treatment of infections worldwide & limits therapeutic options. Hospital acquired infections caused by multidrug resistant strains of *Klebsiella* species are associated with high rates of morbidity and mortality.[3,4]

In the United States, 3-7% of nosocomial bacterial infections are caused by Klebsiella species.[5] Prevalence of *K. pneumoniae* infections has been reported to be 20.96% in Southern India. Antibiotics with broad spectrum of action such as, cephalosporins, fluoroquinolones, aminoglycosides and carbapenems are the treatment options for infections caused by *Klebsiella* strains.[6] *K.pneumoniae* has developed resistance to most of the currently used antibiotics and even resistance to higher antibiotics like carbapenems is on the rise.[7]

Resistance to beta-lactams has been reported to be associated with ESβL,[8] which hydrolyze oxyimino beta-lactams like cefotaxime, ceftriaxone, ceftazidime and monobactams, but have no effect on cephamycins, carbapenems and related compounds.[4] A recent report, from a hospital in rural Southern India, described a high prevalence of ESβL producers,[9] while other report showed 96.1% [4] Within the *Enterobacteriaceae* family, carbapenem resistant *Klebsiella pneumoniae* strains have recently been noted in many parts of the world. Although KPC β-lactamases are mostly found in *K. pneumoniae*, they can also be found in *Enterobacter* and *Salmonella* species.[10] The aim of this study was to assess the diversity and state of resistance of *K. pneumoniae* to antibiotics in a tertiary care hospital.

Materials and Methods

The study was conducted at Sree Balaji Medical College & Hospital, Chrompet, Chennai for a period of 6 months from June 2017 to December 2017. Institutional ethical clearance was obtained.

Klebsiella strains were isolated from numerous clinical samples like pus, urine, blood, sputum, tissue in a teritiary care centre and were identified by Gram stain, colony morphology and standard biochemical techniques. 368 Klebsiella strains were included in the study. Kirby-bauer disc diffusion method was performed to test antibiotic susceptibility pattern of the strains following CLSI guidelines.

2-3 well isolated colonies were suspended in 0.5 ml of sterile broth and the turbidity matched to 0.5 McFarland standard. Using a sterile cotton swab, the broth culture was swabbed on the sterility checked Mueller-Hinton agar plate and the antibiotic discs were placed and incubated at 37^0 C for 24 hours. ATCC

reference *E.coli* strain 25922 was used as control. The diameter of the zone of inhibition was measured and interpreted according to CLSI guidelines
The following antibiotic discs were used for testing:

Samples other than urine:
1. Ampicillin 10mcg
2. Co-trimoxazole – 25mcg.
3. Cefazolin – 30mcg.
4. Ceftriaxone – 30mcg.
5. Gentamicin – 10mcg
6. Ciprofloxacin 5mcg.
7. Amikacin 30 mcg
8. Cefuroxime - 30mcg
9. Amoxicillin clavulanate – 20/10 mcg
10. Piperacillin tazobactum – 100/10mcg
11. Imipenem – 10 mcg
12. Meropenem – 10mcg

Urine samples:
1. Ampicillin 10mcg
2. Co-trimoxazole – 25mcg.
3. Cefazolin – 30mcg.
4. Ceftriaxone – 30mcg.
5. Gentamicin – 10mcg.
6. Nitrofuratoin – 300mcg.
7. Ciprofloxacin 5mcg.
8. Tobramycin 10 mcg
9. Cefuroxime - 30mcg
10. Amoxicillin clavulanate – 20/10 mcg
11. Piperacillin tazobactum – 100/10mcg
12. Imipenem – 10 mcg
13. Meropenem – 10mcg

The data obtained from the study were entered and analysed using SPSS windows version 14.0 software. Pearson's Chi square test was used to find significance of the results. The p value <0.05 is considered statistically significant.

Result

In this study the total no of sample analysed were 2796 of which *Klebsiella* isolates were 368 (13.16%). The various samples analyzed and the distribution of *Klebsiella* among them is described in Table 1.

Table 1: Distribution of sample among Klebsiella isolates

Type of sample	Total no of samples	No of *Klebsiella* isolates	Percentage of *Klebsiella* isolates
Urine	1290	156	12.09%
Sputum	486	80	16.46%
Wound swab/pus	588	108	18.36%
Blood	396	16	4.04%
Ear swab	24	4	16.66%
Tissue	12	4	33.33%

Among the various *Klebsiella* isolates the age distribution showed majority to be of adults (N=334) rather than paediatric age group (N=34). The percentage of males (58.9%) was comparatively higher than the females (41.03%). The distribution of age & sex is among various samples is given in Table 2.

Table 2: Age & Sex distribution among *Klebsiella* isolates

Sample	No of Adults	No of Paediatric sample	No of Male	Percentage of male	No of Female	Percentage of Female
Urine	136	20	72	46.15%	84	53.84%
Sputum	80	0	54	67.5%	26	0.012%
Wound swab/pus	98	10	68	62.96%	40	37.03%
Blood	12	4	16	100%	-	-
Ear swab	4	-	4	100%	-	-
Tissue	4	-	3	75%	1	25%

Among the two major species *Klebsiella pneumoniae* was found to be higher (66.25%) in sputum samples and *Klebsiella oxytoca* (57.35%) was found higher in Urine samples. But on the overview percentage of *Klebsiella pneumoniae* is higher among the various clinical samples. This has been depicted in Fig. 1.

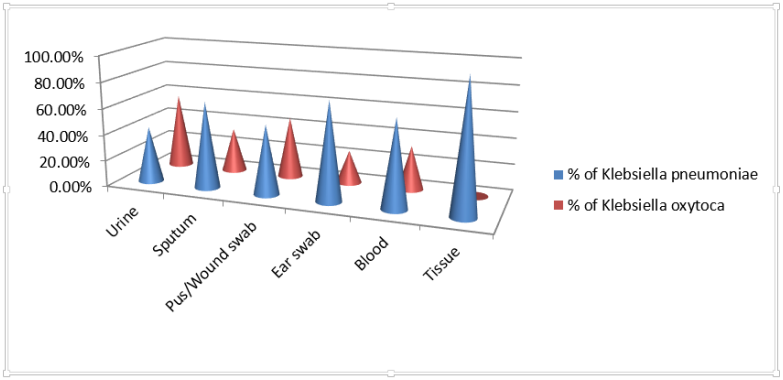

Fig. 1: Speciation among *Klebsiella* isolates

The susceptibility pattern of the various isolates of *Klebsiella* is depicted in Table 3. For urine samples alone susceptibility of Nitrofurantoin (300 µg) was additionally done and readings interpreted.

Table 3: Antibiogram of *Klebsiella* isolates in clinical samples (other than urine) (N=368)

No	Antimicrobial agent	Disk content	No of sensitive Isolates	Percentage of sensitive Isolates	No of Resistant Isolates	Percentage of Resistant Isolates
1	Ampicillin	10 µg	0	0	368	100
2	Cefuroxime	30 µg	133	36	235	64
3	Ceftriaxone	30 µg	214	58	154	42
4	Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	199	54	169	46
5	Amikacin	30µg	15	69	353	31
6	Piperacillin,Tazobactem	100/10 µg	324	88	44	12
7	Imipenem	10 µg	324	88	44	12
8	Meropenem	10 µg	332	90	36	10
9	Gentamycin	10 µg	207	56	161	44
10	Amoxi-clav (nonmeningitis)	20/10 µg	177	48	191	52
11	Cefazolin	30 µg	100	27	268	73
12	Ciprofloxacin	5 µg	199	54	169	46
13	Nitrofurantoin (For urine samples alone) (N=136)	300mcg	60	44	76	56

Discussion

In this study the total no of sample analysed were 2796 of which *Klebsiella* isolates were 368.The prevalence of *Klebsiella* in this study was 13.16%. Another study by priyadarshini et al showed 7.1% prevalence among Klebsiella species.[11] Prevalence of ESBL producing *Klebsiella* was found to be 36.0% in a study by Shireen et al.[12] In India, prevalence of ESBL producing *Klebsiella* spp. is reported varying from 6% to 87.%.[11]

Among the various *Klebsiella* isolates the age distribution showed majority to be of adults (N=334) rather than paediatric age group (N=34).A study conducted in Italy showed 58% of subjects to be of more than 60 years of age.[13] Another study conducted by Shireen rana et al showed majority of the subjects to be between 31-40 years of age. A recent study in South India shows maximum samples to be between 40-60 years of age.[12] A study by *Susethira et al* showed maximum age group to be between 41-50 years.[14]

Our study showed the percentage of males (58.9%) was comparatively higher than the females (41.03%). Another study showed similar findings where males (n=68) were comparatively more than females (n=53).[11] In contrast another study showed females(n=298) to be more than males(n=280).[12]

In our study, *Klebsiella pneumoniae* isolates showed maximum resistance to Ampicillin (100%) which is comparable to E Aktas, N Yigit, H Yazgi *et al* and *Susethira et al* showed 100% resistance to Ampicillin.[14,15] A study by Shireen rana et al showed similar high resistance pattern to Ampicillin among the ESBL producers.[12] Another study conducted by Manjula et al which showed resistance of (75.6%).[11]

Our study showed 53% susceptibility to Quinolones which is quite less than a study conducted by E Aktas, N Yigit, H Yazgi *et al* which showed 92.5% of susceptibility.[15]. Another study showed 95.3% susceptibility to quinolones.[13] A study by Shireen rana et al showed 45.6% resistance pattern among ESBL producing and 39.1% among non ESBL producing *Klebsiella pneumoniae*.[12] A similar study showed 88.8% (ESBL producer) & 68.8% (Non ESBL producer) of resistance pattern to quinolones.[11]

Second and third generation cephalosporins are the common drugs of choice for *Klebsiella pneumoniae* infections.[4] Our study showed a higher rate of resistance (64%) to second generation than third generation cephalosporins (42%). In contrast some studies show a very low percentage of resistance (10%).[15] A study by Manjula N G et al showed 63.4% of resistance which is similar to our study.[4] A remarkable increase of 77.7% resistance of third generation cephalosporins is seen among ESBL producers.[11] In contrast a recent study in 2016 reported only 30.2% resistance among the ESBL producing *Klebsiella pneumoniae*.[12]

Carbapenem resistance rate in our study is around 10-12% found to be increasing compared to earlier studies that show nil resistance.[12,4,15] Some studies show a low percentage in contrast like 3.7%[11] and 3.3%.[16] A study in North India showed an alarming rate of 30% resistance among gram negative isolates.[17] A study conducted by AIIMS showed a very high carbapenem resistance rate of 69% .[17] Due to increase in KPC-producing bacteria, clinicians are becoming dependent on polymyxins and tigecycline for treatment of these infections.[18]

Piperacillin-tazobactum resistance in this study was 12% which is in parallel to another study by A Shetalov et al which reported 12.5% resistance to Piperacillin-tazobactum among *Klebsiella pneumoniae* isolates. A study among ESBL producing Klebsiella showed 26% and 3% resistance among non ESBL producers.[1] A similar study among ESBL & non ESBL producers showed a very high rate of 96.2% &55.9%.[14] Another study showed 39% resistance which is similar to our study.[4]

Resistance to Amoxycillin-clavulanic acid in our study was 52% which is comparable to a study conducted among ESBL producers where 65% of resistance was seen.[12] Another study conducted in 2010 itself shows 44.4% resistance to Amoxy-clav.[19]

Certain studies showed resistance to Nitrofurantoin to be as low as 18-19%.[12] and some showed resistance to be quite high (80%).[4] Another study conducted at 2010 shows 53.08% which is comparable to our study with resistance pattern of 56%.[19]

Conclusion

The prevalence of *Klebsiella* spp in our institute was 13.6% and was found to be resistant to many antibiotics. Hence formulation of a good antibiotic policy and detection of drug resistance mechanisms should be done by all laboratories. A proper antibiotic stewardship program should be incorporated after consulting medical and surgical departments. This helps us to identify and combat emerging multi drug resistance.

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