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Occurrence and antibiotic susceptibility pattern of *Klebsiella species* isolated from different clinical samples in a tertiary care hospital

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Abstract

Background

Klebsiella spp. are common Gram-negative pathogens, frequently encountered in nosocomial infections. The present study was carried out to evaluate the prevalence and development of resistance in *Klebsiella spp* against commonly used antibiotics.

Materials and methods

Klebsiella spp were isolated from various clinical samples such as Miscellaneous (733 which includes sputum, High vaginal swab, Drainage Tube, Bronchial Wash, Stool, eye swab, throat swab, pus) and urine (338) were collected from January 2015 to December 2015 in SSSMCRI. This bacterial species were characterized and identified according to standard bacteriological methods, Gram Staining and biochemical tests. Antimicrobial susceptibility test was performed by Kirby – Bauer disk diffusion method to determine the antibiotic resistance pattern.

Result

Klebsiella spp. were isolated from 733 miscellaneous samples in which 60% were *Klebsiella pneumoniae* and 40% were *Klebsiella oxytoca* whereas from 338 urine samples *Klebsiella pneumonia* 56% were more prevalence than *Klebsiella oxytoca* 44%. Our findings specify that most of the *Klebsiella* infections were caused by *K. pneumoniae* followed by *Klebsiella oxytoca*. Hence *K. pneumoniae* is the most common etiologic agent of nosocomial infection.

Conclusion

In the present study, most of the isolates of *Klebsiella spp* were found to have intrinsic resistance to ampicillin, Cefazolin and Nitrofurantoin in both the samples. But also we proved and recommended Amikacin and Imipenem are the most effective antibiotics being 100% and 96% effective against *K. pneumoniae* strains.

Keywords: Antimicrobial Susceptibility, Clinical samples, *Klebsiella pneumoniae, Klebsiella oxytoca*

1. Introduction

Klebsiella spp. is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family and reported worldwide of all the pathogenic *klebsiella species*, *k. pneumoniae* is the most prevalent and clinically important species. They are the most common organism found in hospitalised patients and is rod shaped bacterium found in the normal flora of the mouth, skin and intestines. *Klebsiella spp*. are known as a cause of community-acquired pneumonia, in particular associated with chronic alcoholism, with a high mortality rate, especially in the absence of adequate treatment [1, 2].

It causes nosocomial infections such as pneumonia (lung infections), wound infections, meningitis, abscesses, urinary tract infections and diarrhoea (Paterson *et al.*, 2007) [3]. It is more prone to cause nosocomial outbreaks because of their ability to disperse rapidly in the hospital environment especially in neonates and Immuno compromised individuals. In addition to being the primary cause of respiratory tract infection like Pneumonia, Rhinoscleroma, Ozaena, Sinusitis and Otitis, they also cause Urinary tract infection, Septicemia, Pyogenic infections and even infection of the alimentary tract [4] *klebsiella pneumoniae* tends to affect people with underlying disease such as alcoholism, diabetes, chronic lung disease. As an opportunistic pathogen, *Klebsiella spp*. occurs in patients with diabetes mellitus or chronic obstructive pulmonary disease. MDR strains are a major problem in nosocomial infections management [5] and moreover, similar resistance profiles were found in *Klebsiella spp* strains isolated from community infections [6] one of the universally recognized major reasons involved in the development of MDR is the widely misused antibiotic therapy [7].

The wide spread use of antibiotics in hospitals has led to the emergence of multidrug resistant organisms of low virulence like *Klebsiella* causing serious opportunistic infections. Over the last 15 years numerous outbreaks of infection with organisms producing extended spectrum β -lactamases (ESBLs) have been observed worldwide [8].

The indications that poor outcome occurs, when patients with serious infections due to ESBL-producing organisms are treated with antibiotics to which the organism is resistant.

The enzyme β-lactamases continue to be the leading cause of resistance to beta lactam antibiotics in gram-negative bacteria. In the recent years there has been an increased incidence and prevalence of ESBLs, enzymes that Hydrolyze and cause resistance to oxyimino-cephalosporins and Aztreonam [9].

In India, the reasons for development of antimicrobial resistance could be due to irrational use of antibiotics, over the counter availability of higher or broader antimicrobial agents, higher prevalence of infection and poor monitoring of antibiotic susceptibility in hospitals [10]. The aim of this study was to identify the *Klebsiella species* isolated from various

specimens and to determine the prevalence of antibiotic resistance.

2. Materials and Methods

2.1 Collection of Samples

Bacterial growth of Clinical samples were collected from 1071 patients of which Miscellaneous (733 which includes sputum, HVS, DT, BW, Stool, eye swab, throat swab, pus) and urine (338) were collected from January 2015 to December 2015 in SSSMCRI. The samples were processed in Microbiology Laboratory immediately after collection for analysis. The samples are obtained by informed consent from the patient were used for this study.

2.2 Examination of Samples

The samples collected were streaked on nutrient agar, Mac Conkey agar and Blood agar. The plates were incubated at 37°C for 24 h, where every specimen that yielded a pure heavy growth of bacterial pathogens were included in this study. Suspected bacterial species were characterized and identified according to standard bacteriological methods, Gram Stains and biochemical tests were done as described by Cheesbrough [11].

2.3 Antibiotic Susceptibility Test

After the identification of *Klebsiella species*, the Kirby-Bauer disk diffusion method was performed to determine the drug susceptibility pattern as followed by CLSI guidelines.(12)

4. Results

Table 1: Bacterial growth in Miscellaneous samples (Jan-Dec-2015)

	Sputum	HVS	Pus	DT	Throat	BW	Fluid	Eye Swab	Stool	Ear Swab	Total
January	28	19	10	1	-	-	-	-	-	-	58
February	30	11	8	2	-	-	-	-	-	-	51
March	31	6	10	1	2	-	-	1	-	-	51
April	50	12	12	2	1	-	1	-	1	-	79
May	38	9	4	2	1	-	-	-	-	1	55
June	45	22	11	3	1	1	-	-	-	1	84
July	33	24	7	1	3	-	-	1	-	-	69
August	56	17	5	-	1	1	2	-	-	-	82
September	28	16	12	2	2	2	-	4	-	-	66
October	16	9	12	2	-	2	1	1	-	-	43
November	19	11	6	4	1	1	-	1	-	1	44
December	18	22	5	2	3	-	-	-	1	-	51
Total	392	178	102	22	15	7	4	8	2	3	733
Percentage %	53%	24%	14%	3%	2%	1%	1%	1%	0.5%	0.5%	100%
Male = 391 = 53% Female= 342 = 47%					*Klebsiella oxytoca* =290 = 40% *Klebsiella pneumoniae* = 443 = 60%						

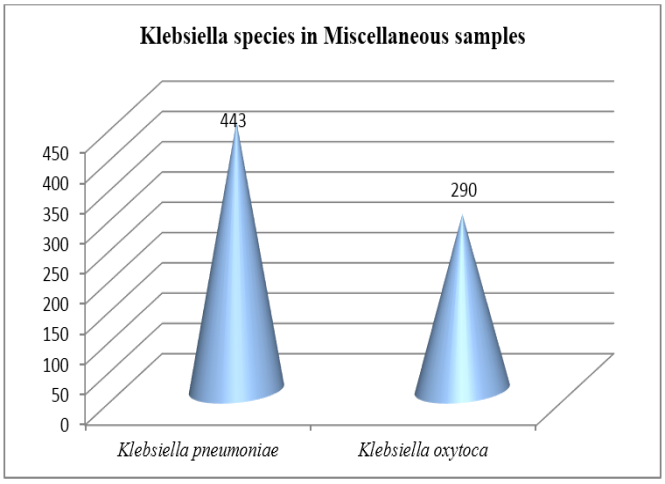

Fig 1: Klebsiella species in miscellaneous samples

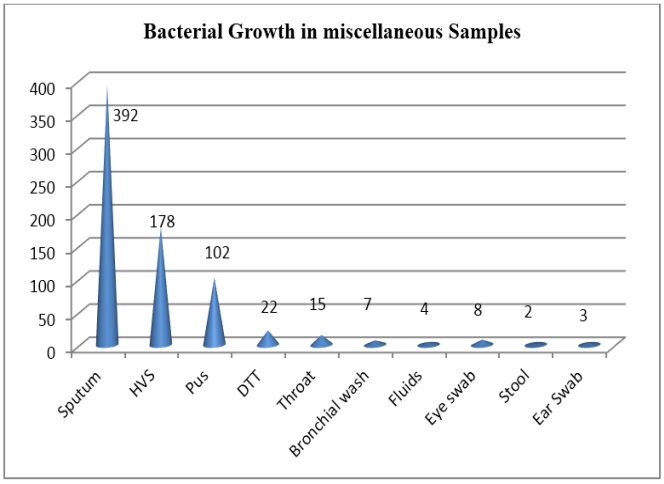

Fig 2: Bacterial growth in miscellaneous samples

Table 2: *Klebsiella pneumoniae* growth in Urine Culture

Duration	Male	Female	No. of organisms isolated
January	6	7	13
February	5	7	12
March	5	4	9
April	6	5	11
May	8	10	18
June	9	12	21
July	12	4	16
August	4	6	10
September	7	6	13
October	5	11	16
November	6	6	12
December	18	20	38
Total	91	98	189
Percentage %	48%	52%	100%

Table 3: *Klebsiella oxytoca* growth in Urine Culture

Duration	Male	Female	No. of organisms isolated
January	4	5	9
February	3	11	14
March	7	3	10
April	2	5	7
May	2	6	8
June	9	3	12
July	9	14	23
August	6	5	11
September	4	2	6
October	7	9	16
November	5	3	8
December	8	17	25
Total	66	83	149
Percentage %	44%	56%	100

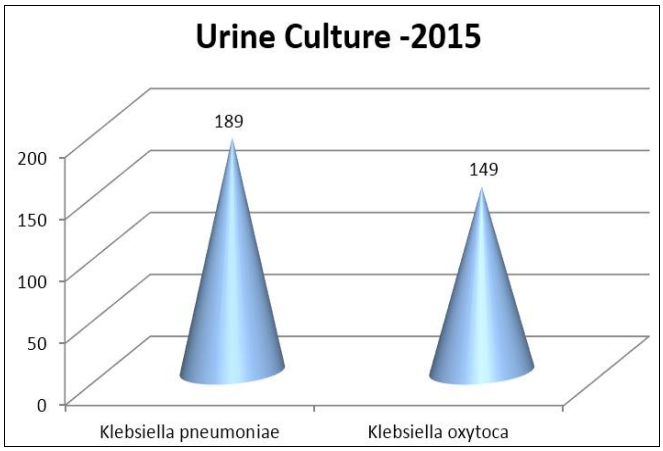

Fig 3: *Klebsiella* species in urine culture

Table 4: Antibiotic Sensitivity pattern for Miscellaneous (733) Samples

Antibiotic Name	No. of Sensitive Drugs	Sensitive (%)	No. of Resistant Drugs	Resistant (%)
Ampicillin	-	-	733	100%
Cefazolin	-	-	733	100%
Cefotaxime	570	78%	163	22%
Ceftazidime	622	85%	111	15%
Ciprofloxacin	598	81%	135	19%
Imipenem	702	96%	31	4%
Amikacin	659	90%	74	10%
Gentamycin	615	84%	118	16%

Table 5: Antibiotic Sensitivity pattern for Urine (338) Samples

Antibiotic Name	No. of Sensitive Drugs	Sensitive (%)	No. of Resistant Drugs	Resistant (%)
Nitrofurantoin	-	-	338	100%
Norfloxacin	302	89%	36	11%
Nalidizic Acid	250	74%	88	26%
Amikacin	338	100%	-	-
Ciprofloxacin	298	88%	40	12%
Gentamycin	299	89%	39	11%
Ampicillin	-	-	338	100%
Cefazolin	-	-	338	100%

In this study a total number of 1071 non-repetitive isolates from various clinical samples (miscellaneous (733) and urine (338)) were collected from the month of Jan-2015 to Dec-2015 in SSSMC & RI.

In Table. 1 & Fig 1: *Klebsiella* spp. Were isolated from 733 miscellaneous samples in which 60% were *Klebsiella pneumoniae* and 40% were *Klebsiella oxytoca.* Of that 733 *Klebsiella* isolates 391 samples were found in males (53%) and 342 samples were from females (47%). The highest prevalence of *Klebsiella spp* were found in miscellaneous samples which includes Sputum(53%) followed by HVS(24%), Pus(14%), DT(3%), Throat(2%), 1% for BW, Eye swab, Fluid and 0.5% for Stool and Ear swab as shown in the Fig 2.

In Table. 2 & Fig 3: *Klebsiella pneumonia* were isolated from 189 urine samples in which 91 samples were from males (48%) and 98 samples from females (52%) as shown in Table. 3. *Klebsiella oxytoca* isolated from 149 urine samples. Among that 149 urine samples, 66 from males (44%) and 83 from females (56%). From the above tables (Table. 2 & Table. 3) *Klebsiella pneumoniae* 56% are more prevalence than *Klebsiella oxytoca* 44%.

These bacteria were tested for their susceptibility in miscellaneous samples against different antibiotics like Ampicillin, Cefazolin, Cefotaxime, Ceftazidime, Ciprofloxacin, Imipenem, Amikacin, Gentamycin among which Ampicillin and Cefazolin showed 100% intrinsic resistance to *Klebisella species* and other antibiotics are sensitivity to *Klebsiella species* as shown in the Table 4. Where as in Table 5, antibiotic susceptibility pattern of *klebisella species* were tested for urine samples, which includes Nitrofurantoin, Norfloxacin, Nalidixic Acid, Amikacin, Ciprofloxacin, Gentamycin, Ampicillin, Cefazolin. Of which Nitrofurantoin, Ampicillin, Cefazolin showed 100% resistant to *klebsiella spp* and remaining drugs are sensitive to the bacterial species.

5. Discussion

Hence, in this study we investigated the occurrence of *Klebsiella species* in various clinical samples collected from Jan 2015 to Dec 2015 in SSSMC & RI.

Our findings specify that most of the *Klebsiella* infections are caused by *K. pneumoniae* followed by *Klebsiella oxytoca*. Our study shows that 60*% K. pneumoniae* and 40% *K. oxytoca* were isolated in miscellaneous samples. Of that 733 *Klebsiella* isolates 391 samples were found in males (53%) and 342 samples were from females (47%).

Out of 338 urine samples, *Klebsiella pneumoniae* were found to be 56% in 189 samples, in which 91 samples were from males (48%) and 98 samples from females (52%), whereas *K. oxytoca* were found to be 44% in 149 urine samples, among which 66 from males (44%) and 83 from females (56%). In our study *K. pneumoniae* is the most common etiologic agent of nosocomial infection.

Our finding therefore indicates that *Klebsiella* infestation is more likely related to miscellaneous samples which includes Sputum (53%) followed by HVS (24%), Pus (14%), DT (3%), Throat(2%) , 1% for BW, Eye swab, Fluid and 0.5% for Stool and Ear swab than the UTI samples. Our results are contrary to Philip O. Orhue *et al.*, [13] where their isolation rate of *Klebsiella spp* was said to be more from urine samples.

Our finding resembles to Al-Gerir, [14] who also reported that 65% prevalence rate of *K. pneumoniae* and 34.5% prevalence rate of *K. oxytoca* in teaching hospitals in Mosul Iraq**.** But in contrast to our study Rasool *et al.* (15) showed high prevalence of *K. oxytoca* (52%) followed by *K. pneumoniae* (42%) among the clinical isolates of *Klebsiella*, collected from various hospitals and laboratories of Karachi city in Pakistan.

On antibiotics susceptibility, our results showed *Klebsiella spp* from miscellaneous samples were found highly sensitivity to Cefotaxime (78%), Ceftazidime (85%), Ciprofloxacin (81%), Imipenem (96%), Amikacin (90%), Gentamycin (84%) whereas 100% resistant to Ampicillin and Cefazolin.

In the same way *klebsiella spp* isolated from urine samples showed maximum sensitivity pattetrn to Norfloxacin (89%), Nalidixic Acid (74%), Ciprofloxacin (88%), Gentamycin (89%) and Amikacin (100%). At the same time, Nitrofurantoin, Ampicillin and Cefazolin showed 100% resistant to the *klebsiella species*.

The antibiotics susceptibility observed in our study were almost similar when compared to the findings by Romanus and Egwu [16] who reported higher susceptibility profiles to Imipenem (100%), Amikacin (100%), Cefoxitin (99.4%), Aztreonam (98%), Ceftazidime (98%), Cefotaxime (96.7%), Amoxicillin/Clavulanic acid (96%), Ciprofloxacin (96%), Tobramycin (93.3%), Kanamycin (90%), Cefuroxime (86.7%), Gentamicin (76%) and Ampicillin (5%).

In the present study, most of the isolates of *Klebsiella pneumoniae* were found to have intrinsic resistance to Ampicillin, Cephazolin and Nitrofurantoin in both the samples.

Farmer, 1999 (17) proved the resistance of *K. pneumoniae* to Ampicillin is not surprising because of the intrinsic structure of *K. pneumoniae* cell. *K. pneumoniae* being a gram-negative bacterium belonging to the enterobactericeae family are known for their high resistance to various antibiotics because of the presence of series of antibiotic resistance genes in their genetic makeup which are easily transferred horizontally to other bacteria SPPS (Piddock, 1996) [18].

This result is similar to that reported by Iroha Ifeanyichukwu *et al.*, who reported *Klebsiella spps*. resistance to Ampicillin.(19) Similarly to our study, Otilia Banu, Coralia Bleotu et al.,(20) observed the increasing prevalence of infections with Klebsiella spp, among their findings of resistant strains, the highest resistance rates were recorded for the first generation cephalosporins (cefazolin - 85%).

In many studies researchers reported that resistant of Klebsiella pneumoniae to routinely used antibiotics is one of the important problems in our country. Like our study, Nastaran Langari Zadeh, (21)had tested antibiotic resistance for 7655 urine samples, The highest antibiotic resistance rate belonged to Amoxicillin (98.6 %) followed by co-trimoxazole, nitrofurantoin.

Klebsiella has been associated with different types of infections. One of the important aspects of *Klebsiella* is the emergence of multi-drug resistant strains particularly those involved in nosocomial infections. In our study Amikacin and Imipenem are the most effective antibiotics being 100% and 96% effective against *K. pneumoniae* strains.

6. Conclusion

The present study reveals the occurrence of infections due to *Klebsiella spp* from different clinical specimens are susceptible to a wide range of antibiotics like Amikacin and Imipenem but are highly resistant to Ampicillin, Cefazolin and Nitrofurantoin. Multidrug resistant bacteria are emerging worldwide which causes major public health problems and challenges to health care. But we recommend Amikacin and Imipenem are the most effective antibiotics being 100% and 96% effective against *K. pneumoniae* strains. We should follow control practices like hand washing, safety measures, isolation of infected patients, experimental use of antibiotics, antibiotic cycling and health education would help to control the emergence and spread of ESBL producing bacteria.

7. Acknowledgment

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