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Isolation, Identification and Antibiotic Sensitivity Pattern of *Klebsiella* species Isolated from Various Clinical Specimens in a Medical College and Hospital

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A B S T R A C T

Klebsiella species are the common cause of community and hospital acquired infections worldwide. *Klebsiella* species are difficult to identify because of improper or insufficient method of detection and identification and one of the important aspects of *Klebsiella* associated infections is the emergence of multi-drug resistant strains particularly those involved in nosocomial diseases. This study was done to determine the isolation Identification and antibiotic sensitivity pattern of *Klebsiella* species. A total of 75 isolates of *Klebsiella* species are identified by standard microbiological techniques and their antibiotic sensitivity pattern was obtained by Kirby-Bauer disc diffusion method. Out of 75 isolates 64 (85.33%) was *Klebsiella pneumoniae* and 11 (14.66%) was *Klebsiella oxytoca*. Out of 75 isolates, maximum sensitivity was shown with polymyxin-B and colistin i.c 100% followed by meropenem 81.33%, imipenem 62.66%, piperacillin/tazobactam 61.33%, 56% sensitivity was shown by amikacin and gentamycin both, ofloxacin 46.66%, cefuroxime 45.33%, ciprofloxacin 42.66%, ampicillin 36%, cefepime 29.33% and minimum sensitivity shown by cefotaxime i.c 17.33%.

Introduction

Friedlander (In 1883) isolated a capsulated bacillus from the lungs of patients who died of *pneumonia* (1). The microorganism was named after him as Friedlander's bacillus. Later on this organism was given the generic name of *Klebsiella*, and is reported worldwide (2). *Klebsiella* is a Gram negative, non motile, encapsulated, lactose fermenting facultative anaerobes belonging to the Enterobacteriaceae family (1,2). Seven species of *Klebsiella* – *K. pneumoniae, K. oxytoca* and *K. granulomatis* are associated with human illness; *K. ozaenae*

and *K. rhinosleromatis* are associated with specific diseases; *K. planticola* (SYN.K. trevisanii), *K. terrigena* and *K. ornithinolytica* are now transferred to a new genus *Raoultella* (3). Among the all *Klebsiella* species *Klebsiella pneumonia* and *Klebsiella oxytoca* are the most commonly isolated *Klebsiella* species in hospital infections (31). The leading *Klebsiella* species giving rise to infection in humans are *Klebsiella pneumoniae* and *Klebsiella oxytoca* (7).

It is the second most popular member of aerobic bacterial flora of the human intestine (1). *Klebsiella* have been considered as one of the major opportunistic human pathogens that causes a range of clinical diseases including *pneumonia*, sepsis, neonatel septicemia, wound infections, urinary tract infection, bacteremia in immunocompromised human, wound infection, infection in intensive care unit (1,2). *Klebsiella pneumonia* is the most common cause of hospital respiratory infections in premature neonates in Intensive Care Units and the second most common cause of urinary tract infections and bacteraemia (9). On the other hand *Klebsiella oxytoca* can also be cultured from skin, mucous membranes, oropharynx, intestines and other tissues in healthy humans. It is thought to be an opportunistic pathogen responsible for nosocomial infections in hospitalized patients and a causative organism for antibiotic-associated hemorrhagic colitis (AAHC) in humans. *Klebsiella oxytoca* may also be especially pathogenic due to the secretion of cytotoxin (10).

Recently, World Health Organization also warned the community that multidrug resistant bacteria are emerging worldwide which is a big challenge to healthcare. If we don't take immediate action then only handful antibiotics will be left to cure diseases (11). Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate by using available antibiotics. Moreover, extensive use of broad-spectrum antibiotics in hospitalized patients has led to increased prevalence of *Klebsiella* species as well as development of multidrug resistant strains of *Klebsiella* species.

Microorganisms are the concealed enemies to the mankind and cause a very profound damage in human body as well as other living organism. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs or antibiotics. There are a lot of antimicrobial drugs of which some are discovered or established (17). Antibiotics were considered to be the most effective therapeutic agents to combat microbial infections. But as a result of the overuse and injudicious use of antibiotics, these have been an emergence and spread of multidrug resistant strains among different groups of microorganisms. Infections resistant bacteria are emerging threat all over the world both as hospital acquired as well as community acquired microorganisms (12).

It is essential to understand the antimicrobial susceptibility pattern of *Klebsiella* which shows variation in different geographical settings, in order to implement effective control measures to prevent rapid spread of drug resistance (3). Years of antibiotic over-prescription and abuse on one hand and a decline in the development of novel antibiotics on the other have led to a tendency among physicians to shy away from prompt and aggressive prescription of the most commonly used drugs. (13, 14) This is especially the case with broad-spectrum antibiotics. The predicted changes in the pathogens and their occurrence make it highly advisable that empirical, first-line antibiotic treatment should be reviewed periodically in every regional tertiary medical centre. First-line antibiotic treatment should be reviewed periodically in every regional

tertiary medical centre (15,16). It is of great importance for the institution to know the local antibiotic resistance patterns in order to implement suitable infection control measures and develop a rational antibiotic policy with local recommendations for antibiotic use.

The correct identification of *Klebsiella* species is not easily accomplished in most clinical microbiology laboratories, because several species share a similar biochemical profile (4). The Microbiology laboratory plays a central role in the decision to choose a particular antimicrobial agent over others. First identification and isolation of the causative organism should be taken place in the microbiology laboratory. Once the microbial species causing the disease have been identified, a rational choice of the class of antibiotics are used for antibiotic sensitivity for the patient (5,6). Therefore this study was carried out with an aim to isolate& identify the pathogenic *Klebsiella* species along with their antibiotic sensitivity patterns from the various clinical specimens.

Materials and Methods

The study was conducted with the 75 isolates of *Klebsiella* species which was carried out from various clinical samples collected from outpatient and inpatients attending in National Institute of Medical Sciences and Research hospital, Jaipur on our study period from July to December 2018. Klebsiella isolates that are considered clinically relevant and all age group and both sex were included. Isolates that are considered as normal flora or commensals were disregarded and repeated isolates from same patients were also excluded.

Culture characteristic were observed on Blood agar and MacConkey's agar, gram stating, motility test and biochemical tests like Catalase, Indole production test, methyl Red test, Voges-Proskaur test, citrate utilization test, urease reaction test, triple sugar iron, sugar fermentation test (Glucose, Sucrose, Lactose, Mannitol) and LAO decarboxylation test (Lysine, Arginine, Ornithine) were performed.

Antimicrobial susceptibility testing was performed on Muller-Hinton agar using Kirby-Bauer disc diffusion method. The antibiotic discs were selected according to the protocol as recommended by Clinical laboratory Standards Institute (CLSI) (18).

Results and Discussion

Our study was carried out in the time period of July 2018 to December 2018.75 *Klebsiella* species were isolated from various clinical specimens of patients of all ages and both sexes attending various outpatients, inpatients at NIMS hospital.

Results analysed as per the clinical and laboratory findings from our hospital. Our statistical analysis carried out based on Demographic details of *Klebsiella* associated Samples analysed for various *Klebsiella* species.

Out of total 75 isolates of *Klebsiella* species, 48(64%) isolates from male while 27(36%) from female patients as shown in table 1 and graph 1.

As shown in table 2 and graph 2 out of total 75 isolates of *Klebsiella* species, 4(5.33%) isolated from age 0-10yrs, 11-20yrs 11(14.66%), 21-30yrs 8(10.66%), 31-40yrs 10(13.33%), 41-50 yrs 11 (14.66%), 51-60 yrs 15 (20%) and above 60 yrs 16 (21.33%).

Table 3 and graph 3 shows that *Klebsiella* species isolated maximum from urine samples i.c 18 (24%) followed by from ET 15(20%), from Pus 13 (17.33%), from Sputum 12(16%), from Blood 9(12%), from wound swab

3(4%), 2(2.66%) from both Swab and Ear swab and from Urethral Discharge 1(1.33%).

Out of total 75 isolates, 44 (58.66%) from IPD and 31 (41.33%) from OPD as shown in Table 4 and graph 4.

As shown in Table 5 and graph 5, Out of 75 isolates of *Klebsiella* species, maximum sensitivity was shown with Polymyxin-B and Colistin i.c. 100% followed by Meropenem 81.33%, Imipenem 62.66%, Piperacillin/ Tazobactam 61.33%, 56% sensitivity was shown by amikacin and gentamycin both, Ofloxacin 46.66%, Cefuroxime 45.33%, Ciprofloxacin 42.66%, Ampicillin 36%, Cefepime 29.33% and minimum sensitivity shown by Cefotaxime i.c 17.33%. On the other hand out of 18 isolates from urine sample Nitrofuratonin shows the maximum sensitivity i.c 61.11% comapare to that of Norfloxacin i.c 44.44% and maximum resistance shown against was Cefotaxime 82.66% followed by Cefepime 70.66%, Ampicillin 64%, Ciprofloxacin 57.33%, Cefuroxime 54.66%, Ofloxacin 53.66%, Amikacin 44%, Pipracilin/ Tazobactam 38.66%, Imipenem 37.33%, and minimum resistance shown by Meropenem i.c 18.66% and No resistance shown by both Polymyxin-B and Colistin.

Out of total 75 *Klebsiella* species 64 i.c 85.33% *Klebsiella pneumonia* and 11 i.c 14.66% *Klebsiella oxytoca* are isolated. On the other hand from various clinical specimens, *Klebsiella pneumonia*e in urine was 83.33%, Blood 88.88%, ET 86.66%%, Pus 76.92%, Sputum 91.66%, Swab 100%, Urethral Discharge 100%, Wound swab 66.66% and Ear swab 100%. whereas *Klebsiella oxytoca* in Urine was 16.66%, Blood 11.11%, ET 13.33%, Pus 23.07%, Sputum 8.33%, Wound swab 33.33% and No *Klebsiella oxytoca* were isolated from swab, Urethral Discharge and ear swab which were shown in the table 6 and graph 6.

Table.1 Sex wise distribution of *Klebsiella* species

Sex	No. of isolates(n=75)	Percentage %
Male	48	64%
Female	27	36%
Total	75	100%

Table.2 Age wise distribution of *Klebsiella* species

Age (yrs)	Isolates(n=75)	Percentage %
0-10	4	5.33%
11-20	11	14.66%
21-30	8	10.66%
31-40	10	13.33%
41-50	11	14.66%
51-60	15	20%
Above >60	16	21.33%
Total	75	100%

Table.3 Clinical samples from which *Klebsiella* species were isolated

Clinical specimens	No. of isolates (n=75)	Percentage (%)
Urine	18	24%
Blood	9	12%
ET	15	20%
Pus	13	17.33%
Sputum	12	16%
Swab	2	2.66%
Urethral Discharge	1	1.33%
Wound swab	3	4%
Ear Swab	2	2.66%

Table.4 Distribution of *Klebsiella* species among patients of IPD/OPD

Department	No. of isolates (n=75)	Percentage (%)
IPD	44	58.66%
OPD	31	41.33%
Total	75	100%

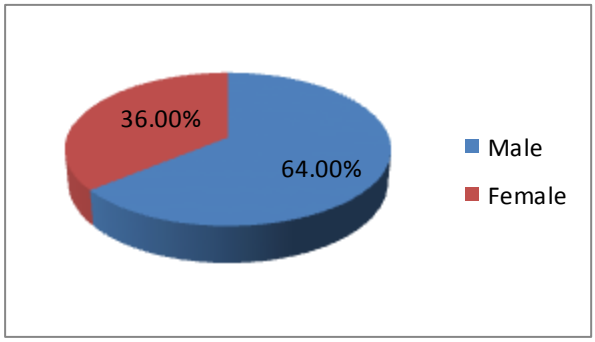
Table.5 Antibiotic sensitivity pattern of *Klebsiella* species

Antibiotic	Disc potency	Sensitive (S)	Percentage (%)	Resistant	Percentage (%)
AK	30µg	42	56%	33	44%
AMP	10µg	27	36%	48	64%
CPM	30µg	22	29.33%	53	70.66%
MRP	10µg	61	81.33%	14	18.66%
CIP	5µg	32	42.66%	43	57.33%
CTX	30µg	13	17.33%	62	82.66%
GEN	10µg	42	56%	33	44%
PIT	100/10µg	46	61.33%	29	38.66%
OF	5µg	35	46.66%	40	53.33%
CXM	30µg	34	45.33%	41	54.66%
IPM	10µg	47	62.66%	28	37.33%
PB	300units	75	100%	0	0%
CL	10µg	75	100%	0	0%
NIT	300µg	11	61.11%	7	38.88%
NX	10µg	8	44.44%	10	55.55%

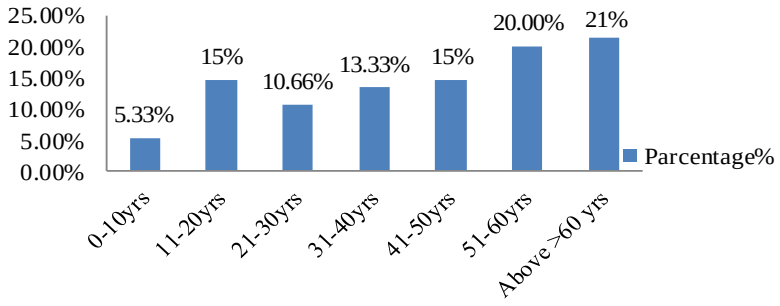
Table.6 Distribution of *Klebsiella pneumonia* and *Klebsiella oxytoca* in various clinical specimens

Specimens	No. isolates (n=75)	*Klebsiella pneumoniae*	Percentage (%)	*Klebsiella oxytoca*	Percentage (%)
Urine	18	15	83.33%	3	16.66%
Blood	9	8	88.88%	1	11.11%
ET	15	13	86.66%	2	13.33%
Pus	13	10	76.92%	3	23.07%
Sputum	12	11	91.66%	1	8.33%
Swab	2	2	100%	0	0
Urethral Discharge	1	1	100%	0	0
Wound Swab	3	2	66.66%	1	33.33%
Ear swab	2	2	100%	0	0
Total	75	64	85.33%	11	14.66%

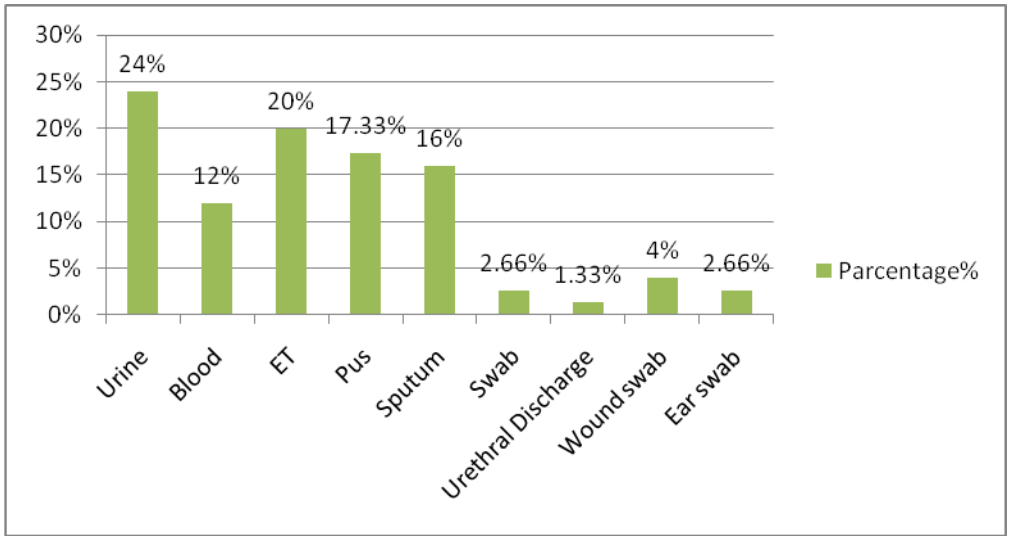
Graph.1 Sex wise distribution of *Klebsiella* species



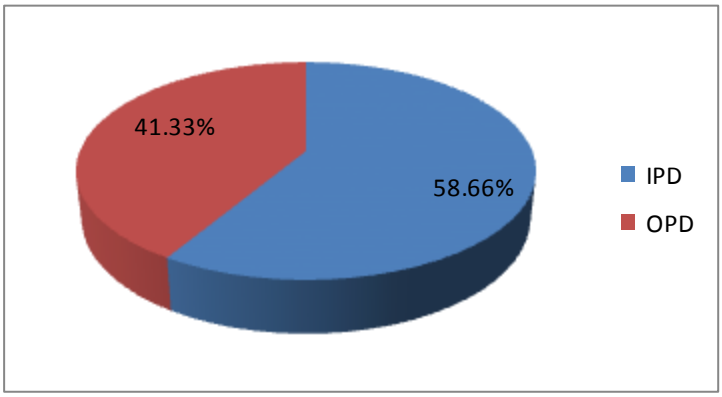
Graph.2 Age wise distribution of *Klebsiella* species



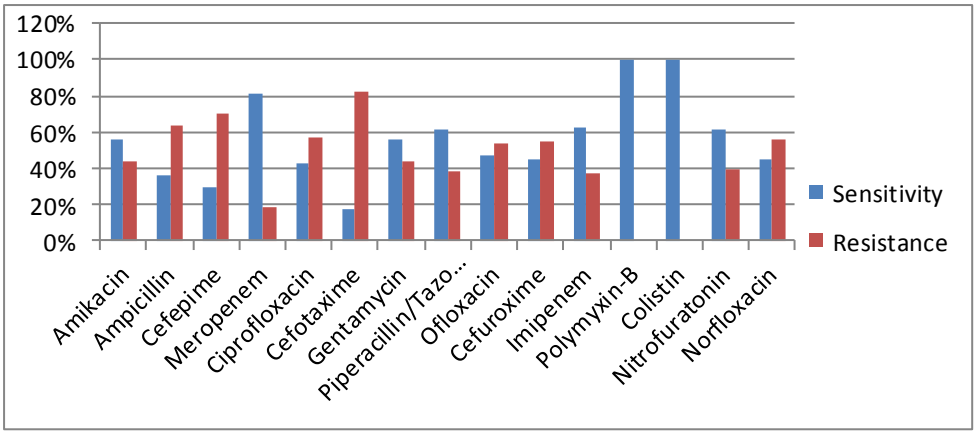
Graph.3 Clinical specimens from which *Klebsiella* species are isolates



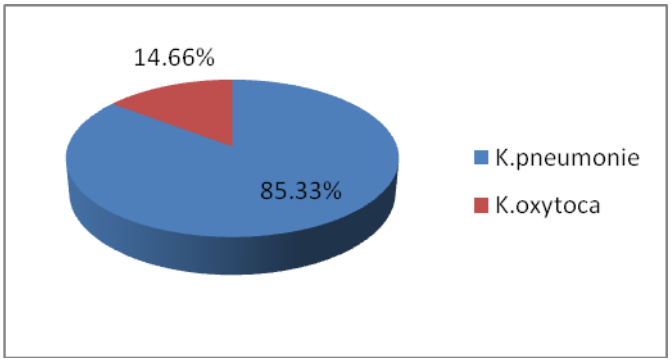
Graph.4 Distribution of *Klebsiella* species among Patients of IPD/OPD



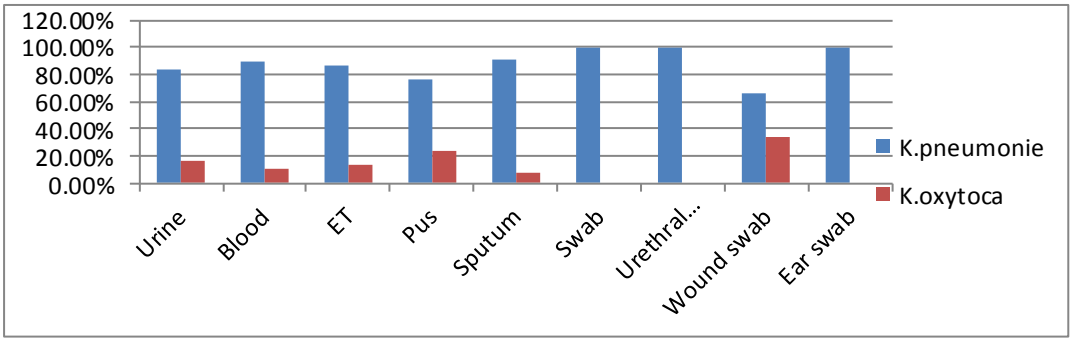
Graph.5 Antibiotic susceptibility pattern of *Klebsiella* species



Graph.6 Percentage of different species of *Klebsiella* isolated from the various specimens



Graph.7 Distribution of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolated from various Clinical specimens



Klebsiella species are the major cause of nosocomial infection. Despite advances in disinfection facilities and the introduction of a wide variety of antimicrobial agents life threatening infections caused by *Klebsiella* species are increasing continuously.

Klebsiella species was common in male patients i.e. 64% as compare to female patients i.e. 36% Similar observation of male preponderance than female was seen by Biradar and Roopa (2015), (19). In an another study by Shilpa *et al.,* (2016) (2) female showed maximum number of *Klebsiella* species infection than the male i.c 45.45% followed by 31.57% which is different than my study.

According to Acheampong *et al.,* (2011), (20), *Klebsiella* species isolated in male are 49.2% and females were 50.73%. In the present study, out of 75 *Klebsiella* species isolated from various clinical samples, maximum no. isolated from urine 24% followed by Pus 17.33%, and blood 12%, which correlate well with Shristi *et al.,* (2015), (21), maximum no. isolated from urine 75% followed by pus 14.1% and blood 5.3%. Whereas maximum no. of *Klebsiella* species isolated was from urine 31.4% followed by pus 27.8% by Manoj Kumar *et al.,* (2015), (22), and maximum number of isolates are from pus 50%, urine 21%, sputum 18%, blood 7% by Sunilkumar *et al.,* (2015) (20).

According to present study out of total 75 *Klebsiella* isolates, from different department maximum isolates from IPD 58.66% followed by OPD 41.33%, whereas Shristi *et al.,* (2015), (21), study that OPD have 13.8%, which is lower than my present study.

According to our study Out of 75 isolates of *Klebsiella* species, maximum sensitivity were shown with Polymyxin-B and *Coli*stin i.c 100% followed by Meropenem 81.33%, Imipenem 62.66%, Piperacillin/Tazobactam 61.33%, 56% sensitivity was shown by amikacin and gentamycin both, Ofloxacin 46.66%, Cefuroxime 45.33%,Ciprofloxacin 42.66%, Ampicillin 36%, Cefepime 29.33% and minimum sensitivity shown by Cefotaxime i.c 17.33%. In comparison to Sunil Kumar *et al.,* 2015, (20), higher sensitivity was found with Meropenem 100% followed by Cefepime 82% Pepracillin/tazobactem 77%, Amikacin 73%, Ceftazidime 63%, Gentamycin 62%, Ciprofloxacin 56%, Ampicillin 6%.

According to Shilpa *et al.,* 2016(2) sensitivity showed by Amikacin was 66.66% followed by Gentamycin 62.50%, Ciprofloxacin 68.90% which are almost similar to our study i.c Amikacin and Gentamycin both are showing 56% sensitive followed by Ciprofloxacin 42.66%.

In the present study sensitivity pattern of amikacin and Gentamycin was 56% followed by ampicillin 36%, Cefepime 29.33%, cefuroxime 45.33%, Norfloxacin 44.44%, cefotaxime 17.33%. According to Asati Rakesh Kumar 2013 (23) which showed somehow difference in the parcentage of 88.1% sensitivity to amikacin which was higher than our study followed by almost similar to our study i.c Gentamycin 57%, ampicillin 42%, Cefepime 33.2%,cefuroxime 22.6%, Norfloxacin 22.6%,Cefotaxime 11.2%.

According to Manikandan *et al.,* (2013)(24) sensitivity of Amikacin was 62% followed by Gentamycin 58%, Imepenem 59%, Nitofuratonin 34%, Norfloxacin 52%, Ofloxacin 56%, Ciprofloxacin 55% and Cefotaxime 43% which was almost similar to our study i.c Amikacin and Gentamycin showed 56% followed by Imepenem 62.66%, Nitofuratonin 61.11%, Norfloxacin 44.44%, Ofloxacin 46.66%, Ciprofloxacin 42.66%, Cefotaxime 17.33%.

In our study Resistance showed against the *Klebsiella* species isolates aganist Ampicillin 64%, Ciprofloxacin 57.33%, followed by Gentamycin 44% which are lower than the study carried out by Sourav *et al.,* (2016)(25) i.c Ampicillin 94% and higher i.c Ciprofloxacin 44%, Gentamycin 25%.

According to the study of Shah *et al.,* (2010)(26) Resistance showed aganist Cefotaxime was 51.66%which was lower than our study i.c 82.66% followed by Imipenem 41.66% which is higher than our study i.c 37.33%.

In our present study out of 75 *Klebsiella* species, *Klebsiella pneumonia* isolated from Blood 88.88% followed by urine 83.33% and pus 76.92% and *Klebsiella oxytoca* isolates from Pus 23.07%.Urine 16.66% and blood 11.11% Whereas study done by Aahuti kumar *et al.,* (2013), (27), *Klebsiella pneumonia*e from Blood 55.6% followed by urine 43.4%, pus 38.5%, and *Klebsiella* species isolates from pus 23.1%, urine 20.2%, Blood 16.7%.

According to Namratha *et al.,* (2015) (3) out of 100 *Klebsiella* species 79% was *Klebsiella pneumonia* and 21% was *Klebsiella oxytoca* which showed almost similar to our study which was out of 75 *Klebsiella* species 85.33% was *Klebsiella pneumonia* and 14.66% was *Klebsiella oxytoca*. In an another study carried out by Maria *et al.,* (2006)(9)out of 120 *Klebsiella* species 84% was *Klebsiella pneumoniae* followed by 16% was *Klebsiella oxytoca* which was also almost similar to our study. In another study carried out by Farha *et al.,* (2015) (28) out of 470 isolates of *Klebsiella* species 57.4% was *Klebsiella*

pneumonia and 42.5% was *Klebsiella oxytoca* which was higher than my study.

In an another study carried out by Sourav *et al.,* (2016) (29) out of 120 *Klebsiella* species isolates 90% was *Klebsiella pneumonia* and 10% was *Klebsiella oxytoca* which are related to our study which is out of 75 *Klebsiella* species 85% was *Klebsiella pneumonia* and 14.66% was *Klebsiella oxytoca*.

In the present study out of each specimen 83.33% urine specimen showed *Klebsiella pneumonia* and 16.66% was identified as *Klebsiella oxytoca* from the urine specimen which is almost similar to the study carried out by Namratha *et al.,* (2015) (3) which was out of each specimen 80.76% urine specimen showed *Klebsiella pneumonia* and 19.24% was *Klebsiella oxytoca*.

In conclusion, microorganisms are the concealed enemies to the mankind and cause a very profound damage in human body as well as other living organism. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs or antibiotics. There are a lot of antimicrobial drugs of which some are discovered or established. The exact detection of Sensitivity pattern of *Klebsiella* species is important for both treatment and epidemiology. Identification of *Klebsiella* species at the species level will increases the tendency to acquire about the related causes with the *Klebsiella* species. Present study will guide the physicians to choose drug which is to be used in the infection caused by both *Klebsiella pneumonia* and *Klebsiella oxytoca*.

The study demonstrates sensitivity pattern of *Klebsiella* species against the resistance pattern of *Klebsiella* species. And there is serious threat of the spread of beta lactamase producers with regard to antibiotic resistance, hence proper procedures is necessary for detection of various beta-lactamase in bacteria.

Therefore it is necessary to take proper strategies to prevent and detect the emergence of sensitivity pattern of *Klebsiella* species for the treatment of *Klebsiella* species.

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