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Original Research Article

Isolation and Antibiogram of *Klebsiella* Species from Various Clinical Samples of Patients Attending a Tertiary Care Teaching Hospital

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Abstract:

Introduction: Klebsiella species is a gram-negative pathogen known to produce community acquired infections and hospital acquired infections. It is caused by Klebsiella pneumoniae followed by Klebsiella oxytoca. There is a development of antimicrobial resistance (AMR) and there is an emergence and spread of extended-spectrum beta-lactamases (ESBLs) in Enterobacteriaceae. Hence, the current study was conducted to identify it from different clinical samples and determine ESBL producers.

Objectives: To isolate and identify the Klebsiella spp in all clinical samples in all age groups, to determine their susceptibility to antimicrobial agents and resistance for ESBL by standard screening and confirmatory methods.

Materials and Methods: Prospective study was done for 1 year. With ethics committee approval and informed consent, clinical samples from 1-70 years from different IPDs and OPDs were included. Samples with incomplete information and contaminants were excluded.

Results: The present study includes 455 non duplicate, consecutive clinically significant *Klebsiella* species. Maximum number of *Klebsiella* species was from pus followed by urine. *Klebsiella pneumonia*e subsp pneumoniae (55.4%)" was" the most common species isolated followed by *Klebsiella* oxytoca" (40.4%) and other *Klebsiella spp* (4.2%). Most of the *Klebsiella* species are isolated from the general surgical ward.

Antimicrobial susceptibility testing has shown highest resistance against ampicillin (74%) and lowest to imipenems (3%). Among 455 isolates, ESBL producer by screening method were 218 isolates and further 105 isolates confirmed by confirmatory method.

Conclusions: This study gave us the information on prevalence of Klebsiella and aids in development of an antibiotic policy for the hospital and as well as limit the spread of multidrug resistant bacteria.

Keywords: Klebsiella, Clinical specimens, Resistance, ESBL, Sensitivity.

Introduction

Klebsiella species is a gram-negative pathogen known to produce bacterial pneumonia, urinary tract infections, wound infection, meningitis, peritonitis, septicaemia and also it can lead to development of community acquired infections and hospital acquired infections.[1,2]

In recent years it has emerged as a major pathogen causing nosocomial infections in India accounting for around 8% of all hospital-acquired infections. Nosocomial Klebsiella infections are mainly caused by Klebsiella pneumoniae followed by Klebsiella oxytoca.[3] But there is a development of antimicrobial resistance (AMR) for various antibiotics among a variety of infectious pathogens. [4]

AMR is exacerbated by the selection pressure exerted by antibiotic usage in animals and human beings. The mutation and transfer of mobile resistant gene is the

major contribution to the rise in antibiotic resistance outbreak, and Klebsiella species are one of the common causes for it.[5] Apart from this there is an emergence and spread of extended-spectrum beta-lactamases (ESBLs) in Enterobacteriaceae leading to major contributor to the global pandemic of antibiotic resistance.[6]

Hence, the current study was conducted to identify Klebsiella species from different types of clinical samples obtained and to determine the susceptibility to various antimicrobial agents including ESBL producers. This study will give us the information necessary for development of an antibiotic policy for our tertiary care multispecialty teaching hospital as well as limit the spread of multidrug-resistant bacteria.

Objectives

- To isolate and identify the Klebsiella spp in all clinical samples in all age groups.
- To determine their susceptibility to antimicrobial agents.
- To determine the resistance for ESBL by standard screening and confirmatory methods

Materials and Methods

Source of data

The patient's samples were received from the different inpatients and out patients visiting, Vijayanagara Institute of Medical Sciences and Medical College Hospital, Ballari.

Study period

This was a prospective study and the samples were collected for 1 year study period and conducted at, Vijayanagara Institute of Medical Sciences and Medical College Hospital, Ballari.

Ethical consideration

The study was initiated after obtaining the institutional Ethics committee approval (**IEC NO. 63/2021**) and after written Informed consent from patients. All the patients satisfying the Eligibility criteria were included.

Eligibility criteria

All *Klebsiella* species isolated and reported from different clinical samples from all age groups (1-70 years), from different IPDs and OPDs of tertiary care teaching hospital received to Central Laboratory seeking for culture and sensitivity were included. The samples with incomplete information and contaminants were excluded.

Materials and Methods

In this **s**tudy 455 clinically significant non-repetitive klebsiella isolates from various clinical samples are assessed. These klebsiella species isolated from various clinical samples were like blood, sputum, urine, pus, vaginal swab, ear swab etc.

The samples were collected aseptically and with universal safety precautions. Samples were inoculated on Blood agar and MacConkey's agar and incubated at 37°C for 24–48 hours. After Gram's staining, colonies were subjected to preliminary tests like catalase, oxidase & to various biochemical tests like IMViC, TSI, urease, sugar fermentation tests-glucose, lactose, sucrose & mannitol.

Antibiotic sensitivity tests were done on Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method according to the recent CLSI guidelines. A broth culture inoculum of the isolate with a turbidity equivalent to McFarland 0.5 standard was used. Lawn cultures on the Mueller-Hinton agar were prepared and allowed to dry. Antibiotic discs were applied to the Mueller Hinton agar surface with the help of sterile forceps. The antibiotics tested were ampicillin (10µg), gentamicin (10µg), amikacin (30µg), amoxicillin/clavulanic acid (20µg)/10µg), piperacillin/tazobactam (100µg)/10 µg), cefepime (30µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), ciprofloxacin (5µg), ofloxacin (5µg), imipenem (10µg), meropenem (10µ g), cotrimoxazole (25µg) nitrofurantoin (300µg). All Klebsiella spp isolates isolated from clinical samples were screened for antimicrobial resistance by ESBL screening test. (Using cefotaxime (30µg, zone size ≤22mm) and Ceftazidime (30µg) zone size ≤ 17 mm.)

ESBL detection by CLSI phenotypic confirmatory test was done using Combined disc diffusion method. In this method a disc diffusion procedure was done by making a lawn culture of the test isolates. Cefotaxime(30µg), Ceftazidime clavulanic acid disc (30/10µg) were placed over the lawn culture between 20-24mm.If the zone size around the beta lactamase inhibitor combo disc was increased by ≥5mm when compared to the zone size without beta lactamase inhibitor, the test isolates were determined to produce ESBL. The test is performed using suitable controls. [7], [8]

Results

Table 1: Distribution of Klebsiella spp obtained from sample sources of Inpatients and Outpatients

S.No	Samples from source	Number of isolates Total = 455
1	Inpatients	310 (68.1%)
2	Outpatients	145 (31.9%)

Table 2: Demographic distribution of Klebsiella species isolates

S.No	Age group (years)	Male 272(59.8%)	Female 183(40.2 %)	Total n=455
1	Newborn -10	20 (64.5 %)	11(35.5 %)	31
2	11-20	25 (78.1 %)	07 (21.9 %)	32
3	21-40	80(51.3%)	76 (48.7 %)	156
4	41-60	134 (62.6 %)	80 (37.4 %)	214
5	61-80	13 (59.1%)	09 (40.9 %)	22
Total		272	183	455

Table 3: Clinical Sample wise distribution of klebsiella species isolates

Klebsiella species isolates obtained from different clinical Samples	Numbers 0f Isolates	Percentage
Pus / wound	176	38.7%
Urine	152	33.4%
Sputum	92	20.2%
Blood	20	4.4%
Endotracheal tube	5	1.1%
Tissues	3	0.7%
Body fluids	7	1.5%
Total	455	100%

Table 4: Department wise distribution of Klebsiella spp isolates samples

S.No	Department	Number of Klebsiella sppisolated	Percentage
1	Surgery	182	40%
2	Obstetrics and Gynaecology	26	5.7 %
3	Orthopaedics	50	11%
4	urology	82	18%
5	ENT	9	2 %
6	ICU	34	7.5%
7	Medicine	70	15.4%
8	Dermatology	2	0.4%
	Total	455	100 %

Table 5: Distribution of Risk factors in patients with Klebsiella spp

Risk factors in patients with Klebsiella infection	Numbers of patients
Diabetes	182
Hypertension	140
Post-surgical	92
Catheterisation	64
Alcohol & smoking	69
multiple risk	154

Table 6: Distribution of Klebsiella species

Type of *Klebsiella* species	Numbers of isolates (Percentage)
Klebsiella pneumonia	252 (55.4%)
Klebsiella oxytoca	184 (40.4 %)
Other klebsiella spp	19 (4.2 %)
Total	455

Table 7: Antibiotic susceptibility pattern in *Klebsiella* species

Antibiotics	Percentage Sensitivity
Ampicillin	26%
Gentamicin	68%
Amikacin	70%
Amox-clav	68%
Piperacillin/Tazobactum	83%
Cefepime	85%
Cefotaxime	69%
Ceftriaxone	65%
Ceftazidime	65%
Ciprofloxacin	58%
Ofloxacin	56%
Imipenem	97%
Meropenem	97%
Nitrofurantoin	72%
Cotrimoxazole	44%

Table 8: Detection of Extended Spectrum Beta Lactamase in" Klebsiella species

ESBL producers	ESBL producers	NON-ESBL producers
Screening test	218 (47.9%)	237(52%)
Confirmatory test	105 (48.16%)	-

Table 1 shows the distribution of Klebsiella spp obtained from sample sources of Inpatients and Outpatients. Table 2 shows Demographic distribution of klebsiella species isolates. The maximum number of" *Klebsiella*, species"" are isolated among"41-60 years age groups followed by 21-40 years of age. Table 3 shows Clinical Sample wise distribution of klebsiella species isolates. Maximum number of *klebsiella* species isolates is obtained from pus sample 176(38.7%) followed by urine 152 (33.4%). Table 4 shows Department wise distribution of Klebsiella spp isolates samples.

The Maximum number of isolates are obtained from surgery department 182(40%), followed by urology department 82(18%). Table 5 shows distribution of various risk factors in patients with Klebsiella spp. The common risk factor was diabetes 182, followed by multiple risk factors in 154 patient isolates. Table 6 shows distribution of Klebsiella species. *Klebsiella pneumonia*e is the most common *Klebsiella* species isolated from various clinical samples accounting for 252(55.4%). Table 7 shows antibiotic susceptibility pattern in *Klebsiella* species. The isolates were Meropenem (97%), Imipenem (97%), Cefepime (85%), Piperacillin/Tazobactum (83%). And the Ampicillin has shown highest resistance (26%) followed by Cotrimoxazole (44%). Table 8 shows the detection of Extended Spectrum Beta Lactamase in" *Klebsiella* species. Of 455 isolates of Klebsiella, 218(47.9%) isolates were found to produce extended spectrum beta-lactamases and among this 105 (48.16%) were further confirmed by CLSI phenotypic confirmation test method.

Discussion

Antimicrobial agents are widely utilized in hospitals and communities. Has resulted in increased antimicrobial drug resistance. Antibiotics must be used rationally. *Klebsiella* infections are a major opportunistic Gram-Negative Bacilli in health-care settings, posing a global healthcare concern. Its importance has grown as a result of its ability to survive in a variety of environments, the development of drug resistance mechanisms, and the rise of multidrug and pan drug resistant strains. The isolation, identification and performing antimicrobial susceptibility testing of *Klebsiella* infection to find the resistance patterns aids in the selection of appropriate antibiotic and it plays the important role in lowering patient mortality and morbidity and also reduces the spread of resistant strains in the community.[8]

In the present study among 455 isolates 272(59.8%) isolates were from male patients and remaining 183(40.2 %) isolates are from female patients. Males are more commonly affected compared to females; this could be due to prevalence of increased risk factors. Our study results are similar to that of study done by Sunilkumar et al and Hera Nirwat et al. [8,9]

Maximum number of *Klebsiella* isolates is obtained from age group between 40-60 years. And this correlates with study done by A. Asha et al And

Zheng et al.[10,11] The maximum number of *Klebsiella* spp were isolated from pus samples and it correlates with the study done by"A. Asha et al and Valarmathi et al.[12]In this study after pus samples, urine samples were isolated more. And is similar to study done by R. Sarath babu et al [13]In the current study, isolation rate of *Klebsiella pneumonia*e was high compared to other *Klebsiella* spp. *Klebsiella pneumoniae* isolated was 80%. This finding is similar to the report of Asmaa et al.[14]

Among 455 isolates, the isolated Klebsiella spp. were found to be "Klebsiella. pneumoniae" (55.4%), "Klebsiella oxytoca"(40.4%), Similar results were suggested by Sandeep Vasikar et al.,[15] Klebsiella pneumonia is one of the leading causes of nosocomial infections seen worldwide causing pneumonia, bloodstream infections, urinary tract infections, surgical site infections and meningitis shown in study by Peleg and Hooper.[16] In this study Klebsiella spp showed highest resistance against ampicillin (74%) followed cotrimoxazole (56 %).This was similar to the study conducted by Mwangi joseph Kikuchi et al.,[17] In our study, Klebsiella isolates showed least resistance against meropenem (3%), imipenrm (3%), cefipime (15%) and this is similar to study conducted by Madahiah et al.[18]

In the present study, ESBLs producers were 218 (47.9%) by screening test and 105 (48.16%) cases using Combined disc diffusion methods. The high occurrence of ESBLs in Klebsiella spp is of great concern since infections caused by this bacterium were very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, easy spread of organism, efficient at acquiring and disseminating resistance plasmid.

This implies the importance of usage of confirmatory test in order to reduce the reporting of false positives because that may lead to usage of higher-level antibiotics leading to contribution of drug resistance. plasmid acquisition and dissemination.[19,20]

Hence the prevalence of Klebsiella infections emphasizes the need for early detection of various beta lactamases which would help in selection of appropriate antibiotic regimen and prevention of emergence and dissemination of MDR strains.

Conclusion

- *Klebsiella* sub spp pneumoniae were found to have a high prevalence than other *Klebsiella* species. And they were isolated more from pus followed by urine.
- It was highly resistant against Ampicillin(74%) and lowest resistance against piperacillin tazobactum (17%) and imipenem (3%).
- ESBL producers were the most common. This will aid in the detection of antibiotic resistance, which will aid in the judicious use of antibiotics

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