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Research Article

PREVALENCE AND ANTIBIOGRAM OF KLEBSIELLA SPECIES DETECTED IN CLINICAL ISOLATES OF TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: We evaluated the prevalence rate and antibiogram of *Klebsiella* species isolated in various clinical samples, for effective management.

Methods: The prospective study was undertaken to evaluate various clinical samples received in the microbiology laboratory for culture and sensitivity testing. Culture showing *Klebsiella* species were further processed for antibiotic susceptibility testing by the Kirby–Bauer disk diffusion test.

Results: During the study period, 332 *Klebsiella* species were isolated in various samples and out of which 98.7% were *Klebsiella pneumoniae* and were predominately from urine samples (37%). All isolates were processed to determine their antibiogram. The highest antimicrobial resistance was observed for third and fourth-generation cephalosporines (more than 85%).

Conclusion: It is concluded from our study that multidrug resistance *K. pneumoniae* are the emerging superbugs which require close monitoring and should be reported regularly which will guide clinicians in effectful management and thus help in preventing the spread of multidrug resistance *Klebsiella* and future threat.

Keywords: *Klebsiella pneumoniae*, Antimicrobial resistance, Antibiogram.

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INTRODUCTION

The genus *Klebsiella* falls under the *Enterobacteriaceae* family is a non-motile, encapsulated Gram-negative bacterium characteristically lactose fermenting and facultative anaerobe in nature [1]. Eight species of *Klebsiella* are identified. In that, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *and Klebsiella granulomatis* are associated with human illness. Out of which, *K. pneumoniae* are a clinically important species accounting for 86% of human infections [2].

Klebsiella species are reported as an important member among hospital-acquired pathogens. Infection caused by *K. pneumoniae* is pneumonia, urinary tract infections, meningitis, wound infections, septicemia, etc. In the case of nosocomial infections, colonization of *Klebsiella* is due to presence of virulence factors and drug resistance [3]. Pathogenicity factors in *Klebsiella* species are acquisition ability for iron, presence of fimbriae, lipopolysaccharide, etc., *Klebsiella* with Types 1 and 3 fimbriaeenhances the urinary tract infections, whereas the presence of lipopolysaccharide and capsule escapes phagocytosis, resulting sepsis and septic shock [4].

They are nearly always naturally resistant to ampicillin. Overuse and misuse of drugs have led to drug resistance as a result of which, 1980 in Germany extended spectrum of beta-lactamase (ESBL) strain was first and foremost isolated in *K. pneumoniae*. Now these strains of *Klebsiella* have become resistant to a wide group of antibiotics such as quinolones and aminoglycoside also. These multidrug-resistant (MDR) *Klebsiella* are responsible in increase surge of morbidity and mortality.

With the emergence of the MDR strain of *K. pneumoniae*, a great threat has evoked to public health and we are left with few therapeutic options, so updated knowledge of the drug resistance pattern in a particular region is necessary for clinical practice [5]. The current study was undertaken to know the prevalence of *Klebsiella*-associated infections and their antibiogram and resistance pattern in our region.

METHODS

Study design

This cross-sectional analytic study was carried out in the Department of Microbiology, People's College of Medical Sciences and Research Centre, Bhopal, Madhya Pradesh after obtaining ethical approval from the institutional ethics committee.

Sample collection

Various clinical samples received in the microbiology laboratory for culture and sensitivity testing from inpatients and outpatients of various departments in the tertiary care hospital during the study period of 18 months, from January 01, 2014, to June 30, 2015 were considered.

Inclusion criteria

All lactose fermenting mucoid colonies on MacConkey agar, further confirmed as *Klebsiella* species were included in the study.

Exclusion criteria

Other than *Klebsiella* species isolates were excluded from the study.

Sample processing

Sputum, pus swab, and miscellaneous samples collected by aseptic precaution along with requisition form received by the department of microbiology were processed as per standard bacteriological techniques for aerobic cultures. After performing, direct Gram-staining samples were inoculated on blood agar and MacConkey agar. Urine samples were inoculated on cystine lactose electrolyte deficient agar (CLED) and incubated overnight at 37°C. For blood culture, an appropriate volume of blood about 08–10 mL was collected in blood broth, and incubated at 37°C for 48 h, and then inoculated on blood agar and MacConkey agar. Colonies were read after overnight incubation.

Klebsiella species were identified by colony characteristic of large dome-shaped colonies on blood agar and lactose fermenting, mucoid colonies on MacConkey agar. Gram staining showed Gram-negative, thick, and stout rod. *K. pneumoniae* were indole negative, whereas *K. oxytoca* was indole positive. Other biochemical tests observed were citrate utilization test and urease test positive, triple sugar iron (TSI) test showed acid/acid reaction with abundant gas production and glucose, lactose, sucrose, and mannitol sugar fermentation tests positive [6].

Antimicrobial susceptibility testing was performed for all isolated organisms on Mueller–Hinton agar by the Kirby–Bauer disk diffusion method. The antibiotic disks were used and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2015 [7].

Statistical analysis

Data were collected in Microsoft Excel and result was analyzed and expressed in frequency and percentage.

RESULTS

In our study, *Klebsiella* species among positive culture samples were 332 in number, maximum positivity of *Klebsiella* species was observed in urine samples 125 (37.6%), followed by pus samples 116 (34.9%), sputum samples 60 (18%), then blood samples 26 (7.8%), and minimum were miscellaneous 05 (1.5%) which included body fluids, swab, tissue, etc.

Among the 332 isolate species, identification was as follows *K. pneumoniae* 328(98.7%) and *K. oxytoca* 04(1.2%).Maximum percentage of *K. pneumoniae* was isolated in urine culture positive samples125 (37%). Similarly, in pus samples, 115 (34.6%), sputum and blood samples *K. pneumoniae* isolated were 59 (17.7%) and 26 (7.8%), respectively. Similarly, in miscellaneous samples, 5 (1.5%). In our study, out of four isolates of *K. oxytoca* ca 02 (0.6%) in urine samples and 01 (0.3%) in pus and sputum samples, respectively, as shown in Fig. 1.

Fig. 2 shows gender-wise positivity for *Klebsiella* species out of total 332 isolates female preponderance was observed in our study with 179 (54%), whereas male gender showed 153 (46%) positivity.

Fig. 3 represents the isolation rate of *Klebsiella* species from various departments. The highest isolation 36.10% (120) was observed in the department of surgery, followed by medicine 24.40% (81). The isolation rate in the obstetrics and gynecology department was observed to be 13.20% (44). There were about 11.70% (39) in the department of pediatrics, followed by 6.30% (21) from the orthopedics department and outpatient department and only 1.80% (6) from the ENT department.

Antibiogram of the *Klebsiella* species isolated from various samples is depicted in Table 1. Among various samples, the highest sensitivity was 81% toward amikacin and imipenem followed by more than 40% to meropenem, piperacillin–tazobactam, and ofloxacin and nearly 40% cefperazone sulbactum, ciprofloxacin, and Amoxyclav. Less than 15% sensitivity was seen for cefotaxim, ceftriaxone, and ceftazidime. In the urine sample sensitivity observed for nitrofurantoin and norfloxacin were 63% and 45%, respectively.

Based on different clinical samples (Fig. 4), it was noted that strains isolated from urine were 100% resistant to ampicillin and more than 90% resistant to third and fourth-generation cephalosporine. β-lactam/β-lactamase-inhibitor showed more than 70% resistance, whereas the weakest resistance was detected in cases of amikacin (18.4%) and imipenem (21.6%).

Among pus and sputum samples, more than 80% resistance to cefotaxime, ceftriaxone, and ceftazidime was observed. In pus more than 45%, whereas in the sputum sample, more than 68% resistance to Amoxy clav, cefperazone sulbactum, and piperacillin–tazobactum was observed. Resistance to amikacin was observed 28.3% in sputum and

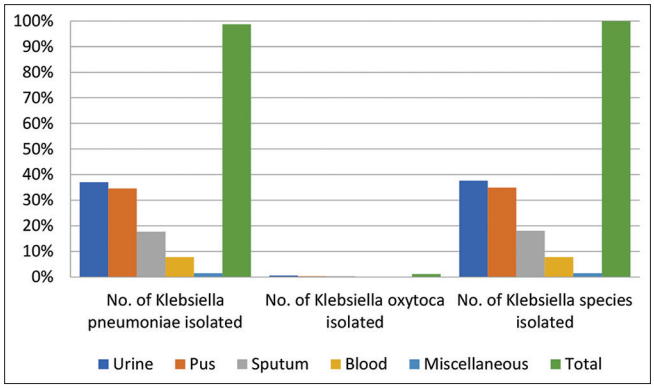

Fig. 1: Specification of *Klebsiella* in various clinical samples n=332

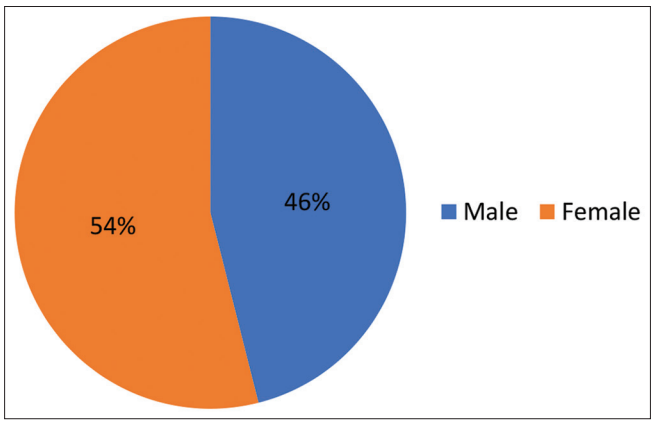

Fig. 2: Gender wise distribution among *Klebsiella* positive isolates n=332

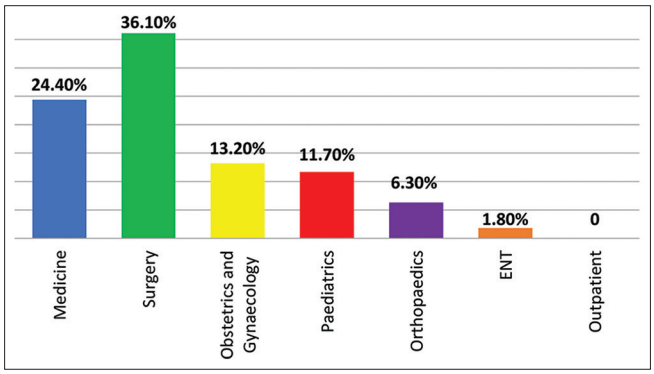

Fig. 3: Department-wise distribution of *Klebsiella* species n=332

15.5% in pus, whereas to imipenem it was 19.8% in pus and 16.6% in sputum.

Isolates in blood and miscellaneous samples showed 70–80% resistance to third and fourth-generation cephalosporine. A 100% sensitivity was detected in miscellaneous sample regarding amikacin, ciprofloxacin, and imipenem.

DISCUSSION

K. pneumoniae are an important member of ESKAPE and are considered most frequently isolated Gram-negative bacteria in the hospitalized patient [8]. Due to the wide use of antibiotics in hospitalized patients, the prevalence of the MDR *K. pneumoniae* is increasing. This study aims to reveals the prevalence and antimicrobial pattern of *Klebsiella* isolates.

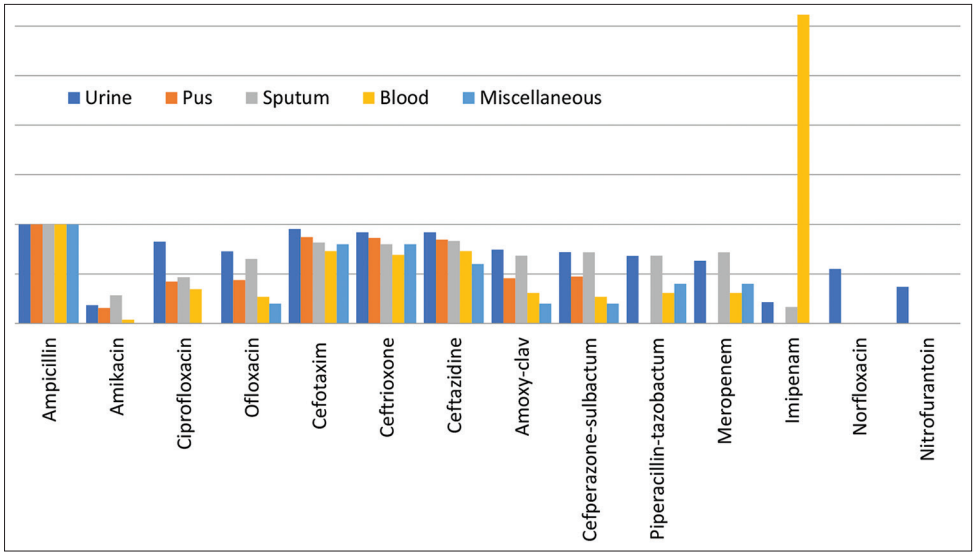

Fig. 4: Antimicrobial resistance observed in *Klebsiella* species in different clinical samples

Table 1: Antibiogram of *Klebsiella* species isolated (n=332)

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	00	332 (100)
Amikacin	269 (81)	59 (17.7)
Ciprofloxacin	132 (39.7)	189 (56.9)
Ofloxacin	137 (41.2)	189 (57)
Cefotaxim	37 (11.1)	292 (87.9)
Ceftrioxone	47 (14.1)	285 (85.8)
Ceftazidine	40 (12)	285 (86)
Amoxyclav	131 (39.4)	196 (59)
Cefperazone-sulbactum	136 (40.9)	196 (59)
Piperacillin–tazobactum	140 (42.1)	189 (56.9)
Meropenem	140 (42.1)	183 (55.1)
Imipenam	269 (81)	63 (18.9)
Norfloxacin (in urine sample only)	56 (45)	69 (55)
Nitrofurantoin (in urine sample only)	79 (63)	46 (37)

The total number of *Klebsiella* isolates from various clinical samples in our study was 332 which is at par with the isolation rate seen in the study by Ravichitra *et al*. [9]. Out of the total *Klebsiella* isolates, 98.70% (328) were *K. pneumoniae* which is still more than Biradar and Roopa who reported 89% in his study [10]. *K. oxytoca* 1.20% (04) were isolated in our study which was less than Sridhar Rao *et al*. [11] 0.9%.

A gender difference of infection was observed in our study. A female dominance of infection was observed among the *Klebsiella*-positive samples. One hundred seventy-nine (53.90%) were from females and 153 (46%) were from males, thus showing female preponderance which is contrary to other studies [10].

In the present study, sample-wise culture positivity for *Klebsiella* species in urine (37.6%), pus (34.9%), sputum (18%), blood (7.8%), and miscellaneous (1.5%) which was similar to other studies. Singh *et al*. also reported most common sample with *Klebsiella* species being urine in their study [12].

The highest isolation rate of *Klebsiella species*, about 36.10%, was in the surgery department, followed by medicine (24.40%), obstetrics and gynecology (13.20%), pediatrics (11.70%), orthopedics and outpatient department (6.30%), and ENT (1.80%). Vijayashree *et al*. also found highest isolate from department of surgery [13].

The antimicrobial pattern of *Klebsiella* isolated showed 100% resistance to ampicillin, due to chromosomal-mediated intrinsic resistance encoding β-lactamases [14]. Markedly, high resistance (86%–88%) was observed to third generation of cephalosporins (cefotaxime, ceftazidime, and ceftriaxone) in our study which corroborates with the report from Chandigarh (87%-89%) by Gupta *et al*. [15]. In our study, ESBL and Amp C beta-lactamase might be the cause of decreased susceptibility to third generation of cephalosporin.

Isolates, in our study, showed resistance to β- lactam and β- lactamase inhibitor combinations were 57% to piperacillin–tazobactam followed by Amoxyclav and cefoperazone sulbactam (59%). This could be due to the production of inhibitor resistance TEM β-lactamase.

A moderate level of resistance was observed in *Klebsiella* isolates from various samples among the fluoroquinolones tested. Ciprofloxacillin and ofloxacin showed 57% resistance. In urine sample, 55% resistance against norfloxacin was reported which was similar to Namratha *et al*. [16]. Mutations in the chromosomal genes encoding DNA gyrase of the bacteria or due to efflux of the drug could be the cause of resistance to flouroquinolones.

Isolates of our study showed the highest susceptibility to amikacin (81%) which is in similarity with the studies done by Pavani [17] and to imipenem, which was similar to the findings by Gupta *et al*. [15]. However, 55% of them were found to be highly resistant to meropenem which might be due to the presence of plasmid-mediated β-lactamases and the loss of porin.

Urine samples showed the highest sensitivity toward amikacin and imipenem highest resistance toward cefotaxime, ceftriaxone, and ceftazidime which is similar to the study done by Kumar [18]. The urine sample showed higher susceptibility to nitrofurantoin (63%) which is at par with Harada *et al*. [19].

CONCLUSION

From the present study, we can conclude, high resistance against commonly used antibiotics for *Klebsiella species* is the major reasons for the high morbidity and mortality associated with its infections in hospitalized patients. Therefore, antibiotic stewardship is required to prevent indiscriminate use of antibiotics which is a major cause of the emergence of drug resistance among pathogens.

AUTHORS CONTRIBUTION

Abhijit Awari: Conceptualization and study designing, manuscript writing, Surender Kaur: Data acquisition, analysis and interpretation. Final version approval done by all authors.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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