

Antibiotic Resistance Pattern of Klebsiella sp. at a Tertiary Care Centre from Garhwal Himalayan Region

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ABSTRACT

Background: Infections due to Klebsiella sp. comes under nosocomial infections. These are difficult to treat due to the multiple drug resistance acquired by organisms. The present study was done to know the antibiotic resistance pattern of Klebsiella sp. isolated from different clinical samples collected at the tertiary care centre of Garhwal Himalayan region of Uttarakhand. **Place and duration:** This study was done in the Base Hospital of Srinagar Garhwal region of Uttarakhand from July 2019 to December 2019. **Methods:** Klebsiella sp. was isolated from different clinical samples (Urine, pus, and sputum samples) in the Base Hospital of Srinagar Garhwal region of Uttarakhand. Identification of Klebsiella sp. was done by biochemical tests. Antibiotic susceptibility test (AST) method was done by Kirby-Bauer disc diffusion method and compared. Data Analysis was done by using a simple percentage method. **Results:** The total number of culture samples collected with Klebsiella sp. isolates were 116 patients. Sample distribution of 116 samples was 63 Pus samples, 48 Urine samples, and 5 Sputum samples. Sensitivity was seen in Colistin and Polymixin B, meropenem, and cefoperazone-sulbactam. Resistance was seen in Ampicillin, 95.68% (N=111), cefalexin, 98.27% (n=114), cefotaxime and cefuroxime, 97.41% (n=113), cefixime, 94.82% (n=110), tetracycline, 88.79% (n=103), cefepime, 89.65% (n=104), ciprofloxacin, 86.20% (n=100), cotrimoxazole, 85.34% (n=99), ticarcillin-clavulanic acid 82.75% (n=96), nitrofurantoin 75% (n=87), piperacillin-tazobactam, 72.41% (n=84), gentamicin, 68.10% (n=79) and aztreonam, 63.79% (n=74). **Conclusion:** This is an awareness issue among the public to take necessary precautions, maintain cleanliness and hygiene to minimize the spread of nosocomial infections due to multi-drug-resistant strains.

Keywords: Antibiotic susceptibility test (AST), Kirby-Bauer disc diffusion method, Klebsiella oxytoca, Klebsiella pneumoniae, Multiple drug resistance (MDR), Nosocomial infections.

INTRODUCTION

Antibiotic resistance is a widely spreading problem in all places due to its excessive use, its control is very important to combat this widespread problem.^[1] Klebsiella sp. are most commonly known to be responsible for nosocomial infections, one-third of all gram-negative infections like Urinary tract infections, pneumonia, cystitis, septicemia, endocarditis, surgical wound infections, pyogenic liver abscesses, necrotizing pneumonia, endogenous endophthalmitis.^[2,3] Infections with these organisms leads to a high rate of motility, prolong hospitalization, and high expenses.^[4] The treatment option for infection with Klebsiella sp. is very less because of ESBL, Ampc, carbapenemases, etc. but also due to frequent co-resistance with other antibiotics.^[5] The formation of a large number of new mutant strains is also a reason for multiple drug resistance in Klebsiella sp.^[6] In the case of a hospitalized patient, a large number of broad-spectrum antibiotic led to increased carriage of

Klebsiella sp. and also the development of multiple drug resistance (MDR) strains that produce extended-spectrum β -lactamase (ESBL).^[7] There are very little data regarding the resistance pattern of antibiotic drugs to Klebsiella sp. in the Garhwal Himalayan region. Thus, we evaluated the trends in antibiotic resistance of Klebsiella sp. at the Garhwal Himalayan region of Uttarakhand, as it connects three different districts (Pauri, Tehri, and Chamoli) in the same tertiary care center.

MATERIALS AND METHODS

The present study was done at Base hospital, Srinagar, Uttarakhand. It begins from July 2019 till December 2019. In this study total of 116 strains of Klebsiella sp. were isolated from different clinical samples (Urine, pus, and sputum samples) at Base Hospital. Identification of Klebsiella sp. was done by biochemical tests. Antibiotic susceptibility test (AST) method was done by Kirby-Bauer disc diffusion method as per CLSI (clinical and laboratory standards institute) criteria.^[8] All the analysis was performed using the simple percentage method.^[8,9]

• Identification of Klebsiella sp.

All clinical samples collected were inoculated aseptically on Mac-Conkey agar and Blood agar

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media plates in case of urine and pus samples while an additional chocolate agar plate was used in case of sputum samples which were incubated at 37°C for 24 Hrs.

Urine samples were inoculated by using a 4 mm inoculating loop. In the case of the urine sample, 10⁵CFU/ml of growth was considered for significant bacteriuria.

Further *Klebsiella* sp. was identified by cultural characteristics, morphological characteristics, and biochemical tests like Indole, methyl red, Voges-Proskauer, citrate, TSI (Triple Sugar Iron), oxidation fermentation (O/F), and urease.^[1,10] Indole test help in the identification of *Klebsiella* sp. (Positive isolates were identified as *K. oxytoca* while negative as *K. pneumonia*). The presence of the capsule was seen by staining of mucoid and smooth colonies with India ink.

• Antibiotic susceptibility test

Antibiotic susceptibility test (AST) method was done by Kirby-Bauer disc diffusion method on *Klebsiella* sp. following the guidelines of the Clinical Laboratory Standards Institute 2015.^[9] First, the inoculum was prepared for AST with the help of nutrient broth by taking 5/6 colony of *Klebsiella* sp. that matched to 0.5 McFarland standard (1.5x10⁸CFU/ml). within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension and pressed inside the wall of the tube above the fluid level and inoculate at 60° Cover the dried surface of Muller Hilton agar(MHA) plate. After 3-5 minutes antibiotic disc was applied and gently pressed down to ensure complete contact with agar.

The antibiotic disc used for AST were Ampicillin, cotrimoxazole, cefalexin, cefixime, tetracycline, aztreonam, cefoperazone-sulbactam, ciprofloxacin, meropenem, Amikacin, nitrofurantoin, cefotaxime, ceftazidime, gentamicin, cefepime, ticarcillin-clavulanic acid, piperacillin-tazobactam, Colistin, Polymixin-B, amoxicillin-clavulanate. Zone of inhibition was measured in mm was concluded with interpretative criteria CLSI 2015,^[9] and isolates were sensitive, intermediate, and resistant. *Klebsiella pneumonia* ATCC62003 was used as a control strain.^[11]

• Identification of extended-spectrum β-lactamase (ESBL) producing strains

ESBL producers isolates were identified as resistant to an antibiotic like ceftazidime, cefotaxime, and ceftriaxone in AST.^[12] Further confirmation for ESBL producing Strains was done by placing the amoxicillin-clavulanate at the center and on sides, ceftazidime and cefotaxime were placed 30 mm apart from center to center on inoculated MHA plate. The incubation of the MHA plate was done at 37°C for 18-24 hrs. The inoculum that showed zone of inhibition with synergism in between Amoxicillin/clavulanic acid and cefotaxime or ceftazidime was confirmed ESBL producers.^[7]

Ethical Clearance:

No ethical clearance was required in this study as it was done on routine laboratory isolates. Patient consent was taken about their participation in the study and also informed to maintain confidentiality about their details.

RESULTS

The total number of culture samples collected with *Klebsiella* sp. isolates were 116 (65 male and 51 females) patients. Sample distribution of 116 samples was 63 Pus samples, 48 Urine samples, and 5 Sputum samples as shown in the [Figure 1].

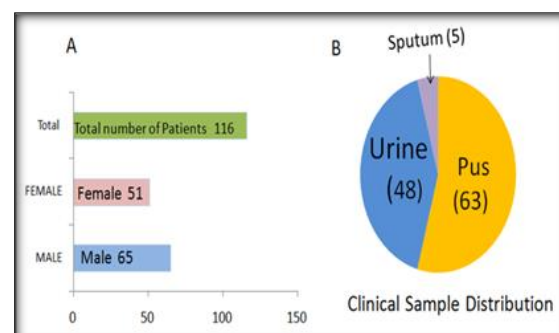


Figure 1: A (Gender wise distribution of samples) B (clinical sample distribution).

Total 30 isolates of *Klebsiella* sp. were collected from Patients with Age intervals between 41 to 50 years, as shown in [Figure 2].

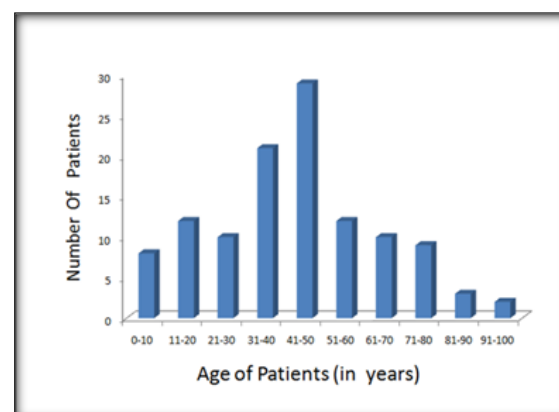


Figure 2: Age-wise distribution of the number of patients

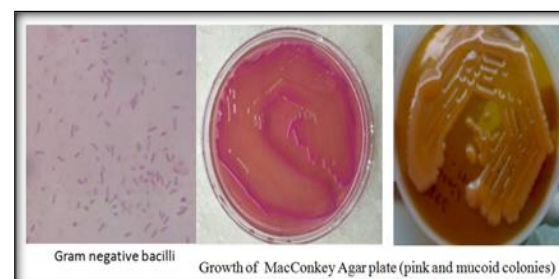


Figure 3: Morphology and culture characteristics of *Klebsiella* sp.

Morphology of *Klebsiella* sp. are gram-negative rods belongs to the family of Enterobacteriaceae. They are non-motile and possess a polysaccharide capsule. The capsule is responsible for the mucoid appearance of the bacterial colonies. The colony characteristics were pink mucoid colonies on the Mac-Conkey agar plate. As shown in [Figure 3]. Identification of *Klebsiella* sp. was done by biochemical tests.

Biochemical tests, as shown in the [Figure 4] Indole test help in the identification of *Klebsiella* sp. Positive Indole test (appearance of the pink ring at the top layer of the tube) isolates were identified as *K. oxytoca* while negative Indole test isolates were identified as *K. pneumonia*, [Figure 4(B)]. The presence of the capsule was seen by staining of mucoid and smooth colonies with India ink. In mannitol test non-motility reaction and ferment mannitol tube. The colour of citrate converted to blue from green indicated a positive reaction. In some species urease tubes remain unchanged indicates a negative reaction. TSI indicates A/A reaction with gas production [Figure 4(A)]. Oxidative-Fermentative reaction was observed fermentative. The Oxidase test indicated a negative reaction in many isolates.



Figure 4: Identification by (A) TSI, urease, citrate, mannitol (B) Indole test.

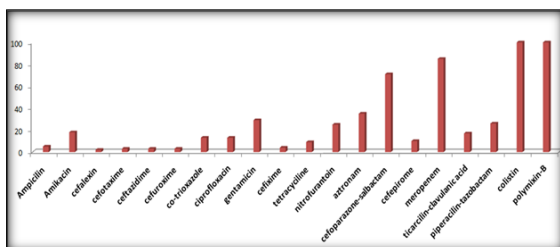


Figure 5: Sensitivity to different Antibiotic drugs by *Klebsiella* sp.

Sensitivity was seen in Colistin and polymixin-B, 100%(n=116), meropenem, 85.34% (n=99), cefoparazonesalbutam, 71.55% (n=83), aztronam, 35.34% (n=41), gentamicin, 28.44%(n=33), piperacilin-tazobactam, 26.72% (n=31), nitrofurantoin,25% (n=29), ticarcilin-clavulanic acid and Amikacin,17.24% (n=20),co-trioxazole and ciprofloxacin, 12.93% (n=15), cefepime, 10.34% (n=12), tetracycline, 9.48% (n=11), Ampicilin,

5.1% (n=6),cefixime,4.31% (n=5), cefotaxime, ceftazidime and cefuroxime,3.44%(n=4)and cefalexin, 1.72% (n=2). As shown in the [Figure 5]. Resistance was seen in Ampicilin,95.68% (N=111), cefalexin, 98.27% (n=114), cefotaxime and cefuroxime, 97.41% (n=113), cefixime, 94.82% (n=110), tetracycline, 88.79% (n=103), cefepime,89.65% (n=104), ciprofloxacin,86.20% (n=100), co-trioxazole85.34% (n=99), ticarcilin-clavulanic acid, 82.75% (n=96), nitrofurantoin,75% (n=87), Amikacin, 73.27%(n=85), piperacilin-tazobactam,72.41% (n=84), gentamicin,68.10% (n=79), aztronam, 63.79% (n=74), cefoparazone-salbutam,25% (n=29), meropenem,12.93% (n=15), Colistin and polymixin-B,0% (n=0),as shown in the [Figure 6].

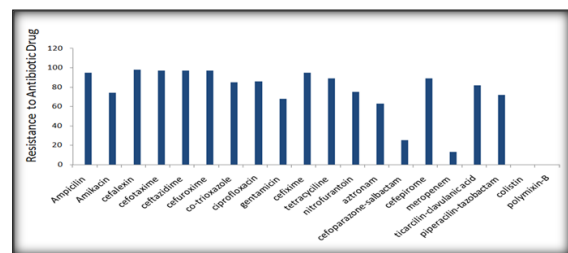


Figure 6: Resistance to different Antibiotic drugs by *Klebsiella* sp.

In the case of male patients, maximum resistance strains were seen in the pus sample whereas in female patients maximum resistance strains were seen in urine samples. As shown in [Figure 7].

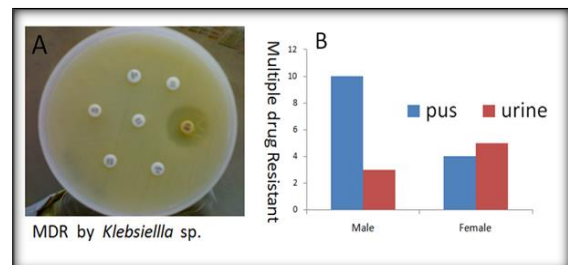


Figure 7: (A) Multiple Drug resistance by *Klebsiella* sp. (B) Gender wise distribution of MDR samples.

The total number of ESBL producing strains was seen higher in urine samples as compared to pus samples. As shown in [Figure 8].

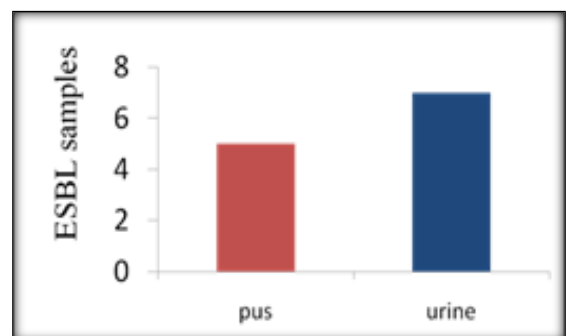


Figure 8: ESBL seen in clinical samples

From the total strains, the number of Klebsiella pneumoniae strains (n=59) was seen as greater than Klebsiella oxytoca (n=41). As shown in [Figure 9].

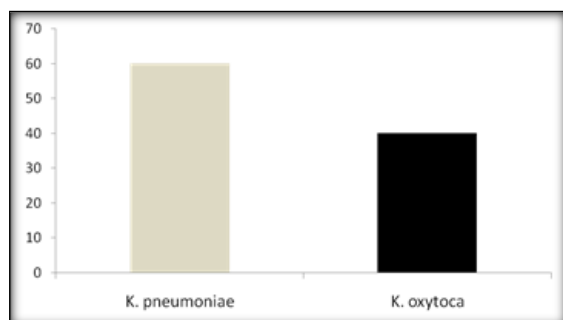


Figure 9: Species of Klebsiella species isolated from clinical samples

DISCUSSION

Most strains of Klebsiella sp. are MDR that cause a wide range of clinical conditions from relatively mild to serious and sometimes life-threatening that can lead to death.^[13] In our study we found the age group between 41 to 50 years had a higher number of Klebsiella sp. findings and it effect almost every age group both male as well as female. In our findings, we noticed that isolates of Klebsiella sp. were seen maximum in case of pus samples as compared to urine samples and very low in sputum samples. In the case of sensitivity 100% sensitivity of Klebsiella sp. was seen in Colistin and Polymixin-B, 85.34% seen in meropenem, and 71.55% in cefoperazone-sulbactam. Resistance was seen in many drugs like Ampicillin (98.2), cefalexin (98.2), cefotaxime, ceftazidime and cefuroxime 97.41%, cefixime 95.68%, tetracycline, and cefepime 89.65%, ciprofloxacin 86.20%, cotrimoxazole 85.34%, ticarcillin-clavulanic acid 82.75%, nitrofurantoin 75.86%, piperacillin-tazobactam 72.41%, gentamicin 68.96% and aztreonam 63.79%. It is very necessary to monitor the consumption of antibiotics and MDR in case of nosocomial infections to decrease the resistance to antibiotics.^[14] In our study, we also observed MDR maximum in the male in case of pus samples than urine while in females MDR was seen maximum in urine samples as compared to pus samples. ESBL producing Klebsiella sp. was seen maximum in numbers in urine samples as compared to pus samples. From the total number of isolates collected the species of Klebsiella pneumonia was higher in numbers than Klebsiella oxytoca as per our study findings.

CONCLUSION

The most effective treatment for isolates of Klebsiella sp. in the Garhwal region is Colistin and Polymixin-B while meropenem and cefoperazone-sulbactam are also effective in treating infections. It is a very important task to monitor the consumption

of drugs and MDR in nosocomial infections to lower resistance drugs. It is an effort to control the irrelevant and excessive use of antibiotics. It is also an awareness issue among the public to take necessary precautions, maintain cleanliness and hygiene to minimize the spread of nosocomial infections. It is the primary role of all health care workers and all clinicians and all patients along with their attendants to promote proper hand washings with soap or sanitization of all areas to lower the spread of hospital-acquired infections. In this way, we can protect many lives from life-threatening MDR strains and also try to keep our society healthy.

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