ABSTRACT

The aim of the study was to investigate the antimicrobial susceptibility pattern of *Klebsiella* species from different clinical samples at Sree Balaji Medical College and Hospital, India. Overall 189 samples out of 980 non-repetitive clinical samples obtained from wound/pus, urine, sputum swab and blood by disc diffusion method and identified as *Klebsiella* and analysed. Out of total 189 *Klebsiella* isolates, 76 out of 155 *K. pneumoniae* (49%) were resistant and none out of 34 *Klebsiella oxytoca* (0%) showed resistance to cefotaxime and ceftazidime by disc diffusion method. Antimicrobial susceptibility testing of *Klebsiella* exhibited 100% resistance to Ampicillin. The present study highlights the need for the continued monitoring of antimicrobial susceptibility patterns of important bacterial pathogens, so that rational antibiotic policies can be formulated.

Keywords: *Klebsiella pneumoniae*; *Klebsiella oxytoca*; antimicrobial susceptibility; cefotaxime.
1. INTRODUCTION

The Enterobacteriaceae Family embraces seven tribes, of which Klebsiella fits into fifth tribe. The genus Klebsiella is the second most common organism among Enterobacteriaceae family next to E. coli, which is a gram negative, lactose fermenting, non-motile, rod shaped, facultative anaerobic bacilli. They are the common causative agents of both nosocomial and community acquired infections. Klebsiella is the second commonest cause of nosocomial bacteremia and lethal sepsis in pediatric wards especially in premature infants and intensive care units (ICU), and often causes neonatal sepsis. Klebsiella spp. cause 3 – 8% of all nosocomial infections and are widely recognized as important pathogens in urinary tract infections, pneumonia, wound, soft tissue and blood stream infections (BSI) [1]. The most common opportunistic nosocomial Klebsiella infections are caused by K. pneumoniae and K. oxytoca [2].

Clinical manifestations by Klebsiella species may range from colonization of the skin and mucous membrane to serious infection leading to substantial morbidity and mortality. The ability of this organism to spread rapidly often leads to nosocomial outbreaks. Since 1982, strains that produce Extended spectrum beta-lactamase (ESBL) have been evolving [2]. Epidemic and endemic nosocomial infection caused by Klebsiella species are a leading cause of high morbidity and mortality. Klebsiella spp is an important sources of transferable antibiotic resistance [3]. Overuse of broad spectrum antibiotics and development of multidrug resistant (MDR) strains, has led to production of ESBL. ESBL producing Klebsiella pneumoniae causes outbreaks of nosocomial infections. They pose serious therapeutic challenge to clinicians due to limited therapeutic options [4]. The ESBL are mutants, plasmid mediated β-lactamases which are derived from the older, broad spectrum β–lactamases and they confer resistance to all extended spectrum cephalosporins and aztreonem, except the cephapymics and carbapenems. The genus Klebsiella are the bacterial pathogens most often found associated with infections in healthcare settings and infections may be endogenous or acquired through direct contact with an infected host [5]. In recent years, many Klebsiella pneumoniae strains have acquired a massive variety of β-lactamase enzymes, which can destroy the chemical structure of β-lactam antibiotics such as penicillins, cephalosporins, and carbapenems. Because carbapenems are conventionally used to treat persistent infections caused by Gram-negative bacteria, the increasing prevalence of Carbapenem-resistant K. pneumoniae (CRKP), with resistance encoded by bla KPC presents a significant challenge for physicians [6]. The study was aimed at giving an insight into the anti-microbial sustainability pattern of Klebsiella sp. Obtained from Sree Balaji Medical College and Hospital.

2. MATERIALS AND METHODS

The study was conducted in central diagnostic laboratory, Department of Microbiology for a period of one year (Jan 2017 to Dec 2017) at Sree Balaji Medical College and Hospital. Overall 980 non repetitive clinical samples (wound/pus, urine, sputum and blood) received and processed from General surgical wards. Out of which, 189 were isolated and identified as Klebsiella by Gram staining, culture morphology on to MacConkey Agar. Blood agar and Nutrient agar plates and biochemical tests and antimicrobial susceptibility testing is done by “Disc Diffusion method” (Kirby-Bauer method).

2.1 Antimicrobial Susceptibility Testing

All the isolated strains were subjected to antibiotic sensitivity testing by the “Disc diffusion” method by Kirby Bauer method [7]. Hi media discs were used. A Mueller-Hinton agar plate was used. The standard drugs used were Ciprofloxacin (5 µg), Gentamycin (10 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30µg), Piperacillin-Tazobactam (100/10 µg), Imipenem (10 µg), Trimethoprim-Sulfamethoxazole (1.25/23.75 µg), Amikacin (30 µg), Ampicillin (10 µg), Meropenem (10 µg), Amoxicillin-Clavulanic acid (20/10 µg) for isolation from Urine- Nitrofurantoin (300 µg).

2.2 Disc Diffusion Susceptibility Testing (Kirby Bauer)

Four to five colonies of same morphology were inoculated into 4-5 ml of trypticase soy broth at 35°C for 2-6 hrs. The turbidity of the organism suspension was compared to commercially available 0.5 McFarland Standard against white background with a contrasting black line which
corresponds to $1.5 \times 10^8$ colony forming units per millimetre. Within 15 minutes of formation the turbidity, a sterile nontoxic cotton swab was dipped in to the inoculum suspension and swab was rotated several times with firm pressure on inside wall to remove the excess fluid. Dried surface of the Mueller-Hinton agar plate which was brought to room temperature was streaked with the swab for 3 times over the entire surface rotating the plate approximately 60 degrees each time for even distribution of inoculum. After rim of the agar was swabbed, lid was replaced in Petri dish and after 3 to 5 minutes, appropriate antimicrobial impregnated discs were placed using forceps. Each disk was gently tamped down onto the agar for uniform contact and was placed uniformly, no closer than 24 mm from Centre to Centre. Plates were inverted and incubated at 35ºC for 18-24 hrs [8].

Using ruler, the zones of complete growth inhibition around each discs were measured to within the nearest millimetre, diameter of the disk were included in the measurement. Results were interpreted as sensitive, intermediate and resistant by comparing the inhibition zone diameters with the ranges recommended by CLSI guidelines 2017. The control strains were obtained from American Type Culture Collection (ATCC) [8].

3. RESULTS AND DISCUSSION

In the present study, a total of 189(19.8%) clinical samples of Klebsiella species were subjected to Antimicrobial susceptibility testing.

Out of total 189 Klebsiella isolates, 76 out of 155 K. pneumoniae (49%) were resistant and none out of 34 Klebsiella oxytoca (0%) showed resistance to cefotaxime and ceftazidime by disc diffusion method.

Out of 155 Klebsiella pneumoniae, 144 were susceptible to imipenem and 141 to meropenem. Out of 34, 32 and 30 of Klebsiella oxytoca were susceptible to imipenem and meropenem respectively.

Klebsiella species in this study showed maximum susceptibility towards Imipenem 93%, Meropenem 91%, Piperacillin-Tazobactam 78.7%, and Amikacin 71%. A study showed susceptibility to imipenem 90.5%, Piperacillin-Tazobactam 77.5% and amikacin 78.4% [9]. It was reported 84.61% of Klebsiella pneumoniae were susceptible to imipenem [10]. Totally 61% of Klebsiella species was sensitive to Nitrofurantoin for urine samples. Another study showed 53% sensitivity to Nitrofurantoin in their study [11].

Table 1. Antimicrobial susceptibility and resistance pattern of Klebsiella species by Disc Diffusion Method (Kirby-Bauer Method)

| S. no | Antimicrobial agents | Abbreviations | Klebsiella pneumoniae n=155 | Klebsiella oxytoca n=34 |
|-------|----------------------|---------------|-----------------------------|-------------------------|
|       |                      |               | S | I | R | S | I | R |
| 1.    | Amikacin             | AK            | 110 | 16 | 29 | 27 | 03 | 4 |
| 2.    | Amoxycillin clavulanic acid | AMC | 52 | 21 | 82 | 19 | 05 | 10 |
| 3.    | Ampicillin           | AMP           | 0  | 0  | 155 | 0 | 0 | 34 |
| 4.    | Cefotaxime           | CTX           | 79 | 0  | 76 | 26 | 08 | 0 |
| 5.    | Ceftazidime          | CAZ           | 79 | 0  | 76 | 26 | 08 | 0 |
| 6.    | Ceftriaxone          | CTR           | 80 | 10 | 65 | 24 | 10 | 0 |
| 7.    | Ciprofloxacin        | CIP           | 88 | 16 | 51 | 19 | 04 | 11 |
| 8.    | Piperacillin tazobactam | PIT | 122 | 06 | 27 | 27 | 07 | 0 |
| 9.    | Gentamicin           | GEN           | 83 | 18 | 54 | 22 | 08 | 04 |
| 10.   | Imipenem             | IPM           | 144 | 03 | 08 | 32 | 02 | 0 |
| 11.   | Meropenem            | MRP           | 141 | 05 | 09 | 30 | 04 | 0 |
| 12.   | Trimethoprim-sulfamethoxazole | COT | 81 | 20 | 54 | 23 | 03 | 08 |
Antimicrobial susceptibility testing of *Klebsiella* exhibited 100% resistance to Ampicillin. A previous study showed a similar resistance towards ampicillin (100%) that depicts *Klebsiella* species are intrinsically resistant to Ampicillin [12].

This study shows, out of total 189 *Klebsiella* isolates, 76 (49%) of 155 *K. pneumoniae* alone were resistant and out of 34 *Klebsiella oxytoca* (0%) showed resistance to cefotaxime and ceftazidime by disc diffusion method. The study showed 44.5% of *Klebsiella pneumoniae* were resistance to both cefotaxime and ceftazidime which was similar to the present study [13].

4. CONCLUSION

In our present study, Imipenem and meropenem are the drug of choice for the above *Klebsiella* isolates. We also noted a high rates of resistance to most classes of antimicrobials, except carbapenems. Cephalosporins are the first line drugs used in the treatment of infections caused by member of the family *Enterobacteriaceae*. The extensive use of third generation cephalosporins has resulted in the increased prevalence of extended spectrum beta-lactamases (ESBLs) and plasmid mediated AmpC among these organisms. ESBL production is frequently accompanied by drug resistance to commonly used 3rd and 4th generation cephalosporins and aztreonam making therapeutic options limited, resulting in need for new measures for management of *Klebsiella* species.

Our study also highlights the need for the continued monitoring of antimicrobial susceptibility patterns of important bacterial pathogens, so that rational antibiotic policies can be formulated. Simple methods of curtailing cross-infections such as Hand washing technique should be emphasized to hospital staff by Hospital infection control committee (HICC).

CONSENT

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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