Isolation, Identification, Speciation and Antibiotic Susceptibility Pattern of *Klebsiella* Species among Various Clinical Samples at Tertiary Care Hospital

K. Sathyavathy¹ and B. Kiran Madhusudhan¹*

¹Department of Microbiology, Sree Balaji Medical College and Hospital Affiliated to Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

To isolate, identify and speciate *Klebsiella* from various clinical samples and their Antimicrobial susceptibility pattern at tertiary care hospital, Chennai. Out of this 76 ESBL producing *Klebsiella pneumoniae*, 64(84%) were positive by Double Disc Synergy Test (DDST), 71(93%) by phenotypic confirmatory disc diffusion test (PCDDT) and 69(90.7%) by Etest strip method. Sensitivity was found to be maximum (93%) by PCDDT method, compared to other phenotypic methods such as DDST and Etest strip method. The Present 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.

Keywords: Penicillin; blood stream infections; *Klebsiella* and beta-lactamase.

1. INTRODUCTION

Enterobacteriaceae are a group of gram negative bacilli most commonly colonizing the gastrointestinal tract causing various human infections. Few genera are recognized as environmental habitats or colonizers of animals. The term “Enterobacteriaceae” was first put

*Corresponding author: E-mail: kiran.b@bharathuniv.ac.in;
forward by Rahn in 1937, which comprises organisms that share subsequent properties. They are gram negative, rod shaped bacilli or coccobacilli, catalase positive, oxidase negative, motile by peritrich flagella or non-motile; capsulated or non-capsulated; mostly non-sporing; and are facultative anaerobes.

The Enterobacteriaceae family confers resistance to frequently used and prescribed antibiotics that share common structures and mechanism of action, inhibition of synthesis of bacterial peptidoglycan of the cell wall. β-lactam antibiotics groups include penicillin, its derivatives and cephalosporins. Enterobacteriaceae are capable of producing beta-lactamase enzymes that hydrolyzes penicillin and cephalosporin and monobactams but not carbapenems. The beta-lactamase enzymes produced by various bacteria cleave the amide b and in the β-lactam ring, thereby rendering β-lactam antibiotics ineffective. These Extended spectrum beta-lactamases producing strains are treated using beta-lactamase inhibitors.

The Enterobacteriaceae family embraces seven tribes, of which Klebsiella fits into the fifth tribe. The genus Klebsiella is the second most common organism among Enterobacteriaceae family which is a gram negative, lactose fermenting, non-motile, rod shaped, and facultative anaerobic bacilli. Klebsiella spp. cause 3 – 8% of all nosocomial infections and are widely recognized as important pathogens in genitourinary infections, bronchopneumonia, 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].

Depending on the site of infections, klebsiella species were isolated from different a clinical sample that causes various infections. K. pneumoniae subspecies pneumoniae is the most common hospital acquired pathogens causing urinary tract infection, wound infections, meningitis, abscesses, lung infections and stated to cause sepsis and death of newborn in the intensive care unit [3]. They account for about 75% to 80% of all Klebsiella species [4]. The rarely encountered species are K. pneumoniae subspecies ozaenae and K. pneumoniae subspecies rhinoscleromatis, which are still retained as separate species producing specific diseases [2]. Both K.ozaenae and K.rhinoscleromatis are considered to be subspecies of K. Pneumoniae as per DNA hybridization technique [5].

The enzyme NDM-1, of klebsiella pneumoniae encoded by bla NDM-1, has increased the rate of Carbapenem-resistant isolates posing threat to antibiotics such as β-lactams, aminoglycosides, and fluoroquinolones [6,7]. Combination therapy for K. pneumoniae infections is commonly used due to the organisms ability to obtain resistance to distinct classes of antibiotics [8].

Due to a drastic increase in the antibiotic resistance pattern encountered among Klebsiella species, it is imperative to know the institutional prevalence and susceptibility profile. Henceforth, this study is initiated to isolate and identify Klebsiella species from various clinical samples and their antimicrobial susceptibility testing. In addition, this study also includes detection of ESBL producer among Klebsiella pneumoniae by various phenotypic and genotypic methods and assessing its sensitivity [9]. Such Extended Spectrum when detected in the clinical sample indicates the need for appropriate and judicious use of antibacterial agents [10].

2. MATERIALS AND METHODS

- Place of study:
  - Central diagnostic laboratory, Sree Balaji Medical College and Hospital, Chennai.
- Type of study:
  - Single Centre, Cross sectional study
- Period of study:
  - January 2017 to December 2017

Inclusion criteria:

1. Klebsiella isolates from all the routine clinical samples (wound/pus, urine, sputum and blood) received for culture and sensitivity testing from General surgical wards were included in this study
2. Patients above 14 years belonging to both gender were included in the study

Exclusion criteria:

1. Bacteria other than Klebsiella isolates were excluded from this study
2. Paediatric age group (0-14 years) were excluded in the study

Total number of bacterial isolates: 189 isolates of Klebsiella.
Standard reference strains (used as controls):

- Escherichia coli ATCC 25922
- Pseudomonas aeruginosa ATCC 27853
- Staphylococcus aureus ATCC 25923
- Streptococcus pyogenes ATCC 19615

2.1 Methodology

This prospective study was conducted in Sree Balaji Medical College and Hospital, Chennai. The period of study was from January 2017 to December 2017. Various clinical samples such as sputum, urine, wound swab/pus and blood received in the central diagnostic laboratory from General surgical wards were processed in microbiology section. Samples were then subjected to microscopy, bacteriological culture, biochemical identification and Antimicrobial susceptibility testing.1

2.1.1 Collection of specimen

Blood samples were collected under strict aseptic precautions by venipuncture in Bactec (BACT/ALERT 3D/60)-Blood culture bottle.

Urine samples were collected after proper instructions to the patients to collect midstream urine by clean catch technique in disposable wide mouthed sterile container.

The specimens of pus and wound exudates were collected in disposable sterile swab stick/sterile syringe.

Coughed out sputum was collected in sterile container

3. RESULTS

The present study was conducted in Department of microbiology, of central diagnostic laboratory during a period of one year (Jan 2017- Dec 2017). A total of 189(19.8%) clinical samples of Klebsiella species were isolated from the overall 980 non-repetitive clinical samples received from General surgical wards of Sree Balaji Medical College and Hospital. These isolates were subjected to Antimicrobial susceptibility testing (Kirby-Bauer method) and various phenotypic ESBL detection methods done and sensitivity compared.

Out of 76 ESBL producing (by disc diffusion method) Klebsiella pneumoniae, 64(84%) were positive by DDST, 71(93%) by PCDDT and 69(90.7%) by Etest strip method. Sensitivity was found to be maximum (93%) by PCDDT method.

Table 1. Preliminary tests for enterobacteriaeae groups

| S. No | Name of the test      | Results for Klebsiella |
|-------|----------------------|------------------------|
| 1     | Catalase             | Positive               |
| 2     | Oxidase              | Negative               |
| 3     | Nitrate reduction    | Positive               |
| 4     | Hanging drop         | Non motile             |

Table 2. Biochemical test used in identification of Klebsiella species are indicated in the chart below [12]

| S.NO | Biochemical test               | Klebsiella pneumoniae | K. oxytoca |
|------|--------------------------------|-----------------------|------------|
| 1    | Indole production test         | _                     | +          |
| 2    | Methyl red test                | _                     | _          |
| 3    | Voges-proskaur test            | _                     | _          |
| 4    | Citrate utilization test       | _                     | _          |
| 5    | Urease production test         | +                     | +          |
| 6    | Triple sugar iron              | A/A                   | A/A        |
| 7    | GAS                             | _                     | _          |
| 8    | Arginine decarboxylase         | _                     | _          |
| 9    | Lysine decarboxylase           | _                     | _          |
Colony Morphology and Gram Staining

Fig. 1. *Klebsiella* colonies on nutrient agar plate

Fig. 2. *Klebsiella* colonies on MacConkey agar plate

Fig. 3. *Klebsiella* colonies blood agar plate

Fig. 4. Gram stain: Short on gram negative bacilli

Blood Culture

Fig. 5(a). Blood culture broth
A. Clear un-inoculated BHI broth  B. Inoculated turbid BHI broth
Biochemical Test

**Fig. 6.** Biochemical reactions of *Klebsiella pneumoniae*

| TSI – Alkaline/Alkaline with gas | Citrate–Utilised |
|----------------------------------|------------------|
| Indole – Negative                | Urease–Hydrolysed|
| Methyl red – Negative            | Sugars–Lactose, Sucrose, Glucose, Maltose and Mannitol fermented. |
| Voges proskauer–Positive         |                  |

**Fig. 7.** Biochemical reactions of *Klebsiella oxytoca*

| TSI – Alkaline/Alkaline with gas | Citrate–Utilised |
|----------------------------------|------------------|
| Indole – Positive                | Urease–Hydrolysed|
| Methyl red – Negative            | Sugars–Lactose, Sucrose, |
| Voges proskauer–Positive         | Glucose, Maltose and Mannitol fermented. |
Fig. 8. Tube catalase test positive by *Klebsiella*

A – Positive control, *Staphylococcus aureus* ATCC 25923; B – Test positive, *Klebsiella* Species; C – Negative control, *Streptococcus pyogenes* ATCC 19615

**Antimicrobial Susceptibility Testing by Disc Diffusion Method (Kirby-Bauer method)**

Fig. 9. Antimicrobial susceptibility testing

*PIT* – Piperacillin - Tazobactam, *IPM* – Imipenem, *AK* – Amikacin, *GEN* – Gentamicin, *CAZ* – Ceftazidime, *CTR* – Ceftriaxone

Fig. 10. Antimicrobial susceptibility testing

*MRP* – Meropenem, *CTX* – Cefotaxime, *CIP* – Ciprofloxacin, *COT* – Cotrimoxazole, *AMP* – Ampicillin
Detection of ESBL by Phenotypic Method

**Fig. 11. Double Disc Synergy Test (DDST)**
A - Ceftazidime (30µg); B - Amoxicillin –Clavulanate (20/10µg); C – Cefotaxime(30µg)

**Fig. 12. PCDDT test**
A ≥ 5mm increase in zone of inhibition of ceftazidime+clavulanic acid and cefotaxime+clavulanic acid versus zone diameter tested alone with ceftazidime and cefotaxime (confirmed as ESBL producing organism)

**Fig. 13. E Test strip method**
Two sides strip containing ceftazidime and ceftazidime clavulanic acid; The ratio of ceftazidime with and without clavulanic acid is ≥ 8 indicating ESBL producer
4. DISCUSSION

The total number of samples taken in this study during the one-year period is 1980. The samples consisted of urine, wound/pus, sputum and blood from inpatients of general surgical wards at Sree Balaji Medical College and Hospital, Chrompet, Chennai, south India. In this study, under proper aseptic conditions samples were collected, sent and processed in the microbiology section of the central diagnostic laboratory. Organisms were identified based on culture characteristics, gram staining, motility test, and biochemical reactions. Antimicrobial susceptibility testing (Kirby-Bauer method) was done by measuring the zone of inhibition recommended for antibiotics by CLSI 2017 guidelines [13-15]. Klebsiella species in this study showed maximum susceptibility towards Imipenem 93%, Meropenem 91%, Piperacillin-Tazobactam 78.7% and Amikacin 71%. A study by Sadaf guldin et al. showed susceptibility to imipenem 90.5%, Piperacillin-Tazobactam 77.5% and amikacin 78.4% [16]. A study by khalid et al., reported 84.61% of Klebsiella pneumoniae were susceptible to imipenem. 61% of Klebsiella species was sensitive to Nitrofurantoin for urine samples. Namratha et al. [12] showed 53% sensitivity to Nitrofurantoin in their study [17,18].

This study shows, out of total 189 Klebsiella isolates, 76(49%) of 155 K. pneumoniae alone were resistant and out of 34 Klebsiella oxytoca K. pneumoniae alone were resistant and out of 34 Klebsiella oxytoca showed resistance to cefotaxime and ceftazidime by disc diffusion method. A study done by sadaf guldin et al. showed 44.5% of Klebsiella pneumoniae were resistance to both cefotaxime and ceftazidime which was similar to the present study [19]. 76(49%) of Klebsiella pneumoniae which was resistant to both ceftazidime and cefotaxime were further tested for ESBL production by various phenotypic methods. Out of 76 ESBL positive Klebsiella pneumoniae(by screening method), 64(84%) were positive by DDST, 71 (93%) by PCDDT and 69(90.7%) by Etest strip method.Sensitivity were found to be maximum (93%) by PCDDT method [20]. This study shows out of 76 ESBL screening positive isolates of Klebsiella pneumoniae, same 64 isolates were found to be ESBL producer by DDST, PCDDT and Etest, 5isolates which were positive by PCDDT and Etest were not detected by DDST and 2 isolate that were positive by PCDDT was not detected by both DDST and Etest [21].

A study by Khalid et al. isolated 46% ESBL producers in Klebsiella pneumoniae by DDST method, whereas 51% were detected by PCDDT method which shows PCDDT is more sensitive than DDST [12]. A study done by Singh et al. [22] showed sensitivity to DDST to be (89%), Etest for cefotaxime and ceftazidime 83.6% and 88.52% respectively. PCDDT was most sensitive (93.44%) in their study also.A study done by Ashok kumar et al. [23] reported 47% of ESBL producing Klebsiella pneumoniae by both DDST and PCDDT [24,25,22]. Dalela et al. [26] in his study detected ESBL in 90% isolates by DDST and 100% by PCDDT method. The ability of PCDDT method to detect ESBL is satisfactory with sensitivity of 93% in his study. A study by singh et al. [22] demonstrated that PCDDT achieved the highest sensitivity 93.44% among all the phenotypic tests applied. All the above studies correlated well with this study [23].

The PCDDT test was verified with DDST and it was found to be more sensitive and alternative to DDST for the detection of ESBL producers. The DDST lacks sensitivity because of the problem in

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**Table 3. Samplewise distribution of Klebsiella isolates**

| S. NO | Sample type   | Total number | Klebsiella isolates |
|-------|---------------|--------------|---------------------|
| 1.    | URINE         | 398          | 51(27%)             |
| 2.    | WOUND/PUS     | 502          | 94(50%)             |
| 3.    | SPUTUM        | 48           | 26(14%)             |
| 4.    | BLOOD         | 32           | 18(9%)              |
|       | TOTAL         | 980          | 189(100%)           |

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**Table 4. Sensitivity for ESBL detection by various phenotypic methods**

| S. No | Phenotypic methods                        | ESBL positive Klebsiella pneumoniae (n=76) |
|-------|------------------------------------------|------------------------------------------|
| 1.    | Double Disc Synergy Test (DDST)          | 64(84%)                                  |
| 2.    | Phenotypic Confirmatory Disc Diffusion Test (PCDDT) | 71(93%)                                  |
| 3.    | Etest Strip method (MIC),                | 69(90.7%)                                |
optimal disc space and storage of Clavulanic-acid containing discs. According to CLSI guidelines, PCDDT is recommended for confirmatory method to detect ESBL producing Klebsiella species [26,27,11]. The limitation of this study was that PCR were not performed due to cost factor. Hence, we performed various phenotypic methods for ESBL detection and compared the sensitivity of each with reference to disc diffusion screening method.

5. CONCLUSION

In conclusion, this study shows klebsiella is most commonly encountered in wound/pus samples, among which K.pneumoniae isolates were maximum than K. oxytoca. Imipenem and meropenem is the drug of choice for the above Klebsiella isolates and also for ESBL producing Klebsiella pneumoniae among hospitalized patients. High rates of resistance to most classes of antimicrobials, except carbapenems were reported in our study. The first line drugs used in the treatment of infections caused by a member of the family Enterobacteriaceae is Cephalosporins. The use of third generation cephalosporins extensively has resulted in the increased prevalence of extended spectrum beta- lactamases (ESBLs) and plasmid mediated AmpC among Klebsiella isolates. ESBL production is frequently accompanied by drug resistance to commonly used 3 rd and 4 th generation cephalosporins and aztreonem making therapeutic options limited, resulting in need for new measures for management of Klebsiella species.

Capsular polysaccharides (CPS) have been the obvious vaccine candidates due to its high immunogenic and nontoxic properties. A serious disadvantage of a Klebsiella CPS vaccine is the great number of K antigens different antigens). However, in a study of the incidence of the capsule types among bacteremia Klebsiella isolates, Cryz et al. [26] observed that only 25 serotypes made up 70% of all bacteremia strains. Based on their epidemiological findings, they formulated a 24 -valent Klebsiella CPS vaccine that subsequently was proven to be safe and immunogenic. To date, this vaccine seems to be the most promising approach for preventing sepsis caused by Klebsiella and has already passed phase I human trials.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard, respondents’ (parents & patients) written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

The study was approved by Institutional Ethical Committee Sree Balaji Medical College.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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