

A study of extended spectrum β -Lactamase (ESBL) producing *Klebsiella* from pus & urine samples from a tertiary care medical college hospital in Coimbatore

¹ Dr Shreeram A Deshpande, ² Nachammai SM, ³ Kousalya Murugesan

Karpagam faculty of Medical Sciences and Research, Othakalmandapam, Coimbatore, Tamil Nadu, India

Abstract

Urinary tract, gastrointestinal, and pyogenic infections are the common hospital-acquired infections caused by members of Enterobacteriaceae. Among Enterobacteriaceae, *Klebsiella* and *Escherichia coli* has been the most commonly isolated species. *Klebsiella* are very well known to exhibit multidrug resistance.

β -lactamase production is perhaps the single most important mechanism of resistance to penicillins and cephalosporins. *E. coli* & *Klebsiella* possess a naturally occurring chromosomally mediated β -lactamase or plasmid mediated β -lactamases. These enzymes are thought to have evolved from penicillin binding proteins. This development was likely to be because of selective pressure exerted by β -lactam producing soil organisms found in the environment. In early 1960s, TEM-1 was the first plasmid mediated β -lactamase described in Gram-negative organisms. Another common plasmid mediated β -lactamase is SHV.

Extended spectrum β -lactamases (ESBLs), enzymes that show increased hydrolysis of oxyimino- β -lactams, which include cefotaxime, ceftriaxone, ceftazidime, and aztreonam, have been reported increasingly in recent years. They belong to Ambler molecular class A and Bush–Jacoby functional group 2be. These enzymes have been identified in large numbers from different regions and are significantly detected in various *E. coli* & *Klebsiella* strains. They have also been found in other members of Enterobacteriaceae such as *Citrobacter spp*, *Enterobacter spp*, *Proteus spp* and non-lactose fermenters like *Pseudomonas aeruginosa*. Today over 200 different ESBLs have been described. Major outbreak involving these resistant organisms has been reported all over the world in many members of the Enterobacteriaceae and *Pseudomonas spp*, resulting in limitation of therapeutic options.

Current knowledge of prevalence and incidence of ESBL production by commonly isolated organism such as *E. coli* and *Klebsiella* is necessary to understand the disease burden and to take necessary action to prevent the spread. Therefore the present study was conducted with an objective to find out the prevalence of ESBL producing *Klebsiella* and its antimicrobial resistance profile to formulate effective antibiotic strategy and plan a proper hospital infection control strategy to prevent the spread of these strains.

Keywords: CDL, Extended spectrum beta lactamase, Phenotypic, *Klebsiella*

1. Introduction

Urinary tract, gastrointestinal, and pyogenic infections are the common hospital-acquired infections caused by members of Enterobacteriaceae. Among Enterobacteriaceae, *Klebsiella* and *Escherichia coli* has been the most commonly isolated species. *Klebsiella* are very well known to exhibit multidrug resistance. Extended antibiotic abuse, Length of stay in hospitals, severe illness, irrational use of third generation cephalosporin, and increased use of intravenous devices or catheters are important risk factors for infection with multidrug resistant *Klebsiella* [1].

ESBL producing strains are probably more prevalent than is currently recognized because they often remain undetected by routine susceptibility testing methods [6]. ESBL strains have been associated with resistance to other non β -lactam antibiotics like the aminoglycosides and chloramphenicol. Another property of these ESBL strains is that they might show a false sensitive zone of inhibition in the Kirby–Bauer disk diffusion method [7].

2. Materials and Methods

Klebsiella isolates recovered from pus & urine samples received in the bacteriology laboratory in the Department of Microbiology, Karpagam faculty of Medical Sciences and

Research Hospital, Coimbatore from inpatient and out-patient during the period from May 2015 to June 2016 were included in the study.

2.1 Isolation and identification

Urine samples collected in universal container, approximately 50 mL in amount, were inoculated using an inoculating loop of 10 μ L volume calibration on cysteine lactose electrolyte deficient (CLED) agar plates. Pus Swabs were first rolled on blood agar plates and MacConkey agar plates and then streaked using an inoculating loop. All inoculated media were incubated aerobically overnight at 37°C. On the basis of colony morphology, gram staining, motility, and biochemical reactions, the organisms were identified as *Klebsiella*. Biochemical reactions were performed by inoculating the colony in a nutrient broth at 37°C for 2–3 hours. Following criteria was used for identification of *Klebsiella* [8].

- **Colony morphology:** small 2-3 mm diameter, circular in shape, regular margin, flat, Mucoid, lactose fermenting, and translucent.
- **Gram staining:** Gram-negative rods, 1–5 \times 0.3–0.5 μ m in size, uniformly stained with no particular arrangement, non-sporeing, and capsulated.

- **Motility:** non-motile bacteria in hanging drop preparation.
- **Biochemical reactions:** Oxidase negative, catalase positive, O/F test showed glucose fermentation, Non motile and abundant gas production, reduces nitrates to nitrites, indole negative, methyl red negative, Voges–Proskauer positive, citrate not utilized, lactose fermenter, triple sugar iron agar showed both butt and slant yellow with abundant gas production, lysine decarboxylase test positive.

2.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines [9]. Commercially available antibiotic disks (HiMedia Labs, India) were used for antimicrobial susceptibility testing. The following antibiotic disks were used, ampicillin (10 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), amoxicillin/clavulanic acid (20/10 µg), cefoperazone/sulbactam (75/10 µg), ceftazidime/clavulanate (30/10 µg), cefoperazone (75 µg), cefoxitin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), ofloxacin (5 µg), norfloxacin (10 µg), and nitrofurantoin (300 µg).

2.3 Procedure

Inoculum of 0.5 McFarland standards turbidity was prepared in a nutrient broth from isolated colony of *Klebsiella* selected from 18–24 hour agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of a Mueller-Hinton agar (MHA) plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60°C over the agar surface. After 3–5 minutes, antibiotic discs were applied and pressed down to ensure complete contact with agar surface. The discs were distributed evenly to ensure a minimum distance of 24 mm from center to center. The plates are then inverted and incubated aerobically at 37°C within 15 minutes of disc application.

2.4 Interpretation

Diameter of zone of inhibitions were measured and recorded in millimeters with the help of sliding calipers and organism was labeled as sensitive, resistant, or intermediate as per CLSI 2012 guidelines [9].

2.5 ESBL detection methods

Klebsiella were first screened for ESBL production by phenotypic method and then phenotypic confirmatory test was done as per CLSI guidelines 2012 [9].

(a) Phenotypic screening of ESBL

CLSI 2012 has recommended the use of any of the following antibiotic discs for screening of ESBL producers. Antibiotic disks of ceftazidime, aztreonam, cefotaxime, and ceftriaxone were used. More than one of these agents was used for

screening to improve the sensitivity of ESBL detection, as CLSI has recommended the method only in 2012 guidelines.

i) Procedure

Inoculum with turbidity equivalent to 0.5 McFarland standards was prepared from colonies on agar plates. MHA plates were inoculated by lawn culture method using a sterile cotton swab. With a sterile forceps ceftazidime, cefotaxime, ceftriaxone, and aztreonam disks were placed on the MHA plate and the plate was incubated at 35°C for 18–24 hours.

ii) Interpretation of results

Zones given below, against respective antibiotic indicate potential ESBL producer. If any strain was suspected as ESBL producer then phenotypic confirmatory tests were done.

1	Ceftazidime	≤ 22 mm or
2	Aztreonam	≤ 27 mm or
3	Ceftriaxone	≤ 25 mm or
4	Cefotaxime	≤ 27 mm

(b) Phenotypic confirmatory methods

Confirmatory test was done by two methods.

i) Double disk diffusion test

Double disk approximation or double disk synergy (DDS) is a disk diffusion test in which 30 µg antibiotic disks of ceftazidime, ceftriaxone, cefotaxime, and aztreonam are placed on the lawn culture plate of *E. coli* on MHA, 30 mm (center to center) from the amoxicillin/clavulanic acid (20/10 µg) disk. This plate was incubated aerobically overnight at 37°C and examined for an extension of the edge of zone of inhibition of antibiotic disks toward the disk containing clavulanate. It is interpreted as synergy, indicating the presence of an ESBL.

ii) Cephalosporin/clavulanate combination disks

Cefotaxime (30 µg) or ceftazidime disks with (30 µg) or without clavulanate (10 µg) was used for phenotypic confirmation of the presence of ESBL as recommended by CLSI 2012 guidelines [9]. A lawn culture of *E. coli* was made on the MHA plate and disks were placed at an appropriate distance from each other and incubated aerobically overnight at 37°C. A difference in zone of inhibition of ≥5 mm of either of cephalosporin disks and their clavulanate containing disks indicates production of ESBL.

2.6 Statistical analysis

Chi-square test is used for statistical analysis of the data. A ‘P value’ less than 0.05 was considered statistically significant.

3. Results

Among all the isolates, only 185 non-duplicate isolates of *Klebsiella* that showed resistance to third generation cephalosporins were included in this study without applying any selection criteria for the patients. *Klebsiella* was isolated in the highest number from urine followed by pus samples. Among 185 isolates, 95 isolates were obtained from in-patients samples and 90 isolates were isolated from out-patients samples.

In our study, it is observed that *Klebsiella* is 100% susceptible to imipenem and meropenem. Susceptibility to third generation cephalosporin is between 40 and 65%, which is quite low. Susceptibility to piperacillin/tazobactam, amoxicillin/clavulanic acid, cefoperazone/sulbactam, ceftazidime/clavulanate, amikacin, norfloxacin (only in urine), and ciprofloxacin was good and between 50 and 90%. There was 100% resistance to ampicillin of the total *Klebsiella* isolates, 125 (67.55%) isolates were ESBL producers and 60 (32.4%) isolates were non-ESBL producers. Among ESBL producers, the maximum number was isolated from urine (54.67%) followed by pus (45.33%) of the 95 organisms isolated from 95 in-patients, 64 (67.3%) were ESBL producers while 31 (34.4%) out of 90 from out-patients were ESBL producers. ESBL producers were more common among in-patients than out-patients. ESBL and non-ESBL producers compared among in- and out-patients give significant result ($P < 0.001$).

Antimicrobial sensitivity pattern of ESBL producing *Klebsiella* from urine and pus showed that it was 100% susceptible to imipenem and meropenem.

4. Discussion

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents. This has increased the hospital stay of the patients, which in turn causes economic burden. In this study overall incidence of ESBL producing *Klebsiella* was studied based on laboratory findings and includes the patients attending the out-patient and in-patient departments of Karpagam Medical College Hospital from May 2015 to June 2016. On screening with third generation cephalosporin, a total of 185 *Klebsiella* isolates were selected and studied for their antimicrobial susceptibility and ESBL production.

In this study, samples were collected from IP/OPDs. All the 185 isolates of *Klebsiella* were tested by Kirby-Bauer disk diffusion method for antimicrobial susceptibility pattern. Highest susceptibility was found to imipenem & meropenem (100%) followed by piperacillin/tazobactam (87.22%), cefoperazone/sulbactam (76.67%), amoxicillin/clavulanic acid (75.55%), and ceftazidime/clavulanate (66.11%). *Klebsiella* were resistant to most of the drugs used as first line drugs. A low susceptibility was observed with third generation cephalosporin (cefotaxime, ceftazidime, and ceftriaxone) (31.11 and 35.55, 38.33%, respectively), cephamycin (cefotaxin) (31.11%), monobactam (aztreonam) (31.11%), piperacillin (33.33%), cefoperazone (27.77%), and cefepime (35.55%).

Akram *et al* and Padmini *et al* also reported 100% susceptibility of urinary isolates of *Klebsiella* to imipenem [11, 11]. Menon *et al.* in their study reported almost similar results of susceptibility for imipenem, piperacillin/tazobactam, cefoperazone/sulbactam, and ceftazidime/clavulanate with slight variation [12]. Similar susceptibility patterns were also observed in studies conducted outside India. Kibret *et al* showed a high resistance to amoxicillin (86.0%) and tetracycline (72.6%) but a significantly high degree of susceptibility to nitrofurantoin (96.4%), norfloxacin (90.6%), and gentamicin (79.6%) [13]. Bamford *et al* demonstrated a significant decline in susceptibility to β -lactam antibiotics and

fluoroquinolones, while susceptibility to amikacin and gentamicin remained significantly high [14].

In the present study, out of 185 *Klebsiella*, 67.55% were ESBL producers by phenotypic confirmatory methods. The incidence of ESBL producing *Klebsiella* varies from country to country and from center to center. In the United States, ESBL producing *Klebsiella* ranges from 0 to 25% with the average being around 3%. [15]. In Japan, the prevalence of ESBL producing *Klebsiella* is <0.1% [16]. In Asia, the percentage of ESBL production is 4.8, 8.5, and up to 12% in Korea, Taiwan, and Hong Kong, respectively [17, 19]. In India, the percentage of ESBL producers ranges from 22 to 75% [20, 23].

In our study, incidence of ESBL among in-patients and out-patients was 67.3% and 34.4%, respectively. Although the incidence of ESBL in out-patients is less than in-patients, it is common in communities. This is because ESBL producing *Klebsiella* isolates were wide spread among both in-patients and out-patients. This observation therefore confirms the assertion by Pitout *et al* that ESBL producers are indeed as much a problem in the communities as in the hospitals [24].

ESBL producers may have spread through communities, especially those with poor hygienic and sanitation conditions, through fecal contamination of soil and water, since most patients with ESBL producers may have had their gastrointestinal tracts colonized for a long period of time by these organisms as was reported by Paterson and Bonomo (2005) [5]. In vitro susceptibility studies of ESBL producing *Klebsiella* isolated from pus and urine showed that drug resistance was higher in ESBL producers than non-ESBL producers. Analysis of antimicrobial susceptibility pattern of ESBL producing *Klebsiella* isolates demonstrated high susceptibility rates to imipenem & meropenem (100%), β -lactam/ β -lactamase inhibitor combination drugs such as piperacillin/tazobactam (80.48, 70%), cefoperazone/sulbactam (70.73, 80%), ceftazidime/clavulanate (70.73, 70%), amoxicillin/clavulanic acid (68.29, 70%), and aminoglycosides such as amikacin (73.17, 60%) and gentamicin (68.29, 60%) from urine and blood, respectively. High resistance rates were observed to penicillins such as ampicillin and piperacillin, third and fourth generation cephalosporins and fluoroquinolones. Norfloxacin and nitrofurantoin have good susceptibility against ESBL producing *E. coli* isolated from urine. So these drugs are recommended for the treatment of infections caused by ESBL producing *E. coli*. The carbapenems, on the other hand, should be used to treat only serious or life threatening infections in order to minimize cases of carbapenem resistance, though rare [5]. In a study conducted by Ankur *et al* on clinical isolates of ESBL producing *E. coli*, resistance found to amikacin was 14.7%, gentamicin 66.7%, trimethoprim/sulfamethoxazole 79.1%, and ciprofloxacin 93.8% [25]. Maina *et al* documented a higher proportion of isolates resistant to ciprofloxacin, levofloxacin, and tetracycline, and approximately 100% sensitivity to carbapenems [26]. Al-Zarouni *et al* also demonstrated high resistance rates to fluoroquinolones and cephalosporins and higher susceptibility rates to carbapenems and amikacin [27].

The ESBL producing *Klebsiella* are a cause of concern to the microbiologist as well as to the clinicians, particularly the multi drug resistant strains. Correct choice of antimicrobial

agents according to the sensitivity profile is essential for appropriate empirical treatment. In the present study, no resistance was shown to carbapenems (imipenem & meropenem). So, we suggest the use of carbapenems as the drug of choice for ESBL producers causing life threatening infections. However, antimicrobial susceptibility testing should be performed for each strain before prescribing antibiotics. The carbapenem should be used as a reserve drug only in cases of multi drug resistant strains. Carbapenem resistance in *Klebsiella* is only beginning to emerge as a clinical issue, yet the attention it has already received serves to underscore the seriousness of the problem.

5. References

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