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Detection of Extended Spectrum Betalactamases in Klebsiella Isolates from Various Clinical Samples in a Tertiary Care Hospital.

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ABSTRACT

Antibiotic resistance is emerging as a major threat to the practitioners. Extended spectrum beta lactamases (ESBL) are one of the common beta lactamases responsible for the resistance. The present study was done to detect the ESBL production among the Klebsiella isolates from various clinical samples. This study was conducted in a tertiary care hospital for a period of 6 months from December 2012 to May 2013. A total of 100 Klebsiella isolates from various clinical samples were included in the study. The diagnosis was confirmed using standard microbiological methods. Antibiotic susceptibility testing was done using disc diffusion technique by Kirby Bauer method. Klebsiella isolates which were resistant to the third generation cephalosporins were tested for ESBL production using double disk synergy test (DDST) using cefotaxime, augmentin and phenotypic confirmatory tests using ceftazidime disc and ceftazidime-clavulanic acid discs. 19% isolates were found to be ESBL producers. Multidrug resistance was observed in most of the ESBL producing Klebsiella. All the isolates were found to be susceptible to imipenem. Careful monitoring and efforts should be taken to prevent the spread of ESBL producers.

Keywords: Extended spectrum beta-lactamases, Klebsiella isolates, Antibiotic susceptibility

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INTRODUCTION

Beta-lactam antibiotic drugs are being commonly used for treating many infections nowadays. Increased and injudicious use of such drugs especially the third generation cephalosporins has lead to the emergence of β -lactamases, which cause resistance to these drugs. The emergence of antibiotic resistance in the commonly prevalent organisms is one of the threats to the treating practitioners. In recent times, Extended spectrum β -lactamase (ESBL) producers are increasing enormously. *Klebsiella pneumonia* and *Escherichia coli* are the leading producers of ESBLs [1,2]. Infections caused by *Klebsiella* species, in particular, have emerged as a serious threat in hospitals with respect to treatment. This may be indicated by the treatment failures in severely ill patients [3].The first report of ESBL producing *Klebsiella pneumoniae* was from Germany in 1983. Since the enzymes are encoded by plasmids, there is a rapid spread of resistance among Gram- negative bacteria [4].

Earlier studies from Germany (1990), Argentina (1992) and France showed that most of the ESBL producers demonstrated resistance to cefotaxime at a higher rate when compared with ceftazidime [5].The increase in resistant organisms may be attributed to the injudicious use of antibiotics particularly β -lactam antibiotics such as penicillin and cephalosporins. The organisms have gained a survival advantage over these antimicrobials. The ESBLs are plasmid-mediated. Conjugative plasmids have a characteristic feature of rapid dissemination of genetic information causing the spread of drug resistance among organisms of the same or different species [6-9].

Hence, ability of the organism to produce ESBL and transfer of resistance genes through the agency of plasmids play an important role in community and hospital acquired infections. As the plasmids which code for ESBLs also code for resistance to aminoglycosides and trimethoprim, the infections caused by them are difficult to control [10]. The actual incidence of ESBLs among the organisms can be determined by the detection of ESBL production by various tests. To the best of our knowledge, there have been only fewer reports of the prevalence of ESBL producing *Klebsiella* spp. from central and south India [11,12]. Hence, this study was performed to determine the susceptibility of *Klebsiella* spp. to several third generation cephalosporins and to detect the ESBL producing *Klebsiella* strains in various clinical samples.

MATERIALS AND METHODS

This study was done in a tertiary care hospital from December 2012 to May 2013. Hundred *Klebsiella* isolates obtained from various samples were identified by standard bacteriological techniques.

Antibiotic susceptibility testing of the isolates was done by Kirby Bauer's disc diffusion method. Antibiotics used were ampicillin (10 μ g), cotrimoxazole (25 μ g), gentamicin (10 μ g), nalidixic acid (30 μ g), nitrofurantoin (300 μ g), amikacin (10 μ g), cephotaxime (30 μ g),ceftriaxone (30 μ g), ceftazidime (30 μ g) and imipenem (10 μ g) . Muller Hinton Agar plates were prepared and the test organism was inoculated forming a lawn culture. The antibiotic discs were placed on the surface of the agar. The zone diameters of

clearance around the discs were measured. The results were interpreted according to guidelines recommended by Clinical and Laboratory Standards Institute (CLSI). Isolates which showed decreased susceptibility or resistance to third generation cephalosporins (3GC) such as ceftriaxone, cefotaxime, and ceftazidime by disc diffusion test were further tested by double-disc synergy test.

Double-disc Synergy test

Muller Hinton agar with 4 mm depth was prepared. The test organism inoculum equivalent to 0.5 McFarland Standard was spread as lawn culture on the agar and allowed to dry for 15 minutes. Cefotaxime disc (30 µg) and augmentin (20 µg amoxicillin /10 µg clavulanic acid) discs were placed on it. If the strain is an ESBL producer, then the zone of inhibition around cefotaxime disc is extended towards the augmentin disc and the distance between the two discs was roughly twice the radius of the inhibition zone produced by cephotoxime tested on its own.

Phenotypic confirmatory test

The Muller Hinton agar with 4 mm depth was inoculated with the test strain (0.5 McFarland Standard) as a lawn culture. Two discs containing ceftazidime (30µg) alone and ceftazidime (30µg) + clavulanic acid (10µg) were placed on the surface of the plate at a distance of 15-20 mm, and incubated at 37°C for 18-24 hours. The isolate was considered to be an ESBL producer if the difference in zone size between ceftazidime alone and ceftazidime + clavulanic acid was ≥ 5 mm.

RESULTS

100 isolates of *Klebsiella* spp. were included in our present study. Out of which 54 were isolated from males and 46 were isolated from female patients.

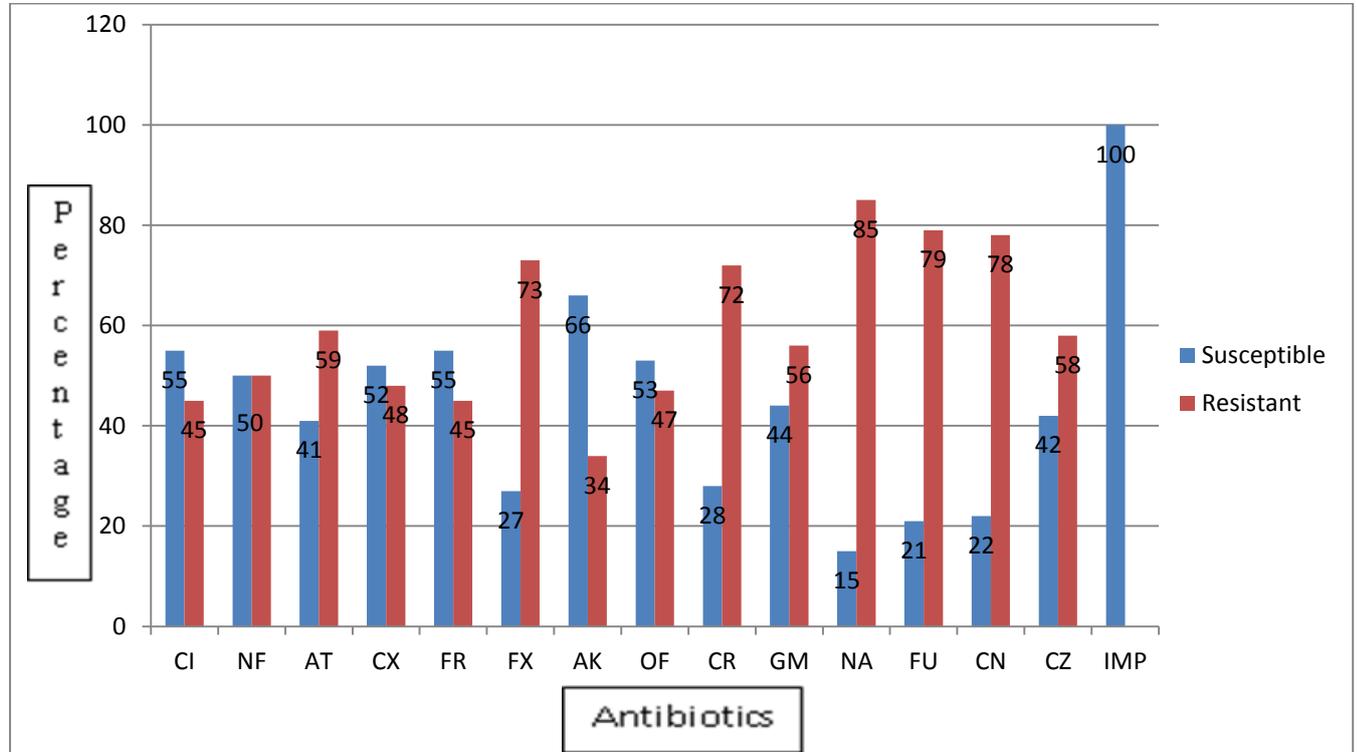
Table 1: *Klebsiella* isolates from various clinical samples

Sample	No. of isolates	Percentage of isolates
Urine	34	34 %
Sputum	28	28%
Pus	15	15%
Blood	8	8%
Wound swab	4	4%
High vaginal swab	3	3%
Throat swab	4	4%
Pleural fluid	1	1%
Ascitic fluid	1	1%
Endotracheal tube	1	1%
Bronchial wash	1	1%

In our study, we found that out of the 100 isolates, 73 were found to be resistant to a minimum of three antibiotics tested. Hence 73% of the isolates were considered to be multidrug resistant. 66% of the isolates showed resistance or decrease in susceptibility to at least one of the third generation cephalosporins. 35% *Klebsiella* isolates were found to show

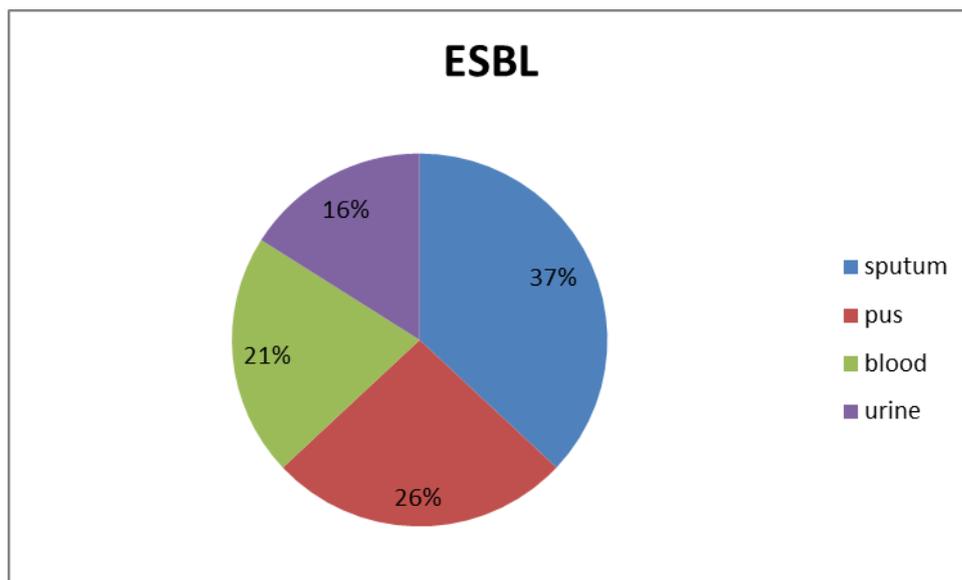
resistance or decreased susceptibility to all the third generation cephalosporins. All the isolates were found to be susceptible to imipenem.

Figure 1: Antibiotic susceptibility pattern of Klebsiella isolates



ESBL production was detected in 19% of isolates, identified by phenotypic disc diffusion test. Out of the 19 Klebsiella spp. isolates, 37% were from sputum, 26% were from pus, 21% from blood and 16% from urine samples.

Figure 2: Distribution of ESBL producing Klebsiella isolates in different samples:





DISCUSSION

Many clinical laboratories have not yet completely understood the importance of ESBLs. Also, the increasing use of broad spectrum cephalosporins in day to day life has evolved the emergence of ESBLs.

Resistance to ceftazidime and susceptibility to the combination of that drug with clavulanic acid confirms ESBL. Cefpodoxime is another sensitive drug [13] used in the detection of ESBLs. Resistance to cefotaxime also indicates the possibility of an ESBL producer. NCCLS guidelines 2000 [14] suggested that all ESBL strains will be reported as resistant to penicillin, cephalosporins and aztreonam. The prevalence of ESBL producing *Klebsiella pneumoniae* is increasingly reported in the literature [15-18]. In India, the range of ESBL producing *Klebsiella* isolates is 4-83% [19]. This wide range may be due to various reasons such as patient factor, antibiotic policy followed by the hospitals, carrier rate, different disinfectants used in the hospital set up. The ESBL producing strains are not easily detectable by routine antibiotic screening methods. The resistance of the *Klebsiella* strains to ceftazidime is found to be a good indicator of ESBL [20].

The production of the β -lactamases is chromosomal or plasmid mediated. Plasmid mediated β -lactamases are usually spread by transfer of genetic information among the organisms. These transferable plasmids are responsible for coding resistant determinants to other antimicrobial drugs. Hence multidrug resistance is commonly found in ESBL producing organisms. In our study, among the 100 *Klebsiella* isolates, 73 were found to be multidrug resistant. Sixty six isolates were found to be resistant to at least one of the third generation cephalosporins. Of these, we found 19% to be ESBL producing. This is in contrast with Shukla *et al* [21] who reported 32% ESBL producing *Klebsiella* in 120 samples and Rodrigues *et al* [22] who reported only 8.5% ESBL producing *Klebsiella* in 47 isolates.

In our study, the rate of ESBL producers was high among the sputum samples followed by pus, blood and urine. ESBL producing *Klebsiella* isolates in blood samples are of much importance because most of them are usually multidrug resistant. In the present study, among the 8 blood samples containing *Klebsiella* isolates, 50% were found to be ESBL producers. This is in accordance with Vemmula *et al* [19] who showed 57.14% of *Klebsiella* in blood samples. But Guptha *et al* [23], reported 69.2% ESBL producing *Klebsiella pneumoniae* from 13 blood samples. There are few studies which showed very high percentage of ESBL producing *Klebsiella* among the septicaemic patients [24].

Many of the ESBL strains were found to be susceptible to amikacin and few to ciprofloxacin similar to a study by Zoltan *et al* [25]. But Subha *et al* found that many strains were resistant to amikacin [26]. In our study, all the ESBL strains were found to be susceptible to imipenem. Sameena *et al*, in their study done on *Escherichia coli* isolates, also reported that all the ESBL producing *E.coli* strains were susceptible to imipenem [27]. But, it should be kept in mind that these carbapenems should not be routinely prescribed, due to fear of emergence of carbapenemases. These drugs should be kept in reserve for the use at final level.

CONCLUSION

The spread of ESBL producing Klebsiella isolates is becoming a great concern for the practising clinicians. The prevalence of ESBLs among the Klebsiella isolates is found to be higher in our hospital set up. Awareness and the knowledge about the ESBLs are needed for the practitioners because multidrug resistance is common among the ESBL producing isolates. Laboratories require quick screening techniques for detection of ESBLs along with the routine antibiotic susceptibility testing. Treatment after studying the antibiotic susceptibility pattern of the organisms will help in preventing the emergence of multidrug resistant organisms. Good infection control measures along with efficient antibiotic control policies emphasising judicious use of antibiotic drugs are recommended. Further in-depth studies at molecular level about the types of ESBLs, prevalence of ESBLs among various organisms and their clinical significance are suggested.

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