Study of Extended spectrum beta-lactamases (ESBLs) producing Klebsiella species in various clinical specimens: A preliminary report

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Abstract Objective

The present study has been undertaken to detect the presence of ESBLs producing Klebsiella species in various clinical specimens and their antibiotic susceptibility pattern.

Materials and methods

The study consists of 60 clinical isolates of Klebsiella species from various clinical specimens submitted to the microbiology laboratory, Kasturba Medical College Teaching Hospital Mangalore over a period of one year, between 1st January 2007 to 31st December, 2007. All isolates were identified morphologically and biochemically by standard procedures and ESBLs production was detected by re-arranging routine discs in a novel predictor disc approximation method. Antimicrobial susceptibility was performed using Kirby-Bauer disc-diffusion method where Imipenem disc, an inducer was placed in center and on either side of it at 15mm distance were placed ceftazidime and cefotaxime (indicator of induction). In addition, another inducer cefoxitin was placed 15mm from cefotaxime (indicator). This was placed opposite to that of ceftazidime + clavulanic acid to avoid any affect of inducible beta-lactamase on the zone of inhibition of the latter.

Results

A total of 16 out of 60 Klebsiella isolates (26.66%) were found to be ESBL producers.

Conclusions

Imipenem was found to be the most effective antibiotic (46.66%) followed by Cefoxitin (31.66%) and Cefotaxime (30.00%).

Key words: Klebsiella species, clinical specimens, ESBLs

Introduction

Klebsiella species, the member of Enterobacteriaceae, are the successful opportunistic pathogens which have always been associated with various clinical ailments mainly in the hospitalized and immunocompromised patients suffering from lower respiratory tract infection, urinary tract infection, wound infection, bacteremia and diarrhoea.[1] The wide spread use of antibiotics in hospitals have led to emergence of multi-drugs resistant organisms causing serious opportunistic infections.[2,3] Beta-lactam antibiotics (cephalosporins) are the most varied and widely used agents accounting for 50 % of antibiotics in use.[4]

The linear increases in resistance to third and fourth generation cephalosporins are the result of

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plasmid mediated extended spectrum beta-lactamases (ESBLs), which are class A enzymes and derivative of beta-lactamases (TEM & SHV beta-lactamases) which have undergone one or more amino acid substitutions near the active site of enzyme. [1,4]

The ESBLs producing organisms are reported worldwide in increasing numbers for which Clinical Laboratory Standard Institute (CLSI) recommends screening for ESBLs producing Klebsiella species, where the clinical microbiological tests are used to detect ESBLs and employ a beta-lactamase inhibitor usually clavulanate in combination with cephalosporins (e.g. ceftazidime + clavulanate) in which clavulanate inhibits ESBLs reducing the level of resistance to cephalosporins and thereby increasing the zone of inhibition.[5]

This study was undertaken to determine the detection and prevalence of ESBLs producing Klebsiella species in various clinical specimens received from Government Wenlock Hospital and Lady Goschen Hospital Mangalore, by using the combined disc method, according to guidelines of CLSI .

Materials and methods

The study was conducted at the Department of Microbiology, KMC Mangalore where the various clinical specimens like urine, sputum, pus, wound swabs, blood and other body fluids were received from the indoor patients of Government Wenlock Hospital and Lady Goshen Hospital, Mangalore.

A total of 280 gram negative bacterial isolates obtained over a period of one year from 1st January 2007 to 31st December 2007 were identified based on standard microbiological methods.[6] Among these, 60 isolates were identified as Klebsiella species and antimicrobial susceptibility was performed by using Kirby-Bauer disc- diffusion method, as per NCCLS guidelines,[7] where the following antibiotics (from Himedia, Mumbai, India) were taken: Imipenem (10µg), Cefotaxime (30µg), Cefoxitin (30µg), Ceftazidime (30µg), Ceftazidime + Clavulanic acid (30/10µg), Aztreonam (30µg), and Cefpodoxime (10µg). The test inocula were matched with 0.5 Mc Farland turbidity standard and lawn cultured onto the Mueller Hinton agar plates (from Himedia, Mumbai, India).The disc placement was designed in novel fashion to detect ESBL production.[8,9,10] The discs of ceftazidime and ceftazidime + clavulanic acid were kept 15mm apart from each other (centre to centre). Imipenem disc, an inducer was placed in center and on either side of it at 15mm distance, ceftazidime and cefotaxime (indicator of induction) were placed. In addition, another inducer cefoxitin was placed 15mm from cefotaxime (indicator). This was placed opposite to that of ceftazidime + clavulanic acid to avoid any affect of inducible beta lactamase on the zone of inhibition of the latter.[8] The remaining discs were placed as shown in the figure A:

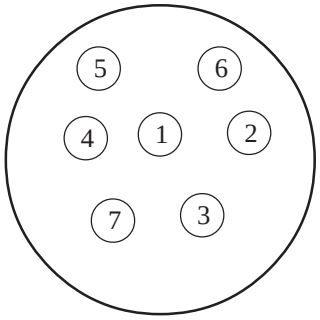


Fig. A

Fig:-A. Showing scheme of disc placement to assess ESBL production.
1.Imipenem(10µg) 2.Cefotaxime (30µg)
3. Cefoxitin (30µg) 4.Ceftazidime (30µg)
5. Ceftazidime + Clavulanic acid (30/10µg)
6. Aztreonam (30µg) 7.Cefpodoxime (10µg)
The agar plates were incubated at 37ÚC for 18 to 24 hours.[9, 10]

The following criteria have been used to decide organisms to be ESBL producers. [11, 12, 13]

Zone diameter of various third generation cephalosporins like Aztreonam(30µg) **d"** 27mm, Cefotaxime(30µg) d" 27mm, Cefpodoxime(10µg) d" 21mm and Ceftazidime (30µg) d" 22mm

2. Susceptible to cefoxitin.

3. Increase in zone size with addition of an inhibitor by **e"** 5mm when tested in combination with an inhibitor clavulanic acid versus zone diameter when tested alone.[13]

Results

Two hundred and eighty gram negative bacterial isolates obtained from various clinical specimens such as urine, sputum, pus, wound swabs, blood and other body fluids were received from Govt. Wenlock Hospital and Lady Goshen Hospital, Mangalore. Out of these sixty (21.42%) clinical isolates of Klebsiella species were detected for the presence of ESBLs whereas sixteen (26.66%) isolates of Klebsiella species were found to be ESBL producers.

Among the antibiotics tested, imipenem was found to be the most effective (46.66%), followed by cefoxitin (31.66%) and cefotaxime (30.00%**Figure B:**

Table-1 Antibiotic susceptibility of Klebsiella species

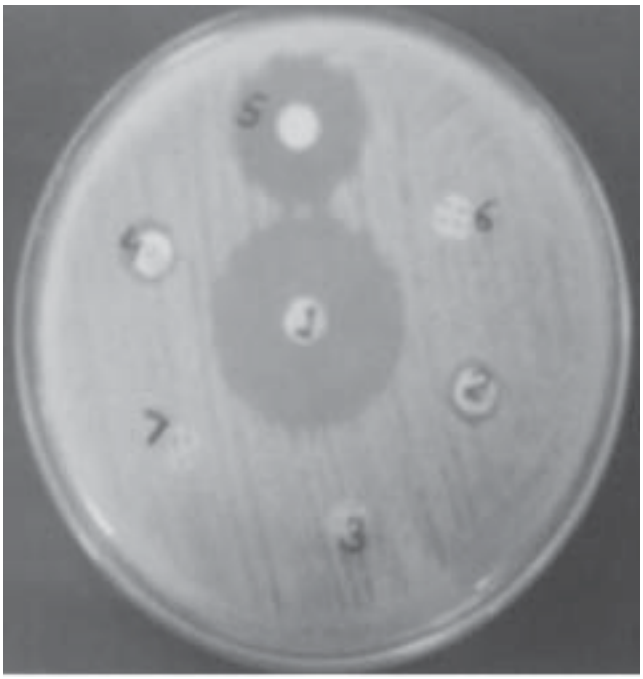
Antibiotics	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)
Imipenem	28 (46.66)	7 (11.66)	25 (41.66)
Cefotaxime	18 (30.00)	11 (15.00)	31 (51.66)
Cefoxitin	19 (31.66)	12 (20.00)	29 (48.33)
Ceftazidime	16 (26.66)	10 (16.66)	34 (56.66)
Ceftazidime + clavulanic acid	17 (28.33)	5(8.33)	38 (63.33)
Aztreonam	7 (11.66)	11(18.33)	42 (70.00)
Cefpodoxime	83 (13.3)	14 (23.33)	38 (63.33)

Table 2: Age distribution of patients in whom ESBL isolates were detected.

Age group	No. (%)
0-1 year	4 (25.00%)
2 years – 18 years	1 (6.25%)
19 years – 35 years	6 (37.50%)
36 years -60 years	13 (18.75%)
Above 60 years	2 (12.50)

Table 3: Sex distribution of patients in whom ESBLs detected.

Sex	No. (%)
Male	10 (62.50%)
Female	6 (37.50%)



Antibiotic susceptibility of Klebsiella spp to detect ESBL production.

Discussion

Beta lactamases continue to be the leading cause of resistance to beta-lactam antibiotics in gram negative bacilli. In recent years there has been an increased incidence and prevalence of ESBLs that hydrolyze and cause resistance to oxymino-cephalosporins and aztreonam.[13] For a number of reasons, the detection of ESBL-producing strains is of significant importance for all major hospitals worldwide. First, these strains are most likely to be even more prevalent than it is currently recognized. Due to the difficulty in their detection by the current clinical methods, many of these strains have been reported to be susceptible to widely used and tested broad-spectrum ß-lactams. Secondly, ESBLs constitute a serious threat to current ß-lactam therapy. Treatment of ESBL infection is difficult as the CLSI recommends that all expanded-spectrum cephalosporins be taken resistant in ESBL producers. Thirdly, institutional outbreaks are increasing because of selective pressure due to the heavy use of expanded-spectrum cephalosporins and also due to lapses in effective infection control measures.[8,9,10]

In our study, sixteen ESBLs producing Klebsiella isolates (26.66%) were detected. However, studies by Jain et al[14] and Babypadmini et al[15] showed 86.6% and 40% of Klebsiella spp to be ESBL-producers respectively. In our study Imipenem showed the highest level of sensitivity (46.66%) against Klebsiella including ESBL producers. Our study differs from the studies of Subha et al and Rodrigues et al.[1,8] Studies by Al-Zahrani et al[16] showed ESBL-producing Klebsiella pneumoniae to be having the highest susceptibility to Meropenem (94.4%).Carbapenems appear to be the drug of choice for serious infections with ESBL producing organisms as recommended earlier.[17] However, these should not be administered as empirical therapy for gram negative infections that are not life-threatening because their over-use can pose a significant problem.[18] Cefoxitin (31.66 %) and cefotaxime (30.00 %) were the other antibiotics which were found to be sensitive against Klebsiella isolates.

This was marginally higher than that reported in studies by Dutta et al[5] but substantially lower in studies by Shivaprakash et al.[19]

Conclusions

Klebsiella isolates have been steadily increasing over the past years and they have been important sources of transferable antibiotic resistance.

Indiscriminate use of third generation cephalosporins to treat gram negative bacterial infections is partly responsible for the emergence of resistance to beta-lactam antibiotics. Strict adherence

to the hospital antibiotic policy and good infection control practices can play a significant role in reducing the emerging drug resistance.

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