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Original Article

Microbiology Section

Extended-Spectrum β-lactamase Production among *Enterobacter cloacae* and *Enterobacter aerogenes* at a Tertiary Care Center in Coastal Karnataka

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ABSTRACT

Introduction: *Enterobacter* spp. have been increasingly isolated as nosocomial pathogens. Multi-drug resistance is more frequently noted among these pathogens. Extended spectrum beta lactamases (ESBLs) are difficult to detect in *Enterobacter* spp. as they also produce inducible AmpC chromosomal enzymes.

Aim: To study the frequency of ESBL production among clinical isolates of *Enterobacter* spp. by modified double disc potentiation test.

Materials and Methods: A prospective study was conducted to analyse the susceptibility profile and to detect ESBL production among 54 *Enterobacter* isolates obtained from various clinical specimens by a phenotypic modified double disc potentiation test to demonstrate synergy between cefepime and amoxicillin-clavulanate discs.

Results: A significant proportion of *Enterobacter* spp. was found to produce ESBLs (44.4%). ESBLs were detected in higher frequency in *E. cloacae* (48.9%) as compared to *E. aerogenes* (14.3%). Imipenem (96.3%) and amikacin (92.6%) were the most effective antibiotics.

Conclusion: Considering the high rates of multi-drug resistance, it is necessary for both clinicians and microbiologists to recognize the clinical and antimicrobial profile of *Enterobacter* spp. so that effective measures may be adopted to control their spread.

Keywords: AmpC, Cefepime, Phenotypic, Susceptibility

INTRODUCTION

Enterobacter spp. are among the most frequent causes of nosocomial infections. The strains which over produce chromosomal AmpC β-lactamase being depressed mutants or infrequently those expressing extended spectrum beta lactamases (ESBLs) are associated with disease outbreaks [1]. Choosing an antimicrobial for treating infections caused by *Enterobacter* spp. exhibiting such resistance mechanisms becomes difficult [2]. The plasmid mediated ESBLs confer resistance to penicillins, cephalosporins and monobactams. They are inhibited by β-lactamase inhibitors like clavulanic acid, sulbactam and tazobactam [3].

The current antimicrobial susceptibility guidelines by Clinical Laboratory Standards Institute (CLSI) focus on ESBL screening for *Escherichia coli*, *Klebsiella* spp. and *Proteus* spp. and their confirmation [4]. The inducible chromosomal AmpC β-lactamase enzymes encoded by certain enterobacteriaceae like *Enterobacter* spp. make it difficult to detect ESBL production in them by the standard double disc synergy test. This test utilizes the synergy between beta-lactam, a third generation cephalosporin and beta-lactamase inhibitor (BLI) like clavulanic acid. The AmpC enzymes which are induced by the BLI, clavulanate in this test hydrolyse the third generation cephalosporin resulting in a false negative ESBL result. Thus AmpC enzymes mask the inhibition of ESBL by clavulanic acid [5,6]. Considering that AmpC β-lactamases have minimal effect on activity of cefepime, third-generation cephalosporins when replaced with cefepime in the double disc synergy test help in accurate detection of ESBLs in bacteria producing AmpC enzymes [5,6]. A study was planned to study the frequency of ESBL production among *Enterobacter* spp. by modified double disc potentiation test including cefepime.

MATERIALS AND METHODS

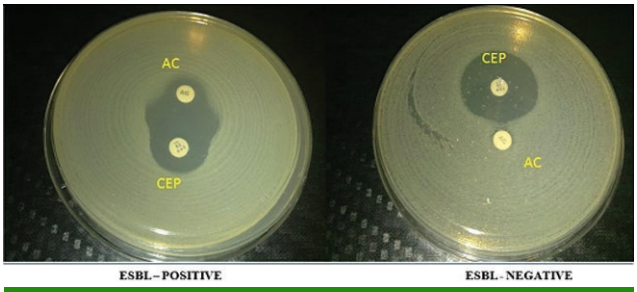
A prospective observational study was conducted in the Department of Microbiology, Kasturba Medical College, Manipal, Karnataka, India, following approval by Institutional Ethics committee. Clinical specimens including blood, wound swab, aspirated pus, tissue, sputum, endotracheal aspirate, urine and sterile body fluids submitted over a period of 6 months from January to June 2010 were included in the study. The specimens were plated on 5% sheep Blood

agar, MacConkey agar and Chocolate agar and incubated at 37°C for 18-24 hours. *Enterobacter* spp. were identified based on colony morphology and biochemical tests following standard bacteriological techniques [7]. Repeat isolates of *Enterobacter* spp. from the same patient were excluded from analysis. Sensitivity testing was performed on Mueller-Hinton agar by Kirby-Bauer disc diffusion method following Clinical Laboratory and Standards Institute (CLSI) guidelines [4]. The isolates of *Enterobacter* spp. were tested against Cefotaxime (30µg), Aztreonam (30µg), Trimethoprim-Sulfamethoxazole (1.25/23.75µg), Ciprofloxacin (5µg), Amikacin (30µg), Gentamicin (10µg), Imipenem (10µg), Cefoperazone-Sulbactam (75/30µg), Piperacillin-Tazobactam (100/10µg) (Span diagnostics, Surat, India), Cefepime (30µg), Netilmicin (30µg) and Ticarcillin-Clavulanic acid (75/10µg) (Oxoid, Thermo Scientific, UK). Quality control of sensitivity testing was performed with *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 [4]. ESBL production was detected by modified double disc potentiation test [Table/Fig-1]. This test was performed to demonstrate synergy between cefepime disc (30 µg; Oxoid, ThermoScientific, UK) placed 15 mm (edge to edge) from amoxicillin-clavulanate disc (20 µg/10 µg, Span diagnostics) [5].

RESULTS

During the study period, a total of 2446 gram-negative bacilli belonging to enterobacteriaceae were isolated from clinical specimens submitted to the laboratory. Among them, 54 (2.2%) isolates of *Enterobacter* spp. were obtained and included in the analysis. Majority of the patients with *Enterobacter* infections were males 37 (68.5%). The median age of the study group was 35 years. The various infection sites from which *Enterobacter* spp. were isolated included, skin and soft tissue 17 (31.5%), urine 12 (22.2%), bone and joint 12 (22.2%), respiratory 7 (13%), blood 3 (5.6%), intra-abdominal 1 (1.8%), eye 1 (1.8%) and central nervous system 1 (1.8%).

E. cloacae 47(87%) was the most common species isolated, followed by *E. aerogenes* 7(13%). Among isolates of *Enterobacter* spp. studied, reduced susceptibility was seen for cephalosporins (39%) and monobactams (43%) including both ESBL and non-ESBL producers. Imipenem (96.3%) and amikacin (92.6%) were the most effective antibiotics. [Table/Fig-2].



[Table/Fig-1]: Showing extended spectrum beta lactamase production in *Enterobacter* sp.
*CEP: Cefepime 30µg; AC: Amoxicillin – Clavulanic acid (20µg/10µg)

ESBL production was noted among 24 (44.4%) isolates of *Enterobacter* spp. ESBLs were detected in higher frequency in *E. cloacae* 23 (48.9%) as compared to *E. aerogenes* 1 (14.3%). ESBL isolates were mostly recovered from aspirated pus/wound swabs 15 (62.5%) followed by urine specimens 4 (16.7%). Majority 13 (54.2%) of the ESBL producing *Enterobacter* spp. were isolated from patients admitted to Orthopaedic wards. All isolates of *Enterobacter* spp. including those showing an increased zone of inhibition towards amoxicillin-clavulanate disc (ESBL producers) in the modified double disc potentiation test with cefepime were sensitive to tigecycline. Considering a susceptibility zone diameter of ≤11 mm, only one isolate of *E. cloacae* (1.9%) was found to be resistant to colistin. However, it is recommended that susceptibility testing for colistin is performed by dilution methods. Isolates of *Enterobacter* spp. which were negative for ESBL production were found to be more susceptible to the antibiotics tested in comparison to strains which were ESBL producers [Table/Fig-2].

DISCUSSION

Enterobacter spp. belong to enterobacteriaceae family which are gram-negative, facultative anaerobic bacilli. The most commonly isolated species include *E. aerogenes* and *E. cloacae* which are usually encountered as nosocomial pathogens [8]. After *E. coli* and *K. pneumoniae*, *E. cloacae* is considered as third most common enterobacteriaceae causing nosocomial infections [8]. We found 2.2% of enterobacteriaceae to be *Enterobacter* spp. Kaur J et al., have reported an isolation rate of 3.7% *Enterobacter* spp. from

Antimicrobial agent (Disk content)	Isolates (No %)		
	ESBL* (24, 44.4%)	Non ESBL (30, 55.6%)	Overall (n=54)
Cefotaxime (30µg)	0	21, 70%	21, 38.9%
Aztreonam (30µg)	0	23, 76.7%	23, 42.6%
Cefepime (30µg)	0	26, 86.7%	26, 48.1%
Co-trimoxazole (1.25/23.75µg)	05, 20.8%	24, 80%	29, 53.7%
Ciprofloxacin (5µg)	09, 37.5%	27, 90%	36, 66.7%
Amikacin (30µg)	22, 91.7%	28, 93.3%	50, 92.6%
Gentamicin (10µg)	06, 25%	25, 83.3%	31, 57.4%
Netilmicin (30µg)	18, 75%	27, 90%	45, 83.3%
Imipenem (10µg)	23, 95.8%	29, 96.7%	52, 96.3%
Cefoperazone-Sulbactam (75/30µg)	16, 66.7%	27, 90%	43, 79.6%
Piperacillin-Tazobactam (100/10µg)	19, 79.2%	26, 86.7%	45, 83.3%
Ticarcillin-Clavulanic acid (75/10µg)	03, 12.5%	26, 86.7%	29, 53.7%

[Table/Fig-2]: Showing the susceptibility pattern of *Enterobacter* spp. to various antimicrobials.
* ESBL: Extended-spectrum β-lactamase

urine specimens [3]. Similar to earlier published literature, *E. cloacae* was found to be the predominantly isolated species (87%). Rizi KS et al., found 78.2% of the *Enterobacter* isolates to be *E. cloacae* [9]. The spectrum of infections caused by *Enterobacter* spp. include, bacteremia, endocarditis, septic arthritis, osteomyelitis, infections of skin and soft tissue, lower respiratory tract, urinary tract and intra-abdominal infections [8].

Enterobacter spp. show inherent resistance to β-lactam drugs including ampicillin, amoxicillin–clavulanic acid, first generation cephalosporins and cefoxitin by virtue of production of low level natural inducible cephalosporinases. They also exhibit drug resistance to other β-lactams by production of various ESBLs belonging to TEM, SHV and CTX-M types; by over production of AmpC β-lactamase enzymes either by derepressed chromosomal gene or by plasmid borne AmpC genes and by expressing NDM, GIM, VIM, and serine carbapenemases. These resistance determinants confer resistance to third generation cephalosporins, β-lactamase inhibitors and even carbapenems. Drug resistance to aminoglycosides and fluoroquinolones are also frequently noted by production of plasmid encoded aminoglycoside modifying enzymes and chromosomal mutations respectively. Altered expression of porins and efflux pumps are the additional resistance mechanisms noted in *Enterobacter* spp. [8]. Co-expression of both ESBL and AmpC enzymes make the strains resistant to both third and fourth generation cephalosporins [10].

According to the current interpretive criteria for susceptibility testing by CLSI, routine ESBL testing may not be necessary for reporting results of cephalosporins and monobactams, however, detection of ESBLs would be of epidemiological importance and for infection control in the hospital settings [4]. Considering that AmpC β-lactamase production will mask ESBL detection, incorporating accurate tests becomes important. Cefepime being a poor substrate for AmpC enzymes makes it a reliable agent for ESBL detection in isolates that co-produce both ESBL and AmpC enzymes [5]. Other options in such isolates would be to include the BLIs, tazobactam and sulbactam which are weak inducers of AmpC β-lactamases or by use of cloxacillin, an AmpC inhibitor [11,12]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines also recommend the use of cefepime as the indicator cephalosporin in isolates producing AmpC enzymes [12]. In our study, by including cefepime as the indicator β-lactam, we found a high proportion of *Enterobacter* spp. as ESBL producers (44.4%). Similar rates of ESBL production have also been reported in earlier studies. Crowley B et al., [5] in their analysis of *E. cloacae* blood culture isolates (n=15), found 33% (n=5) strains to be ESBL producers. Kaur J et al., in their study on ESBL detection in AmpC co-producing enterobacteriaceae found four (44%) isolates of *Enterobacter* spp. (n=9) to be ESBL producers by using piperacillin-tazobactam and cefepime discs [3]. Derbyshire H et al., found cefepime-clavulanate and cefepime discs to detect all enterobacteriaceae (n=32) co-producing ESBL and AmpC enzymes [13]. Tzelepi E et al., studying various phenotypic methods among confirmed ESBL producing strains of *Enterobacter* spp. (n=31) found that use of cefepime along with closer application of amoxicillin-clavulanate and cefepime discs (20 mm) detected the maximum number of ESBL isolates 28, (90.3%) [2].

ESBL producing isolates were found to be more resistant to other classes of antimicrobials than the non ESBL isolates [Table/Fig-2]. There are reports of *Enterobacter* spp. being resistant to carbapenems which are the agents of choice for AmpC producing gram-negative bacilli and also to colistin used for carbapenem-resistant enterobacteriaceae [14,15]. Considering that, *Enterobacter* spp. are among the most commonly isolated nosocomial pathogens and are associated with high rates of multi-drug resistance, it becomes important to obtain local data on susceptibility profile of *Enterobacter* spp. their prevalent resistance determinants and the risk factors for such infections. Institution of timely appropriate antibiotic therapy and infection control measures are the need of the hour to combat these nosocomial pathogens.

CONCLUSION

ESBL production was noted in a significant proportion of *Enterobacter* isolates. In view of high rates of drug resistance among these pathogens, it is necessary for both clinicians and microbiologists to recognize the clinical and antimicrobial profile of *Enterobacter* spp. so that effective measures may be adopted to control their spread.

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