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Antibiotic Resistance Pattern in Extended Spectrum Beta Lactamases among Enterobacteriaceae

KEYWORDS: Extended spectrum beta lactamases (ESBL), Antibiotic susceptibility-testing, Enterobacteriaceae, Double disc diffusion method, E-test.

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ABSTRACT *The emergence of extended spectrum beta lactamases in clinical isolates is a major threat to the community. These beta lactamases are responsible for resistance to the beta lactam antibiotics. Clinical implication of extended spectrum beta lactamases are extremely serious. The information about the probable origin of extended spectrum beta lactamases in patients is useful in controlling the infection and also for epidemiological purposes. Therefore ESBL producers which show resistance to one of the third generation cephalosporins like ceftazidime or cefotaxime are confirmed by various phenotypic methods and E-test.*

Introduction: The emergence of beta-lactamases in the past two decades has resulted in major clinical crises[1,2]. Beta-lactamases are the enzymes which are resistant to beta-lactam antibiotics in enteric bacteria. Point mutation in one or more amino acid can change the specificity of the molecule. These extended spectrum beta-lactamases TEM-1(Temoneire-1) and SHV-1(Sulphydryl variable-1) were first found in Europe, most commonly in isolates of klebsiella species and less commonly in Escherichia coli[3]. The extended spectrum beta- lactamases are capable of hydrolyzing extended spectrum cephalosporins with oxyimino side chain such as ceftazidime, ceftriaxone and cefotaxime and oxyimino monobactam antibiotics such as aztreonam[4]. These are inhibited by beta lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. Extended spectrum beta- lactamase producing organisms are responsible for variety of infections which result in treatment failure[5]. The number of extended spectrum beta- lactamase positive patients are increased from hospital to community due to overuse/misuse of beta- lactam antibiotics. Therefore, microbiologists, infection control team and clinicians are to be notified about extended-spectrum beta-lactamase outbreaks to take appropriate steps. So sensitive diagnostic methods are required to guide therapy, monitor resistance development and implement intervention strategies in routine to curtail these strains. Hence some easy, rapid and reproducible methods are used in routine laboratories to detect these strains.

Materials and Methods: Different biological samples collected from patients attending SVS MBNR over a period of one year were inoculated on blood agar and Mac Conkey agar and incubated overnight at 37^{0}C. The different organisms were identified by various biochemical reactions and Gram-staining. The antibiotic susceptibility testing was done on Mueller Hinton agar by using Kirby-Bauer disc diffusion method according to CLSI guidelines. The Enterobacteriaceae strains which were resistant to both cefotaxime and ceftazidime, and their respective zones of inhibition are 23 mm and 18mm respectively which were further confirmed by phenotypic confirmatory tests and E-test. .

Antibiotic susceptibility testing by Kirby–Bauer Disc diffusion method: The test organism with 0.5 Mc. Farland turbidity was inoculated on Mueller-Hinton agar plate and antibiotic discs were placed 15mm from the edge of the plate and their zones of inhibition were measured with a ruler and interpreted as per CLSI guidelines[6]. Escherichia coli ATCC 25922 used as the quality control strain.

Double disc diffusion method[4,7,8] : In this method discs of ceftazidime, cefotaxime were placed 15mm (center to center) from the amoxicillin / clavulanate disc and incubated overnight. If the test strain had an extended spectrum beta lactamase, the inhibition zone around the cephalosporin disc was extended on the side nearest the co amoxiclav discs.

Disc potentiation test[4]: In this test pair of discs containing cephalosporin with and without clavulanic acid was placed on opposite sides of the same inoculated plate .The test organism was regarded as an ESBL producer if the zone of inhibition around the combination disc was at least 5mm larger than that of cephalosporin alone .

E-test: Confirmation of ESBLs is also done by E-test and performed in accordance with the guidelines of manufacturer. In this method 0.5M inoculum of test organism was swabbed over 90mm Mueller Hinton agar plate and allowed it to dry at room temperature.E-strip with ceftazidime and ceftazidime/clavulanic acid with stored concentration gradients were applied on the test plate and incubated the plate at 37^{0}C overnight. The MIC was obtained at the intersection of bacterial growth with the MIC gradient .This lowest concentration gradient which inhibits bacterial growth was the MIC of the drug. If the MIC was more than one for ceftazidime and if the ratio of MIC was more than 8 the strain was confirmed as an ESBL producer.

Results: In the present study, it was observed that 20.8% strains of Escherichia coli and 21.4% strains of Klebsiella were confirmed to be extended spectrum beta lactamases and percentage of ESBL producers was found to be 20.9%. These ESBL producing isolates showed resistance to all beta lactams but when Ceftazidime and Ceftazidime-clavulanic acid combination discs were placed 15mm apart from centre to centre, synergistic effect was observed.

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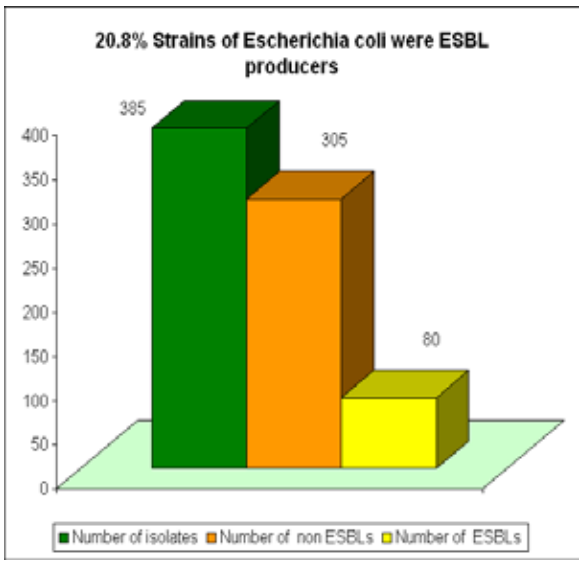
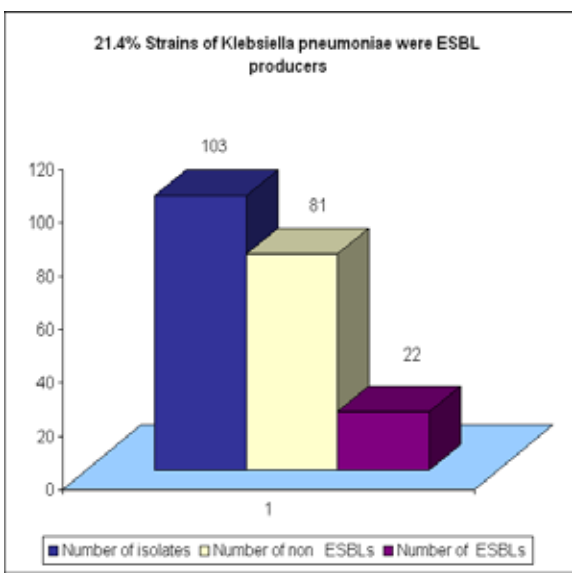
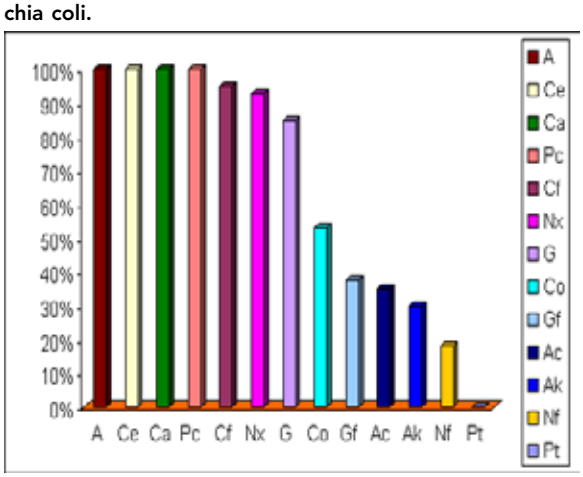
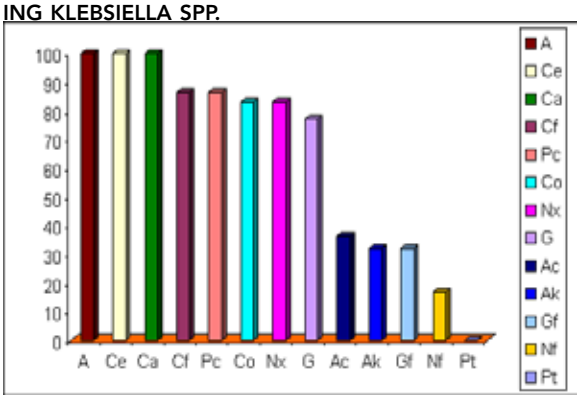


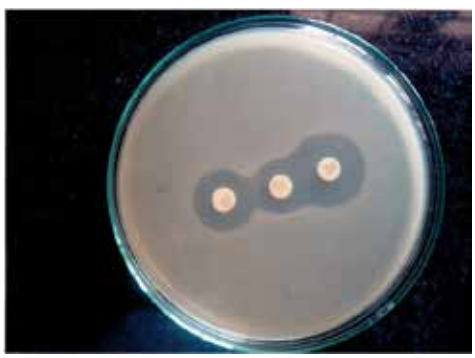


Antibiotic resistance pattern in ESBL producing Escherichia coli.

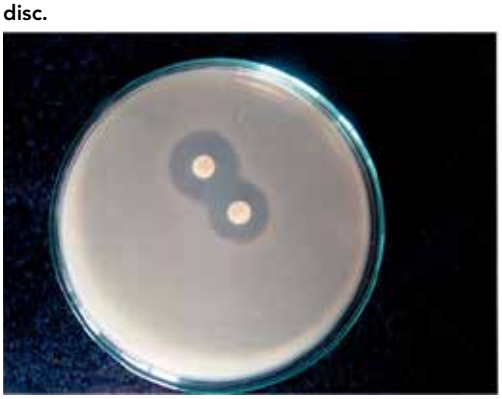


ANTIBIOTIC RESISTANCE PATTERN IN ESBL PRODUCING KLEBSIELLA SPP.

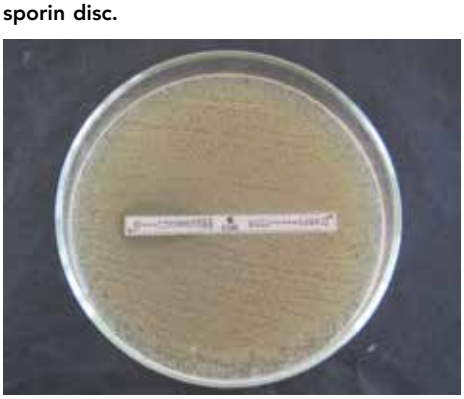




Double disc diffusion method – inhibition zone around cephalosporin disc is extended towards co-amoxiclav disc.



Disc potentiation test showing zone of inhibition around combination disc is 5mm larger than that of cephalosporin disc.



E-test Strip showing ESBL producer showed ceftazidine (TZ) MIC is reduced by 3 $\log_2$ dilution (Ratio TZ/TZL >8)

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in the presence of clavulanic acid.

Discussions: Extended spectrum beta lactamases are matter of great concern in hospitalized patients throughout the world which remains a challenge for the clinical microbiologists. The routine laboratory techniques which monitor a decrease in susceptibility to 3rd generation cephalosporins have not been sensitive enough to detect extended spectrum beta lactamase producing strains. In the present study 44% were found to be culture positives, amongst which 72.5% of them were gram negative bacilli. Out of which 53.3% were Escherichia coli, the predominant organism isolated followed by Klebsiella. The percentage of ESBL producers in the present study was 20.9% which comprised of 20.8% ESBL producing Escherichia coli strains and 21.4% ESBL producing Klebsiella strains that correlates with other studies which showed 19.8%, 20% and 22% ESBL producers[7,5,9]. Incidence of ESBL producing strains were more in females because most of the isolates were from urine due to the anatomy of genitourinary tract[4].The incidence of extended spectrum beta lactamase production is more commonly encountered in Klebsiella and Escherichia coli because these organisms most commonly colonize in the bowel. These strains were seen more commonly in hospitalized patients as compared to out patients so it has been proven the incidence of extended spectrum beta lactamase producing strains are encountered most commonly in hospitalized patients as compared to community due to inadvent use of invasive procedures and prolonged stay in the hospitals. These strains were found to be more resistant to 3rd generation cephalosporins due to extensive use of these drugs by the practitioners without antibiotic susceptibility testing which correlates with the previous studies and these strains were also found to be multidrug resistant[4,9,10,11,12]. High degree of co-resistance to non beta lactam antibiotics such as Gentamicin, Ciprofloxacin, Co-trimoxazole was seen in these ESBL positive isolates which is in par with the earlier study[10]. These ESBL producing isolates showed resistance to all beta lactams but when Ceftazidime and Ceftazidime-clavulanic acid combination discs were placed 15mm apart from centre to centre, synergistic effect was observed due to resistance of the isolates to beta lactam antibiotics alone and in combination with beta lactam inhibitors like Clavulanic acid. This finding of present study correlates with the previous studies[2,9,11,13].

Conclusion: These ESBL producers showed 100% resistance to beta lactam antibiotics- third generation cephalosporins such as Cefotaxime and Ceftazidime due to misuse of these drugs and showed co-resistance to non beta lactam antibiotics like Norfloxacin, Co-trimoxazole, Gentamicin because these strains were isolated mostly from inpatients and possibly there is co-transmission of ESBL and to other antimicrobials within same conjugative plasmids. Beta lactamase inhibitors like clavulanic acid, Amikacin and Nitrofurantoin were found to be more sensitive in these strains. So these drugs can be the alternatives for treating such patients at low cost. These strains also showed good response with third generation cephalosporins and clavulanic acid combination. Although Piperacillin-tazobactam combination was found to be much effective but this combination could not be substituted as prophylactic drug. Antimicrobial therapy in infections is being complicated by emergence of multi drug resistant strains and extensive use of third generation cephalosporins. The high incidence of beta lactamase production due to multiple mechanisms in clinical isolates is alarming and urgent action needs to be taken from both therapeutic and infection control perspective. Hence the spread of infection can be reduced by implementation of standard control measures and restrictive use of third generation cephalosporins so that these strains may not reappear.

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