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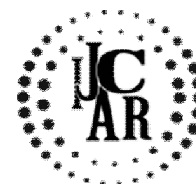

Research Article

RAPID PHENOTYPIC DETECTION OF ESBL PRODUCING ENTEROBACTERIACEAE USING ESBL HICHROME AGAR AND E TEST: A COMPARATIVE STUDY

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A B S T R A C T

Introduction: ESBL producing enterobacteriaceae have been responsible for numerous outbreaks of infection and an increase in ESBL producing enterobacteriaceae has been observed in recent years. This poses a challenging infection control issue.

Objective: Isolation and identification of ESBL producing enterobacteriaceae from clinical samples and to comparatively evaluate ESBL detection by ESBL Hichrome agar and E test. Antibiotic susceptibility testing according to CLSI guidelines.

Materials and Methods: Samples were processed using conventional methods. Bacterial etiology was identified and antibiotic susceptibility testing was done on Mueller Hinton agar according to CLSI guidelines. All enterobacteriaceae isolates were subjected to ESBL Hichrome agar plating and E test.

Results: A total of 548 enterobacteriaceae were isolated. Klebsiella species (48.1%), Escherichia coli (39.7%), Proteus species (7.6%) were the major isolates. 56.02% of all the enterobacteriaceae isolates were found to be ESBL producers by ESBL E strip method. ESBL Hichrome agar was able to detect 53.1% of enterobacteriaceae as ESBL producers.

Conclusion: It is important to identify ESBL producing enterobacteriaceae from clinical samples for the judicious use of antibiotics. For early detection of ESBL producing enterobacteriaceae isolates ESBL Hichrome agar and E tests were found to be equally effective in detecting ESBL production. ESBL Hichrome agar can be used for rapid and presumptive identification of ESBL producing enterobacteriaceae by means of growth on ESBL Hichrome agar and colony color within 24 hours with good sensitivity and specificity.

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INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes capable of conferring resistance to the penicillins, first-, second-, third- generation cephalosporins and aztreonam. But not against cephamycins (cefoxitin and cefotetan). ESBLs are susceptible to β-lactamase inhibitors (clavulanic acid) [1]. Extended spectrum beta lactamase (ESBL) producing enterobacteriaceae have been responsible for numerous outbreaks of infection and an increase in ESBL producing enterobacteriaceae has been observed in recent years[2]. Enterobacteriaceae are the commonest organisms producing extended-spectrum β-lactamases [3]. A total of more than 200 different types of ESBLs have been characterized. Enterobacteriaceae produceESBLs such as SHV, TEM types and have been established since the 1980s as a major cause of hospital-acquired infections [4].

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Treatment of extended spectrum beta-lactamase (ESBL) producing strains of *Enterobacteriaceae* is a major challenge for the clinician both in hospital acquired as well as community acquired isolates. This is because combination of beta-lactam and beta-lactamase inhibitor (co-amoxiclav, piperacillin-tazobactam, etc.) may have significant activity against ESBLs in vitro, but they might be clinically ineffective and are not the optimal therapy for serious infections due to ESBL-producing organisms[5]. And also plasmid coding for ESBL enzymes may carry co-resistance genes for other non-β-lactam antibiotics [6]. ESBLs may not always be detected in routine susceptibility tests and selection of antibiotic or antibiotic combination becomes difficult. The aim of this study is to detect ESBL producing enterobacteriaceae by using detection tests like ESBL E test strips (based on Ceftazidime and Ceftazidimeclavulanate) and rapid phenotypic detection tests like ESBL Hichrome agar and also to compare the efficacy of these tests.

MATERIALS AND METHODS

Study design: This is a cross sectional comparative study.
Studyperiod: From January 2016 to June 2016.

Place of study: Bangalore Medical College And Research Institute, ethical clearance was obtained.

Inclusion criteria: Clinical isolates of enterobacteriaceae.

Exclusion criteria: Clinical isolates other than enterobacteriaceae like gram positive cocci, Gram negative non-fermenters were excluded.

Sample size: A total of 3400 patients were selected and samples urine, pus, sputum, pleural and ascitic fluid were collected and processed for bacteriological investigations.

Sample collection and processing: The clinical history of the patients such as age, sex, relevant history were recorded on a proforma. Samples were processed using conventional methods and Gram staining was also performed. They were incubated aerobically at $37^{0}C$ overnight or maximum of 24 hours and the plates were examined for growth. The organisms were identified on the basis of their Gram staining properties, biochemical reactions. Consecutive non-duplicate isolates of enterobacteriaceae were selected for further processing. Antimicrobial susceptibility was done by means of agar disc diffusion method of Kirby Bauer according to the guidelines of clinical and laboratory standards institute[7].
All enterobacteriaceae isolates were subjected to ESBL Hichrome agar plating (Figure-1) and E test

Colony morphology on ESBL Hichrome agar is as follows

- ESBL producing E coli- pink to reddish colonies
- ESBL producing Klebsiella spp./ Citrobacter spp.- Metallic blue colonies
- ESBL producing Proteus spp.- Brown halo colonies
- ESBL non-producers - Inhibited

ESBL E- TEST STRIP: The ESBL E-test strips carry two gradients, Ceftazidime (TZ) (0.5-32 g/ml) on the one end and ceftazidime plus clavulanic acid (TZL) (0.064-4 g/ml) in a different concentration gradient on the other end, along with a fixed concentration of clavulanic acid (4 g/ml). The test organism was inoculated as a lawn on a Mueller Hinton agar plate and the above mentioned E strip is placed on the plate. The plates were incubated at $37^{0}C$ overnight and they were examined next day (Figure-2). The isolate showing MIC reduction of ceftazidime by 3 two-fold dilutions in the presence of clavulanic acid is considered as ESBL producer. ESBL Hichrome agar allows the detection of ESBL producers by inhibiting the growth of other bacteria as shown in Table-1.

Statistical analysis: The data obtained is in the form of frequencies and percentages and is analyzed using statistical software and projected in form of tables.

RESULTS

A total of 3400 various samples were included in our study. Out of which 548 samples were culture positive for enterobacteriaceae and were selected for further processing. Among them Klebsiella species isolates were 264 (48.1%), Escherichia coli -218 (39.7%), Proteus species - 42(7.6%) and Citrobacter species - 24 (4.3%). Antibiotic sensitivity pattern of the enterobacteriaceae showed that majority of them were resistant to Aztreonam, Ceftazidime, Cefepime. Most of the isolates showed sensitivity to Imipenem (93.4%), Piperacillin/ tazobactam (93%) as shown in table-1. 308 (56.2%) of all the enterobacteriaceae isolates were found to be ESBL producers by ESBL E test method. In the present study 50.9% of E coli, 61.3% of Klebsiella species, 57.1% of Proteus species and 58.3% of Citrobacter species were found to be ESBL producers by E test. In our study 291 (53.1%) isolates yielded growth on ESBL Hichrome agar among 548 isolates of enterobacteriaceae. ESBL E coli showed pink to red colonies, ESBL Klebsiella/ Citrobacter showed metallic blue colonies, ESBL Proteus showed Brown halo on ESBL Hichrome agar. 47.2% of E coli, 58.3% of Klebsiella species, 52.3% of Proteus species and 58.3% of Citrobacter species were found to be ESBL producers by ESBL Hichrome agar as shown in table-2. ESBL Hichrome agar for detecting ESBL production showed total Sensitivity-91.2%, Specificity-95.8%, Positive predictive value-96.5%, Negative predictive value-89.4% when compared to ESBL E test.

DISCUSSION

ESBL producing enterobacteriaceae have been increasing both in number and variety as they are rapidly evolving [1].
In our study we isolated 48.1% of Klebsiella species, 39.7% of Escherichia coli, 7.6% of Proteus species and 4.3% of Citrobacter species from a total of 548 enterobacteriaceae isolates. Commonest isolate in our study was Klebsiella species. In the study done by Kumar S *et al* predominant isolate was Escherichia coli (53.6%) [1]. E. coli was the most common (65.32%) isolate followed by K. pneumoniae (24.9%) in study done by Shashwati N *et al* [6]. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms. Our study showed that 93.4% of enterobacteriaceae isolates sensitivity to Imipenem, which is comparable to studies done by Kumar S *et al*, Dalela G [1,2]. In studies done by Shashwati N *et al* and Mohanty S *et al* they showed that all the isolates were sensitive to Imipenem [6,8].

Clinical and Laboratory Standards Institute (CLSI) provides guidelines for the detection of ESBLs in Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli and Proteus mirabilis. In common to all ESBL-detection methods is the general principle that the activity of extended-spectrum cephalosporins against ESBL-producing organisms will be enhanced by the presence of clavulanic acid. Two different Etest gradient formats have been use in our study based on reduction of ceftazidime MICs by more than 3 two-fold dilutions in the presence of clavulanic acid. It was observed in our study that 56.2% of all the enterobacteriaceae isolates were found to be ESBL producers by ESBL E test.35% of the isolates were ESBL positive and the remaining 65% were ESBL negative according to the study done by Prabha R. *et al* [3]. In the present study 50.9% of E coli, 61.3% of Klebsiella species were observed to be ESBL producers whereas Kumar S *et al* showed that E.coli with 53.6was the largest group of ESBL producers followed by K. pneumonia 32.8% [1]. In study done by Dalela G showed that 73.5% E. coli and 58.1% Klebsiella pneumoniaeisolates were ESBL producers [2]. R. Prabha *et al* showed sensitivity of 100% in detecting ESBL producers by ESBL Hichrome agar whereas in our study we found 94.4% sensitivity for the same [3].

Table 1 Antibiotic sensitivity pattern of the enterobacteriaceae isolates (% of sensitivity).

Name and no of isolates	AMC	CAZ	CTX	CPM	AT	C	CIP	COT	GEN	PIT	IMP
E coli n= 218	121 (55.3%)	131 (60%)	142 (65.1%)	130 (59.6%)	88 (40.3%)	135 (61.9%)	124 (56.8%)	136 (62.3%)	184 (84.4%)	201 (92.2%)	200 (91.7%)
Klebsiella spp. n= 264	141 (53.4%)	129 (48.8%)	136 (51.5%)	132 (50%)	94 (35.6%)	156 (59%)	147 (55.6%)	147 (55.6%)	196 (74.2%)	245 (92.8%)	250 (94.6%)
Proteus spp. n=42	28 (66.6%)	19 (45.2%)	21 (50%)	18 (42.8%)	16 (38.0%)	22 (52.3%)	24 (57.1%)	27 (64.2%)	34 (80.9%)	40 (95.2%)	39 (92.8%)
Citrobacter spp. n=24	16 (66.6%)	10 (41.6%)	14 (58.3%)	12 (50%)	16 (66.6%)	18 (75%)	18 (75%)	20 (83.3%)	19 (79%)	24 (100%)	23 (95.8%)
Total n=548	306 (55.8%)	289 (52.7%)	313 (57.1%)	292 (53.2%)	214 (39%)	331 (60.4%)	313 (57.1%)	330 (60.2%)	433 (79%)	510 (93%)	512 (93.4%)

Amoxicillin-clavulanate acid[AMC] (20/10 µg), Ceftazidime[CAZ] (30µg), Cefotaxime[CTX] (30 µg),Cefepime[CPM] (30µg), Aztreonam[AT] (30µg), Chloramphenicol[C] (30µg), Ciprofloxacin[CIP] (5µg),Trimethoprim- sulfamethoxazole[COT] (1.25/23.75 g), Gentamicin[GEN] (10µg), Piperacillin/tazobactam[PIT] (100/10µg), and Imipenem[IMP] (10µg)

Table 2 Detection of ESBL production by ESBL Hichrome agar and ESBL E test

Organism isolated	ESBL production	
	ESBL Hichrome agar	ESBL E test
E coli n= 218	111 (50.9%)	103 (47.2%)
Klebsiella spp. n= 264	162 (61.3%)	154 (58.3%)
Proteus spp. n=42	23 (57.1%)	22 (52.3%)
Citrobacter spp. n=24	14 (58.3%)	14 (58.3%)
Total n=548	308 (56.2%)	291 (53.1%)

Figure-1 ESBLHichromeagar

Figure-2 Ceftazidime with Ceftazidime + Clavulanic acid ESBL E test

CONCLUSION

It is important to identify ESBL producing enterobacteriaceae from clinical samples for the judicious use of antibiotics. For early detection of ESBL producing enterobacteriaceae pathogens, ESBL Hichrome agar and E tests were found to be almost equally effective in detecting ESBL production. ESBL Hichrome agar can be used for rapid and presumptive identification of ESBL producing enterobacteriaceae by means of colony colour within 24 hours with good sensitivity and specificity.

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