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COMPARISON OF DOUBLE DISC DIFFUSION METHOD AND VITEK 2 COMPACT SYSTEM TO SCREEN THE ESBL PRODUCERS IN INTENSIVE CARE UNIT IN HOSPITAL

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ABSTRACT

Background: It is essential to develop screening methods for the detection of Extended Spectrum of B- Lactamase (ESBL) producing strains in the laboratories, so that the appropriate medication can be started. In this study we compared the ESBL strain detection with the help of double disk diffusion method and Vitek 2 compact (Biomerieux India Pvt. Ltd.).

Material and Methods: 67 clinical isolates of E. coli (33) and Klebsiella spp. (34) were isolated from the intensive care unit of the hospital from India and screened for ESBL production by double disc diffusion method and Vitek 2 compact system (fully automated susceptibility testing and identification system).

Results: Total 67 isolates of E. coli and Klebsiella spp. were screened in this study, out of that 16 isolates E. coli (9) and Klebsiella (7) were ESBL non-producer according to double disk diffusion method, and then these 16 isolates were retested by Vitek 2 compact system. Out of these16, 14 were ESBL non-producer E. coli (7) and Klebsiella (7) and 2 were ESBL producer by Vitek 2 compact.

Conclusion: From the above results we can conclude that Vitek 2 compact is an automated system and gives more accurate results than double disk diffusion method.

Keywords: ESBL, Double disk diffusion, Vitek 2 compact, E. coli, Klebsiella pneumoniae

INTRODUCTION

Oxyimino cephalosporins are commonly used in the hospital since they are introduced in the clinical practice[1, 2]. The effectiveness of these β-lactam antibiotics has been diminished by Klebsiella spp., which has become resistant to their mode of action. This resistance has spread to strains of Escherichia coli and to other gram-negative bacteria as well [3].

Many surveys shows that the presence of extended spectrum β-lactamase (ESBL) enzymes derived from the widespread TEM-1/2 and SHV-1 family, responsible for this resistance. There are over 110 derivatives of TEM β-lactamase and more than 63 derivates of SHV β-lactamases[4-6]. These enzymes are usually less efficient at hydrolysis than their parent enzymes, and consequently their detection by currently used susceptibility tests is difficult. Therefore ESBL-producing Klebsiella spp. and E. coli may falsely appear to be susceptible to newer cephalosporins. Because current breakpoints for cephalosporin sensitivity are set for clinical efficacy, they are too high to detect ESBL mutations; therefore, there is clearly a requirement to detect the resistance mechanism itself rather than to in vitro susceptibility testing.

MATERIAL AND METHODS

Total 67 clinical isolates of E. coli and Klebsiella spp. from intensive care unit were studied. 36 isolates were E. coli and 34 isolates were Klebsiella

spp. Out of that 23 isolates were from urine, 24 from pus, 12 isolates from sputum, 3 isolates from blood, 3 isolates from high vaginal, 1 isolate from CVP tip and 1 from endotracheal tube. Identification of isolates was done on the basis of their cultural characteristics and reactions in standard biochemical tests according to standard Clinical and Laboratory Standards Institute (CLSI) guideline.[7]

Antibiotic Susceptibility Testing: The isolates were subjected to antibiogram study of routine antibiotics by modified Kirby Bauer's method. The antibiotics are cefixime (AZ-5mcg), ceftazidime (MP-30mcg), tobramycin (TT-10mcg), cefoperazone sulbactum (CM-105mcg), cefuroxime (CG-30mcg), cefoperazone (TF-75mcg), piperacillin (GF-100mcg), amikacin (AK-30mcg), cefepime (GM-30mcg), aztreonam (AC-30mcg) netilmycin (NT-30mcg) amoxicillin/ clavulanic acid (AS -30mcg), co-trimoxazole (BA-25mcg), cefotaxime (CI-30mcg), chloramphanicol (CH-30mcg), cephalexin (PR-30mcg), tetracycline (TE-30mcg), ciprofloxacin (RC-5mcg), imipenem (FD-10mcg), sparfloxacin (DC-10mcg), ampicillin (NX-10mcg) and gentamycin (ZN-10mcg) susceptibility and resistance was determined on the basis of interpretative criteria recommended by the Clinical Laboratory Standards. E. coli (ATCC 25922 strain) was used as the quality control strain in disc diffusion method.

Double Disk Diffusion: Test strains were pre-incubated in brain heart infusion broth (BHIB) at 37^0C to an optimal density matching that of 0.5 McFarland turbidity standards. This suspension was then used to inoculate in Muller Hinton Agar (MHA) plates by swabbing them with a sterile cotton swab. Ceftazidime (30µg) versus ceftazidime/clavulanic (30/10µg) and cefotaxime (30µg) versus cefotaxime/ clavulanic acid (30/10µg) are placed at the recommended distance from each other on the plate. The plates were incubated at 37^0 for 18 hours aerobically before the zone size recorded. A positive result was indicated by a zone size difference at $\geq$5 mm diameter between the combination disc and the corresponding and corresponding single disc as recommended by the manufacturer.

Vitek 2 Compact: The Vitek is an automated system for identification and antibiotic susceptibility testing. Vitek susceptibility test results are expressed as Minimum Inhibitory Concentration (MIC) values and interpreted as susceptible, intermediate or resistant by reference to a CLSI. All isolates were tested with gram negative susceptibility cards (ASTGN13).

RESULT

Among 67 isolates of E. coli and Klebsiella spp. sensitivity was 100% with imipenem, 95% and 90% with piperacillin-tazobactam respectively, by Manual method. In automated system, E. coli and Klebsiella spp. sensitivity was 100% with imipenem, 100% and 96.29% with piperacillin-tazobactam respectively. (Table 1 & 2)

Table: 1 Sensitivity Pattern of Drugs in ESBL Producer by Manual Method

Drug name	Sensitivity (%)	
	E.coli	*K. pneumoniae*
Ampicillin	0%	0%
Piperacillin/tazobactum	95%	90%
Ceftazidine	11.5%	3.7%
Cefepime	26.9%	11.11%
Aztreonam	7.6%	0%
Imipenem	100%	100%
Amikacin	65.3%	62.96%
Gentamycin	34.6%	29.62%
Tobramycin	34.6%	14.8%
Ciprofloxacin	7.6%	22.2%
Cefixime	7.6%	3.7%
Cefoperazone Salbactum	61.5%	77.7%
Cefoparazone	7.6%	3.7%
Piperacillin	50%	55.5%
Netilmycin	50%	48.14%
Cefotaxime	11.5%	3.7%
Chloramphenicol	30.71%	25.9%
Cephalexin	3.8%	3.7%
Tetracycline	11.5%	7.4%
Sparfloxacin	3.8%	18.5%

Table 2: Sensitivity Pattern of Drug in ESBL Producer by Automated System

Drug name	Sensitivity (%)	
	E.coli	*K. pneumoniae*
Ampicillin	0%	0%
Ampicillin salbactum	3%	0%
Piperacillin /tazobactum	100%	96.29%
Cefazolin	0%	0%
Cefotetan	19.23%	33.30%
Ceftazidine	0%	0%
Ceftriaxome	0%	0%
Cefepime	3%	0%
Aztreoman	3%	0%
Imipenem	100%	100%
Ertapenem	100%	100%
Amikacin	100%	100%
Gentamycin	46.15%	22.22%
Tobramycin	23.7%	14.81%
Ciprofloxacin	3%	3.7%
Levofloxacin	3%	18.51%
Nitrofurantoin	34.61%	22.22%
Trimethoprim sulfamethoxazole	38.41%	25.90%

Out of 67 strains of E. coli and Klebsiella spp., 16 were negative for ESBL production by double disc diffusion using cefotaxime and ceftazidime with their clavulanic acid combination. These 16 strains (9) E. coli and (7) Klebsiella spp. retested with

Vitek 2 compact (Biomerieux India Pvt. Ltd.) system, and 14 of these strains were subsequently found to be ESBL negative. Two strains still flagged ESBL positive by Vitek. (Table 3)

Table 3: Comparison of Double disc diffusion method Double disc diffusion method and Automated system

Isolate	Double disc diffusion method		Automated system	
	ESBL+ve	ESBL–ve	ESBL+ve	ESBL-ve
E.coli	24	9	26	7
K. pneumoniae	27	7	27	7
Total	51 (76.11%)	16 (23.88%)	53 (79.10%)	14 (20.89%)

DISCUSSION

Currently there is a great need of reliable and efficient tests to detect ESBLs in clinical isolates of Enterobacteriaceae. Conventional susceptibility testing methods, on their own, fail to offer reliable susceptibility results for β-lactam antibiotics when testing those species that harbors ESBLs. Currently most clinical laboratories do not use a standard method for the detection of ESBLs, and clinical laboratories do not routinely identify Enterobacteriaceae to genus and species level. The Vitek system addresses this issue[8]. It will only validate susceptibility result once the organism has been identified to species level. If the system detects the presence of an ESBL resistance mechanism in strains of Klebsiella spp. and E. coli, it then utilizes its expert software and applies it to the final susceptibility results. If β-lactams are found to be susceptible to ESBL activity, the strain is then flagged as resistant, regardless of whether the in vitro test indicates susceptibility.

This study has shown that the Vitek 2 compact system in our hands, while easy to perform. On the other hand, the double disk diffusion test requires careful spacing of discs for accurate result and careful interpretation of zone sizes. It is therefore technically demanding. In previous studies, the double disk diffusion test was able to detect 82 and 88% of ESBL-positive strains[9-10]. A recent study has reported that cefotaxime achieves a 92% and ceftazidime achieves 82% sensitivity rate in detecting ESBLs in tested isolates.

This study shows the importance of identification of E. coli and Klebsiella spp. and usefulness of the Vitek system for routine detection of ESBLs if accurate and consistent results are to be reported to clinicians.

CONCLUSION

The Vitek ESBL test was cost-effective as an ESBL screen in as much as the ESBL test is an integral part of the susceptibility card and is performed simultaneously with the susceptibility tests. In addition, the Vitek test is interpreted by the system itself, which removes any errors of subjectivity. No additional outlay of resources is required but if laboratories can't afford this system so in that case double disc diffusion is also a good manual method for ESBL detection.

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