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Are Disk Diffusion Tests the Answer for Phenotypic Carbapenemase Detection in *Escherichia coli* in Clinical Laboratories?

Microbiology

KEYWORDS: Phenotypic, Carbapenemase, MHT, Carba NP

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ABSTRACT

Introduction-Carbapenem antibiotics are used to treat infections due to multidrug resistant *Enterobacteriaceae*. Carbapenemases are enzymes which hydrolyse lactam antibiotics including β lactam/ β lactam inhibitor combinations and third generation cephalosporins and the Carbapenem antibiotics -Imipenem, Meropenem and Ertapenem. With the widespread use of higher antibiotics in obstetrics and gynaecology and the emergence of multidrug resistant bacteria especially *Escherichia coli*, detection in clinical laboratories becomes essential. There are multiple genes responsible for carbapenem resistance and identifying each in every isolate may not be worth as the treatment does not differ significantly. **Aims and Objectives**-Detection and comparison of carbapenemases by phenotypic methods in *Escherichia coli* isolated from obstetric surgical site infections. **Materials and methods –** AST of the isolates was performed by the Kirby –Bauer technique and the Imipenem zone diameters were interpreted using the CLSI 2015 guidelines.23 randomly selected *Escherichia coli* isolates were further tested by the Modified Hodge Test, the Carba NP test and AmpC production and compared . **Results**-The Imipenem disk was non-susceptible in 16/23(69.57%).MHT was positive in 12/23(52.17%) of the strains, the Carba NP test was positive in 12/23(52.17%) and Amp C in 8/23(34.78%).The Imipenem disk diffusion test was able to detect carbapenemases as detected by Carba NP test and showed non-susceptibility in the presence of AmpC. MHT did not prove to be useful. **Conclusion**- The phenotypic methods detect Carbapenemase, but not its class. Also there are chances of false positive and false negatives and these cannot be recommended for routine diagnostic laboratories.

INTRODUCTION

Carbapenem antibiotics are used to treat infections caused by multidrug-resistant *Enterobacteriaceae.* Carbapenems have a broad spectrum of antibacterial activity and are highly resistant to -lactamases. The main mechanism of resistance is the production of carbapenemases(1,2). Carbapenemases, including enzymes of Ambler classes A (e.g., KPC types), B (metallo-b-lactamases e.g., IMP, VIM, and NDM types), and D (e.g., OXA-23,48) can hydrolyze almost all -lactams and are not inhibited by lactamase inhibitors(1,3). Carbapenem resistance in *Enterobacteriaceae* is mainly due to two different mechanisms: (i) hyper production of ESBL or AmpC enzymes combined with porin loss or alteration or upregulated efflux pump and/or (ii) production of carbapenem-hydrolyzing carbapenemases(4).

Detection of resistance by molecular methods is challenging, also the number of Carbapenemase genes is increasing rapidly(5).It becomes practically impossible to determine each of the genes implicated in resistance to carbapenems and identifying each in every isolate mat not be worth as the treatment does not differ significantly. Phenotypic methods like Modified Hodge test have been recommended but are not without limitations.MHT is known to give false positive results in the presence of AmpC.

Thus this study was conducted to detect Carbapenem resistance using the phenotypic methods MHT, the CarbaNP test and AmpC production in Obstetric surgical site infections and assess its utility in clinical laboratories.

Materials and methods

This study was carried out from the department of microbiology, King George Medical University, Lucknow, U.P ,a tertiary care centre serving as referral centre for the neighbouring districts. This was a hospital based observational study.

Isolates obtained from patients who had undergone caesarean section and developed surgical site infections during September 2015 –July 2016 were included in the study. The isolates were recovered from pus samples sent by obstetricians.

Inclusion criteria-*E coli* isolates from obstetric cases were included. Exclusion criteria –Non *E coli* isolates and isolates from other infections were not included.

Laboratory procedure

AST was performed by the Kirby Bauer technique and Imipenem disk diameters were interpreted using the CLSI 2015 guidelines (6). 23 randomly selected isolates identified as Escherichia coli from Obstetric surgical site infections were further tested by the Modified Hodge test as described by CLSI 2016(7) and Carba NP test as described by Pasteran F et al(8). Briefly the Carba NP test was performed as follows: Two types of solutions were prepared. Solution A-containing only 0.5% phenol red, 1% triton X 100 and 10 Mm zinc sulphate heptahydrate. Solution B consisting of solution A and 12mg/ml Imipenem cilastatin powder (injectable form).A loopful of the isolate was inoculated in both the solutions taken as 100µl in eppendorf and incubated for 2 hours. A colour change of yellow was considered as positive for Carbapenemase production. If the solution remained red coloured in the eppendorf labelled as B ,it was considered positive.

Briefly the MHT was performed by preparing a 0.5 McFarland suspension of *Escherichia coli* ATCC 25912 strain was prepared and diluted 1:10 with broth. This suspension was then used to prepare a lawn culture over the MHA plates and allowed to dry for 10minutes.A 10µg Imipenem disk was then applied in the centre of the disk. The isolates to be tested were then streaked from slightly away from the disk margin till the edge of the plate and incubated .A zone of enhanced indentation at the junction of ATCC strain inhibition zone and the test streak was considered as positive.

AmpC was performed by the combined disk test using cloxacillin as the inhibitor. A 0.5 McFarland suspension of the isolate to be tested was prepared and allowed to reach the log phase for 20 minutes and poured off over MHA plates and the plates allowed to dry. Two disks one containing cefoxitin 30µg and the other containing cefoxitin with cloxacillin (30/100) µg were applied at a distance of 2.5cm and incubated for 18-20 hours at 37°C and the zone diameters compared. A difference of more than 5mm between the two disks was considered as AmpC producer.

RESULTS-

23 strains of *E.coli* randomly selected from the surviving strains and subjected to the phenotypic tests showed the following results.

Table1. *Phenotypic tests done on the test strains and their results.*

Tests	Imipenem disk		MHT		Carba NP		Amp C	
	R(16)	S(7)	P(12)	N(11)	P(12)	N(11)	P(8)	N(23)
Imipenem disk	R(16)		10	6	12	4	4	12
	S(7)		2	5	0	7	4	3
MHT	P(12)	10	2		10	2	3	9
	N(11)	6	5		2	9	5	6
Carba NP	P(12)	12	0	10	2		2	10
	N(11)	4	7	2	9		6	5
Amp C	P(8)	4	4	3	5	2	6	
	N(15)	12	3	9	6	10	5	

16(69.57%) strains were detected to be non-susceptible by Imipenem disk diffusion tests. 12(52.17%) strains of *E.coli* were positive by the modified Hodge test, 12(52.17%) by the Carba NP test and 8(34.78%) for the AmpC.

Among the 16 Imipenem non-susceptible strains, only 10 could be detected by the MHT and concurrently by the Carba NP. Thus there were 10 strains that were positive by all the three tests. Only 2 strains were positive for AmpC. 2 strains susceptible by Imipenem disk were positive by the MHT, but non by Carba NP test. Thus Imipenem disk susceptibles correlated well with Carba NP negatives. In the two MHT positives one strain was AmpC producer.

Among the 12 MHT positive strains, 2 were susceptible by Imipenem disk and negative by Carba NP test implying resistance mechanisms other than Carbapenemases being detected by MHT or false positives. 6 strains were non-susceptible on Imipenem disk and negative by MHT, thus all Carbapenemases were not detected by the MHT. Among the 12 Carba NP positive strains all were non-susceptible by Imipenem disk test. Thus Imipenem disk diffusion test and Carba NP test correlated well in detecting Carbapenemase production.

AmpC was detected in 4 strains with Imipenem non-susceptibility and 4 strains with Imipenem susceptible strains.

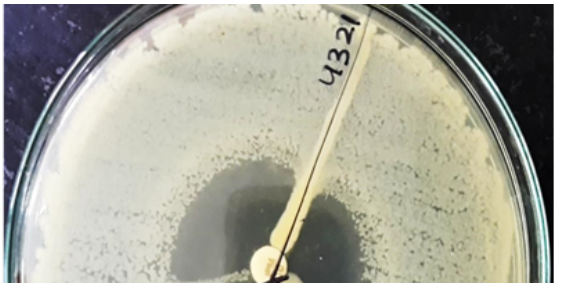

Fig.1:Zone of indentation in a MHT positive strain.

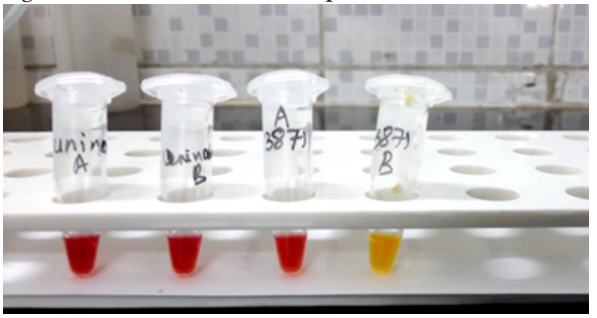

Fig.2:Positive Carba NP test. Yellow coloured eppendorf shows a carbapenemase production by the bacterial isolate which caused hydrolysis of the Imipenem and produced acidic products causing a change in the PH of the solution.

Red eppendorf did not produce a colour change ,hence no hydrolysis indicating a negative test.

DISCUSSION

19 strains were detected to be non-susceptible by Imipenem disk diffusion tests of which 7 were MHT negative and 6 were Carba NP negative which means these strains exhibited non-susceptibility but it could not be detected by either of the tests. Phenotypic methods like MHT give variable results.Studies(3,4) have reported that MHT was useful for detection of KPCs and OXA-48 like enzymes but performed poorly for metallo- -lactamases(NDM,VIMs and IMP).Another shortcoming of MHT is it can give false-positive results for Carbapenem-resistant but non Carbapenemase producing strains(4).In this study also MHT was positive in strains with Amp C phenotype and negative Carba NP and Imipenem disk susceptibility.In this study the Carba NP test correlated well with the Imipenem disk susceptibility test as all the Carba NP positives were resistant by disk test.

Phenotypic detection of AmpC in *E. coli* does not indicate if the enzyme is chromosomal or plasmid mediated, but as a crude guide, lack of multiple drug resistance is suggestive of a chromosomal AmpC whereas multiple drug resistance is consistent with either plasmid-mediated or chromosomal AmpC production.

The automated susceptibility systems may be unreliable for Carbapenemase detection(9,10). Thus the Phenotypic tests were able to detect resistance but as many of the strains may be harbouring more than one mechanism of resistance it may be difficult to predict which test may be superior and the recent CLSI guidelines for Imipenem zone diameters seem to appropriate in detecting resistance.

CONCLUSION

Thus the Imipenem disk diffusion test seems to be sufficient enough to detect Carbapenemases in *E coli* using the current CLSI guidelines from Surgical site infections in obstetric patients but there are chances that some susceptible isolates may be reported as resistant also.

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