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Research Paper

Phenotypic Methods for Detection of AmpC ß-lactamase in Klebsiella Species

Medical Science

KEYWORDS : CDDT, M3DT, AmpC β-lactamase, Klebsiella species

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ABSTRACT

Objective: To find incidence of AmpC β-lactamase in Klebsiella species and comparison of two methods, combined disc diffusion test (CDDT) & Modified three dimensional test (M3DT).

Methods: All the cefoxitin resistant isolates of Klebsiella species were subjected to determine the AmpC β-lactamase by combined disc diffusion test (Cefoxitin + Phenylboronic acid) & Modified three dimensional test (Enzyme extract method).

Result: AmpC β-lactamase was detected in 44 & 55 of the 200 isolates of Klebsiella species by combined disc diffusion test (CDDT) and Modified three dimensional test (M3DT) respectively.

Conclusion: AmpC β-lactamase as a drug resistance mechanism was found in 22% strains by CDDT and 27.5% strains by M3DT method. Hence M3DT method is advised to study AmpC β-lactamase in Klebsiella species.

Introduction

The predominant mechanism for resistance to β-lactam antibiotics in Gram-negative bacteria is by the synthesis of β-lactamases [(1)]. Among the β-lactamases the production of ESBLs and AmpC β-lactamases are the most common [(2)]. AmpC enzymes belong to Class C in the Ambler structural classification of β-lactamases, while in the functional classification scheme of Bush et.al., these are assigned to group 1 [(3,4)]. AmpC β-lactamases have gained importance since the late 1970s as one of the mediators of antimicrobial resistance in Gram-negative bacilli. AmpC β-lactamase production confers resistance to a wide variety of β-lactam antibiotics including 7-α-methoxy cephalosporins (cefoxitin or cefotetan), oxyimino cephalosporins (cefotaxime, ceftazidime, ceftriaxone), monobactam (aztreonam) and these AmpC β-lactamase are not inhibited by clavulanic acid [(5)].

The production of the AmpC beta lactamases was detected and their prevalence in the *Enterobacteriaceae* was found to be 1.7-7.6% in Canada [(2)], 1.91-7.54% in China [(6)] and 1.2% in the US [(2)]. Indian studies have detected prevalence rates of the AmpC beta lactamases of 3.3-24.1% among the *E.coli* isolates [(7-12)], 2.2-37.5% among *Klebsiella pneumonia* [(7-13)] and 37.77% among *Proteus mirabilis* [(14)].

Material & Methods

A total of 200 nonduplicate isolates of *Klebsiella* species were collected from different clinical samples in Microbiology laboratory of MGM Hospital Kamothe, Navi Mumbai, over a period of 2 years. The identification of *Klebsiella* species were done by various biochemical tests. Screening of Cefoxitin resistance were identified by Kirby Bauer disc diffusion method on Muller Hinton agar, as per CLSI guidelines [(15)]. All the cefoxitin resistant isolates were subjected for the detection of AmpC β-lactamase by CDDT & M3DT.

Detection of AmpC β-lactamase

Combined disc diffusion test (CDDT)[(16)]

Procedure: - Two discs cefoxitin (30µg) and cefoxitin + phenylboronic acid (30/400µg) were placed at a distance of 20mm (centre to centre) on Muller-Hinton agar plates inoculated with cefoxitin resistant *Klebsiella* strain and incubated for 24hrs at 37^{0}C.

Interpretation: - If the diameter of the inhibition zone around the cefoxitin + phenylboronic acid disc is ≥ 5mm greater than the diameter of the inhibition zone around the cefoxitin disc alone is considered to be AmpC β-lactamase production.

Preparation of Phenylboronic acid: - 120mg of phenylboronic acid was dissolved in 3ml of dimethylsulphoxide and 3ml of sterile distilled water was added to this solution. 20µl of the stock solution was dispensed onto disc.

Modified three-dimensional test (M3DT)[(2)]

With slight modification

Procedure: -

Fresh overnight culture from the MacConkey agar plate is transferred into a sterile micro centrifuge tube containing peptone water.

Incubate at 37^{0}C for 3-4hrs.

Centrifuged at 3000rpm for 15minutes.

The pellet was subject to repeated freeze-thawing for seven times in the freezer portion of the ordinary Refrigerator and crude enzyme was extracted.

Cefoxitin (30µg) disc placed on Muller-Hinton agar plates containing lawn of 0.5 McFarland of E. coli ATCC 25922 culture.

Linear slits (3cm) was cut using sterile surgical blade, 3mm away from antibiotic disc.

A well was made using sterile standard bacteriological loop having diameter of 2mm.

A total of 20-30µl of enzyme extract was loaded in the well.

The plates were kept upright for 5 to 10 minutes until the liquid dried and incubated at 37^{0}C for 24hrs.

Interpretation: -

Distortion of inhibition zone is considered positive, for the presence of AmpC β-lactamase and no distortion is considered as

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negative for the presence of AmpC β-lactamase.

RESULT

Out of 200 isolates of *Klebsiella*, 87 isolates were resistant to cefoxitin, so they were suspicious for AmpC β-lactamase producer and further subjected to CDDT and M3DT. AmpC β-lactamase detected in 44 (22%) & 55 (27.5%) isolates of *Klebsiella* species by CDDT and M3DT respectively.

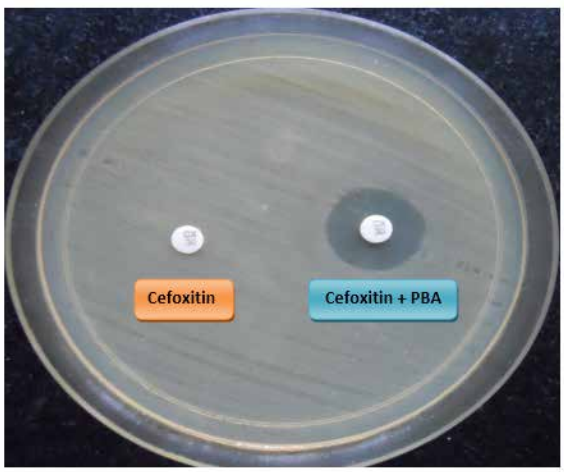


Fig: 1 Combined disc diffusion test (CDDT). (PBA = Phenylboronic acid)

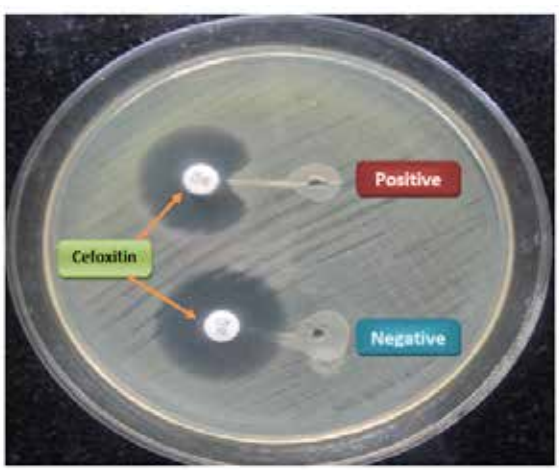


Fig: 2 Modified three dimensional test (M3DT)

1st Chart showing comparison of methods

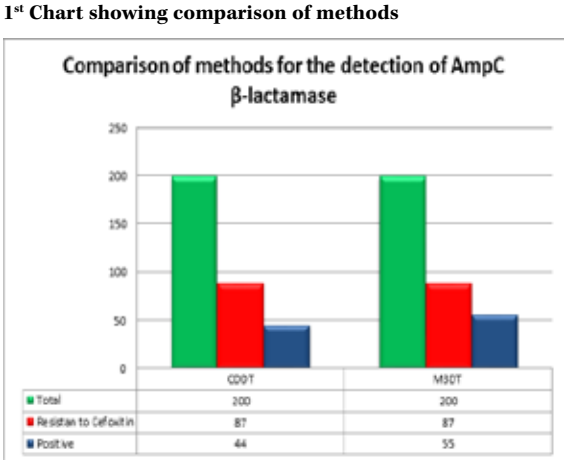


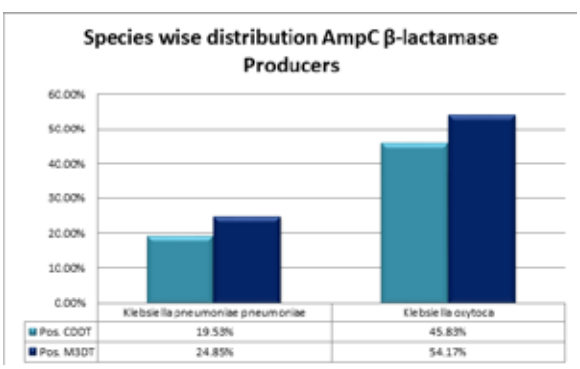
Table 1) Sample wise distribution AmpC β-lactamase Producers

S.No.	Sample	Total	Cefoxitin Resistant	Positive	
				CDDT	M3DT
1	Blood	17	15	8 (47%)	11 (64.7%)
2	Pus	37	21	12 (32.43%)	14 (37.8%)
3	Sputum	51	24	12 (23.5%)	15 (29.4%)
4	Urine	53	14	7 (13.2%)	8 (15%)
5	Endotracheal aspirate	25	10	5 (20%)	6 (24%)
6	Vaginal swabs	2	1	0	1 (50%)
7	Accessory devices	6	2	0	0
8	Throat swabs	5	0	0	0
9	Stool	4	0	0	0
Total		200	87	44 (22%)	55 (27.5%)

Table 2) Species wise distribution AmpC β-lactamase Producers

S.No.	**Species of *Klebsiella***	No. of isolates	Cefoxitin Resistant	Positive	
				CDDT	M3DT
1	Klebsiella pneumoniae pneumoniae	169	66	33 (19.53%)	42 (24.85%)
2	Klebsiella pneumoniae Ozaenae	7	2	0	0
3	Klebsiella oxytoca	24	19	11 (45.83%)	13 (54.17%)
Total		200	87	44 (22%)	55 (27.5%)

2nd Chart showing species wise distribution of AmpC β-lactamase



Discussion

Study of AmpC β-lactamase by CDDT and M3DT was carried out by various researchers. Though results are variable, our results are close to following.

CDDT – Our findings 22% is nearer to Shahal Mansouri et.al. (28%) [17], No. Yilmaz (18.3%)[18]. However Tanushree Barua et.al. (35.9%)[19] reported higher values. Anand Manoharan reported less values (12.54%)[20].

M3DT – Our findings 27.5% are nearer to V. Manchanda et.al. (25.5%)[21], Singhal et.al. (33.3%)[8], Varsha Gupta (32%)[22], Subha et.al. (24.1%)[12], Tanushree Barua et.al. (34.3%)[19]. However, Praveen et.al. (63%)[5], Rudresh et.al. (45.45%)[23] reported higher values. Suranjana et.al. (13%)[24], Neena et.al. (17.33%)[25], No Yilmaz (18.3%)[18] reported lower values.

Differences of findings of AmpC β-lactamase by different researchers could be because of different population of *Klebsiella*

strains which differ in their genomic endowment according to geographic/environmental conditions and usage.

Conclusion

AmpC β-lactamase as a drug resistance mechanism was found in 22% strains by CDDT and 27.5% strains by M3DT method. Hence M3DT method is advised to study AmpC β-lactamase in *Klebsiella* species.

Failure to detect AmpC β-lactamase has contributed to their uncontrolled spread and therapeutic failures so that it is important to identify earliest by simple & cost effective test to avoid irrational use of antibiotics and to prevent further resistance.

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