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Association of Non- molecular Methods to the Detection of Metallo-B-Lactamases

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Abstract
Introduction: *The non-molecular methods used for detection of MBL were the Double Disc Synergy Test (DDST), Combined Disc Synergy Test (CDST). The aim of the study was to evaluate the association of two non-molecular methods (CDST & DDST) for the detection of Metalo-beta-Lactamases (MBL).*
Methods: *This Cross-Sectional study was carried out in the Department of Microbiology, Chittagong Medical College and Hospital, Chittagong. During the period of July 2015 to June 2016 after approval of the protocol by the ethical review committee of Chittagong Medical College. Samples were collected from patients admitted to the Intensive Care Unit (ICU) in Chittagong Medical College. Data were analyzed by using computer software SPSS (Statistical Package for Social Sciences) v. 20.0 and MS-Excel 2016.*
Results: *Among the 66(100%) screening test positive cases 50 (75.8%) were positive for MBL producers by the Combined Disc Synergy Test & 48 (72.7%) were positive for MBL. producers by the Double Disc Synergy Test. The association between CDST and DDST (with y test significance) was shown. The difference in MBL detection by Combined Disc Synergy Test and Double Disc Synergy Test were statistically not highly significant (P < 0.001).*
Conclusions: *High rate of MBL producing gram-negative bacteria in this study emphasizes the need for active surveillance in the microbiology laboratories for the detection of these resistant strains and also stresses the judicious use of Carbapenems to prevent the spread of resistant organisms, CDST was found efficient method to detect MBL. than DDST.*

Keywords: *Non-molecular Method; CDST; DDST; Metallo-B-Lactamase*

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I. Introduction

In the early 1990s, Metallo-β-lactamase (MBL) encoding genes have been reported worldwide in clinically important pathogens, such as Pseudomonas spp., netobacter spp. and members of the Enterobacteriaceae family[1]. MBLS spread easily on plasmids and the acquired resistance mechanisms are attained by bacteria through mutations or mechanisms of horizontal gene transfer such as transformation, conjugation, transduction, transposon and insertion sequence common region (ISCR) elements. A study[2] in Bangladesh 132 Pseudomonas spp. and 76 Acinetobacter spp. were studied. Among them 53(40.1%) Pseudomonas spp. and 29(38.1%) Acinetobacter spp. were resistant to Imipenem. Among 53 and 29 Imipenem-resistant Pseudomonas and Acinetobacter isolates, 44(83.1%) Pseudomonas spp. and 19 (65%) Acinetobacter spp. were found positive for MBL by EDTA-IPM microdilution MIC test. In her study. 36 (67.9%) Pseudomonas spp. were positive by DDST on the other hand, 37(69.8%) Pseudomonas spp. were positive by CDST. In the case of 19(65.5%) Acinetobacter spp. isolates, 15(51.7%) were positive by DDST similarly 16(55.2%) were positive by CDST. However, another study has been conducted in BIRDEM, Dhaka, in which 43% of Pseudomonas spp. isolates from various clinical samples were MBL. Producers[3]. In Bangladesh, 22.86% NDM-1 positive isolates were detected from the Imipenem resistant organisms[4]. In October (2010), at the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB) laboratories, 1,816 consecutive clinical samples were tested for Imipenem resistant gram-negative organisms. Imipenem-resistant isolates were tested for the bla NDM-1 gene. Among 403 isolates, 14(3.5%) were positive for bla NDM-1, and the predominant species were Klebsiella pneumoniae. Acinetobacter baumanni and Escherichia coli. All bla NDM-1-positive isolates were resistant to multiple antibiotics[5]. The non-molecular methods used for detection of MBL

were Double Dise Synergy Test (DDST), Combined Disc Synergy Test (CDST). Later Lee et al[6] described DDST method and Yong et al.[7] described CDST method for easy detection of MBL. in routine laboratories. Several studies document that MBL is already present in our country, the reason of this high rate of MBL producers in Bangladesh might be due to indiscriminate use of antibiotics, especially Carbapenems. So, it is necessary to detect the MBL producing gram-negative bacilli in the routine clinical microbiology laboratories to initiate appropriate antimicrobial therapy and thereby reduce the dissemination of MBL producing gram-negative bacilli in hospitals and communities.

II. Objectives

The objective of this cross-sectional study was to evaluate the association of two non-molecular methods (CDST & DDST) for the detection of Metalo-beta-Lactamases (MBL).

III. Methods

This Cross-Sectional study was carried out in the Department of Microbiology, Chittagong Medical College and Hospital, Chittagong. During the period of July 2015 to June 2016 after approval of the protocol by the ethical review committee of Chittagong Medical College. Samples were collected from patients admitted to the Intensive Care Unit (ICU). Urology Department and Burn unit of Chittagong Medical College. Total 300 hundred samples were collected from both sexes and different age groups; informed written consent was duly taken. The patients wh included in this study were infected wounds, urinary catheterization, and infected bur patients. A wound swab was taken from the wound using two sterile swab sticks. One was used for microscopic examination and the other for culture. Swabs were taken to the laboratory as early as possible[8,9]. About 10 ml of urine sample was aspirated and injected into the sterile test tube. The container was properly labeled with patients' names, ID numbers, etc. Identification of organisms was done on the basis of their colony morphology, staining characteristics, pigment production, oxidase reaction, citrate utilization, hydrozen sulphide production, and other relevant biochemical tests as per standard laboratory methods of identification. Community-acquired infections were excluded in this study. Data collection was done by using a structural questionnaire comprised of general information, history of getting antibiotics, clinical findings and checklist. Data was collected, recorded, edited and analyzed in a predesigned datasheet. The result of the experiment was recorded systematically and statistical analysis was performed by Chi-Square test. Data were analyzed by using computer software SPSS (Statistical Package for Social Sciences) v. 21 & MS-Excel 2016.

IV. Results

A total of 220 samples were studied in this study. Sex distribution of 220 cases of them 70 (31.8) were male and 150 (68.2) were female. The male and female ratio was 1:2.1 [Figure-1]. From the age distribution of the study patients, the highest 83(37.7%) cases were from the 21-30 years age group followed by 33(15.0%) cases from 31-40 years, 31(14.1%) cases from 11-20 years, 27(12.3%) cases were from 41-50 years, 16(7.3%) cases were from 51-60 years, 15(6.8%) cases were from > 60 years and ≤ 10 years [Figure-2]. In the distribution of gram-negative bacterial isolates was studied. Among the 220(100%) isolates 197(89.5%) were gram-negative bacterial isolates of which the majority were Klebsiella species 86 (39.1%) followed by E. coli 50 (22.7%). Pseudomonas species 49(22.3%), Acinetobacter species 7(3.2%), Proteus species 5(2.3%). This table also showed that among the 172(100%) wound swab and pus majority were Klebsiella species 76(44.2%) followed by Pseudomonas species 36(20.9%), E. coli species 28 (16.3%), Acinetobacter species 7(4.1%), Proteus species 3(1.7%). Among the 48 (100%) urinary isolates majority were E. coli 22(45.8%) followed by Pseudomonas species 13(27.1%), Klebsieilla species 10(20.8%). Proteus species 2(4.2%) and Acinetobacter species nil. This table also showed the distribution of gram-positive bacterial isolates. Among the 220 (100%) isolates 23(10.5%) were gram-positive bacteria of which Staphylococcus aureus were 12(5.4%), Coagulase-negative Staphylococcus 10(4.5%), Enterococcus foecalis 1(0.4%). Staphylococcus aureus 12(7.0%) and Coagulase-negative Staphylococcus 10(5.8%) were isolated only from wound swab and pus. No Enterococcus foecalis was isolated from wound swab and pus but 1(2.1%) Enterococcus foecalis was isolated only from urine samples. No Staphylococcus aureus and Coagulase-negative Staphylococcus were found in urine. All bacterial isolates were tested for antimicrobial sensitivity by Kirby Bauer disc diffusion technique against different antimicrobial agents [Table-1]. Out of 197(100%) gram-negative bacteria, 66(33.50%) were resistant to both Imipenem and Ceftazidime by Kirby Bauer disc diffusion technique taken as screening positive for MBL. Among the 66(100%) screening test positive cases 50 (75.8%) were positive for MBL producers by the Combined Disc Synergy Test and 48 (72.7%) were positive for MBL. producers by the Double Disc Synergy Test [Table-2]. In the association between CDST and DDST (with y test significance) was shown. The difference in MBL detection by Combined Disc Synergy Test and Double Disc Synergy Test were statistically not highly significant ($P < 0.001$). So, any of the test can be recommended [Table-3].

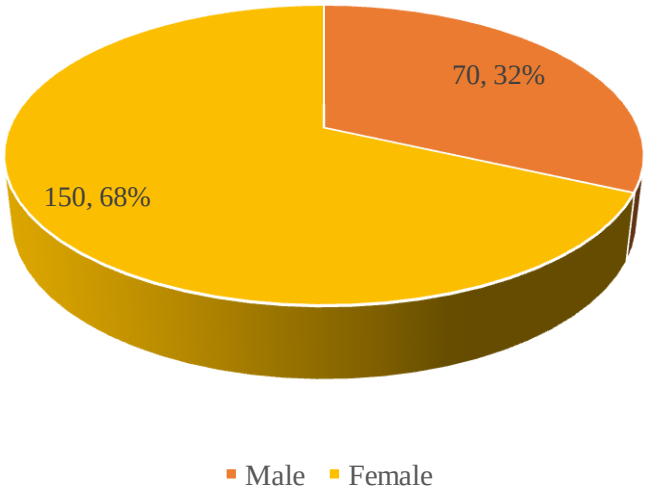

Figure-1: Gender distribution of the study patients (N=220)

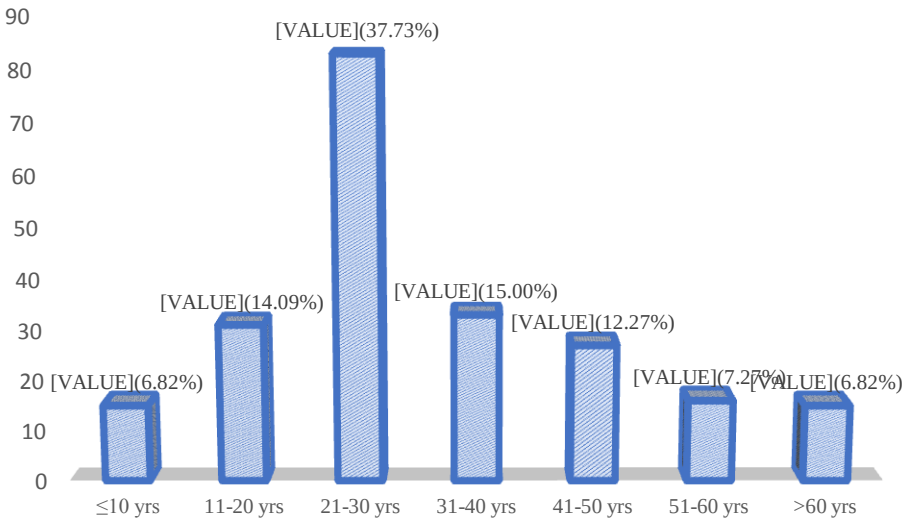

Figure-2: Age distribution of the study patients (N=220)

Table-1: Distribution of gram negative and gram-positive bacteria among the total isolates (n=220)

Number of bacterial species	Wound swab and pus	Urine	Total number of bacteria
Klebsiella spp.	76 (44.2%)	10 (20.8%)	86 (39.1%)
E.coli	28 (16.3%)	22 (16.3%)	50 (22.7%)
Pseudomonas spp.	36 (20.9%)	13 (27.1%)	49 (22.3%)
Acinetobacter spp.	07 (4.1%)	0 (0.0%)	07 (3.2%)
Protenus spp.	03 (1.7%)	02 (4.2%)	05 (2.3%)
Gram Negative Total	**150 (87.2%)**	**47 (97.9%)**	**197 (89.5%)**
Staphylococcus aureus	12 (7.0%)	0 (0.0%)	12 (5.4%)
Coagulase negative Staphylococcus	10 (5.8%)	0 (0.0%)	10 (4.5%)
Enterococcus foecalis	0 (0.0%)	1 (2.1%)	01 (10.5%)
Gram Positive Total	**22 (12.8%)**	**1 (2.1%)**	**23 (10.5%)**
Grand Total	**172 (100.0%)**	**48 (100.0%)**	**220 (100.0%)**

Table-2: Results of CDST and DDST for detection of MBL producing gram negative bacilli among the screening test positive cases (n=66)

Parameter	CDST		DDST	
	n	%	n	%
Positive for MBL	50	75.8	48	72.7
Negative for MBL	16	24.2	18	27.3

Table-3: Association between CDST and DDST (with x^2 test significance)

Parameter		Double Disc Synergy Test			x^2 test Significance
		Positive	Negative	Total	
Combined Disc Synergy Test	Positive	46	4	50	$x^2 = 34.720$
	Negative	2	14	16	
Total		48	18	66	P<0.001 Highly Significance

V. Discussion

Infection with the Metallo-beta-lactamase (MBL) producing organisms is associated with higher rates of morbidity, mortality, and health care costs. Gram-negative bacilli associated with hospital infections are often difficult to eradicate because they are resistant to drugs. Therefore, detection of MBL producing gram-negative bacilli is crucial to control the spread of resistance and for the optimal treatment of patients, particularly the critically ill and hospitalized patients[8].

Among the 220 bacterial isolates, 197(89.5%) were gram-negative bacilli and 23(10.5%) were gram-positive bacilli. This finding was almost similar to the study of Mishra, SK et al.[9] in Nepal, which found 448 (84%) gram-negative bacilli out of total 497 bacterial isolates. In our study among 172(100%) culture-positive wound swab and pus samples, gram-negative bacilli were 150(87.2%) and gram-positive organisms were 22(12,8 %). Pondei, K et al.[10] showed gram-negative bacilli were responsible for 85% of wound infections which was similar to our study. In our study, out of 150(87.2%) gram-negative bacilli, Klebsiella spp. 76(44.2%) was highest followed by Pseudomonas spp. 36(20.9%), E.coli 28(16.3%), Acinetobacter spp. 7(4.1%) and Proteus spp. 3(1.7%). Among the 22(12.8%) gram-positive cocci, we found 12(7.0%) were Staphylococcus aureus, 10(5.8%) Coagulase-negative staphylococcus. In contrast, Pondei. K et al. (2013) found 50% of Pseudomonas aeruginosa was the predominant microorganism isolated from the wound swab and Staphylococcus aureus was the only gram-positive organism isolated. But out of 48(100%) culture-positive urine samples gram-negative bacilli were 47(97.9%), among them E.coli 22(45.8%) was highest followed by Pseudomonas spp. 13(27.1%), Klebsiella spp. 10(20.8%) and Proteus spp. 2(4.2%). and only one (2.1%) gram-positive Enterococcus foecalis was isolated. Guentzel et al.[11] found E.coli was the highest 25% followed by Pseudomonas spp.11%, Klebsiella spp. 8%, Proteus spp. 5% which correlates with my study. An article published in Health and medicine technology journal.[12] found E.coli spp. 63%, Pseudomonas spp. 5%. Klebsiella spp. 15%, and Proteus spp. 5%.

Among the 197-gram negative bacilli, we found 50(25.4%) MBL producers by CDST and 48 (24.4%) by DDST. According to Saini, M et al. (2016) in India among 350 gram-negative bacilli 23.7% were MBL producers by both CDST and DDST which correlates with our study. Agarwal, R. et al[13] showed among the 126 gram-negative bacilli 50,79% were MBL producers by CDST and 48.4% by DDST.

In this study, CDST showed a better the detection of MBL than DDST which is similar to the observation of other workers. According to Gupta, V et al.[14] with the global increase in the types of MBLs, the Clinical Laboratory Standard Institute (CLSI) does not have performance standards documented so far, various screening methods have been employed for screening of clinical isolates for MBL production. Both CDST and DDST are simple, reliable, less cumbersome, and cheap With CDST the positive and negative results were more clearly discriminated and were found to be more superior to DDST. Interpretation of the CDST is more objective than that of DDST results because the DDST depends upon the expertise in discriminating true synergism from the intersection of inhibition zone. So, one major disadvantage of DDST was the subjective interpretation of results. Noyal et al. in India[15], showed. CLSI has recommended modified Hodge. test for detection of carbapenemases activity in Enterobacteriaceae. Tellis, R et al.[16] told us that other methods such as PCR and E test have been used to identify MBL. producers.

LIMITATIONS OF THE STUDY

These non-molecular detection tests may not be cost-effective for routine testing in clinical laboratories. PCR has become more difficult with the increasing number of MBLS and our institute does not have any molecular setup, we were not able to confirm these findings by the genotypic method which was a limitation of our study.

VI. Conclusion

The high rate of MBL producing gram-negative bacteria in this study emphasizes the need for active surveillance in the microbiology laboratories for the detection of these resistant strains and also stresses the judicious use of Carbapenems to prevent the spread of resistant organisms, CDST was found efficient method to detect MBL. than DDST. The main disadvantage in the case of the Double Disc Synergy Test was a subjective interpretation of the result. Hence CDST and DDST can be used as an alternative method for testing in the laboratory to monitor the emergence of MBLs which are economical and easy to perform. Controversies exist regarding the choice of optimal laboratory methods because the two tests are almost similar. In absence of molecular detection techniques, the non-molecular detection method CDST provides a sensible choice for the detection of MBL production & can be implemented in the clinical laboratory on a daily basis. In addition, routine surveillance of MBL producing bacteria is crucial for establishing appropriate empirical antimicrobial therapy and restraining their spread in a hospital environment.

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