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Original Research Article

Evaluation of different phenotypic techniques in detection of metallo-betalactamases in pseudomonas aeruginosa

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ABSTRACT

Background : Metallo-beta-lactamase (MBL) producing Pseudomonas is a worrisome pathogen to hospital infection control due to production of resistance to multiple antibiotics. So, treatment options are narrowed down to only few antibiotics which will result in high morbidity and mortality. This study was conducted with the aim to detect prevalence of MBL producing Pseudomonas aeruginosa and to compare the different phenotypic methods for the detection of MBL production in Imipenem resistant clinical isolates of Pseudomonas aeruginosa.

Materials and Methods: In this prospective study total of 14,145 different clinical samples received from different wards. Out of which 804 Pseudomonas aeruginosa were isolated and antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion method using CLSI guidelines. These were evaluated for Carbapenems resistance and MBL production. Imipenem resistant strains were subjected to screening tests like Double-disc synergy test, Combined disc diffusion test (CDT) and Modified-Hodge test.

Results: In our study out of 804 isolates,153(19.02%) isolates are resistant to Imipenem. Out of 153 Imipenem resistant isolates, Combined disc diffusion test was positive in 100%, Double disc synergy test in 76.5% and Modified-hodge test in 84.15% isolates.

Conclusion: Combined disc diffusion test is very sensitive, cost effective and convenient screening test for detection of MBL producing Pseudomonas aeruginosa. So, all Imipenem resistant Pseudomonas isolates should be regularly screened for detection of MBL by Combined disc diffusion test (CDT) to prevent spread of resistance, longer hospital stay and treatment failure.

1. Introduction

Pseudomonas aeruginosa is well known as a clinically troublesome pathogen causing a wide range of opportunistic infections and nosocomial outbreaks.[1] The infection caused by Pseudomonas species can be seen among patients with cystic fibrosis, burn wounds, acute leukemia, organ transplants, immunocompromised patients and intravenous-drug addicts.[2] The most serious infections include malignant otitis media, endophthalmitis, endocarditis, meningitis, pneumonia and septicaemia.[3]

Because of its intrinsic and acquired mechanisms of drug resistance, it is a cause of concern for treating physicians.[4] Acquired resistance is reported by the production of plasmid mediated AmpC Beta (β)– lactamase, Extended Spectrum Beta(β)–lactamase (ESBL) and Metallobeta (β)–lactamase (MBL) enzymes.[5]

Acquired metallo-(β)–Lactamase(MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyse with

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the exception of aztreonam, all (β)- lactamas including carbepenems and also because their genes are carried on highly mobile elements which allows easy dissemination.[6] In recent years, MBL genes have spread from Pseudomonas aeruginosa to members of Enterobacteriaceae.[7] There is association between MBL producing isolates and morbidity and mortality which is high (ranging between 27 to 48 %) in critically ill patients.[6,7]

There are very few studies that have been done on MBL producing Pseudomonas aeruginosa isolates from our area. This study is undertaken to detect prevalence of MBL producing Pseudomonas aeruginosa from different wards and to compare the different phenotypic methods for the detection of MBL production in imipenem-resistant clinical isolates of Pseudomonas aeruginosa.

2. Materials and Methods

This prospective study was carried out in Department of Microbiology B.J.Medical College, Ahmedabad from October 2017 to June 2018. A total of 14,145 different clinical samples from various sites like pus, sputum, urine, blood and pleural fluid were received from hospitalized patients. Pus, sputum, urine and pleural fluid samples were inoculated on MacConkey agar & Blood agar at 37°C for 18-24 hours. Blood samples were inoculated in automated blood culture bottles. After signalling in BacT/alert, blood samples were subcultured on MacConkey agar and Chocolate agar and incubated at 37° C for 18-24 hours. Pseudomonas was identified as per standard laboratory procedure. The isolates were subjected for antibiotic susceptibility testing on Muller Hinton Agar by employing Kirby Bauer disc diffusion techniques according to recent CLSI guidelines. Pseudomonas aeruginosa ATCC 27853 strain was used as control strain. Colistin screening agar was used to screen Colistin.

All Imipenem resistant strains were further tested for carbapenemase production by Modified Hodge test, screeing for MBL production done by Imipenem (IMP)-EDTA combined disk synergytest (CDST) and Double disk synergy test (DDST). All data entered in an MS Excel data sheet and analyzed.

2.1. Imipenem (IMP) -EDTA combined disk synergy test (CDST)

Muller hinton agar plate was used for lawn culture of test isolate (0.5 McFarland opacity standard). Two 10 μg imipenem discs were placed on inoculated plates.10 μl of 0.5 M EDTA solution was added to one of the imipenem discs. The plate was then incubated overnight at 37°C for 24 hours. If the zone of inhibition of imipenem + EDTA discs compared to imipenem alone is >7 mm, the test was considered as positive.

Fig. 1: Combined disc synergy test

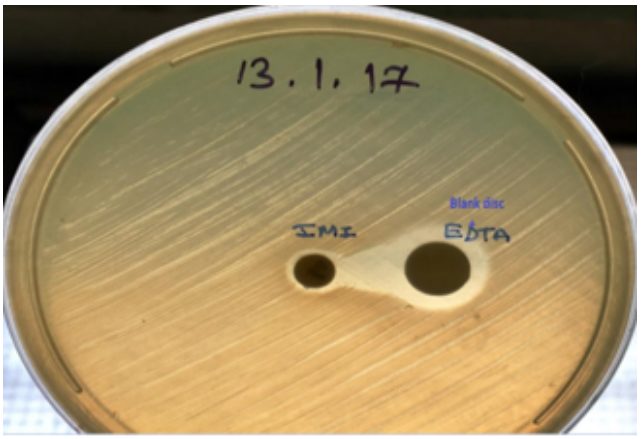

Fig. 2: Double disc diffusion test

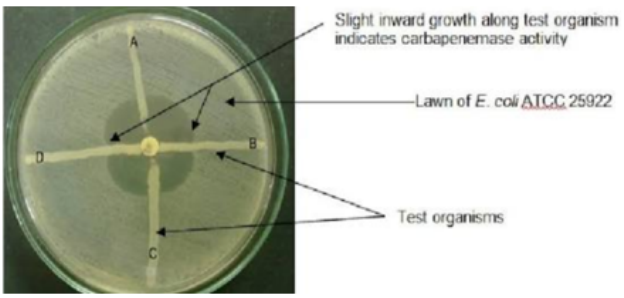

Fig. 3: Modified hodge test

2.2. Double disk synergy test (DDST)

The test isolate (0.5 McFarland opacity standard) was inoculated on a Muller hinton agar plate. After drying, a 10 μg imipenem disc and a sterile blank disc were placed 10 mm apart from edge to edge. A volume of 10 μl of 0.5 M EDTA solution was applied to the blank disc. The plate was then incubated overnight at 37°C for 24 hours. The zone of inhibition around imipenem disc expands toward EDTA disc which is considered as positive result.

2.3. Modified Hodge Test (MHT)

1:10 dilution of 0.5 McFarland's standard Escherichia coli ATCC 25922 was used. A lawn culture of this solution was done on a Muller-Hinton (MH) agar. A 10 μg imipenem disc was placed in the center of the plate. Imipenem-resistant test isolates were streaked from the edge of the disc to the periphery of the plate in four different directions. The plates were incubated overnight and were observed for the presence of a "clover-leaf" shaped zone of inhibition which was interpreted as MHT positive.

3. Results

Out of total 14,145 samples, 804 isolates of Pseudomonas aeruginosa were isolated from different clinical samples. Out of this isolates, 153 were Imipenem resistant-Metallobeta-lactamase positive. Out of 153 MBL producers, highest isolates- 49(32.02%) were in age group of more than 50 years and least 9(5.88%) from 0-10 years age group. Mean age of the patients is 40.45 years. Out of 153 MBL producer cases, males were 106(69.28%) and females were 47(30.72%) with male female ratio is 2:1.

Highest number 63(41.2%) of MBL positivity was seen from Medicine ward followed by Surgery 31(20.2%), Other ward 30(19.6%), Orthopaedics 22(14.4%) and least from Paediatrics 7(4.6%)

In the present study MBL producers showed very high resistance to all antimicrobials compared to non-producers and the difference was statistically significant ($p<0.05$). In MBL positive isolates, Colistin was the most sensitive drug 100%(153) followed by Amikacin 9.2% (14), Aztreonam 3.3%(5), Ciprofloxacin 1.3%(2), Levofloxacin 0.65%(1), Gentamicin 0.65%(1), Piperacillin-Tazobactum0.65%(1), Ticarcillin-Clavulinic acid 0.65%(1) and Tobramycin 0.65%(1). In 651 MBL negative isolates, Aztreonam was the most sensitive 72.04% (469) and Ciprofloxacin is the least sensitive 64.36% (419) antibiotic.

Table 1 shows that out of 153 MBL positive isolates, 125(81.7%) were from urine followed by 11(7.2%) blood, 8(5.23%) sputum, 7(4.57%) pus and 2(1.30%) pleural fluid.

Table 2 shows that out of 153 positively screened Pseudomonas aeruginosa isolates, Combined disc test was positive in 153(100%), Double disc synergy test in 50(76.5%) and Modified-hodge test in 55(84.15%) isolates.

Table 1: IMBL positive isolates from various clinical samples

S.No.	Specimens	MBL positive (%)
1	Urine	125 (81.7%)
2	Blood	11(7.2%)
3	Pus	7(4.57%)
4	Sputum	8(5.23%)
5	Pleural fluid	2(1.30%)
6	Total	153(100%)

Table 2: Comparison of three different phenotypic tests for detection of Metallobeta-lactamases.

Total number of Imipenem resistant Pseudomonas aeruginosa isolates out of 804	MBL isolates detected by Combined disk diffusion test	MBL isolates detected by Double disk synergy test	MBL isolates detected by Modified-hodge test
153	153(100%)	50(76.5%)	55(84.15%)

4. Discussion

Pseudomonas aeruginosa is a pervasive pathogen in hospital acquired infections, especially among critically ill patients.[7] Pseudomonas is mainly associated with multidrug resistant nosocomial infections. One of the commonest causes for multidrug resistance among these species is the production of Carbapenemase. Carbapenemases are beta lactamases which have the ability to hydrolyze penicillins, cephalosporins, carbapenems and monobactams.[8] Carbapenems hydrolysing Metallo-beta lactamases production had emerged as the most important mechanism behind Carbapenem resistance.[1]

MBL producing isolates are associated with a higher morbidity and mortality. Moreover given the fact that MBLs will hydrolyze all classes of ß-lactams and that we are several years away from the development of a safe therapeutic antibiotic; their continued spread would be a clinical disaster living very few antibiotis in the tunnel for the treatment of multidrug resistant organisms. MBL positive isolate poses therapeutic problem as well as serious concern for infection control management. MBL producing organism is difficult to detect and pose significant risks particularly due to their role in unnoticed spread within institutions and their ability to participate in horizontal MBL gene transfer, with other pathogens in the hospital.[9,10] Unfortunately, the emergence of antibiotic resistance bacteria is threatening the effective usefulness of many antimicrobial agents resulting in increased days of hospital stay and also an economic burden.[6]

In present study, 19.02% strains were found to be MBL producers.Similar findings seen in the study done by Hemalatha et al(16%), Shahina et al (22%) and Shikha et al (15%).[11–13] In our findings more MBL producers was

seen as compared to study done by Rajput et al(12%).[14] This difference may be due to variation in sample size(low sample size) studied or differences in study done at different timing may be the cause for the difference in the prevalence of MBL producing Pseudomonas aeruginosa.

In the present study, highest MBL production seen in more than 50 years age group which may due to reduction in immunity in this age group.We have also observed that MBL producers are seen more in males as compared to females which is comparable to study done by Bashir et al.[9] The reason may be males are going outside for work and hence more expose to the agent.

Our study shows that highest numbers of MBL producers were from medicine ward including ICU which is well correlated with study of Deeba Bashir and Varaiya et al.[9,15] The reason behind it may be due to multiple risk factors like more days of hospital stay, use of catheterisation, IV lines or previous antibiotic use in this patients. So ICU stay increased the risk for acquisition of MBL producing Pseudomonas aeruginosa.

The source of isolation of MBL positive Pseudomonas aeruginosa isolates was more in urinary tract which is well correlated with study of Agrawal and Deeba Bashir et al.[9,10] This association is statistically significant.

In this current study, among all isolates MBL producers showed increased resistance to all antimicrobials from 90.8% to 100%. Only 9.2% MBL positive were sensitive to Amikacin and 3.3% sensitive to Aztreonam which is very much consistent with study done by Deeba Bashir et al., Varaiya et al.[9,15]

In present study in MBL negative isolates, 38.3% P.aeruginosa were resistant to Imipenem which was similar to study done by Behera et al(39.56%), S.Soumya et al(26.6%) and Shobha et al(30%) but MBL positive Pseudomonas aeruginosa isolates were 100%(153) resistant to Imipenem.[6,8,16]

In our study, positive results were detected in all isolates in Combined disc diffusion test(100%) , 77(50.32%) isolates in Double disc synergy test and 84(54.9%) with Modified-Hodge test which is comparable to study done by Sachdev et al (97.9%, 82.3% and 62.5 % respectively) Shikha Ranjan et al(79.2%, 100% and 87.5%) and Sonia Sharma(87.7%, 86.15%).[12,17,18] Our study shows that combined disc diffusion test was more effective than double disc synergy test in detection of MBL production. Differences with the above studies is due to different methods used, different number of isolates and different geographical area studied.

5. Conclusion

With increasing use of carbapenems for treating infections with ESBL producing organism, the prevalence of Pseudomonas aeruginosa producing metallo-beta-lactamases (MBLs) is increasing worldwide The dissemination of acquired metallo-beta lactamases genes and the emergence of new variants are becoming an emerging threat to public health.

The development of simple screening tests designed to detect acquired MBL production will be a crucial step towards large scale monitoring of these emerging resistant determinants. As only a fewer drugs are available in the pipeline, the judicious selection of antibiotics to treat MBL producing isolates should be implemented. The early, rapid and accurate detection of MBL producing Pseudomonas aeruginosa may help in appropriate antimicrobial therapy, avoid the future spread of these multi-drug resistant strains and to implement adequate infection control measures to prevent nosocomial spread of MBL. Thus it reduces mortality and morbidity due to indiscriminate usage.

No phenotypic test is considered gold standard for the detection of MBL strain. On comparison of various phenotypic methods, Combined disc diffusion test using EDTA was found to be the simple, easy to perform, cost effective and highly sensitive method to detect MBL producing Pseudomonas aeruginosa. So,we recommend that all IPM resistant P. aeruginosa isolates should be routinely screened for MBL production using Combined disc diffusion test. The positive isolates may further be confirmed by MBL E-test or PCR.

As there are various MBL genes identified that varies from one geographical location to another hence the MBL detection tests should be assessed and followed based on the local condition. Early detection of MBL-producing Pseudomonas aeruginosa may help in appropriate antimicrobial therapy and avoid the development and dissemination of these multi-drug resistant strains.

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7. Conflict of Interest

The authors declare no relevant conflicts of interest

8. Source of Funding

None

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