STUDY OF METALLO-β-LACTAMASE PRODUCTION IN CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA

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Abstract

A study of metallo-β-lactamase (MBL) production was done in clinical isolates of Pseudomonas aeruginosa. Isolates resistant to ceftazidime and imipenem were screened for MBL production by double disc synergy test (DDST) and minimum inhibitory concentration reduction test. There was complete correlation between two methods for imipenem. For ceftazidime, there was correlation between the two methods in all except four strains. In the screening test for MBL, ceftazidime - EDTA combination was better than imipenem - EDTA combination. 8.05% strains were MBL producers. Presence of MBL producer P. aeruginosa is a cause of concern. Simple DDST can be helpful for monitoring of these emerging resistant determinants.

Key words: Metallo-β-lactamase, Pseudomonas aeruginosa, ceftazidime, imipenem

Pseudomonas aeruginosa has one of the broadest ranges of infectivity among all pathogenic microorganisms. In the past few decades, it has been increasingly recognized as a pathogen in a variety of serious infections in hospitalized patients especially with impaired immune defenses.[1] P. aeruginosa exhibits intrinsic resistance to a variety of antimicrobials including beta lactams. Metallo-β-lactamase (MBL) producing P. aeruginosa is an emerging threat and a cause of concern for treating physicians.[2] No NCCLS recommendations exist for MBL detection and reporting.[3]

In order to select the correct antibiotic for treatment and to prevent dissemination of such infection it is needed to study the antimicrobial resistant pattern in P. aeruginosa isolates. There is not much information available on MBL producing P. aeruginosa isolates from India. We therefore undertook this study to detect MBL production in them.

Materials and Methods

A total of 174 consecutive non-repetitive clinical isolates of P. aeruginosa were collected and confirmed in Department of Microbiology, Indira Gandhi Govt. Medical College, Nagpur. All the confirmed P. aeruginosa isolates were subjected to Kirby-Bauer disc diffusion method as per NCCLS guidelines.[3] Minimum inhibitory concentration of all P. aeruginosa isolates was determined for ceftazidime and imipenem.[3] Isolates resistant to ceftazidime (Ca) and imipenem (I) were screened for MBL production by double disc synergy test (DDST)[4,5] and minimum inhibitory concentration (MIC) reduction test.[6]

For double disc synergy test, the inoculum was prepared emulsifying 5-6 colonies of the suspected isolate in Mueller Hinton broth and turbidity adjusted to 0.5 McFarland opacity standard. Lawn culture was made on Mueller Hinton agar and DDST was done. The combinations used in DDST were Ca-EDTA and I-EDTA. A 10µg imipenem or 30 µg ceftazidime disc and a blank filter paper disc (6 mm in diameter, whatman filter paper no. 2) were placed 10 mm apart from edge to edge. Five microlitre of 0.5 M EDTA (Sigma USA) solution was then applied to blank disc. After overnight incubation, the presence of an enlarged zone of inhibition was interpreted as MBL producer.

For MIC reduction test of ceftazidime and imipenem was done by agar dilution method.[3] MIC of I-EDTA and Ca-EDTA was also performed.[6] EDTA (1 mL solution of 0.5 M) was added to 1 mL of the imipenem or ceftazidime solution spanning similar concentrations as done for MIC to imipenem or ceftazidime. Each 2 mL of EDTA and imipenem or ceftazidime was added to 18 mL of molten Mueller Hinton agar and poured on plates that were allowed to set. A fixed inoculum of 10⁴cfu of the test strains was spot inoculated on these plates. The reading was taken after 18-24 hours of incubation. The highest dilution that inhibits the growth of the organism was taken as MIC. A minimum of four fold reduction in MIC of these strains when tested in combination of I-EDTA or Ca- EDTA as compared to MIC for imipenem or ceftazidime alone, confirmed that the strains were MBL producer.

Results

A total of 174 P. aeruginosa strains were isolated. MIC of strains for ceftazidime and imipenem is shown in Table 1 and 2 respectively.

There was complete correlation between diffusion method and dilution method for imipenem. For ceftazidime,
there was a correlation between two methods in all except four strains. These four strains were resistant to ceftazidime by diffusion method but their MIC value was 8 µg/mL, indicating their susceptibility by dilution method.

A total of 18 *P. aeruginosa* strains were resistant to ceftazidime. Fourteen out of 18 strains were resistant to both ceftazidime and imipenem and four were resistant to ceftazidime and sensitive to imipenem. All the 18 strains were subjected to MBL production by double disc synergy test (using Ca-EDTA and I-EDTA) and MIC reduction test (using Ca and Ca-EDTA as well as I and I-EDTA). The comparison of result of these studies is shown in Table 3.

### Discussion

*P. aeruginosa* is one of the most frequent nosocomial pathogen and the infections due to these are often difficult to treat due to antibiotic resistance. *P. aeruginosa* isolates showed resistance to even the most recent antibiotics like third generation cephalosporins, antipseudomonal penicillins, aminoglycosides and β-lactam antibiotics included in group A. In our study, only 10 out of 18 strains showed intermediate sensitivity to ceftazidime. In dilution method, only 18 out of 22 strains were resistant. The remaining four strains had MIC of 8 µg/mL, indicating their sensitivity to ceftazidime. There is a complete correlation between diffusion method and dilution method for imipenem.

All the 174 strains were subjected to MIC determination for ceftazidime and imipenem. By disc diffusion method, 22 strains were resistant and one strain was intermediate sensitive to ceftazidime. In dilution method, only 18 out of 22 strains were resistant. The remaining four strains had MIC of 8 µg/mL, indicating their sensitivity to ceftazidime. There is a complete correlation between diffusion method and dilution method for imipenem.

In MIC determination of ceftazidime, 73.56% of strains were having their MIC as low as ≤2 µg/mL, but 5.71% of the strains were having MIC as high as 1024 µg/mL (Table 1). In MIC determination of imipenem, as many as 77.01% of the strains were having their MIC as low as ≤0.5 µg/mL, while 1.15% of strains were having MIC as high as 1024 µg/mL (Table 2). There is a need to monitor the resistance against these important antibiotics in *Pseudomonas*.

Of the 174 isolates studied, 18 *P. aeruginosa* strains were resistant to ceftazidime of which 14 were resistant to both ceftazidime and imipenem and four were resistant to ceftazidime but sensitive to imipenem. These 18 strains were screened for MBL production by double disc synergy test (DDST) and MIC reduction test with ceftazidime and imipenem along with EDTA. Of these 18 strains, 14 strains could be confirmed for MBL production by DDST and MIC reduction with Ca-EDTA (Table 3). When imipenem was used in place of ceftazidime, only 12 could be confirmed for MBL production. This is consistent with the findings of Sarkar et al. and Pitt et al. who reported 11% and 36.36% resistance against imipenem respectively in their studies.

Carbapenems are often used as antibiotic of last resort against infections caused by gram negative bacteria including *Pseudomonas*. In our study, 8.05% of *Pseudomonas* showed imipenem resistance (Table 2). Sarkar et al. report as high as 36.36% resistance to imipenem.

Piperacillin is another β-lactam antibiotic included in group A. We observed 40.22% resistance against this antibiotic. Sarkar et al. and Pitt et al. observed 12% and 31.9% resistance respectively against piperacillin. Among aminoglycosides, amikacin showed least resistance in our study. Sarkar et al. found least resistance in amikacin (40.90%), as compared to gentamicin (45.45%), tobramycin (59.09%) and Netilmicin (50.00%). In the study of Nagoba et al. and Veenu et al., amikacin was found to be the most effective antipseudomonal agent. Quinolones, in particular ciprofloxacin, is still active against about 50% of *P. aeruginosa*.
MBL production. Thus Ca-EDTA combination could pick up additional two isolates of MBL producer as compared to I - EDTA combination. In the study of Mendiratta[11], 15 MBL producers could be detected by using DDST with Ca-EDTA and Ca-2 mercapto-ethanal combination. When imipenem was used in place of ceftazidime, only 14 could be detected. Similarly Hemalatha et al[6] could detect six MBL producer with ceftazidime and five MBL producer with imipenem. Further, imipenem is less stable than ceftazidime. As we have seen in our study and as noted by Arakawa et al[4] MBL producer may be imipenem sensitive but they tend to have high MIC for ceftazidime. Thus ceftazidime is a better choice for detection of MBL production.

Both DDST and MIC reduction gave same results for both the combinations (Table 3). MIC reduction is a cumbersome, laborious method. DDST can be performed along with the routine antibiotic susceptibility testing. We recommend DDST with Ca-EDTA as a screening method for detection of MBL production in P. aeruginosa. We also recommend testing of all the P. aeruginosa isolates for MBL production by putting an additional EDTA disc (750μg/mL) on routine susceptibility plate.

Of the 174 isolates studied, 14 were resistant to both ceftazidime and imipenem, of which 12 were found to be MBL producers. Of the four strains found resistant to ceftazidime and sensitive to imipenem, two strains were found to be MBL producer by ceftazidime EDTA combination only. Thus of the suspected 18 strains, 14 were found to be MBL producer. The remaining isolates may have other mechanisms of resistance such as permeability of outer membrane and/or active efflux.

MBL production is a significant problem in hospital isolates of P. aeruginosa. With increasing isolation of extended ß lactamase producing isolates in hospital setting, necessitating the use of carbapenems, the problem of MBL production is also increasing. In our study, out of 174 strains studied, 14 (8.05%) strains were found to be MBL producer. MBL producer Pseudomonas isolates in the studies of Navneeth[3], Mendiratta[11] and Hemalatha[6] were 12%, 8.62% and 14% respectively.

This study documents that MBL has appeared in our region. Considering the need to institute correct antibiotics to the patients infected with MBL producer, and to prevent spread of MBL positive organisms, all clinical microbiology laboratories must routinely identify MBL producer. Use of simple screening test like DDST, will be crucial step towards large scale monitoring of these emerging resistant determinants.

Table 3: Comparison of methods of metallo-ß-lactamase production using ceftazidime and imipenem

<table>
<thead>
<tr>
<th>P. aeruginosa strains</th>
<th>No. of strains</th>
<th>No. of metallo-ß-lactamase producing strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant to ceftazidime, Resistant to imipenem</td>
<td>14</td>
<td>12 12</td>
</tr>
<tr>
<td>Resistant to ceftazidime, Sensitive to imipenem</td>
<td>4</td>
<td>2 2</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>14 14</td>
</tr>
</tbody>
</table>

DDST - Double disc synergy test, MIC - Minimum inhibitory concentration

References


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