Co-expression of ESBL, AmpC and MBL in Pseudomonas Isolates from a Tertiary Care Hospital in Central India

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ABSTRACT

Background: Pseudomonas aeruginosa infections have grown more commonplace globally as an opportunistic infection that might have unfavorable effects on hospital and community settings. The three main mechanisms of bacterial resistance to beta-lactam medications are Metalloβ Lactamases (MBL), AmpC β lactamases, and Extended Spectrum Beta-Lactamases (ESBLs).

Methods: Pseudomonas aeruginosa samples were recognized in this investigation using motility, gram stain, colony morphology, and biochemical responses. The CLSI criteria were followed to detect ESBL production, and the Imipenem-EDTA combination disc test was used to test for MBL production. AmpC Disc Test was used to identify AmpC beta-lactamases.

Results: 204 (14.0%) P. aeruginosa were identified from a total of 1457 bacterial isolates. One hundred P. aeruginosa isolates were obtained from various clinical specimens. The 74 MDR isolates were more prevalent in patients receiving care indoors; coproduction of both MBL and AmpC enzymes was detected in 3% of P. aeruginosa isolates, coproduction of ESBL and MBL enzymes was detected in 20% isolates, coproduction of ESBL and AmpC enzymes was detected in 5% of isolates, coproduction of all three enzymes i.e. ESBL, AmpC β-lactamases and MBL was not detected in any of the P. aeruginosa isolates.

Conclusions: It is crucial to identify the presence of ESBL, Amp C, and MBL in hospital & community isolates. The coexistence of distinct β-lactamase classes for a single bacterial strain might present difficulties in diagnosis and therapy.

Key-words: AmpC β lactamases, Extended Spectrum Beta-Lactamases (ESBLs), Metalloβ Lactamases (MBL), MDR, P. aeruginosa

INTRODUCTION

Pseudomonas aeruginosa, a gram-negative bacterium, has emerged as a significant pathogen due to its role in opportunistic infections, particularly in both hospital and community settings [1].

The increasing incidence of infections caused by P. aeruginosa is alarming, primarily due to the bacterium's intrinsic resistance to many antimicrobial agents and its ability to acquire resistance mechanisms. This resistance complicates treatment regimens and contributes to higher morbidity and mortality rates among affected patients [2].

The mechanisms underlying P. aeruginosa resistance to beta-lactam antibiotics are multifaceted, involving the production of various beta-lactamase enzymes [2]. Metallo-β-lactamases (MBLs) are a class of enzymes that hydrolyze a wide range of beta-lactam antibiotics,
including carbapenems, which are often used as a last-resort treatment. AmpC β-lactamases provide resistance to cephalosporins, including cephemycins, and are not inhibited by β-lactamase inhibitors, making them particularly problematic. Extended Spectrum Beta-Lactamases (ESBLs) confer resistance to extended-spectrum cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime, as well as to monobactams like aztreonam\[2,3\].

This study aims to screen for the presence of *P. aeruginosa* in multi-drug resistant (MDR) isolates and to investigate the co-expression of MBLs, AmpC β-lactamases, and ESBLs within these MDR isolates. Understanding the co-expression of these resistance mechanisms is crucial, as it can inform the development of more effective treatment strategies and infection control measures, ultimately helping to mitigate the impact of these formidable pathogens on public health [1].

An example of an infection that is encroaching on humans is *P. aeruginosa*. However, the last two decades have shown an increasing trend of infections by *P. aeruginosa*, which was not frequently found in healthy humans. *P. aeruginosa* has increasingly been isolated as the etiological factor in several potentially fatal infections in hospitalized patients with immunodeficiencies [1].

ESBL-producing organisms are a variety of MDR organisms that are increasingly becoming significant universally in infections linked to hospitals. These superbugs possess altered β-lactamase enzymes, which are encoded on a transferable plasmid and capable of hydrolyzing third-generation cephalosporins. However, these had become isolated in *P. aeruginosa* only a short time ago [2].

*P. aeruginosa* can develop resistance to third-generation cephalosporins, although it is susceptible to carbapenemycins, cefoxitin, and aztreonam. The constitutive hyperproduction for AmpC β-lactamases is the most common method by which this happens. AmpC cephalosporinase activity remains uncontrolled by β-lactamase inhibitors that are often employed in clinical practice, such as tazobactam, sulbactam, and clavulanic acid [3].

These are those *P. aeruginosa* strains that are resistant to the most effective treatment option against them i.e. carbapenems. They are increasingly being isolated lately. MBLs are β-lactamases of class B which need bivalent metal ions, often zinc, to function and are responsible for resistance to carbapenems [4].

**MATERIALS AND METHODS**

The study was conducted using 1,457 clinical samples from patients at the NSCB Health College, Jabalpur, India, specifically from the School of Excellence for Pulmonary Medicine and the Department of Microbiology. This was a prospective study focusing on 100 *Pseudomonas aeruginosa* isolates derived from various samples, including sputum, blood, pus, urine, and other bodily fluids. These samples were chosen without considering the age or sex of the patients. The identification of *P. aeruginosa* isolates was confirmed through biochemical responses, motility tests, and gram staining.

To assess the antimicrobial susceptibility of the 100 *P. aeruginosa* isolates, the Kirby-Bauer disc diffusion method was employed [5]. This method helps determine the resistance of bacteria to various antibiotics. Furthermore, three types of β-lactamases-Extended Spectrum Beta-Lactamases (ESBL), Metallo-Beta-Lactamases (MBL), and AmpC β-lactamases were identified using phenotypic approaches. ESBL production was detected according to CLSI guidelines [5] and also by using sulbactam as an inhibitory agent [6]. Imipenem resistant isolates were tested for MBL detection by using zone enhancement with EDTA impregnated imipenem (Imipenem(IMP)-EDTA combined disc test) [7]. Cefoxitin resistant isolates were tested for AmpC β-lactamase production by AmpC disc test [8].

**Inclusion Criteria-** The study included samples that demonstrated the growth of *P. aeruginosa*.

**Exclusion Criteria-** Isolates other than *P. aeruginosa* were excluded from the study.

By the end of the study, the prevalence of multidrug-resistant (MDR) *P. aeruginosa* clinical isolates was calculated using the following formula:

\[
\text{Prevalence of MDR isolates} = \frac{\text{Total number of MDR positive *P. aeruginosa* isolates}}{\text{Total number of *P. aeruginosa* isolates analyzed in the same time frame}} \times 100
\]
The association between ESBL, MBL, AmpC production in MDR isolates was determined in terms of percentage and the association between the production of ESBL, MBL, and AmpC in MDR isolates was determined and expressed as a percentage.

**Statistical Analysis** - The data collected were analyzed using IBM-SPSS software version 27.0. The analysis included calculating frequencies and percentages for qualitative data, allowing for a comprehensive understanding of the findings.

**Ethical Considerations** - The study was conducted with the approval of the institutional ethical committee, ensuring that all ethical guidelines were followed.

**RESULTS**

Table 1 shows from a total of 1457 bacterial isolates, the sample-wise distribution of different isolates of *P. aeruginosa*, 204 (14.0%) *P. aeruginosa* were obtained. Among the culture-positive samples, *P. aeruginosa* isolates were most frequently derived from pus specimens (22.36%), which was followed by sputum (12.65%), body fluids (10.41%), urine (8.94%) and blood (3.38%).

<table>
<thead>
<tr>
<th>Name of specimen</th>
<th>Total culture positive samples</th>
<th>Total number of <em>P. aeruginosa</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (n)</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>Pus</td>
<td>425</td>
<td>95</td>
</tr>
<tr>
<td>Sputum</td>
<td>601</td>
<td>76</td>
</tr>
<tr>
<td>Urine</td>
<td>90</td>
<td>08</td>
</tr>
<tr>
<td>Blood</td>
<td>148</td>
<td>05</td>
</tr>
<tr>
<td>Body fluids</td>
<td>193</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>1457</td>
<td>204</td>
</tr>
</tbody>
</table>

Table 2 shows *P. aeruginosa* infection being predominant in males, with the largest number of patients in the age group 21-40 years (57%) with male to female ratio being 1.27:1.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>0-20</td>
<td>11</td>
<td>19.64</td>
<td>10</td>
</tr>
<tr>
<td>21-40</td>
<td>28</td>
<td>50.0</td>
<td>29</td>
</tr>
<tr>
<td>41-60</td>
<td>15</td>
<td>26.78</td>
<td>5</td>
</tr>
<tr>
<td>&gt;60</td>
<td>2</td>
<td>3.57</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>56.0</td>
<td>44</td>
</tr>
</tbody>
</table>

Table 3 shows the presence of MDR isolates from among the isolates of *P. aeruginosa*. It also demonstrates the geographic distribution among MDR *P. aeruginosa* isolates across indoor and outdoor patients. Of the 100 *P. aeruginosa* isolates in total, 74 (74%) were MDR isolates. 39 (52.7%) of the 74 MDR *P. aeruginosa* samples that were obtained were from indoor patients and 35 (47.3%) were from outdoor patients.

<table>
<thead>
<tr>
<th>Type of patient</th>
<th>MDR <em>P. aeruginosa</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Indoor patients</td>
<td>39</td>
</tr>
<tr>
<td>Outdoor patients</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
</tr>
</tbody>
</table>

MDR - Isolates demonstrating resistance to a minimum of three antimicrobial medications, such as aminoglycosides, carbapenems, β-lactams, and fluoroquinolones.
Table 4 demonstrates how MDR *P. aeruginosa* infections are distributed throughout several samples. The greatest number of samples from the seventy-four MDR isolates were from pus samples (35, or 47.3%), followed by samples of sputum (21.6%), bodily fluids (18.9%), urine (9.56%), and blood samples (2.7%).

<table>
<thead>
<tr>
<th>Total MDR isolates</th>
<th>Pus</th>
<th>Body fluids</th>
<th>Sputum</th>
<th>Urine</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>74</td>
<td>35</td>
<td>47.3</td>
<td>14</td>
<td>18.9</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Table 5 shows the distribution of MBL, AmpC, and ESBL among isolates who were from indoor patients and outdoor patients. ESBL was detected in isolates from 51 patients out of a total of 100 *P. aeruginosa* isolates. It was seen in 24 (47.05%) of isolates from indoor patients and 27(52.94%) of isolates from the outdoor patients. AmpC was recognized in 9 of the total *P. aeruginosa* isolates and 8(88.89%) were found in patient isolates from indoor settings, while one (11.11%) was found in patient isolates from outdoor settings. MBL was detected in 57 patients and it was seen in 28(49.12%) isolates from the indoor patients and 29(50.87%) of the outdoor patients.

Table 6 shows the distribution of MBL, ESBL, and AmpC, in MDR *P. aeruginosa* isolates. It was observed that out of a total 74 MDR *P. aeruginosa* isolates, 34(45.94%) were ESBL producers, Eight (10.81%) were AmpC producers and 56 (75.67%) produced MBL.

Table 7 shows the distribution of *P. aeruginosa* showing the coproduction of various β-lactamases (ESBL, AmpC, and MBL). It shows that ESBL alone was produced in 26(26.0%) strains of *P. aeruginosa*. Only MBL production was observed in 31(31.0%) of strains. Likewise, sole production of AmpC was observed in only 1(1.0%) strain of *P. aeruginosa*. In 20(20.0%) of the strains, both MBL and ESBL were produced. Five (5.0%) of the strains showed evidence of both AmpC and ESBL dual production. In 3 (4.0%) of the strains, MBL and AmpC production were seen together. In none of the strains, a coproduction of the three β-lactamases was detected.

<table>
<thead>
<tr>
<th>Total <em>P. aeruginosa</em> isolates</th>
<th>ESBL alone</th>
<th>MBL Alone</th>
<th>AmpC alone</th>
<th>ESBL+MBL</th>
<th>ESBL+AmpC</th>
<th>MBL+AmpC</th>
<th>ESBL+MBL+AmpC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
</tr>
<tr>
<td>100</td>
<td>26(26%)</td>
<td>31(31%)</td>
<td>1(1%)</td>
<td>20(20%)</td>
<td>5(5%)</td>
<td>3(3%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>
DISCUSSION

This research's 14.0% detection rate of \textit{P. aeruginosa} isolates from all culture-positive samples is consistent with Motbainor et al.\textsuperscript{[9]} investigation, which found a 12.5% isolation rate. However, compared to our work, Gales et al.\textsuperscript{[10]} and Khan et al.\textsuperscript{[1]} reported a separation rate from cultured positive samples of 9.46% and 6.67%, respectively. The present investigation revealed a greater frequency of \textit{P. aeruginosa} isolates from pus samples, afterwards sputum, body fluids, blood, and urine. The results of our study agreed with Khan et al who showed that the majority of \textit{P. aeruginosa} were isolated from pus samples, urine, and other samples. The different types of specimens received in different laboratories might be the cause of this discrepancy in isolation rates in different geographical regions.

\textit{P. aeruginosa} were more isolated from males in this study as opposed to females, having a male-to-female ratio of 1.27:1, which is comparable to the 1.3:1 male-to-female ratio described in a study by Motbainor et al.\textsuperscript{[9]}. Additionally, Khan et al.\textsuperscript{[1]} found that the male-to-female ratio is 1.6:1, meaning that men are more likely than women to be exposed to a variety of environmental concerns thereby causing a higher incidence of infection among males.

The age group of 21–40 years old accounted for most of the individuals whose \textit{P. aeruginosa} was found in the current study (57%), followed by 0–20 years old (21%) and 41–60 years old (20%). Another investigation by Ruhil et al.\textsuperscript{[11]} found that patients frequently had \textit{P. aeruginosa} infections aged 16-40 years this higher occurrence of infection in this reproductive age group may be due because people in this age range spend more time outside and are therefore more likely to get sick while they are outside. Nonetheless, a majority of \textit{P. aeruginosa} infections were found by Sherertz et al.\textsuperscript{[9]} in patients in the age bracket of 50–80 years, whereas Mahmoud et al.\textsuperscript{[12]} reported additional infections in patients aged over 45.

The current study's prevalence of multidrug-resistant \textit{P. aeruginosa} isolates was 74%, which is comparable to investigations conducted by other authors\textsuperscript{[13,14]} that found prevalences of 84.5% and 68.75% of these isolates. Nonetheless, compared to our investigation, Tavajjohi et al.\textsuperscript{[15]} found 32.5% multidrug-resistant \textit{P. aeruginosa} isolates while Amutha et al.\textsuperscript{[16]} found 45.2% multidrug-resistant \textit{P. aeruginosa} isolates. This discrepancy could result from a delay in initiating the proper treatment, which might lengthen hospital stays and cause multidrug-resistant \textit{P. aeruginosa} isolates to arise.

The majority of the MDR isolates of \textit{P. aeruginosa} in this investigation came from pus and sputum samples followed by urine samples and blood samples. In contrast, Mahmoud et al.\textsuperscript{[12]} study found that the highest percentage of MDR \textit{P. aeruginosa} isolated from urine (44.4%) was higher than that from pus and sputum samples. This may be due to different types of samples in different healthcare facilities.

In the current investigation, 5.0% of \textit{P. aeruginosa} isolates showed evidence of coproduction of the enzymes ESBL and AmpC. Similarly, Upadhyay et al.\textsuperscript{[17]} reported a lower rate of AmpC β-lactamases and ESBL coproduction i.e. 4 (3.3%) out of 60 isolates. However, a similar study by Kumar et al.\textsuperscript{[18]} on the production of β-lactamases showed higher coproduction of the enzymes AmpC and ESBL in 24.5% isolates of \textit{P. aeruginosa}.

Coproduction of the MBL & AmpC enzymes was found in the current investigation in 3.0% of \textit{P. aeruginosa} isolates. Devi et al.\textsuperscript{[19]} detected ceftazidime resistance in fifty one clinical isolates from the indoor patients among a total of one hundred and fourteen \textit{P. aeruginosa} isolates and thirty eight (74.5%) of these \textit{P. aeruginosa} isolates were found to be MBL producers and six(11.8%) of these \textit{P. aeruginosa} isolates were AmpC producers.

Coproduction of both MBL and AmpC was observed in 5.8% strains. The current study's findings are consistent with the research conducted by Umadevi et al.\textsuperscript{[19]}Coproduction of both MBL and AmpC was observed in 5.8% strains. The current study's findings are consistent with the research conducted by Umadevi et al.\textsuperscript{[19]} According to Noyal et al.\textsuperscript{[20]} out of the 32 isolates of \textit{P. aeruginosa} that were resistant to meropenem, 15 (46.9%) produced AmpC β-lactamase, 16 (50.0%) produced MBL by the EDTA disc synergy test, and only 9 (28.1%) tested positive for carbapenemases using the modified Hodge test. MBL & AmpC β-lactamase tests yielded positive results for two isolates. The modified Hodge test revealed a positive result for carbapenemase, however, the EDTA disc synergistic test & AmpC disc test yielded negative results for MBL & AmpC β-lactamase, respectively. Nevertheless, Upadhyay et al.\textsuperscript{[17]} found that 46.6% (56/120) of the 120 \textit{P. aeruginosa} isolates that produced AmpC also showed evidence of co-producing
AmpC and MBL which is a much higher percentage than the present study.

In this investigation, 20.0% of the isolates of \textit{P. aeruginosa} had coproduction of the enzymes ESBL and MBL. According to Umadevi \textit{et al.} \cite{19} of the 44 MBL manufacturers, 26 were found using the EDTA disc synergy test using both meropenem and ceftazidime, while the remaining 14 were found using the EDTA-ceftazidime combination and the remaining 4 were found using the EDTA-meropenem combination alone. Of the \textit{P. aeruginosa} isolates taken from patients receiving care indoors, four isolates (7.84%) were positive for each ESBL and MBL. In 2010, Saha \textit{et al.} \cite{21} conducted another investigation which revealed that 86% of the strains were able to produce both MBL & Amp C ß-lactamases. However, only one strain has been identified to be able to generate MBL and ESBL. Recent research by Pasteran \textit{et al.} \cite{22} & Pellegrino \textit{et al.} \cite{23} from Argentina and Brazil has shown the presence of ESBL & MBL mixtures of ß-lactamases for a single strain. These investigations, which used molecular techniques, demonstrated the coexistence of GES-1 (ESBL) along VIM-11 (MBL) and PER-1 (ESBL) together VIM-2 (MBL).

None of the \textit{P. aeruginosa} isolates in our investigation showed evidence of coproduction with ESBL, AmpC ß-lactamases, and MBL. No \textit{P. aeruginosa} isolates were found to coproduce ESBL, AmpC ß-lactamases, or MBL, according to a related investigation by Basak \textit{et al.} \cite{24}. Thus, the findings of this investigation support the findings of the current study. Nonetheless, research conducted by Oberoi \textit{et al.} \cite{25} found that 19.04% of gram-negative isolates obtained from a Punjabi tertiary care hospital coproduced ESBL, AmpC ß-lactamases, and MBL. It is not similar, though, because all gram-negative bacteria were included in this investigation. \textit{Pseudomonas aeruginosa} which produces ESBL, AmpC, and MBL is becoming more common; this might be a warning indication that an increasing number of organisms are developing resistance mechanisms, making antimicrobial drug’s fewer effective prescriptions inefficient.

**CONCLUSIONS**

Though phenotypic tests for ESBL, AmpC and MBL detection can be conveniently performed in most laboratories, the results of these tests must be confirmed by molecular techniques. It is also desirable to perform tests for additional potential resistance mechanisms that might account for antibiotic resistance in drug-resistant isolates, including the efflux pump, disappearance of OprD, etc. It is critical to identify coproducing strains of ESBL, Amp C, and MBL as soon as possible because the tip of the iceberg is only being detected compared to their actual prevalence. The co-expression of many Therapeutic and diagnostic
intransigence may result from a single bacterial strain possessing multiple types of β-lactamases. The microbes that produce AmpC beta-lactamases might serve as ESBLs’ reservoirs. Paradoxically, their sophisticated demeanor might mask the identification of the ESBLs leading to antibiotic failure.

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Supervision- Sanjay Kumar Bharty
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Data analysis and Interpretation- Jagmohan Singh Dhakar
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Article editing- Sanjay Kumar Bharty
Final approval- Sanjay Kumar Bharty

REFERENCES


