Imp-1 Metallo Beta-Lactamase Producing Pseudomonas Aeruginosa and Resistance to Antibiotics

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ABSTRACT

The frequency of multi-drug resistance Pseudomonas aeruginosa infections are increasingly recognized worldwide. P. aeruginosa isolates resistant to all antimicrobial agents have been detected in many areas. Since IMP1 producers tend to demonstrate a wide range of resistance to various broad-spectrum beta-lactam including oximino cephalosporin, cephamycine and carbapenemes, early recognition of IMP-1 producers is very important for rigorous infection control. The present study was designed to detect the incidence of P. aeruginosa infection, characterize the antimicrobial resistance profiles and screen for the producers is very important for rigorous infection control. The present study was designed to detect the incidence of P. aeruginosa infection, characterize the antimicrobial resistance profiles and screen for the IMP-1 producers strains. From 1st of July 2007 to 30 June 2008, A total of 4031 isolates were obtained of those 837 (20.76%) were identified as Pseudomonas, the great majority of them was P. aeruginosa (n=816; 97.5%) and the most of which was isolated from the wounds (n=256; 30.6%), sputum (n=242; 28.9%) , urine (n=64; 7.6%), Tracheal aspirate (n=55; 6.65) and lastly ear swab (n= 3; 0.35%).

Intensive care unit accounts the most source of infection (n= 171; 20.4%) then burns unit (n= 111; 13.3%) and lastly obstetric department (n= 8; 0.9%). High resistance rates were observed for all antibiotics studied. imipenem appeared to be the most active agent against the majority of isolates (S=78%), then levofoxacin (S=75%), followed by piperacillin / tazobactam (S= 71%), amikacin (S=67%), tobramycin (S=65%), ciprofloxacin (S= 63%), cepefime (S=60%), gentamycin (S= 59%), piperacillin and cefazidime (S = 57% for each), while ceftiraxone and cefotaxime were the least active agents with a sensitivity (35%) only. IMP-1 Metallo-beta-lactamas were detected in 148 (41% of the 360 CAZ-resistant isolates) out of 816 P. aeruginosa isolates. After the results of this study we concluded that the rates of P. aeruginosa infection were high with increasing in IMP-1 Metallo-beta-lactamase producers strains. Infection control procedures for multi drug resistance need to be re-viewed urgently. There is also a pressing need for new, and hopefully novel, compounds active against pan-resistant Gram-negative bacteria a growing problem that needs to be addressed by both governments and industries.

INTRODUCTION

Pseudomonas aeruginosa is a highly relevant opportunistic pathogen. One of the most worrisome characteristics of P. aeruginosa consists in its low antibiotic susceptibility. A recent survey of Gram-negative resistance from North America showed that P. aeruginosa has become increasingly antibiotics resistant1,2,3,4. This is attributable to a concerted action of multi-drug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes. Besides intrinsic resistance, P. aeruginosa easily develops acquired resistance either by mutation in chromosomally-encoded genes, either by the horizontal gene transfer of antibiotic resistance determinants. Development of multi-drug resistance by P. aeruginosa isolates requires several different genetic events that include acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes5,6.

Metallo-beta-lactamases (MBLs) belong to a group of beta-lactamases which require divalent cations of zinc as cofactors for enzyme activity. They have potent hydrolyzing activity not only against carbapenemes but also against other beta-lactam antibiotics. MBLs are not inhibited by the commercially available inhibitors, clavulanic acid, sulbactam and tazobactam6. The first MBL was reported from Bacillus cereus in the 1960s and since then 18 MBLs have been described in different Gram-negative bacteria. Production of most of these MBLs is chromosomally encoded and did not pose a serious threat of spread to other bacteria. However in 1991, the first plasmid-mediated MBL, IMP-1 from P. aeruginosa was reported from Japan6,7. Japan has become a major reservoir for IMP-type MBLs, which now include IMP-1, IMP-2, IMP-3, IMP-6, IMP-10, IMP-11, and these have spread to a number of strains of Pseudomonas spp. Acinetobacter spp. and Enterobacteriaceae8,9. While second type of acquired MBL, VIM-1 was first reported from Italy in 19979. The third type of acquired MBL is SPM-1. Report from Brazil indicates that 20-45% of P. aeruginosa isolates posses the SPM-
1 MBL. And the fourth rarest MBL is GIM-1, which was recovered in 2002 from five P. aeruginosa isolates from Düsseldorf, Germany. There have been increasing reports of IMP and VIM variants in Pseudomonas spp. and Acinetobacters spp. from several countries. The blalMP and blavaIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. Since IMP-1 producers tend to demonstrate a wide range of resistance to various broad-spectrum β-lactams including the oxyimino cephalosporins, cephemycins, and carbapenems, early recognition of IMP-1 producers is very important for rigorous infection control. The worldwide spread of this kind of organism is becoming a general concern, since several MBL producing Gram-negative bacteria have recently been reported outside Japan. Multi-drug-resistant P. aeruginosa infections are increasingly recognized worldwide. The existence of MBL and extended – spectrum β- lactamase- (ESBL) producing isolates exhibiting resistance to most β-lactam antimicrobial agents greatly complicates the clinical management of patients infected with such isolates.

Since 1998, P. aeruginosa isolates to all commercially available antimicrobial agents have been detected at many countries. The aim of this study is to determine the incidence of P. aeruginosa infection, characterize the antimicrobial resistance profiles and detection of IMP-1 producers by it from the patients samples admitted at King Abdul Aziz Hospital & Oncology Center, Jeddah, Saudi Arabia.

MATERIALS & METHODS

For each isolate, the date of sampling, the name of patient’s, sex, age, words and the source of material were recorded only one isolate per patient was included in the study.

Specimen collection and identification of isolates:

All clinical isolates were identified by conventional methods which used in Microbiology laboratory and the Gram – negative bacilli including the pseudomonas isolates were subsequently identified at the species level with API20E and API20NE (BioMerieux Systems, France) and/or the automated machines Microscan. (Siemens health Care Diagnostic, West Sacramento, California, USA).

Anti-P. aeruginosa susceptibility tests:

- Disk diffusion method:

Performed by a Kirby-Bauer method. The antibiotic disks used were amikacin 30µg, gentamycin 10µg, cefepime 30µg , ticarcillin 75µg, piperacillin 100µg, piperacillin / tazobactam 100 µg /10 µg, imipenem 10µg, ceftazidime 30µg, ciprofloxacin 5µg, azتروnam 30µg,and tobramycin 10µg (Becton, Dickinson and company, Sparks, U.S.A).

- MIC determination:

For studying the MIC we used the Microscan, type TN dried panel. The interpretation standards for disk diffusion and MICs of the NCCLS/CLSI were used to determine antibiotics susceptibilities.

- IMP-1 Metallo-β-lactams detection:

Clinically P. aeruginosa isolated, ceftazidime (CAZ) – resistant (MIC, > 64 μg/ml) were evaluated for IMP-1 Metallo-β-lactamase production by a disk approximation test with disks containing CAZ and 2-mercaptopropionic acid (2-MPA, Sigma chemical Co., St. Louis, Mo.) as described by Arakawa et. al. Briefly a colony of each bacteria strain was suspended and diluted with Mueller-Hinton (MH) broth to 10⁶ CFU/ml and spread on an MH agar plate with a cotton swab according to the protocol recommended by the NCCLS/CLSI.

Two commercially supplied Kirby-Bauer (KB) disks, each containing 30µg of CAZ, were then placed on the plates. The distance between the two CAZ disks was kept at about 4 to 5 cm, and a filter disk was placed near one of the CAZ disks within a center-to- center distance of 1.0 to 2.5 cm. Three microliters of a 1.2 g/ml of the inhibitor thiol compound 2-MPA was added to the filter disk on the agar, and each agar plate was incubated at 37°C overnight.

RESULTS

During the one year study a total of 4031 isolates were obtained in the Microbiology laboratory out of these 837 (20.76%) were identified as a Pseudomonas, the great majority of them was P. aeruginosa (n=816;97.5%) then P. stutzeri (n= 9 ; 1.1%) , P. fluorescence / putida (n=1;0.3%) and finally P. species (n=9; 1.1%).

According to the site of infection:

The great majority of P. aeruginosa was isolated from the wounds (n= 256; 30.6%), sputum (n=242; 28.9%), urine (n= 64;7.6%), Tracheal aspirate (n= 55; 6.6%) and lastly ear swab (n = 3;0.35%) as shown in Table (1) and Fig (1).
Table (1): Site of *P. aeruginosa* infection

<table>
<thead>
<tr>
<th>Side of infection</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound swab</td>
<td>256</td>
<td>31.4</td>
</tr>
<tr>
<td>Sputum</td>
<td>242</td>
<td>29.7</td>
</tr>
<tr>
<td>Urine</td>
<td>64</td>
<td>7.8</td>
</tr>
<tr>
<td>Tracheal Aspirate</td>
<td>55</td>
<td>6.7</td>
</tr>
<tr>
<td>Cath. TIP</td>
<td>43</td>
<td>5.3</td>
</tr>
<tr>
<td>Throat swab</td>
<td>34</td>
<td>4.2</td>
</tr>
<tr>
<td>ETT Aspirate</td>
<td>31</td>
<td>3.8</td>
</tr>
<tr>
<td>Blood</td>
<td>25</td>
<td>3.1</td>
</tr>
<tr>
<td>High vaginal swab</td>
<td>17</td>
<td>2.1</td>
</tr>
<tr>
<td>Stool</td>
<td>13</td>
<td>1.6</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>Eye swab</td>
<td>9</td>
<td>1.1</td>
</tr>
<tr>
<td>Axilla swab</td>
<td>6</td>
<td>0.74</td>
</tr>
<tr>
<td>Tissue</td>
<td>3</td>
<td>0.37</td>
</tr>
<tr>
<td>Groin swab</td>
<td>3</td>
<td>0.37</td>
</tr>
<tr>
<td>Ear swab</td>
<td>3</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>816</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Fig (1)**

According to the source of infection:
Intensive care unit accounts the most source of infection (n= 171; 20.4%) then burns unit (n=111; 13.3%) and lastly obstetric department (n=8; 0.9%) as shown in table (2) fig (2).
Table (2): Source of *P. aeruginosa* infection

<table>
<thead>
<tr>
<th>Side of Source</th>
<th>Number of <em>P. aeruginosa</em></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTENSIVE CARE UNIT</td>
<td>171</td>
<td>21</td>
</tr>
<tr>
<td>FEMALE SURGICAL</td>
<td>78</td>
<td>9.6</td>
</tr>
<tr>
<td>MALE SURGICAL</td>
<td>69</td>
<td>8.5</td>
</tr>
<tr>
<td>REHAB WARD</td>
<td>66</td>
<td>8.1</td>
</tr>
<tr>
<td>OUT-PATIENT</td>
<td>51</td>
<td>6.3</td>
</tr>
<tr>
<td>NEONATAL INTENSIVE</td>
<td>42</td>
<td>5.1</td>
</tr>
<tr>
<td>FEMALE MEDICAL</td>
<td>42</td>
<td>5.1</td>
</tr>
<tr>
<td>MALE MEDICAL</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>PEDIATRICS INTENSIVE</td>
<td>30</td>
<td>3.7</td>
</tr>
<tr>
<td>ONCOLOGY</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>RENAL UNIT</td>
<td>21</td>
<td>2.6</td>
</tr>
<tr>
<td>PRIVATE</td>
<td>18</td>
<td>2.2</td>
</tr>
<tr>
<td>D700</td>
<td>15</td>
<td>1.8</td>
</tr>
<tr>
<td>CARDIAC</td>
<td>10</td>
<td>1.1</td>
</tr>
<tr>
<td>OB-GYNE WARD</td>
<td>8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Antibiotic susceptibility rates of *P. aeruginosa* isolates:**

High resistance rates were observed for all antibiotics studies (Fig.3) Imipenem appeared to be the most active agent against the majority of isolates (S=78%), then levofloxacin (S=75%), followed by piperacillin / tazobactam (S=71%), amikacin (S=67%), tobramycin (S=65%), ciprofloxacin (S=63%), cefepime (S=60%), gentamycin (S=59%), pipracillin and ceftazidime (S=57% for each), while ceftriaxone and cefotaxime were the least active agents with a sensitivity (35%) only Fig (3).
FIG. 3: Antibiotic susceptibility rates of *P. aeruginosa* isolates:

**Cross-susceptibility patterns of *P. aeruginosa* isolates:**

In the study, multi-drug resistant in which the *P. aeruginosa* isolates were resistant to more than one group of antibiotics was encountered, when CAZ-resistant strains were considered, about one-third of the isolates (34%) were susceptible to imipenem. Twenty-nine sensitive to levofloxacin, while piperacilline / tazobactam, amikacine, tabramycin, ciprofloxacin, cefepime, gentamycin and piperacillin were active against 18-23% of resistant-CAZ, *P. aeruginosa* strain where as ceftriaxone and cefotaxime have no activity, and in 2% of the total isolates of *P. aeruginosa*, they resistant to all antibiotics.

**IMP-1 Metallo-β-lactamase detection:**

All CAZ- Resistant isolates detected (360 of the 837 isolates included in the study) were assessed for IMP-1 Metallo-β-lactamase. For each IMP-1 producers *P. aeruginosa* a distinct growth inhibitory zone appeared between the KB disk containing CAZ and filter disk containing 2-MPA (Fig 4). No changes evident around the two KB disk containing CAZ with or without 2-MPA for each non IMP-1 producers *P. aeruginosa* isolates.

In this study a clear positive result was observed for 148 (41% of the 360 CAZ-resistant isolates).

**DISCUSSION**

*P. aeruginosa* in the present study is a leading cause of infection as it accounts about 20% of total isolates in contrast to other studies, among 70,067 isolates obtained from patients admitted to hospitals in five different geographic areas and evaluated by the SENTRY antimicrobial resistance surveillance program, the prevalence rates of *P. aeruginosa* infections were higher in the Latin American and the Asia-Pacific regions (11.4% of total isolates in each region) than in Europe (9.3%), the United States (8.7%), and Canada (8.6%) (21).

In this study, wound infections are the most side of infection as shown in Table 1 and Fig 1 followed by respiratory tract infections, urinarily tract and lastly ear infection, and the most were isolated from ICU patients Table (2) and Fig (2), comparison to other studies,
according to center for disease control and prevention (CDC) data collected from 1990/1996, *P. aeruginosa* was the second most common cause of nosocomial pneumonia (17%) isolates, the third most common cause of UTI (11%), the fourth most common cause of surgical site infections (8%), the seventh most common isolated pathogen from the bloodstream (3%), and the fifth most common isolate overall (9%) – obtained from all sites (22).

Regarding to the susceptibility test high resistance rates were observed for all antibiotics studies (Table 3), However imipenem appeared to be the most active agent against the majority of isolates the reasons for such differences in the rate and the side of *P. aeruginosa* infection and susceptibility rates between this study and other studies are exactly unclear and may be related to the use of infection control practices by health care workers, the use of suboptimal aseptic techniques, inadequate cleaning and disinfection of the environment and medical equipments, method of antibiotics therapies and the different in the isolated strains.

Recently, gram-negative bacterial strains that were speculated to produce Metallo-**β**-lactamases very similar to IMP-1 were also isolated (14,15,23). Thus there is a need to distinguish IMP-1 producers *P. aeruginosa* from non-producers showing a similar antibiotic resistance profile through the production of other genes like ESBLs, VIM, SPM and GIM. Indeed, PCR analysis usually gives satisfactory results in the detection of IMP-1 producers (16, 24) used in the present study a convenient test as described by Arakawa et al. (20), in which CAZ used as a substrate for detection of IMP-1 producers strains, because IMP-1 producers proved usually demonstrated high level resistance to CAZ (MIC, > 64 µg/ml) (8,16). There are many materials can be used as inhibitors for IMP-1 such as thiol compounds including 2-MPA, heavy metal salts and EDTA, and in this study 2-MPA used because it proved gave the cleared results, because this chemical agent blocked IMP-1 activity very strongly even at a low concentration (20).

After the result of this study, we revealed that the rates of *P. aeruginosa* infections and IMP-1 Metallo beta-lactamase producers strains were high which means that the treatment of such patient are difficult as at the present time, no MBL inhibitors are available for treating patients, and there are not likely to be any in the foreseeable future. Co-resistance is likely to further diminish other therapeutic options (8). The only therapeutic alternative may be the administration of polymyxins, which have been show recently to be efficient in treating multidrug-resistant Gram-negative bacilli (29). It has been claimed recently that polymyxins are not as toxic as previously thought; however, it would be prudent to consider polymyxins only as part of combination therapy (26, 27). Clearly, there is a need to produce new and efficacious compounds to combat MBL-positive Gram-negative bacteria. However, there appear to be very few new compounds demonstrating potent activity against *P. aeruginosa* and *Acinetobacter* spp., even without an MBL (28). The reasons for this are multifactorial, but largely center on the lack of net return profits that companies will receive once an anti-pseudomonal’ drug has been developed (29,30). We concluded that the infection control procedures for multi drug resistance need to be re-viewed urgently and there is also a pressing need for new, and hopefully novel, compounds active against pan-resistant Gram-negative bacteria a growing problem that needs to be addressed by both governments and industries.

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ومقاومتها للمضادات الحيوية

1. علاء عبد الهادي، عزل عمرو العربية، محمد الفهمي
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المقاومة المتعددة لميكنوبات السودوموناس إيرجينوزا للمضادات الحيوية أصبحت كثيرة الشبيه في العالم كله وكذلك مقاومتها لجميع أنواع المضادات الحيوية تم تطبيقها في كثير من مناطق العالم، ومن دون استخدام ذلك قد نرى مثل أنواع أخرى من البكتيريا، إذا اعتمدت الميكنوبات لاكتاماز والتي تأتي إلى مقاومة مجموعات واسعة من مضادات الحياة تشمل الأوكسيكلامينوسورين وسيفين مع جموعة الكارباماتيوم والذين فإن استدراك إقامة الأنزيمات معها جداً نخاطة في مكافحة العدو بالمضادات، ولذا تم عمل تلك الدراسة لتحقيق نتائج تلك الأنزيمات من ميكنوبات السودوموناس أيرجينوزا مع تكنولوجيا عنقود الميكنوبات المعادن الحيوية وذلك من الفترة (1) يوليو 2007 إلى (31) يوليو 2008 حيث تم خلال تلك الفترة عزل 430 عينة منهم 202 (71%) من ميكنوبات السودوموناس معظمهم السودوموناس أيرجينوزا (87% عزلة نسبة 97.5%) من كل أنواع السودوموناس) وكان معظم العزلات من الجروح (56% عزلة نسبة 53%) ثم القلق (36% عزلة نسبة 28.9%) ثم أبولا (24% عزلة نسبة 18.6%) ثم عينات البالن من مرضى الجهاز الهضمي (4% عزلة نسبة 3.2%) وثلاثة عزلات بنسبة 3% من الأذن وكانت معظم تلك العزلات من عنقات مرضي الجهاز الهضمي (4% عزلة نسبة 3.2%) ثم قسم الميداني (8% عزلة نسبة 6.2%) ثم قسم المركز (9% عزلة نسبة 7.5%) وكانت نسبة الميكنوبات الصوديومية عالية على وجة العين، ووجد أن عقار الإبيناميد أحسن العلاجات فاعلية ضده، وتعمل التعليمات السماوية 5% ثم اليساروبامدين 5% ثم كان إفريكان 7% ثم السترونوكسانين 2% ثم السيفايلين 2% ثم الستروفوكسانين 2% ثم تجارب 2% ثم الصوديوم 2% لكل منهما.

بما أننا نثق في مستلزمات السودوموناس أيرجينوزا المتنجة الواضح أن مقاومة ميكنوبات السودوموناس أيرجينوزا في كثر من الحالات، فإن رفع الميكنوبات المعادن الحيوية من الميكنوبات يمكن أن يوحي مع انتشار هذا الميكنوبات، وكذلك التعامل مع الحكمة والشركات الأساسية لإنتاج مضادات حيوية جديدة خاصة ضد الميكنوبات سالبة الجرام.

بالأخلاص، ميكنوبات السودوموناس أيرجينوزا


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