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Dissemination of Carbapenem Resistant *Pseudomonas aeruginosa* among Burn Patients in Karbala Province\ Iraq

Aseel A. Shilba[1*], Raghad H. Al-Azzawi[1], Salwa J. Al-Awadi[2]
[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[2]Forensic DNA Training Center, Al-Nahrain University, Baghdad, Iraq

Abstract

In this study, 158 clinical samples were collected from hospitalized burn patients during the period from December 2012 to June 2013 in Karbala province\ Iraq. Bacterial isolates were identified using conventional biochemical tests and then identification was confirmed by using Vitek-2 compact system. *Pseudomonas aeruginosa* recovery was 60 isolates in this study. These isolates were analyzed for antibiotic susceptibility by the disk diffusion test (DDT) according to Kirby Bauer's method using seven clinically important antipseudomonal agents: carbapenems (Imipenem and Meropenem), pencillins (Piperacillin), cephalosporins (Ceftazidim), monobactam (Aztreonam), quinolones (Ciprofloxacin) and aminoglycosides (Gentamicin). The results of resistance were as following: Imipenem 58.33%, Meropenem 66.67%, Piperacillin 86.67%, Ceftazidim 51.67%, Aztreonam 43.33%, Ciprofloxacin 46.67% and Gentamicin 91.67%. Antibiotic susceptibility test was confirmed by using VITEK-2 compact system. Differences between antibiotic susceptibility levels were calculated by Chi-square for each antibiotic. Results were highly significant for all antibiotic groups, p <0.01. The prevalence of increasing resistance rate to carbapenems, the final drug choice for the treatment of *P. aeruginosa*, among the immunocompromized burn patients is due to the increasing usage of this group especially Meropenem.

Keywords: *P. aeruginosa*, immunocompromized, burn, carbapenems.

إنتشار بكتريا *Pseudomonas aeruginosa* المقاومة للكاربينيم بين مرضى الحروق في محافظة كربلاء/ العراق

أسيل عبدالرضا شلبه[١*]، رغد حربي العزاوي[١]، سلوى جابر العوادي[٢]
[١]قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.
[٢]مركز الدنا العدلي، جامعة النهرين، بغداد، العراق.

الخلاصة

في هذه الدراسة تم جمع ١٥٨ عينة سريرية من مرضى الحروق الراقدين خلال الفترة الواقعة ما بين كانون الأول ٢٠١٢ و حزيران ٢٠١٣ في محافظة كربلاء/ العراق. تم تشخيص العزلات البكتيرية باستخدام الاختبارات الكيموحيوية التقليدية ثم تم تأكيد التشخيص بواسطة استخدام جهاز Vitek–2 compact. تم الحصول على ٦٠ عزلة عائدة لبكتريا *Pseudomonas aeruginosa*. أجريت فحوصات الحساسية للمضادات الحياتية على هذه العزلات بواسطة طريقة انتشار الأقراص (DDT) لكيربي بوير، وذلك باستخدام سبعة مضادات للزوائف الزنجارية *P. aeruginosa* مهمة سريرياً هي الكاربينيم (إميبينيم وميروبنيم) ، البنسلينات (بيراسلين)، السيفالوسبورين (سيفتازيديم) ، المونوبكتام (أزترونام) ، الكينولونات (سبروفلوكسين)

*Email: asl_msdy@yahoo.com

والامينوكلايكوسيدات (جنتمايسين). كانت نتائج المقاومة المستحصلة من الاختبار كالآتي : 58.33% للإميبينيم ، 66.67%, ميروبينيم ، 86.67% ببراسلين ، 51.67% سيفتازيديم ، 43.33% أزترونام، 46.67%سبروفلوكسين و 91.67% للجنتمايسين. تم تأكيد اختبار الحساسية للمضادات الحياتية بواسطة استخدام جهاز Vitek-2 compact. إن الفروقات بين مستويات الحساسية حسبت بواسطة مربع كاي (Chi-square) لكل مضاد حيوي والتي أظهرت قيمة معنوية عالية لجميع المضادات الحياتية المستخدمة في هذه الدراسة، بمستوى $p < 0.01$. إن ظهور نسبة المقاومة المتزايدة للكاربينيم (وهو الخيار العلاجي الأخير لعلاج الزوائف الزنجارية *P. aeruginosa*) بين مرضى الحروق هابطي المناعة هو نتيجة الاستخدام المتزايد لهذه المجموعة، خصوصاً الميروبنيم.

Introduction

Pseudomonas aeruginosa is an important opportunistic pathogen for humans, animals, and plants [1]. It is an aerobic Gram-negative rod, possessing a strictly respiratory metabolism. The organisms are usually (1.5-5) µm in length and (0.5-1.0) µm in width, and are motile due to the presence of flagella [2]. This bacterium present in soil and aquatic environments [3]. It is an important pathogen in immunocompromised patients, such as patients suffering from AIDS, cancer, burn wounds and cystic fibrosis (CF) [4]. Infections caused by *P. aeruginosa* are often difficult to eradicate because it requires minimal nutrition and can tolerate a wide range of temperatures. Also, it is resistant to many antibiotics, disinfectants and has the ability to acquire resistance [5] besides that it exhibits intrinsic resistance to several antimicrobial agents [6]. Bacterial infections in burned and wounded patients are common and are difficult to control. Sepsis as a consequence is common and the sepsis is often fatal [7, 8]. Burn injury is a major problem in many areas of the world and it has been estimated that 75% of all deaths following burns are related to infection [9]. The typical burn wound is initially colonized predominantly with gram-positive organisms, which are fairly quickly replaced by gram-negative organisms like *P. aeruginosa*, usually within a week of the burn injury [10]. *P. aeruginosa* developes antimicrobial resistance rapidly, which complicates medical treatment of infections. It is frequently isolated from patients and hospital environments and has been implicated as the cause of nosocomial infections in burn patients [11]. Carbapenem compounds such as (Imipenem and Meropenem) are highly potent broad-spectrum antimicrobial agents. They play an important role in the treatment of infections caused by *P. aeruginosa* [12]. Carbapenems still as the main antimicrobials for treating infections due to multidrug-resistant (MDR) *P. aeruginosa*, but the development of carbapenem resistance may significantly compromise their efficacy [13]. Therefore, these antibiotics remain as the last therapeutic option for treatment of serious infections caused by *P. aeruginosa*. As a result, the recent appearance of carbapenem resistant *P. aeruginosa* isolates is considered a major healthcare problem [5].

This study aimed to detect the dissemination of carbapenem resistance in *P. aeruginosa*, isolated from burn patients, phenotypically depending on the antibiotic profile of these bacterial isolates.

Materials and Methods

Bacterial Isolates:

Sixty *P. aeruginosa* isolates were recovered from 158 skin sample collected by using sterile cotton swabs from burn patients between December 2012 and June 2013. Bacteral isolates were identified as *P. aeruginosa* by the standard microbiological tests such as Gram stain, oxidase test, catalase test, growth on MacConkey agar, growth on cetrimide agar, blood haemolysis, motility, liquefaction of gelatin, growth at 42°C and at 4°C, pigment production, Kligler's Iron test and IMViC tests (indole, methyl red, Voges-Proskauer and citrate) [14]. Then identification was confirmed by using Vitek-2 compact system according to the manufacturer company, bioMérieux (France). The isolates were maintained in nutrient broth medium containing 40% glycerol at -20ºC [15, 16].

Antimicrobial Susceptibility:

The Antimicrobial susceptibility profiles of *P. aeruginosa* isolates were determined by the disk diffusion test (DDT) according to Kirby Bauer's method depending on the recommendations made by the Clinical and Laboratory Standards Institute (CLSI) [17]. The following antimicrobial discs were used: Imipenem (10 µg), Meropenem (10 µg), Piperacillin (100 µg), Ceftazidime (30 µg), Aztreonam

(30 µg), Ciprofloxacin (5 µg) and Gentamicin (10 µg). Then antibiotic susceptibility test of the isolates was confirmed by VITEK-2 compact using (AST-N222) card, susceptible and resistant interpretations were automatically recorded.

Statistical analysis:

The Statistical Analysis System (SAS) [18] was used to analyze the antibiotic susceptibility results. Chi-square test was used to significant compare between percentages in this study. *P- Values* equal to or less than 0.01 were considered statistically highly significant.

Results and discussion:

Cultural and biochemical identification revealed that sixty three (63) *P. aeruginosa* isolates were recovered from 158 samples. The 63 bacterial isolates were found to be gram negative, lactose non fermenter and they had the ability of growth on cetrimide agar. They were catalase positive, oxidase positive and most of them were producing pyocyanin (a water - soluble pigment on king A medium), agreeing with De la Maza, *et al*. [19] who have described *P. aeruginosa* as Gram negative bacilli producing bluish green coloration. The bacterial isolates were motile, able to grow at 42°C and no growth was found at 4°C and they were able of liquefaction of gelatin. Blood hemolysis results were in three types: alpha (α), beta (β) and gamma (γ). β-hemolysis revealed in most of the bacterial isolates followed by α-hemolysis and γ-hemolysis, subsequently. Results were negative for indole test, Voges-Proskauer test and methyl red test while they were positive for citrate test. In kligler's iron test, the slant was alkaline while the butt showed no color change and there were no products of H_2S and gas.

Confirmed identification by using VITEK-2 compact resulted in sixty (60) *Pseudomonas aeruginosa* isolates (37.97%) were recovered from the 158 samples and three (3) out of sixty three (63) previously identified by the morphological and biochemical tests were considered as *Pseudomonas putida*. This study showed that most of the sixty *P. aeruginosa* isolates were highly resistant to all antibiotics used particularly the β-lactams and the aminoglycoside (Gentamicin) and resistance levels rates were largely variable for each antibiotic and revealed highly significant values (p <0.01) for all antibiotics as shown in the table-1 and figure-1 depending on DDT and as shown in table-2 using VITEK-2 compact.

Table 1- Antibiotic susceptibility of R, I and S (distribution in total sample) according to DDT.

Antibiotic	R % (No.)	I % (No.)	S % (No.)	*P value* [a]
IPM	58.33 (35)	10.00 (6)	31.67 (19)	0.0023
MEM	66.67 (40)	6.67 (4)	26.67 (16)	0.0019
PIP	86.67 (52)	–	13.33 (8)	0.0001
AT	43.33 (26)	43.33 (26)	13.33 (8)	0.0028
CTZ	51.67 (31)	31.67 (19)	16.67 (10)	0.0013
CIP	46.67 (28)	15.00 (9)	38.33 (23)	0.0025
GEN	91.67 (55)	1.67 (1)	6.67 (4)	0.0001

Total No. of samples = 60.

a: ***P-value*** was calculated using the Chi-square test in terms of the R, I & S group.

Abbreviations: R: resistant; I: intermediate; S: sensitive; IPM: Imipenem; MEM: Meropenem; PIP: Piperacillin; AT: Aztreonam; CIP: Ciprofloxacin; CTZ: Ceftazidime; GEN: Gentamicin.

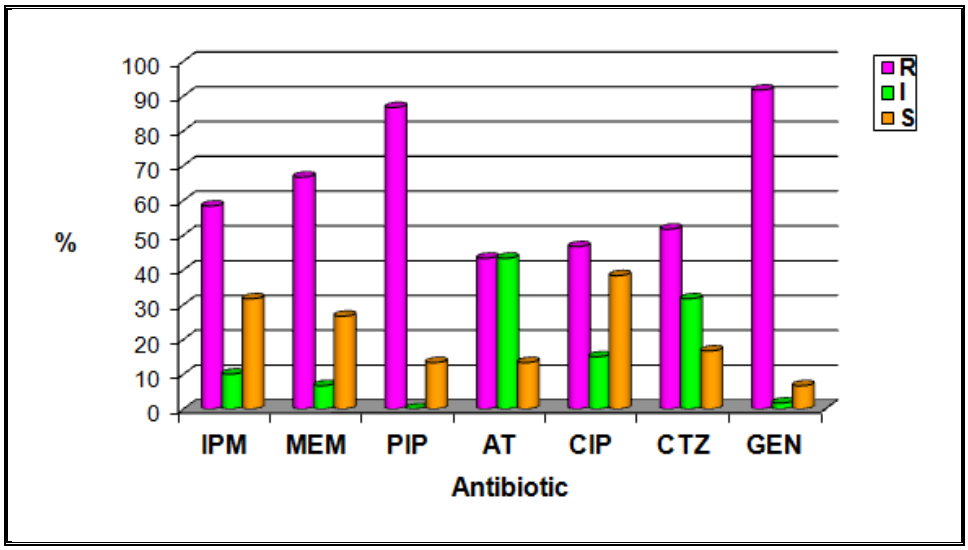

Figure 1- Antibiotic susceptibility of R, I & S by DDT.
Abbreviations: R: resistant; I: intermediate; S: sensitive; IPM: Imipenem; MEM: Meropenem; PIP: Piperacillin; AT: Aztreonam; CIP: Ciprofloxacin; CTZ: Ceftazidime; GEN: Gentamicin.

Table 2-Antibiotic susceptibility of R, I and S (Distribution in total samples) according to VITEK-2

Antibiotic	R % (No.)	I % (No.)	S % (No.)	*P value* [a]
IPM	60 (36)	(٥) 8.33	(١٩)31.67	0.0134
MEM	66.67 (40)	(٣)5	(١٧)28.33	0.0029
PIP	(٥٢)86.67	–	(٨)13.33	0.0001
AT	(٢٦)43.33	(٢٦)43.33	(٨)13.33	0.0149
CTZ	(٣١)51.67	(١٩)31.67	(١٠)16.67	0.0125
CIP	(٢٩)48.33	(٨)13.33	(٢٣)38.33	0.0147
GEN	(٥٥)91.67	(١)1.67	(٤)6.67	0.0026

Total No. of samples = 60.
a: ***P-value*** was calculated using the Chi-square test in terms of the R, I & S group.
Abbreviations: R: resist; I: intermediate; S: sensitive; IPM: Imipenem; MEM: Meropenem; PIP: Piperacillin; AT: Aztreonam; CIP: Ciprofloxacin; CTZ: Ceftazidime; GEN: Gentamicin.

It was confirmed by Lyczak, *et al*. [20] and Ulku, *et al.* [21] that *P. aeruginosa* resistance to many antibiotics and antiseptics and it's so commonly occurrence in the environment make it extremely likely that an individual suffering severe burns or wounds will be challenged with this opportunistic microorganism before the wounds can heal.

As shown in table-1 and table-2, this study resulted in high carbapenem (Imipenem and Meropenem) resistant *P. aeruginosa*; Imipenm reading was 58.33% by Disk Diffusion Test (DDT) and 60% by VITEK-2 and Meropenem resistance was 66.67% in both methods. Piperacillin rsistance was 86.67%, Aztreonam resistance 43.33%, Ciprofloxacin resistance rates were 46.67% and 48.33% by DDT and VITEK-2, respectively. Ceftazidim resistance was 51.67%, and the highest resistance rate was shown by Gentamicin 91.67%.

The differences in the results of antibiotic susceptibility between the manual standardized DDT according to Kirby Bauer's method and automated method by VITEK-2 system were insignificant which made the study depending on the classic DDT results due to its identical replicates while the high cost of VITEK-2 mediated test obstructing the possibility of making replicates led to make it less preference by this study. The variable results between the manual and automated antibiotic susceptibility tests are expected as shown by previous findings like Gagliotti *et al.* [22] who found the

difficulties encountered in testing beta-lactam susceptibility including carbapenem in automated systems. The results obtained in this study revealed that Piperacillin resistanc rate is 86.67 which is similar to Mohammed [23] findings that reported 93.3% of *P. aeruginosa* isolates were resistant to Piperacillin and close to Haran [24] who found that resistance to Piperacillin is 88%. *P. aeruginosa* is naturally resistant against penicillins such as Piperacillin as reported by Ibezim [25] making this study result's rate of resistance against Penicillins reasonable. In contrast, the result of this study crossed with Al-Doory [26] finding, 35.8% resistance showing high considered difference.

P. aeruginosa isolates have shown low resistance against Ciprofloxacin 46.67% but still higher than 20.6% belonging to Al-Doory [26] results. The result of our study is close to Al-Muhannak [27] who found that Ciprofloxacin resistance 40% and similar to the result of Mohammed [23] which was 54.6%. In 2002, Lambert mentioned that Ciprofloxacin, belong to fluoroquinolone, inhibits bacterial growth by binding to A subunit of DNA gyrase [28]. Alterations in the quinolone resistance-determining regions in the genes coding for DNA gyrase and topoisomerase IV play an important role in quinolone resistance in *P. aeruginosa* according to Henrichfreise *et al*. [29]. In this study, the sensitivity of *P. aeruginosa* isolates to Gentamicin belonging to aminoglycosides group has shown notably high resistance to this group of antibiotics 91.67%. This result agree with AL-Khazali [30] who has found that the resistence of *P. aeruginosa* isolated from burns and wounds to Gentamicin was 89.% and disagree with Mohammed [23] and Al-Doory [26] results that were 60% and 35.9 respectively.

The aminoglycosides inhibit protein synthesis in bacterial cell by binding to 30S subunit of the ribosome and the Aminoglycoside-resistance in *Pseudomonas sp*. is primarily due to changes in the target enzymes and inactivation of the antibiotics as Lambert [28] and Matsuo *et al*. [31] have mentioned. In this study, *P. aeruginosa* isolates has revealed high resistance (51.67%) for the fourth generation of cephalosporin Ceftazidime. This result coincides with the finding of Al-Doory [26] who reported 61.6% Ceftazidime resistance rate and disagrees with resistance rates, 89.8% and 82.6% of Al-Muhannak [27] and Mohammed [23], respectively. But in contrast with Gailiene *et al*. [32] who have found that resistance of *P. aeruginosa* to Ceftazidime is 12.8%, the differences seem to be significant. The increased prevalence of Ceftazidime resistant *P. aeruginosa* may be related to the increased use of beta lactam antibiotics such as amoxicillin and ceftazidime. Selective pressure resulted from the use of antimicrobial agents is a major determinant for the emergence of resistant strains. The elevated resistanc of *P. aeruginosa* isolates in burn unit to carbapenems, Imipenem 58.33% and Meropenem 66.67% in this study is so close to Al-Doory [26] finding which resulted in resistance 53.3% and 53.2% for Imipenem and Meropenem, respectively. However, the difference in result is apparent compared with Al-Shwaikh [33] who found that all *P. aeruginosa* isolated from burn and wound infections were sensitive to Imipenem 100%, also this result differs than AL-Khazali [30] who has found that *P. aeruginosa* isolated from burn and wound infections have low resistance to Imipenem 42.1%, and disagree with Gailiene *et al*. [32] finding that resulted in resistance of *P. aeruginosa* to Imipenem and Meropenem 23.9% and 11.3% respectively. Disagreement is continued to the results obtained by Mohammed [23] who showed extremely low resistance rate to Imipenem and Meropenem, 8% both. Pseudomonads may develop resistance to carbapenems through combined mechanisms such as target inaccessibility, stable derepression of AmpC β-lactamase, overexpression of efflux systems and production of Metallo-β-lactamases (MBLs) as reported by Livermore [34].

Aztreonam has potent activity against gram-negative organisms and it is stable to the β-lactamases. It is inactive against gram-positive organisms and anaerobes. This study revealed that *P. aeruginosa* isolates had a moderate resistance level against the monobactam (Aztreonam) which reached 43.33% conflicting with the elevated resistance rate obtained by Al-Muhannak [27] who found that resistance level of bacteria to this antibiotic was 59.3% and differs than the high results, 84% and 81.3% of Haran and Mohammed, respectively [22, 21].

Moazami-Goudarzi and Eftekhar reported in 2013 that the increase in antibiotic resistance is mostly due to extensive use of antibiotics such as ciprofloxacin, β-lactams and aminoglycosides in the burn centers as well as non-availability and high costs of other effective drugs [35].

In this study, out of total (60) *P. aeruginosa* isolates, carbapenem (Imipenem and Meropenem) resistant isolates were 41 (68.34%), while the intermediate-sensitivity isolates were 2 (3.33%) and the sensitive isolates were 17 (28.33%) as shown in figure-2.

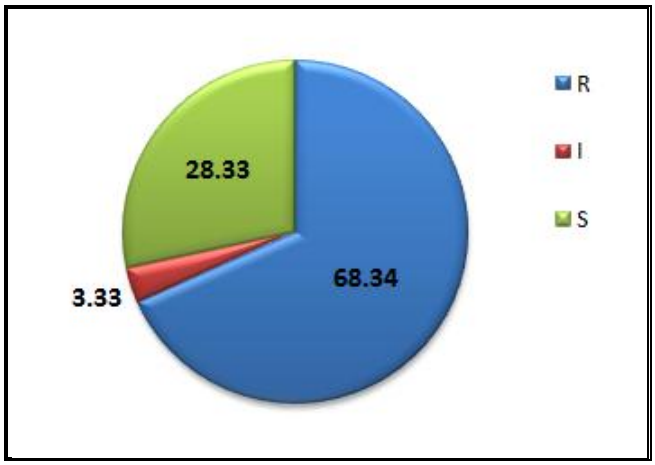

Figure 2- Carbapenem (Imepenem and Meropenem) susceptibility rates of *P. aeruginosa* by Disk Diffusion Test (DDT). R: resistant; I: intermediate; S: sensitive.

These results are elevated in comparison with the result obtained by Mohammed [23] who found that carbapenem resistace in 75 *P. aeruginosa* isolated from different source cases in Baghdad province were 16 (21.3%) and the sensitive ones were 59 (78.7%). In this study, the prevalence of carbapenem resistance in burn unit is high which agrees with Yousefi *et al*. [5] in Iran who found that out of 160 *P. aeruginosa* isolate, 93 (58.1%) isolates were sensitive to Imipenem, 61 (38.1%) were resistant and 6 (3.8%) of isolates showed intermediate resistance and have observed that hospitalization in burn units and ICU wards had significant association with Imipenem non-susceptible isolates. Thus, they concluded that the high prevalence of antimicrobial resistance observed among *P. aeruginosa* isolates underlines the strict consideration in antibiotics use at clinical settings.

Conclusions

Our study has concluded that the prevalence of increasing resistance rate to antibiotics, especially carbapenems, the final drug choice for the treatment of *P. aeruginosa*, among the immunocompromized burn patients which is a threatening matter, is due to the increasing usage of this group especially Meropenem. Therefore, it is important to emphasize the control of hospital contamination with resistant strains especially at burn unit which is the focus to prevent nosocomial resistance dissemination and it is recommended to limit the use of carbapenems as a prompt to reduce the threatening fate of carbapenem resistance.

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