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Antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from different clinical sources

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ABSTRACT

Pseudomonas aeruginosa (P. aeruginosa) is a Gram negative opportunistic pathogen which is responsible for many infections in humans. It is the causative agent of nosocomial pneumonia, urinary tract infection, surgical site infections and burn infection. Bacterial resistance to antibiotic is an increasing problems worldwide. Emergence of bacterial resistance to antimicrobials pose a challenge in treating pyogenic infection hence periodical monitoring of bacterial profile and their antibiotic susceptibility pattern is important. In current study, P. aeruginosa isolates obtained from different clinical sources were identified according to traditional biochemical tests. Antibiotic susceptibility testing was performed by the disc diffusion method. Importantly, present results show that 53% out of P. aeruginosa isolates exhibited multi drug resistance (MDR) pattern. P. aeruginosa isolates showed higher resistance to ciprofloxacin, gatifloxacin and meropenem and intermediate resistance to cefoperazone, cefepime, pipracillin, tobramycin, pipracillin-tazobactam, ceftazidime and azetreonem while Low bacterial resistance was noted against colistin only. The inappropriate use of antibiotics has led to the development of resistant bacteria which led to ineffective antibiotic therapy. Current study suggests applying of strict policies for antibiotic prescription and dispensing.

Key words*: Pseudomonas aeruginosa, Antimicrobial susceptibility, Antibiotic resistance, Multidrug resistance*

INTRODUCTION

P. aeruginosa is a Gram-negative opportunistic pathogen. It is a ubiquitous bacterium that is found and isolated from various environments including plants, animals, soil and humans **(Filiatrault *et al.* 2006)**. *P. aeruginosa* accounts for 10 - 15% of nosocomial infections worldwide **(Strateva and Yordanov 2009)** and is considered the third most-common organism associated with hospital-acquired infections such as urinary catheter-associated infections ventilator-associated pneumonia as well as blood, burn and wound infections. In addition, *P. aeruginosa* is the causative agent of a wide variety of life-threatening infections, particularly in immune compromised patients **(Moreau-Marquis *et al.* 2008; Stover *et al.* 2000).**

Bacterial resistance to antibiotics is a global health problem that limits the therapeutic options. *P. aeruginosa* develops resistance against almost all antibiotics by several mechanisms like, multi-drug resistance efflux pumps, resistance genes, biofilm formation, aminoglycoside modifying enzymes and mutations in different chromosomal genes. Further more exposure to broad spectrum antibiotics and patient to patient spread have added the rapid increase in the isolation of rapid strains **(Gill *et al.* 2011)**. This study aims to investigate the antimicrobial resistance pattern of *P. aeruginosa* isolated from different sources such as urine, sputum, burn and wound.

Materials and Methods

Bacterial isolation and identification

A total of 300 clinical specimens were collected from patients admitted to Zagazig University Hospital and from Al-Ahrar Hospital in Zagazig from different sources. Handling of specimens and isolation were performed following the standard microbiological procedures. Samples were cultured on nutrient agar (LabM, UK) and incubated overnight at 37°C. Colonies appeared on nutrient agar were further examined by Gram-stain; only Gram-negative isolates with green color on nutrient agar were further sub cultured overnight at 37°C on selective medium cetrimide agar (Lab M, UK). Pure colonies on cetrimide agar were further identified by biochemical tests including oxidase test, oxidation-fermentation test (O/F), gelatin liquefaction, motility, growth at 42°C and growth on triple sugar iron (TSI) agar **(Winn *et al*. 2006).**

Antibiotic susceptibility testing of bacterial isolates

The antibiotic susceptibility test was performed using Kirby-Bauer disc diffusion method **(Bauer *et al*. 1966)**. The antimicrobial discs were purchased from Oxoid (Hampshire, England) and include; piperacillin (PRL, 100µg), piperacillin-tazobactam (TPZ, 110µg), cefoperazone (CEP, 75µg), cefepime (FEP, 30µg), ceftazidime (CAZ, 30µg), ciprofloxacin (CIP, 5µg), gentamycin (CN, 10µg), colistin sulfate (CT, 10µg), aztreonam (ATM, 30µg), meropenem (Mem,10 µg),tobtamycin(TOB, 10µg), gatifloxacin (GAT, 5µg) and amikacin (AK, 30µg). Bacterial suspensions were prepared from overnight cultures on Muller-Hinton agar (Oxoid, Hampshire, England). Suspensions densities were adjusted to 0.5 McFarland standard that correspond to approximately (1.5×10^8 CFU/mL).The surface of Muller-Hinton agar plate was inoculated with suspensions using sterile cotton swabs. The plates were dried before applying the antibiotic discs and incubated overnight at 37º C. The diameters of inhibition zones around the discs were measured. The results were interpreted according to Clinical Laboratory Standards Institute guidelines **(CLSI, 2018).**

RESULTS

Isolation and identification of *P. aeruginosa*

P. aeruginosa was isolated and identified in 33.3% of clinical samples. One hundred *P. aeruginosa* isolates were recovered from different sources which are shown in (**Table 1**). *P. aeruginosa* was identified as Gram-negative rods, lactose non-fermenting, appearing as large colonies with characteristic grape-like odor and greenish pigment (pyocyanin) on nutrient agar in addition to pyocyanin non-producing colonies on cetrimide agar. Suspected as *P. aeruginosa* bacteria were collected and further identified based on biochemical characteristics as shown in **(Table 2) (macFaddin 2000).**

Table 1: Sources of P.aeruginosa isolates.

Source	No. of *P. aeruginosa* isolates
Burn	25
Urine samples	27
Endotracheal aspirates	29
Wound & pus	11
Eye	5
Ear	3
Total Number	100

Table 2. Biochemical characteristics of clinical P. aeruginosa isolates

Biochemical test	Result
Oxidase	**+***
Oxidation-Fermentation test (O/F)	O^{+}/F^{-}**
Gelatin liquefaction	+***
Motility	Motile
Growth at 42°C	+
Growth on triple sugar iron (TSI) agar	K/K****

**Color change to violet within 15-30 second*

***Only aerobic tube (O) turned yellow the fermentative tube (F) remain green*

**** Partial or total liquefaction of the inoculated tube (control tube must be solid)*

***** K: alkaline slant/ K: alkaline butt reaction,*

Antibiotic Susceptibility Test:

The antibiotic resistance profile of *P. aeruginosa* showed varying antibiotic resistance patterns to different antibiotics **(figure1)**. High bacterial resistance was found against ciprofloxacin,and gatifloxacin (63% each), gentamycin (62%) and meropenem (60%). Intermediate bacterial resistance was found against cefoperazone, cefepime, pipracillin, and tobramycin (57%each), amikacin (56%), pipracillin-tazobactam, ceftazidime and azetreonam (53% ,46%, and 34% respectively). Low bacterial resistance was noted only with colistin (7%). Twenty isolates were found to be susceptible to all tested antibiotics (20 %)

and only two isolates was pan-drug resistant being resistant to all tested antibiotics. The antibiotic susceptibility testing showed that 53 out of 100 *P. aeruginosa* isolates in this study were MDR (non-susceptible to at least one agent in three or more antimicrobial categories) as shown in **Table 3**.

Figure (1): Percentage of isolates resistance against tested antibiotics.
Abbreviations; amikacin (AK), gentamycin (CN), ciprofloxacin (CIP), meropinem (M EM), pipracillin (PRL), cefoperazone (CEP), piperacillin/tazobactam (TPZ), cefepime (FEP), ceftazidime (CAZ), Aztreonam (ATM), colistin (CT), Tobramycin (TOB), Gatifloxacin (GAT)

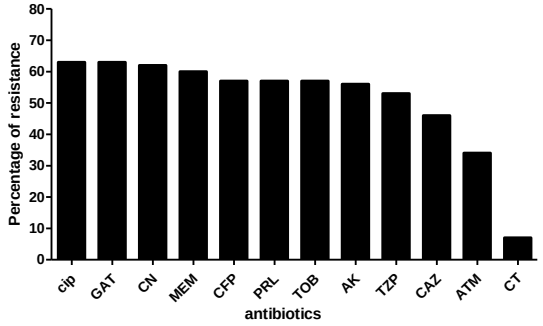

Table 3: Frequency of multidrug resistant isolates of P. aeruginosa

Number of resistant isolates	Number of Antibiotic classes	Classes of antibiotics
4	4	B-lactams, aminoglycosides, fluoroquinolones and lipopeptide
47	3	B-lactams, aminoglycosides and fluoroqinolones.
2		B-lactams,lipopeptide and fluoroqinolones.

DISCUSSION:

P. aeruginosa is a significant opportunistic pathogen that causes many fatal infections in persons with serious medical conditions such as immunocom-promised persons **(Gellatly and Hancock 2013)**. *P. aeruginosa* is considered to be very dangerous because it can easily colonize epithelial surfaces, weaken host defenses, induce systemic toxicity and is associated with elevated morbidity and mortality rates **(Juayang *et al.* 2017)**. Improper use of antimicrobials resulted in the development of MDR strains which are quite hard to be treated due to their higher resistance to various antibiotics **(Lister *et al.* 2009)**. The present study was performed to investigate the antimicrobial resistance of *P. aeruginosa* isolated from different sources.

The prevalence of MDR isolates among the overall collected isolates was 53/100 (53%). This was similar to a previous study that reported a prevalence rate of 52% **(Mahmoud *et al.* 2013)**. Other global studies exhibited lower MDR rates; 5.9% in Canada **(Zhanel *et al.* 2010)** & Germany **(Narten *et al.* 2012).** The obtained results may be attributed to numerous factors that participated in the spread of MDR isolates in Egypt; primarily the increased disaster of antibiotic misuse without proper prescriptions **(Daniel *et al.*, 2015).** This elevated MDR rate in Egypt in comparison to other countries gives us an alarm to the necessity of the application of rigorous antibiotic prescription strategies.The variability observed in the distribution of MDR *P. aeruginosa* isolates, the resistance rates and susceptibility profile against different antibiotics between the present study and the other studies could be attributed to the variation of the antibiotics usage policy applied in each country **(Bekele *et al.*, 2015).**

In current study, the resistance rates against different antibiotics were variable. The overall 100 isolates showed highest resistance against fluroqinolones (ciprofloxacin, gatifloxacin (63% each),

the aminoglycosides gentamycin (62%), followed by carbapenem (meropenem; 60%). The resistance rate of ciprofloxacin in our study (63%) was in accordance with (Senthamarai 2014) who reported (61.53%). While another study showed low resistance (25%) by **(Ogbolu *et al*., 2008)**. The difference in the rate of ciprofloxacin resistance is usually related to the frequency of use of fluoroquinolones and availability of oral doses.

Aminoglycosides are significant members of broad spectrum antibiotics. They act mainly by inhibiting protein synthesis and breaking bacterial cell membrane **(Shakil *et al*. 2008)**. This study showed the resistance rate of gentamycin (62%) was higher than that of amikacin and tobramycin (56% for each).These findings were in agreement with was a previous study by **(Raytaker *et al*. (2017)** that showed higher resistance rate to gentamycin (57.8%) than that of amikacin (35%). On other hand, another study by **Khan *et al*. (2014)** showed that amikacin was the most effective antibiotic with resistance rate (10%). Resistance of clinical isolates to aminoglycoside antibiotics was found to vary with specific drug, the microorganism, its mechanism of resistance, the geographic area and many other factors **(Vakulenko and Mobashery 2003).**

Carbapenems are considered the most significant group of antibiotics against MDR *P. aeruginosa* but the development of carbapenems resistance is becoming a challenge for health care professionals and has limited the therapeutic options. Sufficient measures are required to prevent the spread of carbapenemase encoding gene **(Rodríguez-Martínez *et al*. 2009)**. The current study demonstrated that 60% *P. aeruginosa* were resistant to carbapenem antibiotic (meropenem).This finding was in agreement with that reported by **(Rodríguez-Martínez *et al*. 2009, and Khan *et al*. 2014)** where resistance rates were found to be 60% and 87%; respectively. It is very obvious that efficacy of this particular antibiotic is declining. The reason for the high resistance to meropenem in our study is that the drug is commonly used in the treatment of many infections. This warrants a need to de-escalate therapy based on cultures, as it is not just *Pseudomonas* that will be resistant, but many members of *Enterobacteriaceae* would be resistant, including emergence of carbapenem-resistant *Enterobacteriaceae* **(Khan and Faiz 2016).**

In the present study, increased bacterial resistance to β-lactam antibiotics has been detected. The high resistance to β-lactam in nosocomial *P. aeruginosa* has become a serious threat particularly against third and fourth generation cephalosporins. There are many molecular mechanisms to develop resistance against these antibiotics; including generation of extended-spectrum betalactamases (ESBL), incorporation of *bla* genes in integrons and inability of porin genes to enhance their expression level and/or alteration of antibiotic target sites **(Pfeifer *et al*. 2010)**. *P. aeruginosa* isolates were resistant to the expanded spectrum penicillin, pipracillin (57%). Similar result founded in a recent study by **Pokharel *et al*. (2019)** showing resistance rate of 56.5%. However lower resistance rate (28%) was reported by **Abbas *et al*. (2018)**. The isolates were also resistant to the pipracillin-Tazobactam combination (53%) which was slightly less than the 56.6% that reported by **Khan *et al*., (2014)**, while it was different from the low resistance (4.9%) reported by **Khan and Faiz (2016)**.

For the 3rd & 4th generation cephalosporins, *P. aeruginosa* isolates exhibited resistance rate of 57% to each cefoperazone and cefipime. This result is less than that reported by (Mahmoud ***et al***. 2013) who showed a resistance rates of 73.3% and 98.2%; respectively. While Khan and Faiz (2016) showed low resistance rate for cefepime (8.3%).

In the present study, *P. aeruginosa* isolates showed resistance rate of 46% to

ceftazidime. This result is higher than that of **Khan and Faiz (2016)** who reported resistance rate 14% while **Mahmoud *et al*. (2013) and Pokharel *et al*. (2019)** reported high resistance rate of 91.2% and 63%; respectively. For the monobactam, azetronam, the isolates exhibited low resistance rate (34%), **Mahmoud *et al*. (2013)** showed high resistance rate (82.5%)**.**

Our results showed that colistin was the most effective antibiotic against *P. aeruginosa* with resistance rate of (7%). This result is comparable with **Afifi *et al*.(2013)**, who reported resistance rate of 3% while 0% resistance was reported by **Pokharel *et al*. (2019)**. This study indicates that colistin is an efficient therapy against MDR *P. aeruginosa* isolates followed by aztreonem among all tested antibiotics. It is very obvious that efficacy of particular antibiotic is declining as fluroquinolones and carbapenems in treatment of *P. aeruginosa* infections.

CONCLUSION:

The emergence of MDR *P. aeruginosa* and its continual spread is out of debate. Antibacterial research is not sufficient to keep pace with the clinical challenges of MDR bacterial crises. Failure of antibiotic treatment could result from misuse and abuse of antibiotics in addition prescription of antibiotics without performing susceptibility testing beside the extensive use of broad spectrum antibiotics. New therapeutic agents with maximum efficacy, lesser toxicity and cost effective in nature are urgently needed to overcome the problem of antibiotic resistance. In addition, strict laws regarding antibiotic policies should be constructed to limit the unnecessary use of antibiotics so that spread of multidrug resistance can be avoided.

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اختبار الحساسيه للمضادات الحيويه لعزلات سودوموناس ايروجينوزا المعزوله من مصادر مختلفه

قسم الميكروبيولوجي والمناعه –كليه الصيدله –جامعه الزقازيق- مصر

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سودوموناس ايروجينوزا هي عباره عن بكتيريا سالبه الجرام انتهازيه العدوى.وهي المسؤوله عن مجموعه متنوعه من العدوى مثل عدوى المسالك البوليه والجهاز التنفسي والحروق والجروح .مقاومه المضادات الحيويه مشكله متزايده في جميع انحاء العالم .يظهر السودوموناس ايروجينوزا مقاومه عاليه للمضادات الحيويه.تم التعرف على عزلات السودوموناس ايروجينوزا وفقا للاحتبارات البيوكيميائيه. كما تم اجراء اختبار الحساسيه للمضادات الحيويه بطريقه انتشار القرص.

تم تجميع مئه عينه من بكتيريا السودوموناس ايروجينوزا. وتم التعرف على عزلات السودوموناس ايروجينوزا وفقا للاحتبارات البيوكيميائيه. كما تم اجراء اختبار الحساسيه للمضادات الحيويه بطريقه انتشار القرص تم ملاحظه مقاومه عاليه مع السيبروفلوكساسين ,الجاتيفلوكساسين والميروبينيم.ولوحظت مقاومه متوسطه ضد السيفيبيم ,السيفتازيديم,السيفوبيرازون,الازترونام,الاميكاسين,الجنتاميسين,التوبراميسين, البيبراسيلين والبيبراسيلين / التازوبكتام.تم ايجاد مقاومه منخفضه مع الكوليستين فقط.تم العثور على المقاومه للادويه المتعدده في 53% من العزلات .الاستخدام الغيرسليم للمضادات الحيويه يؤدي الى ظهور البكتيريا المتعددة المقاومه مما يجعل العلاج بالمضادات الحيويه غير فعال ولذا يجب تطبيق سياسات صارمه على وصفات المضادات الحيويه وصرفها