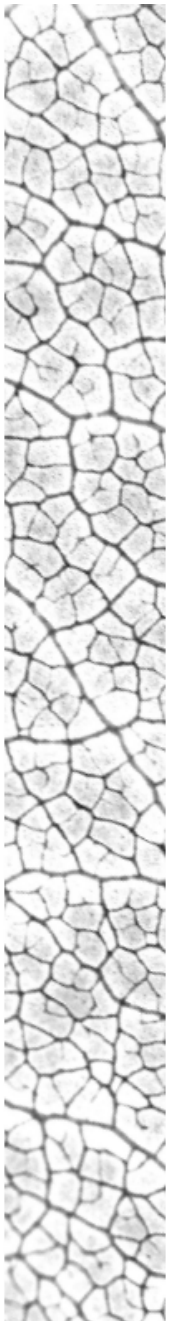

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Isolation and identification of multi-drug resistant "pseudomonas aeruginosa" from burn wound infection in Kirkuk City, Iraq

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Abstract

Background: "*P. aeruginosa"* is considered as ubiquitous bacteria that can rapidly obtain resistance against various wide spectrum antibiotics *It* can rapidly obtain resistance against various wide spectrum antibiotics which lead to problematic conditions. This study was proceeded with an aim to determine the antibacterial resistance pattern and prevalence of "MDR P. aeruginosa" infection among burns patients.

Material and Methods: This study was carried out on the Burn patients in Azadi Teaching Hospital in Kirkuk city / IRAQ from January, 2016 to June, 2016. The clinical samples were collected using sterile cotton swabs from 100 patients with burn wound infections. *"P. aeruginosa"* was identified by using standard microbial methods. The drug susceptibility pattern using 10 different antibiotics (Augmentin, Amoxicillin, Ceftriaxone, Cefotaxime, Ceftazidime, Tetracycline, Gentamycin, Imipenem, Ciprofloxacin and Amikacin) was performed for all the isolates using Kirby Bauer's Disc Diffusion Method.

Results: *"P. aeruginosa"* were isolated from 36clinical burn samples and88.88% of these isolates were Multidrug Resistance *"P. aeruginosa"* (MDRPa). Resistance rates to different antibiotics were as follows: 36 (100%) resistant to (Augmentin, Amoxicillin, Ceftriaxone and Cefotaxime), 19 (53%) isolates showed resistance towards Ceftazidime, 33 (92%) isolates were resistant towards (Tetracycline, Gentamycin and Imipenem). Ciprofloxacin and Amikacin resistance were seen in 31 (89%) and 24 (67%) isolates respectively.

Conclusion: Wide prevalence of MDRPa and nosocomial infections submit continuous monitoring of burn infections and evolve new strategies for drug resistance control and treatment of infections.

Keywords: *Pseudomonas aeruginosa*, burn patients, multidrug resistance

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INTRODUCTION

Burn injury is considered as a serious public health problems worldwide, is at high venture for nosocomial infections (Church et al. 2006). Denatured and dead tissues with moist environment makes the burn wound susceptible to infection with *P. aeruginosa*, breaking the defensive skin barrier, reducing the immunity, and prolonged hospital staying are significant factors responsible for infection of burn wound with such opportunistic pathogens specially multi-drug resistant (MDR) *"P. aeruginosa"* (Naqvi et al. 2005), that considered as ubiquitous bacteria that can rapidly obtain resistance against various wide spectrum antibiotics (Indu et al. 2014).

"Multidrug resistant of *P. aeruginosa"* is a significant cause of mortality and morbidity in burn unit patients', causes 4-60% of nosocomial infections in various countries (Carmeli et al. 1999).

"MDR P. aeruginosa" phenotype is defined as a bacterium which is resistant to at least three classes of antibiotics (penicillins / cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides) by using special enzymes that make carbapenems and beta-lactams insufficient, like the metallo-β-lactamases (MBLs) and extended spectrum beta lactamases (ESBLs) (Marilee and Obritsch 2005, Vahdani et al. 2012).

Intrinsic MDRPa is associated with production of inducible β- lactamase, limited permeability of outer membrane and Multidrug Efflux system (Asma and Noura 2004, Nikaido 1989, Poole et al. 1993). Among

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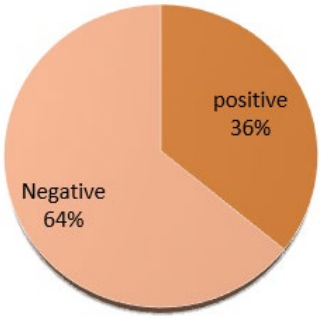

Fig. 1. Prevalence of *"Pseudomonas aeruginosa"* in samples studied

four MDR, MexAB-OprM and MexXY-OprM efflux system in *"P. aeruginosa"* are responsible for providing the intrinsic resistance while hyperexpression of MexCD-OprJ and MexEF-OprN leads to obtain integron and MDRPa.13 Plasmid have a critical role in procuration of mobile elements (Japoni 2009)

The present study was proceeded with an aim to determine the antibacterial resistance pattern and prevalence of "MDR P. aeruginosa" infection among burns patients at Azadi Teaching Hospital in Kirkuk city, Iraq.

MATERIALS AND METHODS

Our study was processed in the Burn Unit of Azadi Teaching Hospital, Kirkuk city, Iraq. The present study included 100 burn patients with different ages, over a period January to June 2016.

Sample Processing

The clinical samples were cultured on Blood Agar, PIA and Mac Conkey's Agar. The identification of *"P. aeruginosa"* was happened by depending on the colony characteristics, grape like odour, pigment production, motility, oxidase positivity, gram staining (gram negative bacilli), non-fermentative character, their ability of reducing the nitrates to nitrites, its ability to decarboxylate arginine, liquefy gelatin and to grow at 42 ℃ (Collee et al. 1996).

Table 1. The rate of *"P. aeruginosa"* isolates according to patients' age group and their gender

Variable		No of *"P. aeruginosa"* strains	Percentage%
Age (Years)	1-25	22	61.1%
	26-45	10	27.8%
	<45	4	11.11%
Sex	Female	22	61.1%
	Male	14	38.9%

Antibiotic Sensitivity Test

This test made on Mueller Hinton agar by Kirby Bauer Disc Diffusion method, by CLSI 2011 Guidelines (Clinical and Laboratory Standards Institute 2011). Antibiotics were tested, which included amoxicillin (10mcg), Augmentin (10mcg), ceftriaxone (30mcg), cefotaxime (30mcg), ceftazidime (30mcg), amikacin (30mcg), gentamicin (10mcg), ciprofloxacin (5mcg), imipenem (10mcg), and tetracycline (10mcg) . In this study, we used *"Pseudomonas aeruginosa"* ATCC 27853 strain for quality control. Multi Drug Resistant *"P. aeruginosa"* isolates were detected by their resistant to three or more antibiotic classes (Magiorako 2011).

Statistical Analysis

Chi squire tests and p-values were used for the statistical analyzing of our results.

RESULTS

Out of 100 clinical samples collected from the burn patients, *"P. aeruginosa"* was isolated from 36 (36%) samples (**Fig. 1**).

Table 1 showed the rate of *"P. aeruginosa"* isolates according to patients' age group and their gender. Our study demonstrated high rate of *"P. aeruginosa"* among the female than male (61.1% and 38.9%, respectively), and the high prevalence of these isolates were reported among 1 to 25 years old group (61.1%) and the low prevalence among <45 years old group (11.11%).

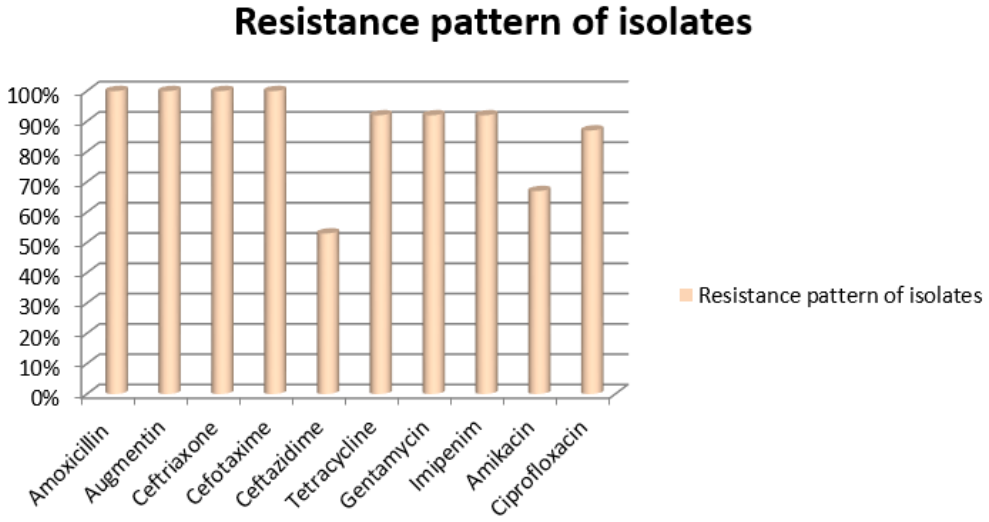

Fig. 2. Resistance pattern of isolates

Table 2. Antibiotyping of the *"P. aeruginosa"* isolates

Type	No. of resistant antibiotic	No. of isolates (%)	Resistance of antibiotics
1	10	8 23%	AMC,AX,CTX,CAZ,CRO,TE,GM,IP,CRP,AK.
2	9	14 39%	AMC,AX,CTX,CRO,IP,GM,TE,CRP,AK.
3	9	9 25%	AMC,AX,CTX,CRO,CAZ,IP,GM,TE,CRP.
4	9	2 6%	AMC,AX,CTX,CRO,CAZ,IP,GM,TE,AK.
5	4	3 9%	AMC,AX, CTX,CRO.

Antibiotic susceptibility tests of *"P. aeruginosa"* toward 10 various kinds of antimicrobial agents is showed in **Fig. 2**. It shows that 36 (36%) isolates were resistant to beta - lactams (Augmentin, Amoxicillin, Ceftriaxone and Cefotaxime), while 19 (53%) isolates showed resistance towards Ceftazidime, 33 (92%) isolates were resistant toward (Tetracycline, Gentamycin and Imipenem).

Ciprofloxacin and Amikacin resistance were seen in 31 (89%) and 24 (67%) isolates respectively.

Table 2 show the Antibiotyping of the *"P. aeruginosa"* isolates which was made by studying the antimicrobial susceptibilities of bacterial isolates and by allotting these isolates under various groups (Type 1-5), depending on resistant patterns. Common antibiotype was the type 2 which demonstrated resistance toward 7 antibiotics, resembled 39%of isolates. This study recorded 91.66% (33/36) of "MDR *P. aeruginosa"* isolates (**Fig. 2**).

DISCUSSION

"Pseudomonas aeruginosa" is one of the most critical causes of healthcare-associated infection and is responsible for 10% of hospital-acquired infection. (Obritsch et al. 2004). *P. aeruginosa* can cause critical infections in patients with immunodeficiency like those with severe burns or post-operative wounds (Dale et al. 2004, Sule et al. 2002).

"Pseudomonas aeruginosa" has great potency to become resistant against many antibiotics , therefore there has been an immediate development of MDR *"P. aeruginosa"* lately, which considered as serious complication for the doctors.

In this study, the most prevalent bacterium isolated from burn patients was *"P. aeruginosa"* (36%). Similarly, other studies such as Ekrami and Kalantar (2007) and Okon et al. (2009) also showed a prevalence of *"P. aeruginosa"* infection among burn patients to be 37.5% and 39.1% respectively.

However, studies of Ekrem et al. (2014) and Naqvi et al. (2005) showed a prevalence of 17.85% and 59.6% respectively. These variations in prevalence average among numerous studies could be associated with the disparities in hygienic practices of population and geographical location.

According to gender, our study recorded the highest prevalence of *"P. aeruginosa"* was in female (61.1%), and according to age group , the highest prevalence of *these isolates* was (61.1%) in patients with young ages (1 to 25 years)in contrast to the elderly patients age groups, whereas the lowest prevalence (11.11%) was found among forty five years age group and above, this could be due to the fact that the young old group is the most active group, and most involved in outdoor activities.

This result is agreed with studies in Ethiopia and Iraq by Shewatatek et al. (2014) and Ekrem and Rokan (2014) respectively, the results of their studies were showed highest prevalence of *"P. aeruginosa"*in female but disagreed with them in most infected age group were from elderly patients.

Otherwise, these results disagreed with study in Nigeria by Okon et al. (2009), in which the males were the most infected gender 52.8% but agreed with them in the most infected age group because the highest rate of these isolates (20.7%) was recorded among 29 years age group and below.

Results in **Fig. 2** show the antibiotic susceptibility testing profile of *"P. aeruginosa"* that all the isolates were resistance towards beta-lactams (Ampicillin, Amoxicillin, Augmentin, Ceftriaxone and Cefotaxime), while 53% of isolates were resistance against Ceftazidime and this agreed with study of Iraj et al. (2013) in Iran that showed high level resistance against Ceftazidime (68.6%), cause of the pervasive use of it in hospitals.

"P. aeruginosa" showed higher resistance against the "Beta-lactam antibiotics" than that of "non Beta-lactam", this result can be associated with the hyper production of "Beta lactamase" through the mutational processes and genes of resistance (CDC 2010, Lister et al. 2009, Okon et al. 2009).

Among aminoglycosides, 62% isolates were resistant towards amikacin and 92% to gentamicin, study of Naqvi et al. (2005) showed 70.5% isolates resistant to amikacin and 93.2% to gentamicin.

Carbapenems are good option of antibiotics against MDR *P. aeruginosa* infection but increasing resistance against carbapenems has now become a significant concern (Pier et al. 2010). In this study, it was found that 92% isolates were resistant to imipenem. However, Moazami-Goudarzi and Eftekhar, in their study, found 94.7% isolates resistant to imipenem (Moazami and Eftekhar 2012).

"P. aeruginosa" showed high resistance against ciprofloxacin (87%). Study by Siva Gowri (2009) showed (83.5%) resistance against the Quinolone (Ciprofloxacin) (Pathmanathan et al. 2009).

The variation in the prevalence rate of bacterial resistance among various studies may be associated with various points such as type of clinical specimen

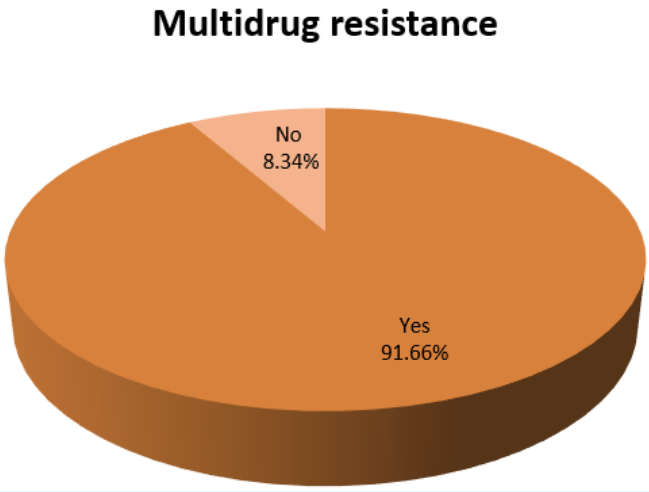

Fig. 3. Multidrug resistance pattern of isolates

examined, hygienic culture of population and exposure to antibiotics.

In this study, 91.66% of isolates were MDR (**Fig. 3**), which is slightly more than the finding of Saderi et al. (2014) in who found 69% isolates to be MDR. While, Moazami-Goudarzi and Eftekhar (2012) showed 100% MDR isolates in burns patients.

Antibiotyping was so instructive in this work, see **Table 2**. *"P. aeruginosa"* isolates were specified five different resistance types. MDR was assorted in antibiotypes 1, 2, 3 and 4. Antibiogram is a susceptible phenotypic significance; but also has disadvantage because it is non-reproducible in numerous occasions, cause of the alternating of R factor among isolates (Ramprasad et al. 2010).

High rate resistance to various antimicrobial agents particularly among nosocomial organisms were revealed and become serious challenge in diseases treating (Jones et al. 2002, Orrett 2004), Report of WHO agrees with the truth of wide use of antimicrobial agents in outside and inside of medicine has a critical role in dissemination of bacterial resistant strains by involving various resistance mechanisms like production of Beta-lactamase enzymes that destruct these drugs (WHO 2002). The great difficulty of the bacterial resistance is the misuse and overuse of antimicrobial agents by patients in addition to the doctors (Goossens et al. 2005, Iduh et al. 2015), and it could be because of the erratically uses of antimicrobial agents without laboratory diagnosis and antimicrobial sensitivity test.

These findings further confirm the need for antibiotic precision and to follow the recommended hospital antibiotic policy to prevent the increasing of MDR strains of *"P. aeruginosa"* in the community.

CONCLUSION

Wide prevalence of MDRPa and nosocomial infections submit continuous monitoring of burn infections and evolve new strategies for drug resistance control and treatment of infections. The choice of therapy for MDRPa often becomes so limited, in addition to the fact that no new antimicrobial agents, active against MDRPa are in advanced stages of development as therapeutic options.

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