Antimicrobial susceptibility of extensively drug-resistant (XDR) and multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolated from patients in Erbil city

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Abstract

Background and Objectives: Bacterial infections including antibiotic resistant Gram negative non lactose fermenter such as *Pseudomonas aeruginosa* has emerged as major threats to human. Isolates show extreme or complete resistant to all except one or two classes of potentially effective antibiotics were considered as extensively drug-resistant (XDR) and those that were resistant to at least three classes of effective antibiotics recommended for treatment were referred as MDR. The purpose of the current study was to determine the occurrence of extensive drug resistance and pandrug resistant in isolates of *P. aeruginosa.*

Materials and Methods: During a one-year period, 91 *P. aeruginosa* were isolated from various clinical samples from infected patients in hospitals. Antimicrobial susceptibility testing was performed on all isolates by Vitek II to identify extensive drug resistance.

Results: Out of 91 isolates of *P. aeruginosa* studied 71 (78%) were found to be XDR and 56 isolates (61.5%) were MDR. of which 36 isolates exhibited resistance to all groups of antimicrobials except one group, rest 20 isolates exhibited resistance to all groups except two groups. However, XDR producing isolates showed 100% resistance against cefoxitin cefuroxime, cefotaxime, Trimethoprim/ Sulfamethoxazole and ampicillin. The resistance rate towards cefepime, imipenem, tobramycin and ciprofloxacin was 78.8%, 67.6%, 81.6%, and 66.1%, respectively, and all isolates were susceptible to colistin. Thus, colistin appeared to be most effective antimicrobial agent against *P. aeruginosa*. The findings of the current study reveal increased burden of XDR and PDR *P. aeruginosa* in our situation.

Conclusion: The majority *P. aeruginosa* isolates were found to be resistant to commonly available antimicrobial agents. Therefore, surveillance and proper antibiotic administration based on culture and sensitivity are all essential for preventing incidence of MDR and XDR *P. aeruginosa*

Keywords: Antimicrobial susceptibility**;** Extensive drug resistance**;** Pandrug resistance; *P. aeruginosa*, Multi-drug resistant

Introduction:

P. aeruginosa is a major opportunistic human pathogen associated broad spectrum of infections mainly bacteremia, wound burn, urinary tract infections, pneumonia and cystic fibrosis particularly in immunocompromised, debilitated, hospitalized patients and those in the intensive care units (ICUs) that contributing to greater morbidity and mortality rates [1]. The anti-Pseudomonal anti-microbial classes with activity against *P. aeruginosa* strains are penicillins /cephalosporins, monobactams, quinolones, aminoglycoside and carbapenems [2]. However, some strains of *P. aeruginosa* have been found resistant to most previously prescribed antibiotics [3]. The rapid and irrepressible increase use in antimicrobial chemotherapy of pathogenic bacteria is widely accepted as a major problem in hospitals followed by the emergence of drug resistance and rapid clonal spread which is becoming a challenging problem worldwide over the past several decades [4,5]. These capabilities have allowed MDR pathogens to be abundant in the hospital environment as well as the community and have made it one of the frontline pathogens threatening the existing antibiotic era [6]. The mechanism and spread of resistance is a complex process that is acquired either through mutations or via horizontal transfer of mobile DNA elements [7]. Antibiotic resistance is a particular problem in *P. aeruginosa* that can be the result of the production of enzymes such as beta-lactamase, alterations in Penicillin- binding proteins, decreased expression of porins, overexpression of efflux Pumps, that makes *P. aeruginosa* a pathogen with a high propensity to becoming resistant to antibiotic therapy [8]. Consequently، treatment options are narrowed down to only a few antibiotics [9]. Currently, terms such as extensive drug resistance (XDR), multi-drug resistance (MDR) and pan drug resistance (PDR) are used to describe the depth of resistance [10]. Unfortunately, no comprehensive data about the occurrence of XDR and MDR in Erbil, Kurdistan are available. The aim of the current study was to evaluate the drug resistance profiles among *P. aeruginosa* to different classes of antibiotics as well as detecting the presence of resistance determinants including XDR and MDR *P. aeruginosa* isolates collected from the different clinical specimen in Erbil city.

Materials and Methods:

A total of 91 consecutive, non-duplicate isolates of *P. aeruginosa* recovered from various clinical specimens between December 2014 to May 2015 submitted to three different hospitals in Erbil city. All clinical isolates were reviewed for XDR, MDR and PDR status. Antimicrobial susceptibility testing was done by Vitek II to the following antimicrobial agents ciprofloxacin, cefoxitin, cefepime, cefotaxim, ceftazidime, cefuroxime, gentamycin, imipenem, meropenem, piperacillin tazobactum, tobramycin, norfloxacin , nitrofuranton and colistin as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC breakpoints were used to interpret susceptibility to antimicrobial agents [11]. Isolates were considered as Multi-drug resistant (MDR) when they were non-

susceptible more than 3 potentially effective of commonly used antimicrobials classes (penicillins, cephalosporins, aminoglycosides, quinolones, β lactamase inhibitor combinations and Carbapenems), and extensively drug-resistant (XDR) when they were non-susceptible to all except two or fewer classes antimicrobial categories while PDR" as those resistant to all classes of antimicrobial agents available [12,13].

Results:

During the study period, out of 91 *P. aeruginosa* samples screened, the distribution of samples was as followed: the highest percentage of isolates were from burn,27; followed by urine, 19; pus,16; blood, 10;aspiration,8; sputum, 4; and others,3 (Table. 1). Of these, 91 samples 71 (78%) were confirmed as XDR *P. aeruginosa.*

Table1. Distribution of *P. aeruginosa* isolates divided into clinical specimens.

Specimen type	**Number of Specimens (%)**
Sputum	4(4.4%)
Pus	16(17.6%)
Blood	10(10.9%)
Urine	19(20.9%)
Aspiration	8(8.8%)
CSF	4(4.4%)
Burn	27(29.7%)
Others	3(3.3%)
Total	91

Antimicrobial susceptibility pattern and details of the antimicrobial agents used against the entire MDR and XDR *P. aeruginosa* isolates and its resistance profile toward all the antibiotics are shown in Table 2. Then the highest resistance attributing pathogenic *P. aeruginosa* showed 100% resistance toward ampicillin, cefuroxime, cefoxitin cefotaxime, trimethoprim/sulfamethoxazole then, tobramycin, ceftazidime, cefepime imipenem and meropenem 64.8%, 65.9%, 61.5%,59.3%, 51.6% respectively. Meanwhile, all isolates of *P. aeruginosa* were susceptible only to colistin.

Table 2. Antimicrobial resistance profile of 91 *P. aeruginosa* isolates.

Antibiotic	Susceptible *n* (%)	Intermediate *n* (%)	Resistant *n* (%)
Ampicillin	0(0%)	0(0%)	91 (100%)
Piperacillin/Tazobactam	1(1.09%)	30(32.9%)	60 (65.9%)
Cefuroxime	0	0	91 (100%)
Cefoxitin	0	0	91 (100%)
Cefotaxime	0	0	91 (100%)
Ceftazidime	27(29.7%)	4(4.3%)	60(65.9%)
Cefepime	27(29.7%)	8(8.7%)	56(61.5%)
Imipenem	24(26.3%)	13(14.2%)	54(59.3%)
Meropenem	34(37.3%)	10(10.9%)	47(51.6%)
Tobramycin	32(35.1%)	0	59(64.8%)
Ciprofloxacin	37(40.6%)	6(6.5%)	48(52.7%)
Gentamicin	19(20.8%)	2(2.1%)	70(76.9%)
Nitrofurantoin	1(1.09%)	0	70(76.9%)
Norfloxacin	39(42.8%)	0	52(57.1%)
Trimethoprim/ Sulfamethoxazole	0	0	91 (100%)
Colistin	91 (100%)	0(0%)	0(0%)

Clinically, XDR *P. aeruginosa* showed 100% resistance toward cephalosporins (cefuroxime, cefoxitin and, cefotaxime), quinolones (ciprofloxacin 47 (66.1%) and Aminoglycosides tobramycin 58 (81.6%) As shown in Table 3, carbapenem (meropenem and imipenem) were used as a last resort of antimicrobial agents found to be resistant against XDR producing isolates, with efficacy rate reached 67.6% and 61.9%, respectively Meanwhile, colistin was best sensitive drug against XDR producing *P. aeruginosa* with efficacy rate that reached 100%.

Table 3. Antimicrobial susceptibilities of XDR producing *P. aeruginosa* isolates.

Antibiotic categories	Susceptible *n* (%)	Intermediate *n* (%)	Resistant *n* (%)
Extended-Spectrum Penicillins			
Piperacillin/Tazobactam	1(1.4%)	14(19.7%)	58(81.6%)
Cephalosporins			
Cefuroxime	0(0%)	0(0%)	71 (100%)
Cefoxitin	0(0%)	0(0%)	71 (100%)
Cefotaxime	0(0%)	0(0%)	71 (100%)
Ceftazidime	8(11.2%)	4(5.6%)	58(81.6%)
Cefepime	8(11.2%)	7(9.8%)	56(78.8%)
Carbapenems			
Imipenem	10(14%)	13(18.3%)	48(67.6%)
Meropenem	17(23.9%)	10(14%)	44(61.9%)
Aminoglycosides			
Tobramycin	13(18.3%)	0	58(81.6%)
Quinolones			
Ciprofloxacin	18(25.3%)	6(8.4%)	47(66.1%)

A total number of 91 *P. aeruginosa* isolates 56 (61.5%) isolates were observed to be MDR and 71 (78%) isolates were found to be XDR. All 56(100%) isolates of MDR *P. aeruginosa* were susceptible to colistin (Fig 1).

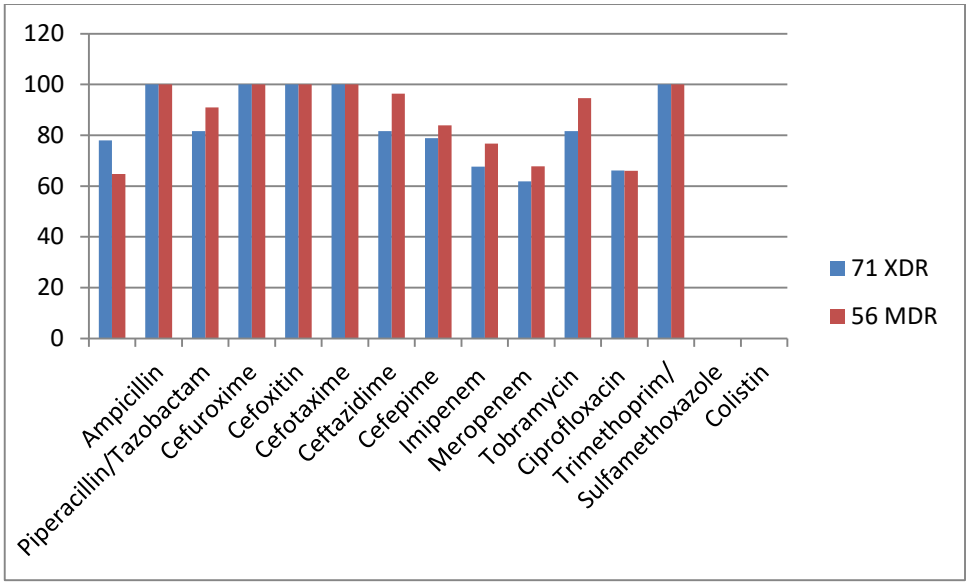

Figure 1: Antimicrobial resistance profile of 71 XDR and 56 MDR strains of *P. aeruginosa*.

Discussion:

The study determined the antimicrobial susceptibility profile and the occurrence of the XDR and MDR –producers among *P. aeruginosa* isolates. In the current study, 27 patients had burn wound infection, 19 had UTI, 16 had pus. 10 patients with bacteremia and 8 *P. aeruginosa* isolates *from* aspiration of patients who had no prior hospitalization. The highest prevalence 27(29.7%) of *P. aeruginosa* infections among burn wound patients could be due to an environmental spread of this organism in the hospitals, besides the fact that burn wounds are a perfect body site for bacterial survival and growth. These are frequently resistant to the most routinely used antimicrobial agents in burn units [14].Antimicrobial resistance among *P. aeruginosa* has increased alarmingly in the past few decades[15]. Definitions of multidrug-resistant *P. aeruginosa* against antimicrobial agents may be illuminated by the organism's relatively impermeable outer membrane, selective pressure, and environmental acquaintance to a large reservoir of resistance genes[16]. Present study followed the most common definition of (MDR) multidrug resistance, extensively drug-resistant (XDR) and pan-drug resistant (PDR) *P. aeruginosa* identified by (ECDC) European Centre for Disease Prevention and Control [17]. Presently, *P. aeruginosa* infections are treated with aminoglycosides, third and fourth generations of cephalosporins, and carbapenems, either alone or in combination and MDR, and extensively resistant *P. aeruginosa* have already emerged [18]. XDR producing isolates are capable of hydrolyzing broad-spectrum cephalosporins and carbapenems [5]. In the present study, 71(78%) of *P. aeruginosa* were XDR producers that exhibited sensitivity to only 1 group or 2 groups of antimicrobial categories. The XDR-producing isolates exhibit resistant to many other classes of frequently used antibiotics resulting in restriction of their therapeutic options[2]. A study in indicated

that previous usage of quinolones was one of the independent risk reasons for the emergence of XDR *P. aeruginosa* infection [12]. No previous study has investigated the prevalence of XDR from patients in Erbil hospitals. The incidence of MDR in *P. aeruginosa* isolates was 61.5% (56 out of 91 isolates) which is comparable to several investigations, particularly from Iran and India who identified 99 strains out of 150 (66%) of isolates as MDR and 73.1% respectively [2,5]. The phenomena of occurrence of MDR in *P. aeruginosa* have also been traced to alteration in the drug target sites, gaining of drug resistance genes, or development of newly acquired mechanisms. Our result is in agreement with the findings of other studies conducted over the world that High level of resistance by *P. aeruginosa* was shown against cephalosporin and medium level against carbapenems which was in harmony with the earlier study that stated 100% of the isolates were resistant to cephalosporin ceftazidime, cefotaxime, cefepime and 45.5% to imipenem [19]. The high level of resistance (>50 %) seen against most tested antimicrobial classes could be due to abuse or misuse of these antibiotics in our country, accessibility of these antibiotics purchase without any prescription. Colistin is the drug of choice and most effective for treatment of patients having severe infections due to XDR [20,21].

Conclusion:

Emergence of MDR and XDR strains of *P. aeruginosa* suggest continuous surveillance and improvement of plans for antimicrobial resistance control in Kurdistan, because surveillance of antibiotic resistance patterns and antibiotic use acts a vital role for giving better information in guiding the clinicians to choose appropriate therapy of infected patients as well as continuous monitoring changes in antimicrobial susceptibility over time is the best preventive and therapeutic strategies.

Conflicts of Interest: The author reports no conflicts of interest.

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Outcome of Corneal Collagen Cross-Linking In Keratoconus Patients In Erbil

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Abstract

Background and objective: Keratoconus is a disease characterized by progressive corneal thinning and associated with myopia and astigmatism, the shape of the cornea change from sphere to conical shape result in blurring of vision. Corneal collagen cross-linking will stop the progression of keratoconus

The current study aimed to report the visual, refractive and topographic outcome after Trans-epithelial (TE) or epithelial removal (ER) corneal collagen crosslinking in keratoconus patients.
Methods: A convenient sample of 45 eyes with keratoconus in different age (men and women) were included, 25 eyes underwent Trans-epithelial and 20 eyes epithelial removal corneal collagen crosslinking. Detailed ophthalmic examination done for all patients which include, Uncorrected visual acuity, best-corrected visual acuity, Spherical Equivalent, cylinder, K, central corneal thickness, topographical parameters, slit-lamp biomicroscopy finding of cornea and IOP were performed before cross-linking, first, second, and third month after crosslinking.
Results: There were significant differences in mean values between preoperative Uncorrected visual acuity (0.29±0.18) and three month postoperative (0.38±0.17) ($p< 0.003$), preoperative best-corrected visual acuity (0.56±0.25) and three month postoperative (0.65±0.19) ($p< 0.01$) in TE procedure, Preoperative UCVA (0.36±0.19) and three month postoperative (0.43±0.24) ($p< 0.001$), preoperative BCVA (0.47±0.22) three month postoperative (0.77±0.22) ($p< 0.01$) in ER procedure. There were significant differences in mean values between preoperative (42.99±1.7) and three month postoperative (42.11±1.2) in TE type of CXL regarding peripheral zone ($p<0.001$). In TE CXL keratoconus probability index significantly decreased at third month ($p<0.03$).
Conclusion: Collagen crosslinking is safe and good option for stopping the progression of keratectasia in patients with keratoconus. Improvement in UCVA and BCVA with stability in refraction and topographic parameters indicate that keratoconus did not progressed.

Introduction

The cornea is a complex structure, which is responsible for about three quarters of the optical power of the eye.[1] The average corneal diameter is 11.5 mm.[2] Central corneal thickness varies between individuals. It is 0.52 mm thick centrally on average, and thicker towards the periphery, which may reach 0.67mm. [3] The mean corneal power is 43 diopters (D) .[4] The cornea consists of the following layers, each of which is critical to normal function: The epithelium, stroma, Descemet membrane, basement membrane, endothelium**.** [4]

Keratoconus is a disorder characterized by progressive corneal steepening, most typically inferior to the center of the cornea, with eventual corneal thinning, induced myopia, and both regular and irregular astigmatism. [4] Presentation is typically during puberty with unilateral impairment of vision due to progressive myopia and astigmatism, which subsequently becomes irregular. (R)

Retinoscopy shows an irregular 'scissoring' reflex. Slit-lamp biomicroscopy shows very fine, vertical, deep stromal stress lines (Vogt striae), which disappear with pressure on the globe. Epithelial iron deposits may surround the base of the cone (Fleischer ring). Progressive corneal thinning (maximal at the apical zone) associated with poor visual acuity resulting from marked irregular myopic astigmatism with steep Keratometry readings. Bulging of the lower lid in down gaze (Munson sign). [1]

Spectacles or soft contact lenses are generally sufficient in early cases. Rigid contact lenses are required for higher degrees of astigmatism to provide a regular refracting surface. Corneal collagen cross-linking**,** using riboflavin drops to photosensitize the eye followed by exposure to ultraviolet-A light, is a newer treatment which offers promise of stabilization or reversal of ectasia in at least some patients. Keratoplasty may be necessary in patients with advanced disease, especially those with significant corneal scarring. [1] The current study aimed To identify the outcome of visual acuity, and to identify the topographic parameters after crosslinking including Keratometry K readings, keratoconus prediction index (KPI) and probability of keratoconus (KProb).

The study aimed to identify the central corneal thickness (CCT) after crosslinking, as well.