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Antimicrobial Sensitivity Patterns of *Pseudomonas aeruginosa* Isolates Obtained From Foot Ulcer Diabetes Patients in Tripoli, Libya.

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Abstract

Background: Pseudomonas aeruginosa is one of the most invasive organism that causes severe tissue damage in diabetic foot ulcers. A major problem in P. aeruginosa infection because of that it is commonly exhibits a high degree of resistance to antimicrobial agents. To improve appropriate antimicrobial therapy and reduce the incidence of antibiotics resistant bacteria, information on the antibiotic susceptibility to this bacterium is urgently needed. Therefore, the aim of this study was to isolate and determinate the antimicrobial susceptibility of the P. aeruginosa in diabetic foot ulcers patients. **Methods:** This study was carried out over the period between June 2014 to April 2015 at Tripoli Medical Center. A total of 120 bacterial isolates were cultured onto bacteriological media such as nutrient agar, MacConkey agar and blood agar. Identification of retrieved bacterial isolates was done using standard diagnostic microbiological laboratory methods and antibiogram was determined by VITEK ® 2 compact automated system. **Results:** Twenty one strains of P. aeruginosa from 120 diabetic foot ulcers were detected. P. aeruginosa isolates exhibited multidrug resistance to Ampicillin, Augmenting, Cefuroxime, Cefoxitin, Cefazolin, Ceftriaxone, Trimethoprim/sulfamethzole, Piperacillin. However, all isolates of P. aeruginosa were 100 % sensitive to Imipenem. **Conclusion:** P. aeruginosa infections of diabetic foot ulcers patients have multi-drug resistant. Imipenem is the empirical antibiotic of the choice.

Key words: Pseudomonas aeruginosa, diabetic foot ulcer, antibiotics resistance.

Introduction

Pseudomonas aeruginosa is an important human opportunistic bacterial pathogen that frequently causes severe tissue damage in diabetic foot ulcers. Often these infections are hard to treat due to the natural resistance of the species, as well as to its remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents.

Diabetic foot infections are more severe and hard to treat than infections in non-

diabetics. Many types of microbes have been implicated in the infected diabetic foot. Gram-negative infections are three-times more frequent in the diabetic than in non-diabetic individuals (1).The pathogenicity of P. aeruginosa is based on its ability to produce a variety of toxins and proteases and also on its ability to resist phagocytosis (2). P. aeruginosa is commonly resistant to antibiotics, and because of this, it is a dangerous and dreaded pathogen (3). Approximately 44% of P. aeruginosa are multi drug resistant (4). It has also an array of chromosomal and plasmid-mediated antibiotic resistance factors, making antibiotic treatment difficult and potentially unsuccessful (5). The antimicrobial susceptibility patterns of P. aeruginosa from diabetes patients with foot ulcers have rarely been documented in Libya. Therefore, the present study has been carried out to study the prevalence of P. aeruginosa and their antimicrobial susceptibility. In this study 21 P. aeruginosa strains were tested against Ampicillin/sulbactam, Amoxicillin/calvulinc acid, Imipenem, Cefoperazone, Cefuroxime, Cefotaxime, Cefepime, Cefoxitin, Cefazolin, Amikacin, Ceftriaxone, Meropenem, Trimethoprim/sulfamethzole, Gentamicin, Piperacillin, Ciprofloxacin, Ceftazidim, Tobramycin, Nitrofurantoin and Levofloxacin.

Materials and Methods

This study was carried out over the period between June 2014 to April 2015 at Tripoli Medical Center, Tripoli, Libya. The study was based on 120 pus specimens received for the screening of *P. aeruginosa* from diabetes patients with foot ulcers attending Tripoli Medical Center. The collected samples were transported immediately to the Microbiology Laboratory.

The specimens were cultured onto nutrient agar, MacConkey agar and blood agar (Oxoid, Basingstoke, and Hampshire, UK, England). Plates incubated aerobically at 37^{o}C for 24-48 hours. Primary cultures were sub cultured according to the standard procedures (6). Growth on culture plates were identified using standard diagnostic microbiological laboratory methods like Gram stain, oxidase test, catalase test and pigment production. The isolates were also detected by VITEK ® 2 compact automated system (Biomeriux, North Carolina/USA), using Gram positive GP identification card and antimicrobial susceptibility testing card AST P580, AST P586, AST-N222 and AST-GN75. Antibiogram results were expressed as susceptible, intermediate or resistant according to the criteria of the clinical laboratory standards institute (CLSI) M100-S23 (2013) (7).

Results

The present study represent 120 pus specimens collected from diabetic patients with foot ulcers. All pus samples were cultured and detected for *P. aeruginosa*.

The 21 strains of *P. aeruginosa* from 120 diabetic foot ulcers were detected and subsequently the isolates were subjected to 20 different antibiotics using standard procedures. The antimicrobial drug resistance profile of isolated *P. aeruginosa* against antibiotic agents is summarized in Table 1. The bacteria isolate resistance profile illustrated that most of *P. aeruginosa* isolates were highly resistant (80-100%) to Ampicillin, Augmentin, Cefuroxime, Cefoxitin, Cefazolin, Ceftriaxone, Trimethoprim/sulfamethzole, Piperacillin and moderate (45%) to Cefotaxime and Amoxicillin; some had low resistance (30-35%) to Meropenem and Amikacin. Interestingly, all isolates of *P. aeruginosa* were however, 100 % sensitive to Imipenem.

Discussion:

The majority of diabetic foot ulcers are superficial colonized by aerobic bacteria (8). The choice of antibiotic therapy is influenced by the sensitivity of the encountered bacterial pathogen. *P. aeruginosa* infection is potentially unsuccessful to treat with different antibiotics due to the presence of an array of chromosomal and plasmid-mediated antibiotic resistance factors. In addition to mediator activation via release of endotoxin, *P. aeruginosa* possesses a repertoire of exotoxins and enzymatic products designed to evade host defences (9).In this study isolated of *P. aeruginosa* were showed 100 % sensitive to Imipenem. This result might be due to the strong antibiotic activities of Imipenem against many species, including *P. aeruginosa* (10,11). Due to the high resistance of *P. aeruginosa* to levofloxacin the wound; which makes the treatment of this multidrug resistant pathogen both difficult and expensive. The outcome of this research is somehow expected and compatible with other recent researches carried out elsewhere (15,16,17); which in this study, a combination therapy of levofloxacin with gentamicin is recommended for the treatment of pneumonia caused by *P. aeruginosa* (12). *P. aeruginosa* showed no effect of Ampicillin in susceptibility testing in this study. Therefore Ampicillin is not recommended as a monotherapy for the treatment *Pseudomonas*. Similar to other studies, ceftazidime and Amikacin showed some affectivity for the treatment of *P. aeruginosa* (13,14).Patients with uncontrolled diabetes often develop diabetic complication such as foot ulcers; which constitute a major public health problem worldwide. Unfortunately, in most medical clinics in Libya, General Practitioners and Clinicians usually treat clinically infected diabetic foot ulcers without performing antibiotic sensitivity tests for the causative agent cultures of concluded that clinically infected diabetic foot ulcers that treated with antibiotics have predominant mono-antimicrobial and multi drug-resistant infection and indicated that imipenem was among the most effective antibacterial agent.

In conclusion,

diabetic foot ulcer infections is common among diabetic patients in Libya, and many studies worldwide indicated that the majority of isolates were multi-drug resistant. However, the results concluded in this study will be beneficial for future

determinations of empirical therapy policies for the management of diabetic foot ulcers. This study highlight that Imipenem and amikacin, Gentamicin could be used for initial therapy for *P. aeruginosa* mediated foot ulcers infections.

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Antibiotics	*P. aeruginosa* resistance %
AMS	100
AMC	93
IMP	0
CFZ	71
CXM	90
CTX	44
FEP	67
FOX	92
CZ	80
AK	33
CRO	79
MEM	38
SXT	87
CN	27
PIP	83
CIP	24
CAZ	38
TOB	50
F	71
LEV	83

Table1 Antibiogram Pattern of *Pseudomonas aeruginosa* isolated among diabetes patients with foot ulcers.

AMS: Ampicillin/sulbactam;AMC:Amoxicillin/calvulinc acid;IMP: Imipenem; CFZ: Cefoperazone; CXM: Cefuroxime; CTX: Cefotaxime;FEP: Cefepime; FOX: Cefoxitin; CZ: Cefazolin; AK: Amikacin; CRO: Ceftriaxone; MEM: Meropenem; SXT: Trimethoprim/sulfamethzole; CN: Gentamicin; PIP: Piperacillin; CIP: Ciprofloxacin; CAZ: Ceftazidim; TOB: Tobramycin; F: Nitrofurantoin; LEV: Levofloxacin.