PSEUDOMONAS AERUGINOSA, AN EMERGING PATHOGEN AMONG BURN PATIENTS IN KURDISTAN PROVINCE, IRAN

Enayat Kalantar[1], Shadi Taherzadeh[2], Tayeb Ghadimi[3], Fariborz Soheili[4], Heiman Salimizand[5] and Alireza Hedayatnejad[6]

[1]Department of Microbiology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj; [2]Department of Microbiology, Azad University, Zanjan Brach, Zanjan; [3]Kurdistan University of Medical Sciences, Sanandaj; [4]School of Medicine, Kurdistan University of Medical Sciences, Sanandaj; [5]School of Medicine, Mazandaran University of Medical Sciences, Iran

Abstract. This study was conducted to determine the incidence of *Pseudomonas aeruginosa* infections among burn patients at Tohid Hospital, Iran. A total of 176 clinical specimens were obtained from 145 burn patients admitted to the burn unit of Tohid Hospital to detect the presence of *P. aeruginosa*. Antimicrobial susceptibility testing was conducted to detect extended spectrum beta-lactamase (ESBL) producing *P. aeruginiosa* using Clinical and Laboratory Standards Institute guidelines with the double disc synergy test (DDST). A polymerase chain reaction was used to detect PER-1 and OXA-10 among the isolates. The mean age, total body surface area and length of hospital stay among patients were 29 years, 37.7%, and 10 days, respectively. Kerosene was the commonest cause of burn (60%), followed by gas (30%). During the study, *P. aeruginosa* was detected in 100 isolates. The antibiotics they were most commonly resistant to were cefotaxime, ceftriaxone and ciprofloxacin. Of the 100 *P. aeroginusa* isolates, 28% were positive for ESBL production with the DDST, 48% and 52% were PER-1 and OXA-10 producers, respectively. The high frequency of PER-l and OXA-10 producers at this hospital is of concern considering their potential spread among burn patients.

Keywords: *P. aeruginosa,* burn patients, ESBL

INTRODUCTION

Burns are serious injuries often complicated by colonization with bacteria, particularly *Pseudomonas aeruginosa*. Bacterial colonization of wounds is a major concern in the treatment of burn victims. The spread and systemic invasion of pathogens introduced through burn wounds is the primary cause of severe complications and death (Pruitt *et al*, 1998; Orenstein *et al*, 2006). It is estimated 75% of deaths following burn injuries are due to infection (Lessa, 2005). Burn injuries in Iran, like other developing countries,

Correspondence: Dr Kalantar Enayat, Department of Microbiology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.
Tel: 0098 871 6131415; Fax: 0098 871 6664654
E-mail: kalantar_enayat@yahoo.com
Department of Pathobiology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran.

are more common than in the USA and Europe (Atiyeh, 2009).

Patient factors, such as age, extent of injury, and depth of burn, along with microbial factors, such as type and number of organisms, enzyme and toxin production, and motility determine the likelihood of invasive infection of burns. Mortality increases with severity of burn injury and increasing age of the patient (Gamer and Magee, 2005; Brusselaers *et al*, 2010).

Several bacterial species are commonly encountered in burns: *Staphylococcus aureus* is the most common gram-positive pathogen and *P. aeruginosa* is the commonest gram-negative species (Batra, 2003; Orenstein *et al*, 2006).

P. aeruginosa develops antimicrobial resistance rapidly, which complicates medical treatment of infections. *Pseudomonas aeruginosa* is frequently isolated from patients and hospital environments and has been implicated as the cause of nosocomial infections in burn patients (Qarah *et al*, 2008).

The purpose of this study was to evaluate the prevalence of extended spectrum beta-lactamase (ESBL) producing *P. aeruginosa* strains isolated from burn patients admitted to Tohid Hospital, Kurdistan Province, Iran, a referral hospital.

MATERIALS AND METHODS

Between April 2009 and April 2010, a total of 176 clinical specimens were collected from burn patients at the burn unit of Tohid Hospital to determine the incidence of *P. aeruginosa* based on biochemical tests (Pat *et al*, 2007).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using the disc diffusion Kirby Bauer method (Bauer *et al*, 1966) on Muller-Hinton agar (Merck, Darmstadt, Germany). The antibiotics tested were: carbenicillin (CB), ciprofloxacin (CIP), ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO), gentamicin (GM) and piperacillin (PIP).

Detection of extended spectrum beta-lactamases (ESBLs)

Detection of ESBL producing *P. aeruginiosa* strains was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2006). Briefly, we used cefepime and cefepime/clavulanic acid, ceftazidime and ceftazidime/clavulanic acid and cefotaxime and cefotaxime/clavulanic acid disks (MAST Diagnostic, Merseyside, UK). After inoculating isolates on Muller-Hinton agar (Merck, Darmstadt, Germany) they were incubated for 24 hours. Clear zones for compound disks ≥5 mm compared to single disks were considered to be producing ESBLs.

Genotypic detection of PER-1 and OXA-10 by polymerase chain reaction (PCR)

Polymerase chain reaction was used to detect PER-1 and OXA-10 among isolates which had a positive ESBL confirmatory test. In this procedure, DNA was extracted by the boiling method (Yan *et al,* 2006) and used as a template for PER-1 and OXA-10 primers. The primers (5′-TAT CGC GTG TCT TTC GAG TA- 3′) were used as a forward primer and (5′-TTA GCC ACC AAT GAT GCC C-3′) was used as a reverse primer for blaOXA-10 and for blaPER-1 (5′-ATG AAT GTC ATT ATA AAA GCT- 3′) was used as a forward primer and (5′-TTA ATT TGG GCT TAG GG-3′) was used as a reverse primer. The DNA amplification was carried out as follows: initial denaturation (94ºC, 5 minutes, 31 cycles) (94ºC, 45 seconds),

annealing (58ºC for OXA-10 and 45ºC for PER-1), extension (72ºC, 30 seconds) and a single final extension (7 minutes at 72ºC). The reactions were carried out in 0.2 ml PCR tubes (Bioneer, AccuPower PCR preMix tubes, 20 µl reaction). The PCR results were electrophoresed on 0.8% (w/v) agarose gel (Sigma, St Louis, MO).

RESULTS

During the study period 145 burn patients were admitted to the burn unit at Tohid Hospital. Their mean age was 29 years old (range: 4-74 years). The age distribution and data regarding injury and outcomes are shown in Table 1. The mean percent of total body surface area (TBSA) was 37.7% (range: 5-95%).

Kerosene was the commonest cause of burn (60%), followed by gas (30%). The mean length of hospital stay was 10 days (range: 4-18 days).

Of 176 clinical specimens obtained, 100 were positive for *P. aeruginosa.* Table 2 shows the antibiotic resistance patterns of the *P. aeroginusa* isolates. The antibiotics these bacteria were most commonly resistant to were cefotaxime, ceftriaxone and ciprofloxacin.

Of the 100 *P. aeroginosa* isolates, 28% were positive for ESBL production using the double-disc synergytest (DDST). Out of the 100 *P. aeruginosa* isolates, 48 (48%) and 52 (52%) were PER-1 and OXA-10 producers, respectively (Fig 1).

Table 1
Demographics of burn patients at Tohid Hospital, Iran.

	Number	%
Sex		
Male	68	68
Female	77	77
Age (years)		
<20	46	46
20-29	37	37
30-39	28	28
≥40	34	34
Etiology of burn		
Kerosene	87	60
Gas	43	30
Electricity	6	4

Table 2
Antibiotics resistance patterns among *P. aeruginosa* isolates from burn patients at Tohid Hospital, Iran.

Antibiotic	Number	%
Carbenicilin (CB)	10	19.2
Ciprofloxacin (CIP)	23	43.0
Ceftazidime (CAZ)	15	28.8
Cefotaxime (CTX)	27	50.0
Ceftriaxone (CRO)	22	43.3
Piperacillin (PIP)	15	28.8
Gentamicin (GM)	13	25.0

DISCUSSION

P. aeruginosa is an important cause of infections in humans; many isolates are resistant to commonly used antibiotics (Giamarellou, 2002, Naiemi *et al,* 2006). Early identification of infections due to this organism is important and may reduced morbidity and mortality among hospitalized patients.

P. aeruginosa resistance has been documented to be due to various factors, particularly ESBL (Ullah *et al,* 2009); we screened isolates for ESBL production by DDST.

The results of this study show contamination of burn wounds is almost the

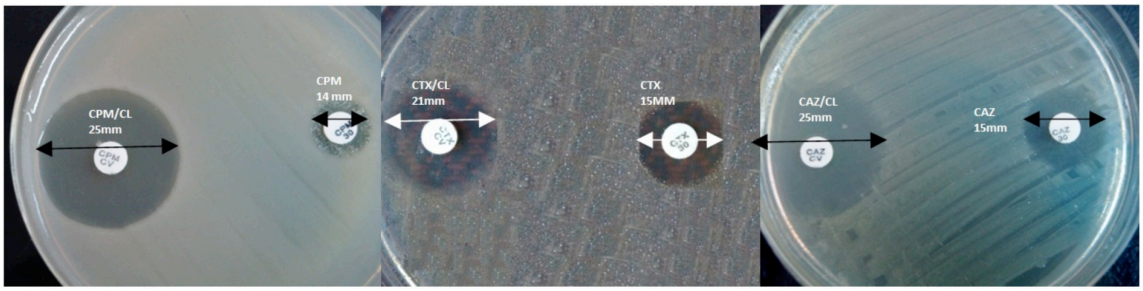

Fig 1–Phenotypic detection of ESBL by DDST among *P. aeruginosa* isolates among burn patients at Tohid Hospital, Iran.

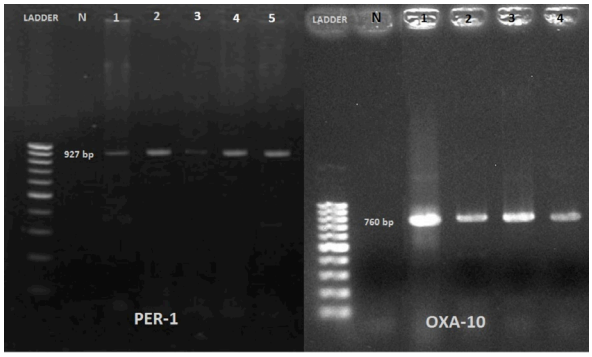

Fig 2–PCR products of PER-1 (left) and OXA-10 (right) producing strains of *P. aeruginosa* isolates.

rule rather than an exception in major burns. Combating infection in burns must remain a priority. *P. aeruginosa* is a "classic pathogen" among burn patients in the referral burn unit in Kurdistan. Similar findings have been reported at a referral burn center in Fars Province, southwestern Iran (Rastegar *et al*, 2002).

Multi-drug resistance by *P. aeruginosa* is a major problem and has been reported in other studies from Iran (Estahbanati *et al*, 2002; Rastegar *et al*, 2005). In this study, the percents of *P. aeruginosa* resistant to cefotaxime, ceftriaxone and ciprofloxacin were 50, 43.3 and 43%, respectively. These resistance rates are lower than other reports from Iran (Estabanati *et al*, 2002; Rastegar, 2005).

Testing for ESBL production using a CLSI phenotypic confirmatory test, and conventional DDST for ceftazidime, cefotaxime, revealed 28 isolates were positive.

The frequency of ESBL-producing strains with amplified $bla_{\text{OXA-10}}$, and $bla_{\text{PER-1}}$ were 52% and 48%, respectively, similar to the rates of other studies carried out in Iran (Weldhagen *et al*, 2003; Mirsalehian *et al*, 2010). Numerous studies have investigated the occurrence of ESBL production among *P. aeruginosa* isolates with varying results (Vahabbolu, 1998; Weldhagen *et al*, 2003; Ben-Hamouda *et al*, 2004). A previous study in Iran found PER-1 and OXA-10 in 49.3% and 74.6%, respectively, of *P. aeruginosa* isolates (Mirsalehian, 2010). Studies from Turkey found PER-1 in 11-23.7% of *P. aeruginosa* isolates and OXA-10-type β-lactamases in 5.3-17% of *P. aeruginosa* isolates (Vahaboglu *et al*, 1997).

In summary, *P. aeruginosa* is the main source of infection at the referral burn center at Tohid Hospital, Iran. Isolates

of *P. aeruginosa* producing PER and OXA β-lactamases were encountered frequently in this hospital. Their high prevalence indicates a considerable risk for spread among patients.

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