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Prevalence of MDR *Pseudomonas aeruginosa* of war-related wound and burn ward infections from some conflict areas of Western Yemen

Mahfouz Nasser[a], Mahmoud Ogali[b], Arun S. Kharat[a,c,*]

[a] Department of Biotechnology, Dr. Babasaheb Ambedkar Marathwada University, Subcampus Osmanabad, 413 528, MS, India
[b] Medical Science College, University of Hodiedah, Yemen
[c] School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

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ABSTRACT

Background and objectives: The antibiotic resistant *Pseudomonas aeruginosa* affecting war-related wound injuries is a serious problem with regional and global implications, which requires antibiotic susceptibility testing on a regular basis. The primary aim of this study was to determine the prevalence for multi-drug resistance *Pseudomonas aeruginosa* of war-related wound infections from conflict areas in Hodiedah, Western Yemen.
Methods and materials: A study was undertaken with ninety-nine wound swabs from war-related wound infections patients those were hospitalized to Wound Burn Centre at Organization The General AL-Thawra Hospital, Hodiedah Western Yemen. The isolates were cultured and identified by standard microbiological techniques and with the use of Kirby- Bauer disc diffusion antibiotic susceptibility testing was done.
Results: Out of 99 swab isolates 46 were *P. aeruginosa* (46.46%). Highest incidence of 63.1% was found in the age group of 20–40 years. Isolates exhibited resistance to aminoglycosides; Gentamycin (87%), Tobramycin (78.2%) and Amikacin (82.6%). Curiously, 65.2% *P. aeruginosa* isolates were multidrug resistance (MDR).
Conclusion: This is the first report documenting enhanced MDR *P. aeruginosa* from war-related wound infections from Yemen. The sampling, timing of medical intervention plays crucial role in preventing nosocomial transmission of MDR *P. aeruginosa* strains.

1. Introduction

The Middle East region is frequently associated with wars conflicts and military conflicts of which the recent Arab uprisings are good examples [1]. Yemen is an Arab country plagued with repeated armed conflicts that affect both civilians and soldiers. Injuries sustained during a war are common and frequently associated with multiple life-threatening complications. In Yemen current Arab uprisings occurred during February 2011, followed by Yemen war in March 2015. As of 2016, October 10th, at least 4125 civilians had been killed and 7207 wounded since the start of the campaign, the majority by coalition airstrikes, according to the United Nations Office of the High Commissioner for Human Rights (OHCHR) [2]. United Nations Office of the High Commissioner for Human Rights(OHCHR)(2017), Zeid R. al-Hussein UNH Commissioner (https://www.hrw.org/world-report/2017/country-chapters/yemen). There is a strong association between military conflicts and bacterial infections. Infectious complications in those that do survive, however, are a major concern, cause nosocomial transmission and may emerge multidrug-resistance infection. Wherever man, warfare, and organisms coincide, wound infection is apparent. Wound infection is defined as the invasion and multiplication of microorganisms in a wound resulting in tissue injury. In 1942 during world war 2 was notable for the emergence of penicillin use on the battlefield, an act that changed the management of combat wounds [3]. The mortality rate from lower extremity wounds decreased from 7.7% during WW1 to 2.1% during WW2, in part owing to the use of penicillin. Tong (1972) provides a temporal analysis from microbiological samples taken at initial debridement, and at days three and five post-injury [4]. An initially mixed picture of the growth of Gram-positive and Gram-negative organisms was seen, with *Pseudomonas* species being the most frequent isolate identified in the later swabs. All those with evidence of bacteremia had strains reported to be resistant to antibiotics. These findings of mixed wound flora, an ultimate predomination by *Pseudomonas* sp. and emerging drug resistance corroborated previous anecdotal reports from wounds in injured servicemen [5]. Wounds analyzed during evacuation from Vietnam to Japanese Base Hospitals showed the presence of *Pseudomonas* sp., but in lesser than *Staphylococcus aureus* [6]. All organisms showed increased resistance to nearly all antibiotics against which they were tested [7]. The microbial aetiology of combat-related injuries differs between various stages of management. Bacterial

* Corresponding author at: School of Life Sciences, Jawaharlal Nehru University, Near Mehrauli Road, New Delhi 110 67, India.
E-mail address: askharat@mail.jnu.ac.in (A.S. Kharat).

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transition occurs over time, from in the early stages an even balance of Gram-positive and Gram-negative flora, to the later predominance of antibiotic resistant Gram-negative species during treatment. The *P. aeruginosa* is an opportunistic gram-negative bacillus, non-spore forming, measuring 0.5–0.8 μm by 1.5–3.0 μm. All strains are motile by methods for a single polar flagellum frequently isolated from hospitalized patients [8]. *P. aeruginosa* rarely causes infections in healthy individuals however it is one of the leading causes of hospital-acquired (nosocomial) infections; it can cause severe infections in immunocompromized hosts [9]. and patients with extreme burn wounds [10]. The ability of *P. aeruginosa* to survive under various ecological conditions, combined with its inherent resistance to several antimicrobials, permits it to colonize and multiply inside the burned tissues. Wound infection is one of the significant medical issues on the planet since it postures genuine and unfortunate confusions that outcome in many could be fatal [11]. Wound infections are significant consequences of these war injuries. The bacteria of war-related wound infections are variable with a predominance of Gram-negative bacteria in particular *P. aeruginosa*. Antimicrobial agents that prevent microscopic organisms from growing are bacteriostatic and bactericidal [12]. Antimicrobial agents can either be natural products or synthetic chemical items, designed to destruct microbial cells' selectively. They particularly meddle with the biochemical processes of microorganisms, and thus they can be securely utilized as a part of mammalian hosts [13]. The occurrence of multidrug-resistant (MDR) *P. aeruginosa* strains is on rise worldwide and restricting effective alternatives or posing threats while managing treatment. An MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories; carbapenems, fluoroquinolones, penicillins/cephalosporins, and aminoglycosides [14]. The emergence of antimicrobial resistance among isolates affecting war- related wound injuries is a serious problem with major regional and global implications.

The purpose of current study was to determine the prevalence MDR *P. aeruginosa* of war-related wound infections from conflict areas in western Yemen. The present study was designed to determine the effect of activities of 10 commonly prescribed antimicrobial agents which were used to treat *P. aeruginosa* infections in wound and burn ward patients. It is important to prevent the over-zealous use of broad-spectrum antimicrobials with the risk of development of MDR organisms.

2. Materials and methods

Over a period of four months, January to April 2017 present study was undertaken with ninety-nine samples (wound/burn swabs); 10 Female and 89 Male which were taken from war-related wound infections patients who were admitted to Wound Burn Centre at Organization The General AL-Thawra Hospital, Hodiedah western Yemen. Patients who were included within an age range of 6–66 years. All clinical isolates were collected from patient wound and Burn infection streaked on Pseudomonas Isolation Agar (PIA), Mac Conkey's Agar, and Blood Agar, (Himedia), plates were incubated overnight at 37 °C, Then the *P. aeruginosa* isolates streaked on Mueller-Hinton agar (Himedia)for susceptibility test, and were further characterized to confirm *P. aeruginosa* identity.

2.1. Sample processing

The samples were cultured on Pseudomonas Isolation Agar (PIA), MacConkey's Agar, and Blood Agar and the plates were incubated overnight at 37 °C. *P. aeruginosa* was identified by its colony characteristics, pyocyanin pigment production, grape-like odor, oxidase positivity, motility, Gram staining, ability of reducing nitrates to nitrites, isolated were processed and identified by standard microbiological techniques [15].

Table 1
Distribution of 46 *P. aeruginosa* strains as per age isolated from the war-related wound infections patients.

Sr. No.	Age (Years)	No. of *P. aeruginosa* strains	Percentage%
01	> 0–20	9	19.6
02	> 20–40	29	63.1
03	> 40–60	6	13
04	> 60	2	4.3

2.2. Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed on all 46 isolates of *P. aeruginosa* by using Kirby-Bauer Disc Diffusion method on Mueller-Hinton agar, by following CLSI 2011 Guidelines [16] and by using Himedia antibiotic discs. About10 antibiotics were tested, which included aminoglycosides; Gentamin (10 μg), Tobramycin(10 μg) and Amikacin(10 μg), β-lactams; Ceftazidime(30 μg), Cefepime(10 μg) and Aztreonam(30 μg), piperacillin-tazobactam(100/10 μg), Ciprofloxacin (5 μg) and carbapenems; Imipenem(10 μg), Meropenem(10 μg). In this study, MDR *P. aeruginosa* was detected as a bacterium which was resistant to three or more anti-Pseudomonal antimicrobial categories [14].

3. Results

Microbial characterization of 99 bacterial isolated obtained from the war-related wound infections and burn wards indicated that 44 isolates (46.46%) were *P. aeruginosa.* Two out of 46 isolates (4.3%) were recovered from Female patient while remaining 44 isolates (95.7%) were recovered from Male. It can be seen from the data in Table 1 that the mean age of the war-related wound infections patients who were included in present study was 33.5 years. The incidence rate of *P. aeruginosa* was highest (63.1%) in the age group of 20–40 years. Antibiogram results have been described in Table 2. It is evident from the Table 2 that 87% isolates exhibited resistance to gentamycin (10 μg), 78.2% Tobramycin (10 μg) and 82.6% Amikacin (10 μg) aminoglycosides. Resistance exhibited to β-lactams by these isolates was; 76.1 Ceftazidime (30 μg), 71.7 Cefepime (10 μg), 58.7% were Aztreonam (30 μg), 52.2% were resistant to piperacillin-tazobactam (100/10 μg), while 65.2% were resistant to a fluoroquinolone antibiotic Ciprofloxacin (5 μg). Resistance exhibited to carbapenms by the *P. aeruginosa* was 21.7 and 17.5% respectively to Imipenem (10 μg) and Meropenem (10 μg). In this study MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories; carbapenems, fluoroquinolones, penicillins/cephalosporins, and aminoglycosides as described (Magiorakos et al., in 2012) [14]. According to this definition, 30 (65.2%) of the 46 *P. aeruginosa* isolates showed resistance to at least three antimicrobial agents and are qualified to be MDR strains.

4. Discussion

The present study was designed to determine the prevalence MDR *P. aeruginosa* of War-Related Wound Infections from conflict areas In Western Yemen. Prevalence of *P. aeruginosa* was documented in a few reports from middle eastern countries including Yemen however this is the first report documenting prevalence of MDR *P. aeruginosa* of war-related wound infections from the Yemen. The results presented in this manuscript isolated 46 *P. aeruginosa* isolates from total 99 samples, Hodiedah Western Yemen. This study reports the highest existence of MDR *P. aeruginosa*; 30 out of 46 isolates i.e. 65.2%. Among the β-lactams, *P. aeruginosa* showed highest resistance to ceftazidime (76.1%) and sensitivity to other β-lactams i.e., piperacillin+ tazobactam (43.5%), imipenem (65.2%) and meropenem (71.7%), Table 2. These

Table 2
Antibiotic susceptibility results of 46 clinical isolates of *P. aeruginosa* from the war-related wound infections patients.

Sr. No.	Categories.	Antimicrobial	Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
01	Aminoglycosides	Gentamicin	40 (87)	2 (4.3)	4 (8.7)
02		Tobramycin	36 (78.2)	3 (6.6)	7 (15.2)
03		Amikacin	38 (82.6)	3 (6.6)	5 (10.8)
04	Carbapenems	Imipenem	10 (21.7)	6 (13.1)	30 (65.2)
05		Meropenem	8 (17.5)	5 (10.8)	33 (71.7)
06	Cephalosporins	Ceftazidime	35 (76.1)	4 (8.7)	7 (15.2)
07		Cefepime	33 (71.7)	6 (13.1)	7 (15.2)
08	Fluoroquinolone	Ciprofloxacin	30 (65.2)	5 (10.8)	11 (24)
09	Penicillins	piperacillin-tazobactam	24 (52.2)	2 (4.3)	20 (43.5)
10	Monobactams	Aztreonam	27 (58.7)	10 (21.7)	9 (19.6)

Table 3
Comparative account of war-related wound infection *P. aeruginosa* prevalence from Western Yemen to that of Middle Yemen and Eastern Yemen.

Sr. No.	*P. aeruginosa* Prevalence	In%
01	Western Yemen—This study	46.46
02	Middle Yemen	25
03	East Yemen	30

Table 4
Percent Prevalence MDR *P. aeruginosa* strains of Wound Infections from a few countries.

Country	Present study	Yemen	India	Pakistan	Egypt	Saudi Arabia
MDR *P.aeruginosa*	65.2%	42.3%	76.8%	22.8%	56%	10.7%

results are in agreement with those of previous studies carried out on war-related wound infections of *P. aeruginosa*, in Syria was23%, Iraq was 34% and Lebanon 30.5% [17–19].

Our results were found to be consistent with earlier reports on war-non-related wound infections in Yemen, the percent prevalence of *P. aeruginosa* war-non-related wound infections in Hadrmout City (East Yemen) was 30% [20], while in the Ibb City (Middle Yemen) it was 25% [21], see Table 3.

In the present study, MDR *P. aeruginosa* rate was determined to be 65.2% (30/46). A study was done by Bhatt et al. (2015) in India it was 67.8% [22]. Significantly low MDR *P. aeruginosa* were reported in Pakistan, 22.8% [23]. Earlier report by Al-amari et al. (2015) from Yemen stated that MDR *P. aeruginosa* were 42.3% [20]. The MDR percent prevalence for *P. aeruginosa* was reported by Hassuna et al. (2015) in Egypt was56% [24]. Curiously studies conducted by Khan & Faizb (2016) reported that in Saudi Arabia lowest MDR *P. aeruginosa* did exist, which were only10.7% [25], shown in Table 4.

The high rate of *P. aeruginosa* from war-related wound infections in this study may be attributed to the ability of *P. aeruginosa* to colonize in damaged skin due to loss/impairment of the first defense barrier against bacteria [26]. Also, the high rate in this study might also be due to the fact that *P. aeruginosa* has the ability to with stand tough environment and rather make use of vast range of substrates. More over increase in MDR *P. aeruginosa* may also include timing and type of surgical management, extensive use of antimicrobial drugs, and the presence of metallic or organic fragments in the wound. Nosocomial transmission is the most important factor in the spread of MDR *P. aeruginosa*. This increase in MDR *P. aeruginosa* has also translated to novel Resistance at the Molecular level, which needs to be dissected in near future.

5. Conclusion

Important conclusions drawn from this work include increase in the rate of MDR *P. aeruginosa.* Future research should therefore concentrate on the investigation of Molecular characterization of MDR *P. aeruginosas.*

Conflict of interest

Authors have conflict of interest to declare.

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