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Alarming Prevalence of Nosocomial *Pseudomonas aeruginosa* among Patients with Infected Burn Wounds

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ABSTRACT

Recently, the prevalence of nosocomial Pseudomonas aeruginosa has become a concern due to its involvement in increased morbidity and mortality, especially those highly virulent strains associated with burn wounds in burn centers. Therefore, this cross-sectional study aimed to isolate and determine the prevalence of nosocomial Pseudomonas aeruginosa among burned inpatients. This study was carried out between September 2023 and March 2024. A total of 355 pus, purulent fluid, and necrotic tissue swabs were obtained from burn victims with burns at the Burn Centers in Najaf City, Iraq. 85/355 (23.9%) of isolates under study were detected as Pseudomonas aeruginosa based on laboratory methods (microscopic, biochemical and culture). All test isolates appeared to grow optimally on cetrimide and chromium agar media, and colonies appeared green on these media when examined by the naked eye and greenish fluorescence under ultraviolet light. However, colonies on blood agar were bright and metallic, and the most common type of lysis was β-hemolysin followed by α-hemolysin. On Nutrient agar, most of the isolates were characterized by positive multi-pigment production 57/85 (67.1%), while 28/85 (32.9%) of the isolates were negative pigment production.

Keywords: Prevalence, *Pseudomonas aeruginosa*, Nosocomial infection, Burn wound.

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1. INTRUDUCTION

Burns are a problematic situation linked with a significant risk of infections globally due to the impaired immune functions of the skin, post-thermal injury inflammatory responses, and prolonged hospital stays [1]. *Pseudomonas aeruginosa* is typically described as ubiquitous, Gram-negative, opportunistic, and commonly related to hospital-acquired infections [2]. The moist environment of burn wounds provides an ideal environment for bacterial growth and colonization, producing the infection and translocation through the vasculature into the bloodstream, resulting in un control propagation and spread of *P. aeruginosa*, leading to bacteremia, which is primarily the consequence of impaired body immunity caused by thermal injuries [3]. As well as *P. aeruginosa* has an arsenal of

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virulence factors that contribute to production pathogenicity, for instance, biofilm formation, efflux pumps, and the production of β-lactamases enzymes resulting in severe acute and chronic infections among thermal injury victims [4]. Recently, the increase in the emergence of newly emerged multidrug resistance strains has become a significant problem worldwide, and in Iraq, resulting in a decrease in treatment options has made effective treatment challenging [5]. Despite the continuous improvements in health care services in burn wards, *P. aeruginosa* remained most prevalent pathogen isolated from burn wound infections resulting in sepsis and death if not treated with an appropriate antimicrobial agent; in this regard, it is responsible for 75% of mortality among burn victims worldwide [6].

Moreover, several vital determinants must be taken into consideration when selecting appropriate treatment and management of Gram-negative infections, including *P. aeruginosa*, particularly nosocomial infections: the typical isolation and identification of species suspected of causing the infections, the identification of some specific characteristics of these isolates, and previous knowledge of their prevalence [7]. Therefore, comprehensive studies are needed to highlight the isolation, identification, and detection of outbreaks of *P. aeruginosa* among burn inpatients to identify better the epidemiology of these isolates in burn units [8]. This study aimed to isolate, identify, and detect the prevalence of nosocomial *P. aeruginosa* among inpatients in the Burn Center in Najaf City, Iraq.

2. MATERIALS AND METHODS

2.1. Bacterial Isolation and Study Design

The period time of the cross-sectional study is extended from September 2023 to March 2024. A total of 355 clinical specimens were obtained from burn victims with infected burn that were recumbent in the Burn Center at Najaf City, Iraq. Swabs were taken from the suspected infected burnt skin areas and immersed in Tryptic Soy agar tubes (Himedia, India), and each swab collected from patients was immediately to the Microbiology laboratory for further investigation, detection, and preservation [9].

2.2. Bacterial Identification

2.2.1. Microscopic Examination

Gram stain was applied to all previously prepared smears from test isolates, and the staining reaction, as well as the arrangement, shape, and size of bacterial cells, were assessed using an oil immersion lens on a light microscope adjusted at 100X [10].

2.2.2. Macroscopic Identification

All swab specimens were subsequently cultured on a variety of media, including Nutrient agar (NA), Blood agar (BA) and Cetrimide agar (CA), MacConkey agar (MAC) (Oxoid, UK), and incubated at 37^{o}C for 24 hours,

several times purified isolates under study until purified isolates were obtained. According to MacFaddin [9], the detection of the isolates depends on general Morphology colony characteristics including edge, size, shape, color, pigment production, hemolysis type. Suspected *Pseudomonas* colonies were also inoculated into CA and then incubated aerobically for overnight at 37 °C. The growing colonies were examined under UV light. Colonies that were fluorescent under UV were recorded as *P. aeruginosa.*

2.2.3. Biochemical Identification

According to MacFaddin [9]. The detection of test isolates was carried out using series of standard biochemical tests including oxidase test, catalase test, hemolysin test, growth at 42°C, and Motility test.

3.RESULTS

3.1. Bacterial Distribution

Among 355 specimens collected from burn wound infections of burn victims, this study observed that 32/355 (9%) specimens did not produce any bacterial growth on any of the routine culture media. While the bacterial growth percentage was 323/355 (91%) distributed as follows: 250/323 (77.4%) of isolates were Gram-negative, and 73/323 (22.6%) of isolates were Gram-positive. Depending on microscopic, cultural, and biochemical characteristics, 85/355 (23.9%) of isolates were identified as *P. aeruginosa* (Figure 1).

3.2. Macroscopic and Biochemical Identification of Pseudomonas aeruginosa.

The morphological characteristics of *P. aeruginosa* colonies under the study on MAC agar appeared to be non-lactose fermenters, prominent, opaque, and flat. This study observed that a total of 85/355 (23.9%) of the isolates appeared to grow ideally on CA agar, which showed fluorescent colonies under UV light. In addition, *P. aeruginosa* isolates tested on NA agar showed that the colonies were smooth, transparent, large, convex, low, and 2-4 mm in diameter with irregular edges (Figure 2). While on BA agar the colonies exhibit swarming, a metallic sheen and as well as *β*-hemolytic producer *P. aeruginosa* isolates were the most frequent (Figure 3). Moreover, this study observed that 57/85 (67.1 %) of *P. aeruginosa* isolates were pigment producers, while 28/85 (32.9%) of isolates were not producers (Figure 4). And Some of cultural and biochemical tests of *P. aeruginosa* were listed in Table (1).

3.4. Demographics of Burn Patients Infected with Pseudomonas aeruginosa

This study revealed that the largest percentage of local *P. aeruginosa* was isolated from burned inpatients in the age group 60-70 years: 11.6% female and 10.6% male, followed by the percentage in the age group 50-60 years: 10.5% female and 7.1% male; while the lowest infection percentage was in the age group 10-20 years: 3.5% female and 2.3% male (Figure 5).

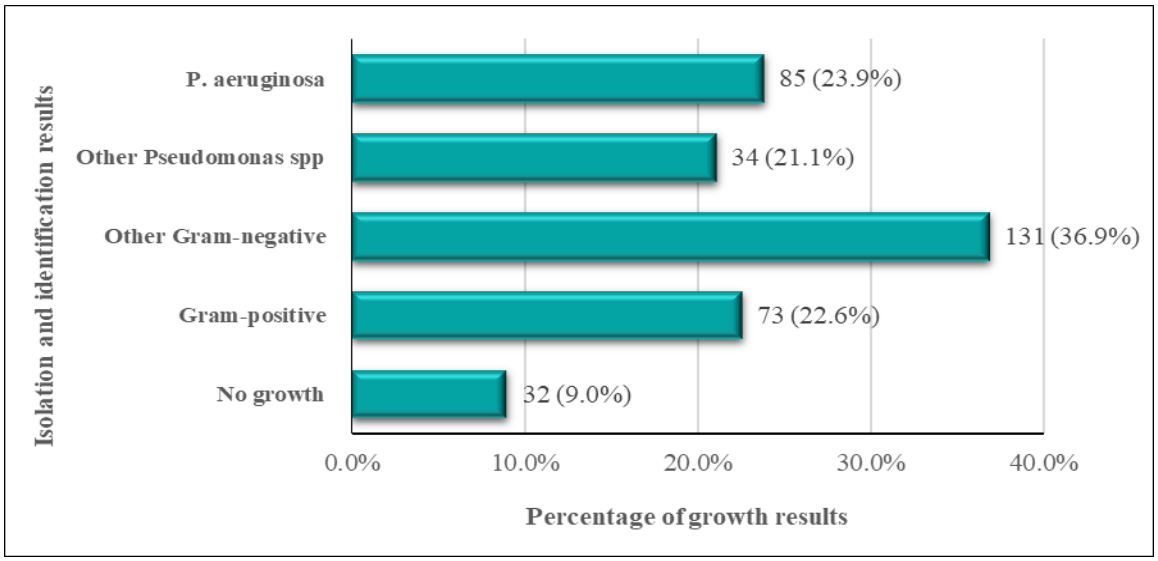

Fig 1: Bacterial distribution of clinical specimens collected from burn wound infections of 355 patients at Burn Center in Najaf City.

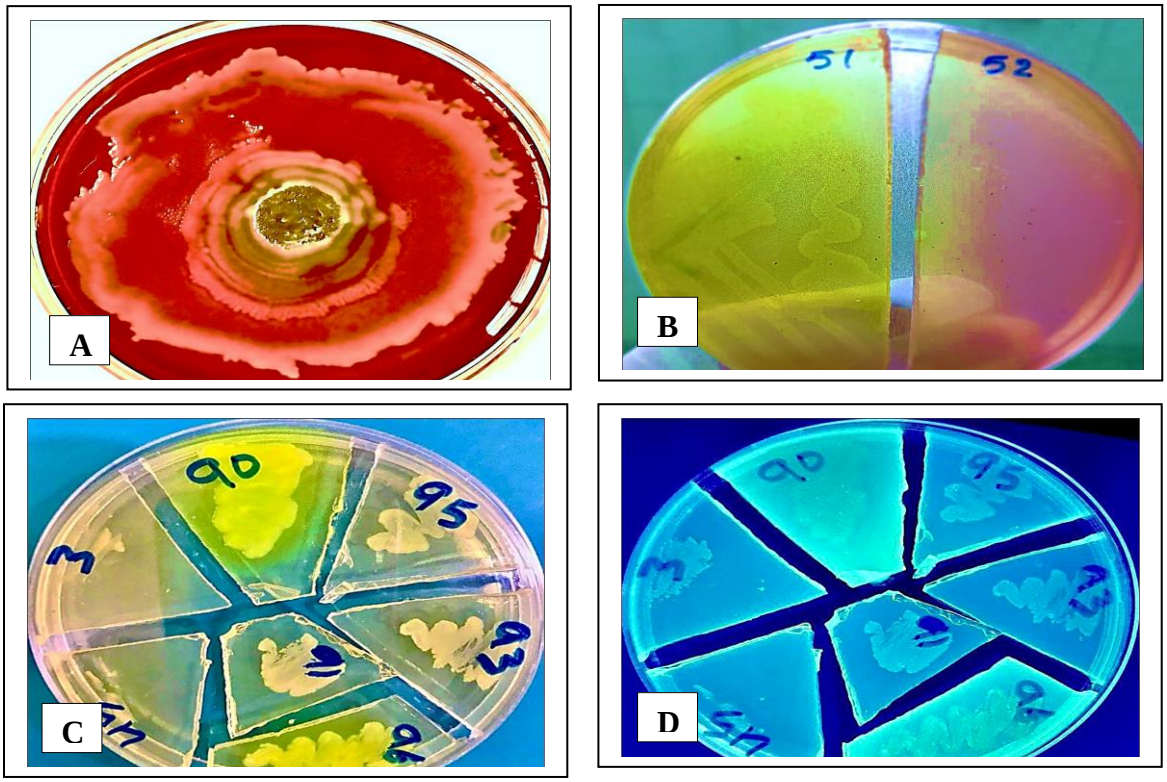

Fig 2: Morphological characteristics of P. aeruginosa on different types of media. (A) P. aeruginosa colonies showed shiny metallic appearance, swarming phenomenon and β-hemolysis on Blood agar. (B) P. aeruginosa isolates on MacConkey agar showed non lactose fermentation. (C)) Colonies of P. aeruginosa on cetrimide agar appeared pale yellow to green when examined by naked eye. (D). Colonies of P. aeruginosa on Cetrimide agar appeared fluorescent under UV light.

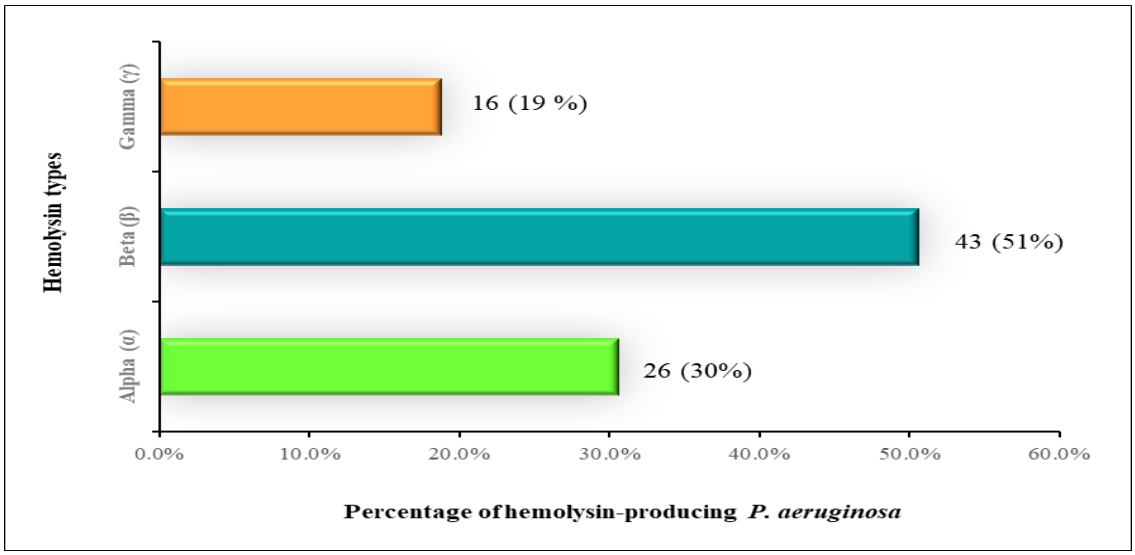

Fig 3: Types and percentage of hemolysis on Blood agar among 85 P. aeruginosa isolates.

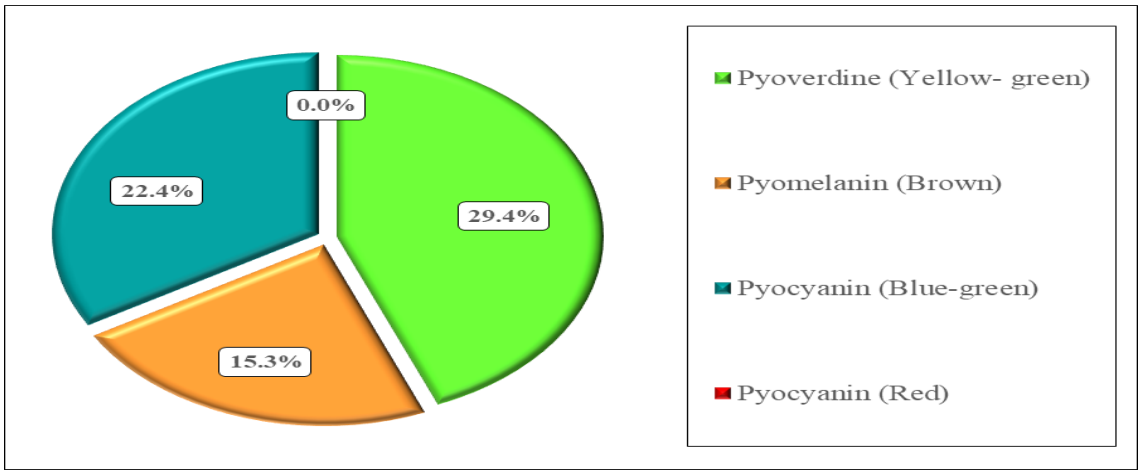

Fig 4: Type and percentages of pigments productions among 85 P. aeruginosa isolates on Nutrient agar.

Table 1. Some of the cultural and biochemical tests among 85 P. aeruginosa isolates

Test	Response	Test	Response
Catalase	+ ve	Motility	+ ve
Growth at 42° C	+ ve	Oxidase	+ ve
Hemolysin	variable	Pigment production	variable
Lactose fermentation	- ve	Swarming	+ ve
		Growth on Cetrimide agar	+ ve

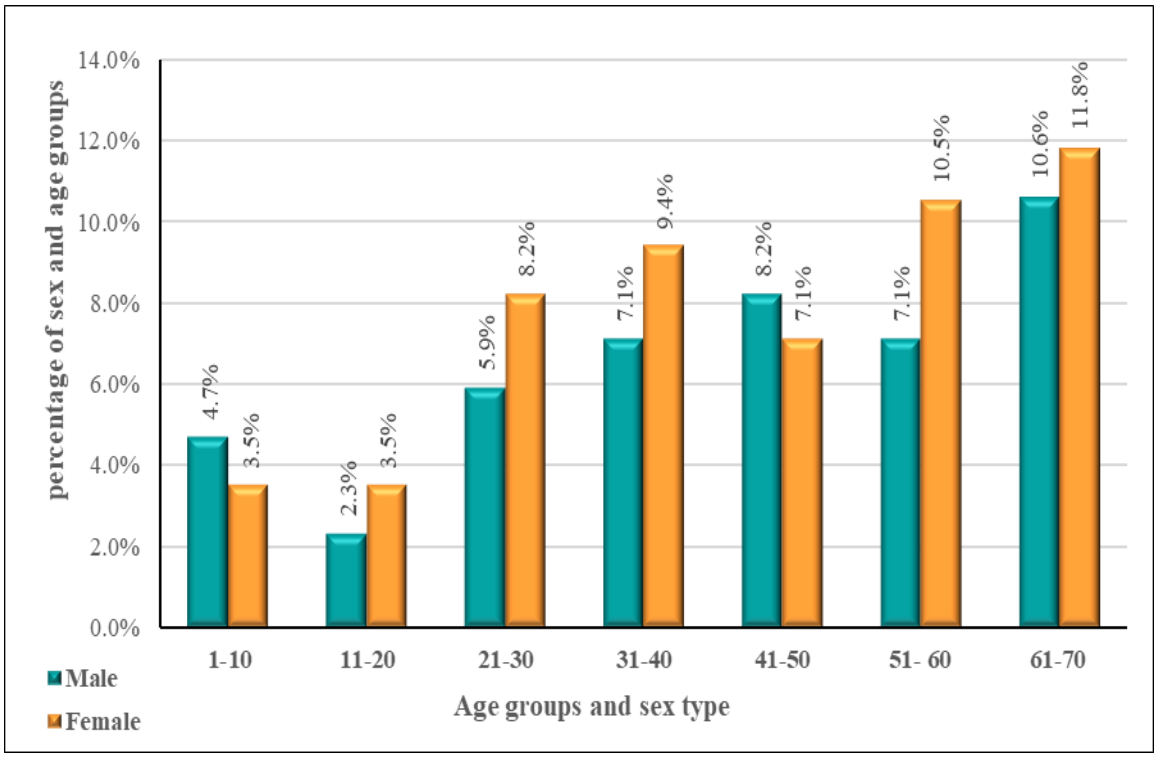

Fig 5: Distribution of 85 nosocomial P. aeruginosa isolates among sex types and age groups.

2. DISCUSSION

Burn infections are a global problematic situation that contributes to 50-75% of mortality among burn patients because they are immunosuppressed [11]. *P. aeruginosa* is a cause of many infections linked with burn patients and those suffering from chronic diseases [12]. Its presence has been associated with increased mortality rates in burn patients because burns remove the protective skin barrier and provide an appropriate environment for the growth of microorganisms such as moisture, dead tissue, and various food sources such as blood, plasma, and other nutrients and require immediate medical intervention to maintain body temperature and internal balance and prevent loss of biological fluids and electrolytes [13]. Although gaining experience in improving the management of burn victims, like fluid resuscitation, healthcare-acquired infections remain the most frequent cause of death among burn victims [14].

Several risk factors are associated with the high prevalence of bacterial infections among burn victims, for instance, the burn wound's area and depth, age, and comorbidities [15]. Although the prevalence rates of bacterial burn wound infections vary depending on the clinical setting and geographical location, this study observed a high prevalence of 323/355 (91%) of bacterial growth in burn wounds, the crucial situation in the burn center near one

previous study by Pondei et al. [16], reported a significant prevalence (86.1%) of pathogenic bacteria in infected burn wounds. In the same regrade, this investigation revealed that *P. aeruginosa* isolates were the most predominant among Gram-negative pathogens among burn infections, with a prevalence percentage of 85/355 (23.9%), consistent with one previous study conducted by Alkhudhairy and Rasool [17], showed the prevalence of (25.6%). While less than those of Ikram et al [18], which observed that virulent *P. aeruginosa* isolates were the most predominant among Gram-negative bacteria isolated from burn infections by a percentage of 65.6%. One of the essential results of the study is that all *P. aeruginosa* isolates appeared to grow ideally on CA agar, which showed fluorescent colonies under UV light, these results were consistent with several earlier studies, for instance, Hosu et al. [19]. and Dukhan et al. [20].

While on BA agar, the colonies exhibit swarming and a metallic sheen. In addition, *β*-hemolytic producer *P. aeruginosa* isolates were the most frequent; these findings were also reported by Shukla and Gupta [21]. As well as the study observed that 57/85 (67.1 %) of *P. aeruginosa* isolates were pigment producers on NA agar, while 28/85 (32.9%) of isolates were not producers; the findings were similar to those of one previous study conducted by Alkhudhairy and Azeez [22], which showed that 70% of isolates were pigment producers. The predominance of bacterial infections and sepsis among elderly burn patients may be related to immunosenescence [23]. Therefore, the present investigation observed that *P. aeruginosa* infections were more prevalent in elderly patient groups than in other age groups overall; several studies reported high prevalent of burn infections among the elderly; for instance, one previous study in China by Qin et al. [24] showed an increased prevalence of pathogenic *P. aeruginosa* among elderly burn inpatients. In addition, the study also observed that the percentage of *P. aeruginosa* infections among females was slightly higher than that of males in most age groups under study, these outcomes consistent with one previous report carried out in India by Sharma et al. [25], showed that female 56% were more affected than male 44%, their interpretation of these results was that accidental burns are more common in females as they tend to stay more time near the fire in the kitchen. The strength of this study is represented by the use of several techniques to isolate and identify *P. aeruginosa* isolates. This results in a comprehensive understanding of the epidemiology of *P. aeruginosa* isolates in burn centers, which provides information about the control of transmission dynamics of these isolates among burn patients in the burn center.

One of the most important goals of this investigation was to evaluate the prevalence rate of *P. aeruginosa* locally and globally to determine the extent of the danger of this pathogen to burn patients. According to the prevalence rate of this bacteria in the current study, which

was estimated at 23.9%, this percentage cannot be underestimated because *P. aeruginosa* has virulence factors that increase the aggravation and complications of burn wound infections. These factors include: pyocyanin triggers proinflammatory activities, Pyoverdine is an iron carrier implicated in iron acquisition and chelation, proteases, and elastases, which contribute to the damage of host tissues elastin. Complications of *P. aeruginosa* infections often result in chronic infections through biofilm formation that delays or prevents patient healing and increases the likelihood of death in burn patients [13]. This pathogen has gained widespread notoriety due to its intrinsic and acquired virulence factors such as resistance to antibiotics. Its virulence has been attributed to its large genome size (~5–7 MB), which makes it exceptionally adaptable and resistant to unfavorable environments [26].

By comparing the prevalence of local *P. aeruginosa* in this investigation with its prevalence in burn wounds in other studies and according to the governorates of Iraq, it can be concluded that the rates range between 15-55%, which are worrying rates, especially since they relate to burn patients because they increase the rates of contamination of burn wounds for hospitalized patients and increase the possibility of an increase in the rate of mortalities. According to the few studies conducted in the governorates of Iraq. The prevalence rates of virulent *P. aeruginosa* isolated from patients with infected burn wounds were as follows: 27% in Sulaymaniyah [27], 15% in Misan [28], 37.9% in Karbala [29], 19.6% in Nasiriyah [30], 45.9% in Al-Diwaniyah [31], 36% in Kirkuk [32], 17.6% in Diyala [33], 53% in Basrah [34], 38.6% in Babylon [35], 34% in Baghdad [36], 55% in Al-Muthanna [37], and 25.7% in Najaf, [17]. While the prevalence rates in countries neighboring Iraq were 12% in Turkey, 57% in Iran [38], 22.7% in Saudi Arabia [39], 18٪ in Syria [40]. Surprisingly, there are no reports, to our knowledge, about the current study, whether in Jordan or Kuwait, despite the importance of the subject.

The prevalence percentage in the current investigation was higher than the rates conducted in India (12%), Kenya (13.7%), Tanzania (12.6%), South Africa (14.5%), Nepal (6.25%), Ethiopia (4.8%), and China (21%). In contrast, the prevalence percentage in this study was lower than the percentages of other studies conducted (22.4%), Malaysia (24.9%), Pakistan (24.9%), and Ghana (30.2%). Such differences in local and global rates between studies may be due to the size of the study samples, laboratory methods, study sample collection protocols, nature of care services provided in burn centers, different population structures of countries, number of health centers dealing with burn cases, type of burns, degree of burns, and season of sample collection [38].

CONCLUSIONS

The rate of virulent *P. aeruginosa* in the current study raises the degree of

alarm, especially among burn patients because they do not have the means of defense that qualify them to resist this virulent pathogen. Also, the older age groups were more affected by it, which increases the levels of risk because elderly patients have weak immunity in addition to most of them being infected with other diseases such as diabetes, which increases the complications associated with burn infections. The impact of Pseudomonal infections on females in most age groups was one of the results of utmost importance and the real reasons behind it must be known. According to numerous studies and reports, the possession of this dangerous pathogen of multiple virulence factors such as its ability to form biofilms delays or prevents the patient's recovery and increases the likelihood of death in burn inpatients. Also, the possession of these virulent bacteria with a large genome makes them exceptionally adaptable and resistant to unfavorable environments.

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