Abstract

*Pseudomonas aeruginosa* is widely present in many diverse environments. It can be found in various biotic sources, including water, plants, intestinal tracts of human and animals, and most importantly the hospital environment. The organism is an important cause of nosocomial infections, such as septicemia and pneumonia, life-threatening infections in immunocompromised persons, and chronic infections in cystic fibrosis patients. Recent studies report that hospitalized patients infected with multidrug resistance (MDR) *P. aeruginosa* have increased hospital length of stay and mortality.

This short review focus on the human infections, potential virulence factors and antimicrobial resistance mechanisms found generally in *P. aeruginosa* as well as its current common occurrence and antimicrobial susceptibility pattern in Jordan.

Keywords

*Pseudomonas aeruginosa*; Opportunistic Pathogen; Antibiotics Resistant; Jordan.

Introduction

*P. aeruginosa* is a non-fermenting Gram-negative bacilli, non-spore-forming, strictly aerobic, catalase and oxidase positive and belonging to the family *Pseudomonadacea*. The organism can grow well at temperature between 4 and 42°C, and at pH values between 5 and 8 [1-2]. It has also the ability to survive on minimal nutritional requirements and to tolerate a variety of physical conditions which allow the organism to persist in both the natural environment and hospital settings [3].

*P. aeruginosa* is a common organism found in moist environments and it can be found in soil, green vegetables, water and sewage. It...
can survive for long periods in water and also on the surface of suitable organic materials in contact with water [4].

*P. aeruginosa* forms biofilms on wet surfaces and in hospital environments such as sinks, humidifiers, and respiratory therapy equipment [4]. Therefore, the organism can be often isolated from a variety of sources in hospital environments, including respiratory therapy equipments, antiseptics, soaps, sinks, mops, medicines, and physiotherapy-hydrotherapy pools and feces of patients [5].

Antiseptics and disinfectants are commonly used in hospital for prevention of nosocomial infections. The constant use of some disinfectants has led to the development of resistance among many Gram-negative and positive bacteria including *P. aeruginosa* and *Acinetobacter baumannii* with a cross resistance to antibiotics [6-8]. Therefore, the selection of proper and adequate concentration of antiseptics and disinfectants is crucial to control nosocomial infections produced by these resistant organisms [9].

**Virulence factors**

*P. aeruginosa* produces a variety of virulence factors, including elastase, various exotoxins (exotoxinA, exotoxinS, exoT, exoU, cytotoxin), and alkaline protease, and it can produce during growth the non-fluorescent bluish pigment pyocyanin or the fluorescent green pigment pyoverdin [10-14]. All these virulence factors of *P. aeruginosa* can cause a range of infections, but rarely cause serious illness in healthy individuals without some predisposing factor [11-14].

*P. aeruginosa* synthesizes an exopolysaccharide called alginate (*alg D* gene) in response to environmental conditions. Alginate is an important virulence factor in *P. aeruginosa*, those strains which produce this particular exopolysaccharide almost exclusively in association with chronic respiratory infections in cystic fibrosis. Evidence suggests that the respiratory tract is initially colonized by non-mucoid *P. aerugi- nosa* strains, which are converted into the mucoid phenotype by production of alginate Alginate serves to protect the bacteria as biofilms from adversity in its surrounding and also enhances adhesion to solid surfaces [15]. The development of biofilms is advantageous to the survival and growth of the bacteria. In addition, *P. aeruginosa* may produce an alginate lyase enzyme under certain conditions, which cleaves the polymer into short oligosaccharides. Both alginate biosynthetic and degradative enzymes are important for the development, maintenance and spread of *P. aeruginosa* biofilms [15-17]. Therefore, control measurements should be designed to minimize biofilm growth *P. aeruginosa* in devices and instruments.

The virulence factors of *P. aeruginosa* play an important pathological role in the colonization, survival of the bacteria and invasion of tissues [18]. The bacterium possesses a large number of cell-associated and extracellular virulence factors, which are highly regulated by cell-to-cell signaling systems. In addition, cell-surface associated structures are acting as virulence factors and enhancing its recurrence and chronic infection [10, 14, 16].

In short, *P. aeruginosa* produces a spectrum of virulence factors. Flagella and type 4 pili are the main adhesins, capable of binding to host epithelial gangliosides, along with lipopolysaccharide. These surface appendages are also highly inflammatory. Once contact with host epithelia has occurred, the type 3 secretion system (T3SS) can be activated, which is able to inject cytotoxins directly into the host cell. Later several virulence factors will be secreted by *P. aeruginosa* which have varying effects on the host. Especially proteases are produced, which can degrade host complement factors, mucus, and disrupt tight junctions between epithelial cells leading to the dissemination of the bacteria through previously impermeable tissues. The release of lipases and phospholipases can affect host cell membranes, and pyocyanin which is a fluorescent siderophore, can interfere with host cell electron
transport pathways and redox cycling system, while pyoverdin can absorb iron from the environment as shown in Figure 1 [19].

Human infections
Pseudomonas aeruginosa is an opportunistic human pathogen that causes chronic and acute infections in any part of human body [11]. The organism predominantly colonizes damaged tissue sites such as burn and surgical wounds, the respiratory tract causing pneumonia, and can cause keratitis in damaged eyes [13, 20-21]. At infected body sites, P. aeruginosa may cause destructive lesions, increase lung epithelial permeability and inflammation, sepsis, and meningitis [10,16]. Water-related folliculitis and ear infections are associated with warm, moist environments such as swimming pools and spas. Additionally, any part of the body can be infected with this organism [2, 15-16].

Patients with cystic fibrosis and immunocompromised patients are highly susceptible to colonization with P. aeruginosa, which may lead to serious progressive or chronic pulmonary infections resulting in high mortality rates [17].

The main route of P. aeruginosa infection is by exposure of susceptible tissue, especially burns, wounds and injured mucous membranes with contaminated water or contamination of surgical instruments [20-21]. P. aeruginosa acquired infections in hospitals are associated with significant morbidity and mortality due to the organism's capacity to adapt easily to changes in the environment, rapidly developing resistance to antibiotics and producing a variety of virulence factors during lung infection [21-23].

P. aeruginosa causes about 20% of ventilator-associated pneumonias (VAP) in intensive care units (ICUs), and it is one of the most difficult pathogens to control with antimicrobial agents. Overall mortality rates due to P. aeruginosa are high and may reach 40% [23].

Ingestion of drinking-water with low numbers of cells of P. aeruginosa does not cause human gastrointestinal infection, but presence the organism in high numbers in potable water, can be associated with complaints about taste, odour and turbidity and may causing diarrhea in children [24].

Antimicrobial resistance
P. aeruginosa is considered to be one of most harmful pathogens because of its high level of resistance to many antimicrobial agents used in clinical medicine. The organism exhibits intrinsic resistance to many clinically useful antimicrobial agents, and it is often difficult to control its infection in hospitalized patients [25-27].

The prevalence of multidrug-resistant (MDR) P. aeruginosa strains recovered from various clinical specimens has been increasing worldwide including Jordan and many Arab countries [12, 28-32]. Therefore, it is expected that high mortality could be associated with its infections in Jordan.

Acquired antibiotic resistance of P. aeruginosa
Acquisition of antibiotic resistance genes mainly refers to transferable resistance genes between
various bacteria species. These genes are located mostly in transferable genetic elements such as plasmids, transposons, and integrons, which carry genes encoding determinants of resistance to aminopenicillinas, cephalosporins, aminoglycosides, carbapenemases and other antibiotics, conferring multidrug resistance in Gram-negative enteric and non-enteric bacteria including *P. aeruginosa*. These genetic elements also increase the spread of antimicrobial resistance, mostly in gut flora of hospitalized patients and general population [33-34].

The original beta-lactamase enzymes were plasmid-encoded and only active against hydrolyzed penicillins and older narrow-spectrum cephalosporins. However, more recently the emergence of acquired beta-lactamases in *P. aeruginosa* includes the extended-spectrum beta-lactamases (ESBLs) enzymes that are able to hydrolyze a wider range of beta-lactams, including the broad-spectrum cephalosporins and monobactams [35-36]. ESBLs are hydrolytic enzymes active against penicillins, extended-spectrum cephalosporins, and aztreonam, but these cannot hydrolyze carbapenemases and cephemycins, and their activities are inhibited by clavulanate, sulbactam and tazobactam [36-37]. ESBLs were initially found in *Klebsiella pneumoniae* and *Escherichia coli*, but have been later detected in *P. aeruginosa* at a low frequency [37-39]. Currently, there are more than 200 different known ESBLs in Gram-negative bacteria, and of these 32 are detected in *P. aeruginosa* [38].

**Resistance to carbapenems in *P. aeruginosa***

Carbapenems, eg: meropenem, imipenem and ertapenem, are an important class of anti-pseudomonal beta-lactams used frequently in the treatment of Gram-negative infections due to their effectiveness and stability to most beta-lactamases, including ESBLs which commonly develop resistance in *P. aeruginosa* during treatment [40-42]. Many carbapenemase varieties have been identified in *P. aeruginosa*, including most recently detected important types; *Klebsiella pneumoniae* carbapenemase (KPC) and metallo-β-lactamases (MBLs) and oxacillinas [40]. However, it is important to note here that all of the carbapenemases enzymes classes A, B, and D can hydrolyze most beta-lactams, including the carbapenems, but not aztreonam [41-42]. *P. aeruginosa* carbapenem resistance has been responsible for several nosocomial outbreaks in medical centers worldwide, and has been associated with failure of therapy with carbapenems [43-45].

**Epidemiology of antimicrobial resistance of *P. aeruginosa* in Jordan**

Most recent studies from Jordan over a period of 14 year, have shown that *P. aeruginosa* isolates from clinical specimens were commonly multidrug resistant (MDR) to many often used antibiotics in clinical medicine such as amikacin, aztreonam, gentamicin, cefotaxime, piperacillin-tazobactam and ceftazidime and colistin B [5, 12, 44-49]. In general, the Jordanian studies (Table 1) showed similar antimicrobial resistance patterns in *P. aeruginosa* isolates from clinical studies as has been reported from neighboring Arab countries [20, 28-29, 30, 32, 49-50].

A new Jordanian study published in 2018 [12], has reported that *P. aeruginosa* isolates are frequently recovered from respiratory samples of patients (61/284; 21.5%). The percentage of MDR among *P. aeruginosa* isolates was 52.5%, and all isolates were susceptible to colistin with lower rates of susceptibility to other tested antibiotics. Positive genes of blaCTX-M, blaVEB, blaTEM, blaGES and blaSHV were detected in 68.9%, 18.9%, 18.9%, 15.6% and 12.5% of isolates, respectively. The percentages of the virulence genes algD, lasB, toxA, exoS,
Table 1. Studies on \textit{P. aeruginosa} in Jordan (2004-2018).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Source of \textit{P. aeruginosa}</th>
<th>No. of tested isolates</th>
<th>[R]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Battikhi &amp; Ammar</td>
<td>2004</td>
<td>Ear discharge</td>
<td>75</td>
<td>49</td>
</tr>
<tr>
<td>Shehabi</td>
<td>2005</td>
<td>Water &amp; human feces</td>
<td>107</td>
<td>5</td>
</tr>
<tr>
<td>Al-Nassar &amp; Al-ASeel</td>
<td>2009</td>
<td>Clinical isolates</td>
<td>100</td>
<td>48</td>
</tr>
<tr>
<td>Masaadeh &amp; Jaran</td>
<td>2009</td>
<td>Wound isolates</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>Shehabi</td>
<td>2011</td>
<td>Water</td>
<td>212</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clinical</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Al Dawodeyah</td>
<td>2018</td>
<td>Respiratory</td>
<td>61</td>
<td>12</td>
</tr>
<tr>
<td>Aljaafreh</td>
<td>2018</td>
<td>Ear-discharge</td>
<td>28</td>
<td>45</td>
</tr>
<tr>
<td>Shishtawi</td>
<td>2018</td>
<td>Infant feces</td>
<td>16</td>
<td>46</td>
</tr>
</tbody>
</table>

\textit{R}: Reference.

and \textit{exoU} among \textit{P. aeruginosa} isolates were 98%, 98%, 80%, 33% and 33%, respectively, and 87% of isolates produced pyocyanin.

A second recent study carried out in 2018 [45], has also showed that \textit{P. aeruginosa} is commonly causes otitis media. A total of 28/128 (22%) \textit{P. aeruginosa} isolates were recovered from ear discharge of patients at the Jordan University Hospital. The antimicrobial susceptibility of these isolates indicated that the lowest susceptibility rate was for gentamicin (25%) and the highest (75%) for each ciprofloxacin, norfloxacin, imipenem and 68% for aztreonam among 9 tested anti-pseudomonal drugs, and 39% of the isolates were multidrug-resistant. In addition, it is important to note that one of \textit{P. aeruginosa} from ear discharge was resistant to colistin [45].

The study also has reported that metallo-\textit{β}-Lactamase-producing \textit{P. aeruginosa} (\textit{KPC genes}) was frequently found in 57% of the isolates. The rates of the potential virulence genes found among 28 \textit{P. aeruginosa} isolates were as follow: \textit{lasB}, \textit{algD}, \textit{toxA}, \textit{exoU}, \textit{PilB} and \textit{exoS} at 100%, 100%, 82%, 72%, 54% and 25%, respectively. All isolates produced either the pigment pyoverdin (57.1%) or pyocyanin (42.8%).

A previous Jordanian study published in 2011 [47], showed that a high percentage of \textit{P. aeruginosa} isolates from patients and sewage effluent water were multidrug-resistant to more than three antimicrobial drug classes, and commonly carried class 1 integrons and metalo-\textit{β}-lactamases (\textit{MBL genes}). The antimicrobial resistance markers; metalo-\textit{β}-lactamase genes (\textit{bla-OXA2}, \textit{bla-VIM2a}, \textit{bla-VIM2b}) and aminoglycoside genes (\textit{aacA}) were detected at high rates in these isolates [49]. Another Jordanian study published in 2009 [47] reported that \textit{P. aeruginosa} isolates from post-operative wound infection were susceptible to amikacin, gentamicin, tobramycin, ciprofloxacin, aztreonam, ceftazidime, piperacillin, meropenem and cefepime in the range between 9% and 78%.

The occurrence of \textit{P. aeruginosa} in feces of the Jordanian healthy population and patients has been found at rates of 7% and 12%, respectively [5]. The same study also reported that \textit{P. aeruginosa} can be frequently isolated from chlorinated and non-chlorinated water sources at 15% and 44%, respectively, and all \textit{P. aeruginosa} isolates from water sources were highly susceptible in the range 87% to 100% to amikacin, aztreonam, ceftazidime, ciprofloxacin, imipenem gentamicin and pepracillin-tazobactam, whereas isolates from human stools were less susceptible (81-98% ) to the same antimicrobial drugs [5].

A study performed in 2009 by Elnasser and Al Aseel at King Abdullah University Hospital, Irbid, reported that 100 tested \textit{P. aeruginosa} isolates from various clinical specimens were multidrug resistant at least to three antibiotics [48].

\textbf{Conclusions}

Overall the results of Jordanian studies show clearly that \textit{P. aeruginosa} isolates have developed gradually
increased levels of resistance over the last 14 years. Results also indicate that *P. aeruginosa* isolates from Jordanian patients exhibit high incidence rates of antimicrobial resistance. Therefore, all medical professions and medical authorities should strive to make their best efforts to prevent further misuse or overuse of antibiotics. It is strongly recommended that antibiotic treatment of *P. aeruginosa* infections must be based on proper antimicrobial susceptibility test in the laboratory.

References


6. Alonso A, Campanario E, Martinez J L. Emergence of multidrug-resistant mutants is increased under antibiotic selective pressure in *Pseudomonas aeruginosa*. Microbiology 145, 2857-2862.


