REVIEW ARTICLE

Pseudomonas aeruginosa: Burn Infection, Treatment and Antibacterial Resistance

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen causing severe, acute and chronic nosocomial infections in immunocompromised, catheterized or burn patients. Various types of virulent factors have been identified in *P. aeruginosa*, suggesting their contribution to the pathogenesis of the disease. The organism is generally resistant to numerous antimicrobial agents due to natural resistance in particular impermeability or mutations and acquisition of resistant determinants. Plasmid and integron have a crucial role in acquisition of mobile elements. Most treatment failures are related to inappropriate initial antibiotic therapy with insufficient coverage of multidrug resistant (MDR) pathogens, the rationale for using combinations of antibiotics to cover MDR gram-negatives. However, clinical data supporting this strategy are limited. In fact, systematic combination therapy may have contributed to the overuse of antibiotics and to the emergence of MDR microorganisms. Nevertheless, combination therapy is the best strategy to treat severe infections due to suspected MDR *Pseudomonas*. Optimally, therapeutic strategies should be sufficiently broad to cover relevant pathogens while minimizing the risk for emergence of antimicrobial resistance. Polymyxin E (colistin) and carbapenems are the most effective antibiotics against MDR isolates.

Keywords: *Pseudomonas aeruginosa*; Multidrug-resistance; plasmid; Integron

Introduction

Pseudomonas aeruginosa is a gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Almost all strains are motile by means of a single polar flagellum. It is a free-living bacterium, commonly found in soil and water. This bacterium, a member of the gamma proteobacteria, is a gram-negative, aerobic rod belonging to the bacterial family pseudomonadaceae.[1] Based on conserved macromolecules (e.g. 16S ribosomal RNA), the family includes only members of the genus *Pseudomonas* which are cleaved into eight groups. *P. aeruginosa* is a typical species of its group which contains 12 other members.[2] Almost all the clinical cases of *P. aeruginosa* infection can be associated with the compromise of host defense such as burn patients. While many cases of *P. aeruginosa* infection can be attributed to general immunosuppression (e.g. AIDS patients),[3,4] in neutropenic patients undergoing chemotherapy,[5] such scenarios predispose the host to a variety of bacterial and fungal infections, and therefore do not yield information which is specific to the pathogenesis of *P. aeruginosa*. In this respect, three of the more informative human diseases caused by *P. aeruginosa* are: 1) bacteremia in severe burn victims; 2) chronic lung infection in cystic fibrosis patients; and 3) acute ulcerative keratitis in users of extended-wear soft contact lenses. Observations and experimental evaluation of various bacterial virulence factors have shed a great deal of light on how *P. aeruginosa* is able to cause disease in a wide variety of organs, secondary to disruption of the normal physiologic function. Such insights provide an understanding at the molecular and cellular level of how and why *P. aeruginosa* has become such an important pathogen in human infection.

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P. aeruginosa Bacteremia in Severe Burn Victims

Bacterial infection following severe thermal injury can be most simplistically attributed to extensive breaches in the skin barrier. The fact that *P. aeruginosa* occurs so commonly in the environment makes it extremely likely that an individual suffering severe burns will be challenged with this microorganism before the burns can heal. Burn hospitals often harbor multidrug-resistant *P. aeruginosa* that can serve as the source of infection. *P. aeruginosa* has been found to contaminate the floors, bed rails, and sinks of hospitals, and has also been cultured from the hands of nurses.[6] Besides transmission through fomites and vectors, bacterial flora can be carried into a hospital by the patient and can be an important source of infection for the same individual after injury.[7] Regarding multidrug resistance, Hsueh et al.[8] reported single multidrug-resistant strain of *P. aeruginosa* over a period of several years, and concluded that the strain was carried by some patients asymptomatically through several rounds of antibiotic treatment which were administered to treat *Pseudomonas* and non-*Pseudomonas* infections. This scenario can be worse during the spread of *P. aeruginosa* from one patient to another; the persistence of this strain takes place in patients throughout several courses of antibiotic treatment. It has been proved that during admission of patients in burn centers, a limited number of common strains cross-contaminate burn victims mostly when their lesions scrubbed in the bathroom.[9]

P. aeruginosa Virulence Factors in Burn Infection

Numerous *P. aeruginosa* virulence factors contribute to the pathogenesis of burn wound infection. Rahme et al. highlighted the occurrence of virulence factors of *P. aeruginosa* contributing to pathogenesis in burn wound infection of rodents.[10] A significant role has also been established for *P. aeruginosa* pili and flagella. Experiments comparing infection of burn wounds by pilus and flagellum deficient *P. aeruginosa* strains clearly demonstrate that the bacteria deficient in either of these structures have reduced virulence, both in their ability to persist at the wound site, and in their ability to disseminate throughout the host organism.[11] Dissemination of *P. aeruginosa* throughout the host is also partially dependent upon production of bacterial elastase and other proteases.[12] Elastase has been shown to degrade collagen and non-collagen host proteins, and to disrupt the integrity of the host basement membrane.[13] Proteases can have adverse effects on several aspects of the innate and acquired host immune response. For example, elastase inhibits monocyte chemotaxis,[14] which could adversely affect early clearance of *P. aeruginosa* from wound sites by phagocytosis, as well as subsequent presentation of bacterial antigens to the host immune system.[15] The *lasR* gene encodes a protein critical for initiation of the quorum sensing response involved in virulence factor production and biofilm formation, indicating that other factors controlled by *lasR* are critical determinants of *P. aeruginosa* pathogenesis in burn wound infection.[16] Other *P. aeruginosa* virulence factors reported to be involved in pathogenesis of burn wound infection include phospholipase C,[17] the ferripyochelin-binding protein,[18] lipopolysaccharide (LPS),[19] and exoproducts secreted by type III secretion apparatus.[20] While the loss of the skin's barrier function is certainly an important factor in burn wound infection, its compromise fails to explain the relatively narrow range of bacterial pathogens which are typically cultured from infected burn wounds.[21] It is, therefore, likely that additional host defense mechanisms specific to some pathogens are more compromised in severe burns. A reduction in infection following local application of polyclonal human antibody to burn sites has been reported,[22] suggesting that in the untreated burn wound, immunoglobulin exists at subprotective levels. The possibility of a local deficiency of antibody-mediated immunity in burn wounds is further supported by an earlier report[23] stating that Fc receptor expression by polymorphonuclear leukocytes (PMNs) decreases by the fifth day post-injury in burn victims. Complement has also been shown to be depleted in burn wounds,[24] probably due to local consumption of complement components. Local deficiencies in protective antibody complement proteins, and PMN Fc receptors may explain the defects in random migration and chemotaxis of PMNs observed at burn wound sites. Taken together, these data suggest that the ability to colonize a burn wound depends upon the concerted impairment of several host immune mechanisms, and that the importance of *P. aeruginosa* in such infections is due to its ability to take advantage of the host immune compromise and secrete a variety of important virulence factors. *P. aeruginosa* produces two extracellular protein toxins, exoenzyme S and exotoxin A. Exoenzyme S has

the characteristic subunit structure of the A-component of a bacterial toxin, and it has ADP-ribosylating activities.[25] Exoenzyme S is produced by the bacteria growing in the burned tissue and may be detected in the blood before the bacteria are present. It has led to the suggestion that exoenzyme S may act to impair the function of phagocytic cells in the blood-stream and internal organs as a preparation for invasion by *P. aeruginosa*.[26] Exotoxin A has exactly the same mechanism of action as the diphtheria toxin; it causes the ADP ribosylation of eucaryotic elongation factor 2, resulting in inhibition of protein synthesis in the affected cell.[27] Although it is partially-identical to diphtheria toxin, it is antigenically distinct. It utilizes a different receptor on host cells than diphtheria toxin does; otherwise, it enters the cells in the same manner and has the exact enzymatic mechanism. The production of exotoxin A is regulated by exogenous iron, but the details of the regulatory process are distinctly different in *C. diphtheriae* and *P. aeruginosa*.[28] Exotoxin A appears to mediate both local and systemic disease processes caused by *P. aeruginosa*. It has necrotizing activity at the site of bacterial colonization and is therefore thought to contribute to the colonization process.[29,30] Table 1 is a summary of the virulence determinants of *P. aeruginosa*.

Candidate Vaccines for High Risk People

Although antibiotic therapy has considerably improved the management of infectious diseases in general, many *P. aeruginosa* infections are not fully treated or eradicated by the application of anti-pseudomonal drugs and can, thus, become chronic infections. For instance, burn patients that survive the initial burn trauma can become colonized with antibiotic-resistant, hospital-derived *P. aeruginosa* strains that are not easily eradicated with antibiotic therapy.[31,32] In cystic fibrosis patients, when the strains are eventually selected out by antibiotic therapy to become multiply-resistant, an increase in the rate of decline in lung function is seen when compared to patients infected with antibiotic susceptible strains.[33-35] Several *P. aeruginosa* antigens are used for vaccine development including lipopolysaccharide alone, polysaccharides alginate, extracellular proteins, exotoxin A, and killed whole cell.[36-39] However, none of them are clinically available to use for people who are at risk such as firefighters or infected patients (Immunocompromised and cystic fibrosis patients). Nevertheless, at the present time some candidate vaccines are under first to third stage of experimental clinical trials.[40]

Table 1: Summary of the virulence determinants of pathogenic *Pseudomonas aeruginosa*

Adhesins
pili (N-methyl-phenylalanine pili)
polysaccharide capsule (glycocalyx)
alginate slime (biofilm)
Invasins
elastase
alkaline protease
hemolysins (phospholipase and lecithinase)
cytotoxin (leukocidin)
siderophores and siderophore uptake systems
pyocyanin diffusible pigment
Motility/chemotaxis
flagella
pili
Toxins
Exoenzyme S
Exotoxin A
Lipopolysaccharide
Antiphagocytic surface properties
capsules, slime layers
LPS (Lipopolysaccharide)
Biofilm construction
Defense against serum bactericidal reaction
slime layers, capsules, biofilm
LPS
protease enzymes
Genetic attributes
genetic exchange by transduction and conjugation
inherent (natural) drug resistance
R factors and drug resistance plasmids
Ecological criteria
adaptability to minimal nutritional requirements
metabolic diversity
widespread occurrence in a variety of habitats

Treatment of Infections

Topical antimicrobial therapy

It has been proved that an effective topical antimicrobial agent substantially reduces the microbial load on the open burn wound surface and reduces the risk of infection.[41,42] Selection of topical antimicrobial therapy should be based on the agent's ability to inhibit the microorganisms recovered from burn wound surveillance cultures and monitoring of the nosocomial infections acquired in the burn unit. Prescription is also based on the individual preparation of the topical agent (e.g., ointment or cream versus solution or dressing) and its pharmacokinetic properties. Burn

units may rotate the use of various topical antimicrobial preparations on a regular basis to decrease the potential for development of antibiotic resistance.[43-45] Topical antibiotic agents should first be applied directly to the patient's dressings before application to the burn wound to prevent contamination of the agent's container by burn wound flora. The inhibitory action of silver is due to its strong interaction with thiol groups present in the respiratory enzymes in the bacterial cell.[46,47] Silver has also been shown to interact with structural proteins and preferentially bind with DNA nucleic acid bases to inhibit replication.[48,46] For this reason, silver has recently been shown to be highly toxic to keratinocytes and fibroblasts and may delay burn wound healing if applied indiscriminately to debrided healing tissue areas.[48-50] Moist exposure therapy, using a moisture-retentive, has been shown to act as an effective antibacterial agent while promoting rapid autolysis debridement and optimal moist wound healing in partial-thickness injury.[51,52] Moisture-retentive ointment also resulted in earlier recovery of keratinocytes with improved wound healing and decreased scar formation.[53] Silver nitrate is most effective before the burn wound becomes colonized. The burn wound needs to be cleansed of emollients and other debris before a multilayered dressing is applied to the burn wound and subsequently saturated with silver nitrate solution. Effective use of this preparation, therefore, requires continuous application with secondary occlusive dressings, making examination of the wound difficult. The silver ion in $AgNO_3$ also quickly binds to elemental chlorine ions so that repeated or large-surface application of this solution may lead to electrolyte imbalance (e.g., hyponatremia and hypochloremia).[40,54] Silver nitrate antibacterial activity is limited to the burn wound surface.[55,56] This agent demonstrates the bacteriostatic activity against gram-negative aerobic bacteria such as *P. aeruginosa* and *E. coli*, but it is not active against other genera, including *Klebsiella*, providencia, and Enterobacter.[40,57] Silver nitrate also has limited antifungal activity so that nystatin should be used concomitantly.[58,59]

Silver Sulfadiazine

This agent is a combination of sodium sulfadiazine and silver nitrate. The silver ion binds to the microorganism's nucleic acid, releasing the sulfadiazine, which then interferes with the metabolism of the microbe.[46] It is easy to use and painless when applied and can be used with or without a dressing. Limited systemic toxicity with repeated daily or twice-daily application has occurred aside from the development of leukopenia.[60,61] Silver sulfadiazine has excellent broad-spectrum antibacterial coverage against *P. aeruginosa* and other gram-negative enteric bacteria, although some resistance has recently been reported.[41,62] In addition, this agent has some activity against *Candida albicans*, but enhanced antifungal activity can be achieved by using nystatin in combination with silver sulfadiazine.[58] Although silver sulfadiazine dissociates more slowly than silver nitrate, there is still poor penetration into the wound.[54,55] Silver sulfadiazine is only absorbed within the surface epidermal layer, which limits its effectiveness in some patients with severe injuries. In Europe, a combination of cerium nitrate and silver sulfadiazine has been used to combat this problem.[63,64] It has been shown to reduce the inflammatory response to burn injury, decrease bacterial colonization, and provide a firm eschar for easier wound management.[64]

Mafenide Acetate

Topical mafenide acetate cream allows open burn wound therapy and regular examination of the burn wound surface because it is used without dressings. Mafenide acetate is applied a minimum of twice daily and has been shown to penetrate the burn eschar.[54] The 5% solution must be applied to saturate gauze dressings, and these need to be changed every 8 hours for maximal effect. Mafenide acetate solution appears to be as effective as the cream preparation when used in this way.[41,65] Mafenide acetate (Sulfamylon) cream has a broad spectrum of activity against gram-negative bacteria, particularly *P. aeruginosa*, but it has little activity against gram-positive aerobic bacteria such as *Staphylococcus aureus*.[41] This agent also inhibits anaerobes such as *Clostridium spp.* Because protracted use of mafenide acetate favors the overgrowth of *C. albicans* and other fungi, this agent should be used in combination with nystatin to prevent this complication due to its limited antifungal activity.[58,59] This compound is converted to p-sulfamylvanzoic acid by monoamide oxidase, a carbonic anhydrase inhibitor, causing metabolic acidosis in the burn patient.[41,42] In burn patients with inhalation injury and a concomitant respiratory acidosis, the use of mafenide acetate over a large burn surface area or

the repeated application of this compound can be fatal. Mafenide acetate also decreases the breaking strength of healed wounds and delays healing.[66]

Acticoat AB Dressing

This product is a specialized dressing, consisting of two sheets of high-density polyethylene mesh coated with nanocrystalline silver (e.g., ionic silver with a rayon-polyester core).[67-69] The more controlled and prolonged release of nanocrystalline silver to the burn wound area allows less-frequent dressing changes, reducing the risk of tissue damage, nosocomial infection, patient discomfort, and the overall cost of topical therapy.[67,70] Acticoat AB provides the most comprehensive broad-spectrum bactericidal coverage against important burn wound pathogens of any topical antimicrobial preparation currently marketed.[67,70] These dressings have a potent antibacterial activity against most aerobic gram negatives, including *P. aeruginosa* and antibiotic resistant members of the family *Enterobacteriaceae* as well as aerobic gram-positive bacteria, including MRSA and vancomycin resistant *Enterococci*.[67,69,70] If the burn wound surface has minimal exudates, these specialized dressings can remain in place for several days and retain antibacterial activity.[70]

Resistance to Antimicrobial Agents

Resistance to topical antimicrobial agents

Although resistance to silver sulfadiazine in *P. aeruginosa* was reported, its resistance mechanism has not been determined.[62] It is suggested that resistance of *Pseudomonas* to silver based topical antimicrobials in part is based on the mutation of outer membrane proteins that transport ions including silver across bacterial membrane.[71,72] Gentamicin-resistant strains of *P. aeruginosa* which were isolated from burned patients have been reported.[73] These stains showed cross-resistance to silver sulfadiazine but their resistance was unstable and did not persist on subculture media. According to a report in USA, an epidemic sepsis of *Enterobacter cloacae* in burned patients occurred and resulted into 13 deaths.[74] The MIC values of silver sulfadiazine for these strains were 3200 μg/ml whilst the strains isolated from non-burned patients were all sensitive to silver sulfadiazine. Similarly, Rosenkranz et al. isolated two silver

sulfadiazine resistant strains of *Entrobacter cloace* in a burn unit where silver sulfadiazine was in use. These strains showed high resistance to silver sulfadiazine (MIC= 400 μg/ml) and were cross-resistant to silver benzoate but not to silver nitrate.[75] Recently we also demonstrated that *P. aeruginosa* isolated from burned patients were resistant to silver sulfadiazine while most of them were sensitive to silver nitrate solution.[76]

Resistance to Antibiotics

Resistance due to mutations

Various penicillins, cephalosporins, carbapenems, monobactams, aminoglycosides, fluoroquinolones, and polymyxins have been used to treat patients infected with *P. aeruginosa* and are active against most isolates. All, however, are prone to being compromised by mutational resistance. Mutations to topoisomerases II and IV confer fluoroquinolone resistance more readily in *P. aeruginosa* than in *Enterobacteriaceae*, because *P. aeruginosa* has a poorer inherent susceptibility.[77] Derepression of the chromosomal AmpC β-lactamase reduces susceptibility to penicillins and cephalosporins although the level of resistance depends on the degree of derepression, which is more variable than that in Enterobactermutants.[78] The up-regulation of *MexAB-OprM* compromise*s* the fluoroquinolones, penicillins, cephalosporins, and, to some extent, meropenem (although not imipenem), and it also enhances resistance to many other drugs that lack useful antipseudomonal activity.[78-79] Up-regulation of other efflux systems, for example *MexCD-OprJ* and *MexEF-OprN* confers resistance to fluoroquinolones and some β-lactams; up-regulation of *MexXY-OprM* also affects aminoglycosides.[80] There is better evidence that increased impermeability is a mechanism of aminoglycoside resistance, for example in the "small-colony variants" which are sometimes selected during gentamycin therapy and in isolates with reduced susceptibility to all aminoglycosides, carbapenems and fluoroquinolones.[81-84]

Multidrug Resistance due to Mutations

No single mutation compromises every antipseudomonal drug. Nevertheless, up-regulated efflux can simultaneously compromise fluoroquinolones and most β-lactams, leaving only the aminoglycosides (which lack reliable efficacy as antipseudomonal monother-

apy) and imipenem (to which mutational resistance evolves at high frequency). A combination of upregulated efflux, loss of OprD and impermeability to aminoglycosides compromises every drug class except the polymyxins. Each of the necessary mutations arises in 1 cell per 107 to 109 cells, and, although simultaneous emergence is mathematically and biologically improbable, sequential emergence is all too likely because infections resistant to the first antibiotic administered are likely to be treated with a second antibiotic, and so on. Mutations that up-regulate efflux may act additively with those effecting permeability, β-lactamase expression, or topoisomerase susceptibility so as to exacerbate resistance.[85] Accumulation of sequential mutations may be facilitated by hypermutators, which either lack the ability to perform DNA proofreading or mismatch repair, or which use DNA polymerases with a reduced copying fidelity. Because resistance is most likely to emerge in hypermutators, antibiotics may select for hypermutators, thereby increasing the probability that further resistance will emerge.[86]

Acquisition of Genes and Multidrug Resistance

Many acquired β-lactamases and aminoglycoside-modifying enzymes have been noted in *P. aeruginosa*.[87-89] Resistance to oxyimino-aminothiazolyl cephalosporins, monobactams, and penicillins but not to carbapenems has been reported as a result of expression of potent aminoglycoside-modifying enzymes.[90] Metallo-β-lactamases enzyme rapidly hydrolyzes penicillins, cephalosporins, and carbapenems but not aztreonam.[91] Resistance to penicillins and cephalosporins usually accompanies production. A variety of enzymes have been identified from Japan, Taiwan, France, Greece, South Korea, Italy and Canada.[91-93] The genes for resistance are often carried as cassettes within integrons, which are natural recombination systems that assemble series of acquired genes behind a single promoter. This organization facilitates gene recombination. Critically, the β-lactamase genes are often adjacent to aminoglycoside 6-N acetyltransferase [*aac(6)-1b*] determinants.[91,94,95]

Prevalence of Multidrug Resistance

P. aeurginosa isolated from patients in burn center were resistant to most classes of antibiotics. According to a survey conducted in Ghotbeddin Burn hospital (Shiraz, Iran) almost all *P. aeurginosa* isolated from burn patients were resistant to all tested anti-Pseudomonal antibiotics except carbapenems (meropenem and imipenem).[96] Moreover, several reports from Iran confirmed multidrug resistance of burn's isolates.[97-99] It seems likely that most of this multidrug resistance reflects the accumulation of multiple mutations, although this surmise remains to be confirmed by molecular studies, and although reports from other parts of the world document extreme multidrug resistance associated with acquired resistance genes. In a hospital in Thessaloniki, Greece, a serotype with cross-resistance to aztreonam, aminoglycosides, and ciprofloxacin persisted for 3 years, with 1211 isolates of this strain recovered.[100] In South Korea, resistant isolates of *P. aeurginosa*, with the hydrolyzing enzyme being found in organisms at 9 out of 29 hospitals were surveyed. Moreover, a detailed study at one Korean Hospital revealed dissemination in multiple *P. aeruginosa* lineages.

Prevention and Management of Multi-Drug Resistance

The selection of resistant mutants, a risk associated with any antipseudomonal therapy, varies with the type and dosage of antibiotic used and the infection site. It revealed a 2-fold greater risk of selection for resistance when imipenem, rather than ciprofloxacin, ceftazidime, or piperacillin, was used.[101,102] It is often assumed that combination therapy prevents the selection of mutational resistance, but evidence for this is scanty. In addition, single efflux mutations may affect both the β-lactams and the fluoroquinolones, thereby undermining the use of combinations of these drugs. The original emergence of multi-drug resistance in association with plasmids and integrons is less predictable than mutational resistance because it depends on the random escape of genes to mobile DNA. However, once such resistance emerges, either the host strain can spread among patients or the resistance can disseminate among strains. When strains have multiple mutational or acquired resistance, the choice of therapy is often frighteningly limited, especially because most clinicians would prefer to use a synergistic combination for serious Pseudomonal infections. No new fluoroquinolone offers better antiPseudomonal activity than ciprofloxacin, and none retains activity against ciprofloxacin-resistant isolates.

Where resistance is mutational, tobramycin and meropenem are the drugs most likely to retain activity, because they are the aminoglycoside and the β-lactam with the best inherent activity against *P. aeruginosa.* Isolates with efflux-mediated resistance to meropenem, penicillins, and cephalosporins might, however, retain susceptibility to imipenem. Although meropenem is usually a more active carbapenem, this possibility should always be considered. In instances in which all chances of β-lactam, aminoglycoside, and quinolone use are lost, the polymyxins remain the drugs of last resort. Despite their significant toxicity, they have been used with some success. Levin et al.[103] reported that the use of intravenous polymyxin E (colistin) was successful in 35 (58%) out of 60 patients treated for multidrug-resistant *Pseudomonas* and acinetobacter infections although it was associated with a failure rate of 75% when used for the treatment of pneumonias. Perhaps most disturbing is the dearth of new drug options. Clinafloxacin was slightly more active than ciprofloxacin, but its development has been suspended, and no other antiPseudomonal antibiotic is in advanced development. For the long term, multi-drug efflux inhibitors are promising for use with fluoroquinolones or β-lactams[104], and metallo-β-lactamase inhibitors[105] are the focus of laboratory investigation. Unless new drugs are developed, it is hard to escape the conclusion that multi-drug-resistant *Pseudomonas* strains will be an increasing reality and that the use of polymyxins will increase, despite their toxicity.

Conclusion

P. aeruginosa infections identify those of a pathogen with many potentially virulent factors that allow it to colonize and infect essentially any mammalian tissue. The organism possesses a multitude of factors that promote adherence to host cells and mucins, damage host tissue, elicit inflammation and disrupt defense mechanisms. Due to impairment of the skin barrier in burn patients and frequent scrubbing, debridement and manipulation of the burn site, cross-contamination of MRD strains of *Pseudomonas* and colonizing of MDR strains is more likely. In spite of the ubiquitous nature of this microorganism and the frequency with which it is encountered, most human hosts counteract the infectious process effectively via the innate immune system. A more detailed molecular and cellular understanding of the bacterial and host factors is crucial to an overall comprehension of the pathogenic process of *Pseudomonas*, and will be of increasing importance to the development of preventative strategies to be sought for this major human pathogen. Selection of multi-drug resistant *Pseudomonas* in burn centers can be facilitated through transmission from person to person as well as extensive applications of antipseudomonal antibiotics. To overcome inappropriate treatment of burn patients infected with *P. aeruginosa*, periodical antibacterial susceptibility surveys for the bacteria isolated from burn patient are warranted.

Conflict of interest: None declared.

References

1 Krieg, Noel *Bergey's Manual of Systematic Bacteriology, Volume* 1. Baltimore: Williams & Wilkins, 1984.

2 Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* 2000;**50**:1563-89. [1093 9664]

3 Franzetti F, Cernuschi M, Esposito R, Moroni M. *Pseudomonas* infections in patients with AIDS and AIDS related complex. *J Intern Med* 1992;**231**:437-43. [1588272]

4 Kielhofner M, Atmar RL, Hamill RJ, Musher DM. Life-threatening *Pseudomonas aeruginosa* infections inpatients with human immunodeficiency virus infection. *Clin Infect Dis* 1992;**14**:403-11. [1554824]

5 Bendig JW, Kyle PW, Giangrande PL, Samson DM, Azadian BS. Two neutropenic patients with multiple resistant *Pseudomonas aeruginosa* septicaemia treated with ciprofloxacin. *J R Soc Med* 1987;**80**:316-7. [3112380]

6 Chitkara YK, Feierabend TC. Endogenous and exogenous infection with *Pseudomonas aeruginosa* in a burns unit. *Int Surg* 1981;**66**:237-40. [6797982]

7 Phillips LG, Heggers JP, Robson MC, Boertman JA, Meltzer T, Smith DJ Jr. The effect of endogenous skin bacteria on burn wound infection. *Ann Plast Surg* 1989;**23**:35-8. [2764460] [doi:10.1097/00000637-198907000-00007]

8 Hsueh PR, Teng LJ, Yang PC, Chen YC, Ho SW, Luh KT. Persistence of a multidrug-resistant *Pseudomonas aeruginosa* clone in an intensive care burn unit. *J Clin Microbiol* 1998;**36**:1347-51. [9574703]

9 Japoni A, Farshad S, Alborzi A, Kalani M, Mohamadzadegan R. Comparison of arbitrarily primed-polymerase chain reaction and plasmid profiles typing of *Pseudomonas aeruginosa* strains from burn patients and hospital environment. *Saudi Med J* 2007;**28**:899-903. [17530107]

10 Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 1995;**268**: 1899-902. [7604262] [doi:10.1126/science. 7604262]

11 Sato H, Okinaga K, Saito H. Role of pili in the pathogenesis of *Pseudomonas aeruginosa* burn infection. *Microbiol Immunol* 1988;**32**:131-9. [2897617]

12 Pavlovskis OR, Wretlind B. Assessment of protease (elastase) as a *Pseudomonas aeruginosa* virulence factor in experimental mouse burn infection. *Infect Immun* 1979;**24**:181-7. [110690]

13 Bejarano PA, Langeveld JP, Hudson BG, Noelken ME. Degradation of basement membranes by *Pseudomonas aeruginosa* elastase. *Infect Immun* 1989;**57**:3783-7. [2509368]

14 Kharazmi A, Nielsen H. Inhibition of human monocyte chemotaxis and chemiluminescence by *Pseudomonas aeruginosa* elastase. *APMIS* 1991;**99**:93-5. [1899578]

15 Rumbaugh KP, Griswold JA, Hamood AN. Contribution of the regulatory gene lasR to the pathogenesis of *Pseudomonas aeruginosa* infection of burned mice. *J Burn Care Rehabil* 1999;**20**:42-9. [9934636]

16 Rumbaugh KP, Griswold JA, Iglewski BH, Hamood AN. Contribution of quorum sensing to the virulence of *Pseudomonas aeruginosa* in burn wound infections. *Infect Immun* 1999;**67**:5854-62. [10531240]

17 Ostroff RM, Vasil ML. Identification of a new phospholipase C activity by analysis of an insertional mutation in the hemolytic phospholipase C structural gene of *Pseudomonas aeruginosa*. *J Bacteriol* 1987;**169**:4597-601. [2820937]

18 Sokol PA. Surface expression of ferripyochelin-binding protein is required for virulence of *Pseudomonas aeruginosa*. *Infect Immun* 1987;**55**:2021-5. [3114141]

19 Goldberg JB, Coyne MJ Jr, Neely AN, Holder IA. A virulence of a *Pseudomonas aeruginosa algC* mutant in a burned-mouse model of infection. *Infect Immun* 1995;**63**:4166-9. [7558335]

20 Nicas TI, Iglewski BH. Contribution of exoenzyme S to the virulence of *Pseudomonas aeruginosa*. Antibiot Chemother 1985;**36**:40-8. [2988426]

21 Bowen-Jones JR, Coovadia YM, Bowen-Jones EJ. Infection control in a Third World burn facility. *Burns* 1990;**16**:445-8. [2073344] [doi:10.1016/0305-4179(90)90075-8]

22 Felts AG, Giridhar G, Grainger DW, Slunt JB. Efficacy of locally delivered polyclonal immunoglobulin against *Pseudomonas aeruginosa* infection in a murine burn wound model. *Burns* 1999;**25**:415-23. [10439150] [doi:10.1016/S0305-4179(99)00017-0]

23 Jeyapaul J, Mehta LN, Arora S, Antia NH. Fc and complement receptor integrity of polymorphonuclear (PMN) cells following thermal injury. *Burns Incl Therm Inj* 1984;**10**:387-95. [6434163] [doi:10.1016/0305-4179(84)90077-9]

24 Deitch EA, Dobke M, Baxter CR. Failure of local immunity. A potential cause of burn wound sepsis. *Arch Surg* 1985;**120**:78-84. [3917665]

25 Engel J, Balachandran P. Role of *Pseudomonas aeruginosa* type III effectors in disease. *Curr Opin Microbiol* 2009;**12**:61-6. [19168385] [doi:10.1016/j.mib.2008.12.007]

26 Deng Q, Zhang Y, Barbieri JT. Intracellular trafficking of *Pseudomonas* ExoS, a type III cytotoxin. *Traffic* 2007;**8**:1331-45. [17714437] [doi:10.1111/j.1600-0854.2007.00626.x]

27 Jørgensen R, Wang Y, Visschedyk D, Merrill AR. The nature and character of the transition state for the ADP-ribosyltransferase reaction. *EMBO Rep* 2008;**9**:802-9. [18583986] [doi:10.1038/embor.2008.90]

28 Kohno K, Uchida T, Mekada E, Okada Y. Characterization of diphtheria-toxin-resistant mutants lacking receptor function or containing nonribosylatable elongation factor 2. *Somat Cell Mol Genet* 1985;**11**:421-31. [3862242] [doi:10.1007/BF01534836]

29 Wolf P, Elsässer-Beile U. *Pseudomonas* exotoxin A: from virulence factor to anti-cancer agent. *Int J Med Microbiol* 2009;**299**:161-76. [18948059] [doi:10.1016/j.ijmm.2008.08.003]

30 Moller PC, Evans MJ, Fader RC, Henson LC, Rogers B, Heggers JP. The effect of anti-exotoxin A on the adherence of *Pseudomonas aeruginosa* to hamster tracheal epithelial cells in vitro. *Tissue Cell* 1994;**26**:181-8. [8023323] [doi:10.1016/0040-8166(94)90093-0]

31 Agnihotri N, Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms: five-year study. *Burns* 2004;**30**:241-3. [15082351] [doi:10.1016/j.burns.2003.11.010]

32 Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. *Burns* 2004;**30**:357-61. [15145194] [doi:10.1016/j.burns.2003.12.013]

33 Pedersen SS, Koch C, Hoiby N, Rosendal K. An epidemic spread of multiresistant *Pseudomonas aeruginosa* in a cystic fibrosis centre. *J Antimicrob Chemother* 1986;**17**:505-16. [3086274] [doi:10.1093/jac/17.4.505]

34 Cheng K, Smyth RL, Govan JR, Doherty C, Winstanley C, Denning N, Heaf DP, van Saene H, Hart CA. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. *Lancet* 1996;**348**:639-42. [8782753] [doi:10.1016/S0140-6736(96)05169-0]

35 Lechtzin N, John M, Irizarry R, Merlo C, Diette GB, Boyle MP. Outcomes of adults with cystic fibrosis infected with antibiotic-resistant *Pseudomonas aeruginosa*. *Respiration* 2006;**73**:27-33. [16113513] [doi:10.1159/000087686]

36 Lang AB, Rudeberg A, Schoni MH, Que JU, Furer E, Schaad UB. Vaccination of cystic fibrosis patients against *Pseudomonas aeruginosa* reduces the proportion of patients infected and delays time to infection. *Pediat Infect Dis J* 2004;**23**:504-10. [15194830] [doi:10.1097/01.inf.0000129688.50588.ac]

37 Pennington JE, Reynolds HY, Wood RE, Robinson RA, Levine AS. Use of a *Pseudomonas aeruginosa* vaccine in patients with acute leukemia and cystic fibrosis. Am J Med 1975;**58**:629-36. [805535] [doi:10.1016/0002-9343(75)90498-2]

38 Manafi A, Kohanteb J, Mehrabani D, Japoni A, Amini M, Naghmachi M, Zaghi AH, Khalili N. Active immunization using exotoxin A confers protection against *Pseudomonas aeruginosa* infection in a mouse burn model. *BMC Microbiol* 2009;**9**:23. [19183501] [doi:10.1186/1471-2180-9-23]

39 Cripps AW, Peek K, Dunkley M, Vento K, Marjason JK, McIntyre ME, Sizer P, Croft D, Sedlak-Weinstein L. Safety and immunogenicity of an oral inactivated whole-cell *Pseudomonas aeruginosa* vaccine administered to healthy human subjects. *Infect Immun* 2006;**74**:968-74. [16428742] [doi:10.1128/IAI.74.2.968-974.2006]

40 Döring G, Pier GB. Vaccines and immunotherapy against *Pseudomonas aeruginosa*. *Vaccine* 2008;**26**:1011-24. [18242792] [doi:10.1016/j.vaccine.2007.12.007]

41 Heggers JP, Hawkins H, Edgar P, Villarreal C, Herndon DN. Treatment of infections in burns. *In:* Herndon DN. Total burn care. WB Saunders, London, England. 2002; pp 120-169.

42 Murphy KD, Lee JO, Herndon DN. Current pharmacotherapy for the treatment of severe burns. *Expert Opin Pharmacother* 2003;**4**:369-84. [12614189] [doi:10.1517/14656566.4.3.369]

43 Altoparlak U, Erol S, Akcay MN, Celebi F, Kadanali A. The time related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. *Burns* 2004;**30**:660-4. [15475138] [doi:10.1016/j.burns.2004.03.005]

44 Elliott TS, Lambert PA. Antibacterial resistance in the intensive care unit: mechanisms and management. *Br Med Bull* 1999;**55**:259-76. [10 695090] [doi:10.1258/0007142991902240]

45 Namias N, Samiian L, Nino D, Shirazi E, O'Neill K, Kett DH, Ginzburg E, McKenney MG, Sleeman D, Cohn SM. Incidence and susceptibility of pathogenic bacteria vary between intensive care units within a single hospital: implications for empiric antibiotic strategies. *J Trauma* 2000;**49**:638-45. [11038080] [doi:10. 1097/00005373-200010000-00010]

46 Lansdown AB. Silver. 1. Its antibacterial properties and mechanism of action. *J Wound Care* 2002;**11**:125-30. [11998592]

47 Lansdown AB, Williams A, Chandler S, Benfield S. Silver absorption and antibacterial efficacy of silver dressings. *J Wound Care* 2005;**14**:155-60. [15835225]

48 Lansdown AB. Silver. 2. Toxicity in mammals and how its products aid wound repair. *J Wound Care* 2002;**11**:173-7. [12055941]

49 Boyce ST, Supp AP, Swope VB, Warden GD. Topical sulfamylon reduces engraftment of cultured skin substitutes on athymic mice. *J Burn Care Rehabil* 1990;**20**:33-6. [9934634]

50 Cooper ML, Boyce ST, Hansbrough JF, Foreman TJ, Frank DH. Cytotoxicity to cultured human keratinocytes of topical antimicrobial agents. *J Surg Res* 1990;**48**:190-5. [2314091] [doi:10.1016/0022-4804(90)90212-K]

51 Atiyeh BS, Dham R, Kadry M, Abdallah AF, Al-Oteify M, Fathi O, Samir A. Benefit-cost analysis of moist exposed burn ointment. *Burns* 2002;**28**:659-63. [12417161] [doi:10. 1016/S0305-4179(02)00075-X]

52 Atiyeh BS, Ioannovich J, Magliacani Masellis GM, Costagliola M, Dham R, Al-Farhan M. Efficacy of moist exposed burn ointment in the management of cutaneous wounds and ulcers: a multicenter pilot study. *Ann Plast Surg* 2002;**48**:226-7. [119 10244] [doi:10.1097/00000637-20 0202000-00032]

53 Atiyeh, BS, El-Musa KA, Dham R. Scar quality and physiologic barrier function restoration after moist and moist-exposed dressings of partial-thickness wounds. *Dermatol Surg* 2003;**29**:14-20. [12534506] [doi:10. 1046/j.1524-4725.2003.29002.x]

54 Klasen HJ. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. *Burns* 2000;**26**:131-8. [1071 6355] [doi:10.1016/S0305-4179 (99)00116-3]

55 Herruzo-Cabrera R, Garcia-Torres V, Rey-Calero J, Vizcaino-Alcaide MJ. Evaluation of the penetration strength, bactericidal efficacyand spectrum of action of several antimicrobial creams against isolated microorganisms in a burn centre. *Burns* 1992;**18**:39-44. [1558672] [doi:10.1016/0305-4179(92)90118-E]

56 Stefanides MM Sr, Copeland CE, Kominos SD, Yee RB. *In vitro* penetration of topical antiseptics through eschar of burn patients. *Ann Surg* 1976;**183**:358-64. [1267492]

57 MacMillan BG, Hill EO, Altemeier WA. Use of topical silver nitrate, mafenide, and gentamicin in the burn patient. A comparative study. *Arch Surg* 1967;**95**:472-81. [4962314]

58 Heggers JP, Robson MC, Herndon DN, Desai MH. The efficacy of nystatin combined with topical microbial agents in the treatment of burn wound sepsis. *J Burn Care Rehabil* 1989;**10**:508-11. [2600098] [doi:10.1097/00004630-198911000-00009]

59 Wright JB, Lam K, Hansen D, Burrell RE. Efficacy of topical silver against fungal burn wound pathogens. *Am J Infect Control* 1999; **27**:344-50. [10433674] [doi:10.1016/ S0196-6553(99)70055-6]

60 Choban, PS, Marshall WJ. Leukopenia secondary to silver sulfadiazine: frequency, characteristics and clinical consequences. *Am Surg* 1987;**53**:515-7. [3631764]

61 Fuller FW, Engler PE. Leukopenia in non-septic burn patients receiving topical 1% silver sulfadiazine cream therapy: a survey. *J Burn Care Rehabil* 1988;**9**:606-9. [3220867] [doi:10.1097/00004630-198811000-00006]

62 Pirnay JP, De Vos D, Cochez C, Bilocq F, Pirson J, Struelens M, Duinslaeger L, Cornelis P, Zizi M, Vanderkelen A. Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. *J Clin Microbiol* 2003;**41**:1192-202. [12624051] [doi:10.1128/JCM.41.3. 1192-1202.2003]

63 Garner JP, Heppell PS. The use of Flammacerium in British Burns Units. *Burns* 2005;**31**:379-82. [157 74299] [doi:10.1016/j.burns.2004. 12.001]

64 Garner JP, Heppell PS .Cerium nitrate in the management of burns. *Burns* 2005;**31**:539-47. [15955636] [doi:10.1016/j.burns.2005.01.014]

65 Falcone PA, Harrison HN, Sowemimo GO, Reading GP. Mafenide acetate concentrations and bacteriostasis in experimental burn wounds treated with a three-layered laminated mafenide-saline dressing. *Ann Plast Surg* 1980;**5**:266-9. [6985504] [doi:10.1097/00000637-198010000-00003]

66 Boyce ST, Supp AP, Swope VB, Warden GD. Topical sulfamylon reduces engraftment of cultured skin substitutes on athymic mice. *J Burn Care Rehabil* 1999;**20**:33-6. [9934634]

67 Dunn K, Edwards-Jones V. The role of Acticoat with nanocrystallinesilver in the management of burns. *Burns* 2004;**30**:S1-9. [15327800] [doi:10. 1016/S0305-4179(04)90000-9]

68 Tredget EE, Shankowsky HA, Groeneveld A, Burrell R. A matched-pair, randomized study evaluating the efficacy and safety of Acticoat silver-coated dressing for the treatment of burn wounds. *J Burn Care Rehabil* 1998;**19**:531-7. [9848045] [doi:10.1097/00004630-199811000-00013]

69 Yin HQ, Langford R, Burrell RE. Comparative evaluation of the antimicrobial activity of ACTICOAT antimicrobial barrier dressing. *J Burn Care Rehabil* 1999;**20**:195-200. [10342470] [doi:10.1097/00004630-199905000-00006]

70 Heggers, J, Goodheart RE, Washington J, McCoy L, Carino E, Dang T, Edgar P, Maness C, Chinkes D. Therapeutic efficacy of three silver dressings in an infected animal model. *J Burn Care Rehabil* 2005;**26**:53-6. [15640735] [doi:10.10 97/01.BCR.0000150298.57472.26]

71 Vasishta R, Saxena M, Chhibber S. Contribution of silver ion resistance to the pathogenicity of *Pseudomonas aeruginosa* with special reference to burn wound sepsis. *Folia Microbiol (Praha)* 1991;**36**:498-501. [1821876] [doi:10.1007/BF0288 4072]

72 Daikos GL, Lolans VT, Jackson GG. Alterations in outer membrane proteins of *Pseudomonas aeruginosa* associated with selective resistance to quinolones. *Antimicrob Agents Chemother* 1988;**32**:785-7. [313 4852]

73 Bridges K, Kidson A, Lowbury EJ, Wilkins MD. Gentamicin and silver-resistant pseudomonas in a burns unit. *Br Med J* 1979;**1**:446-9. [10 6914] [doi:10.1136/bmj.1.6161.446]

74 Hendry AT, Stewart IO. Silver-resistant Enterobacteriaceae from hospital patients. *Can J Microbiol* 1979;**25**:915-21. [526888]

75 Rosenkranz HS, Coward JE, Wlodkowski TJ, Carr HS. Properties of silver sulfadiazine resistant *Enterobacter cloacae*. *Antimicrob Agents Chemother* 1974;**5**:199-201. [4840432]

76 Japoni A, Hayati M, Farshad SH, Abbasia SA. *In vitro* Susceptibility of *Pseudomonas aeruginosa* Isolated

from a Burn Center to Silver Sulfadiazine and Silver Nitrate in Shiraz, South of Iran. *Iran J Med Sci* 2005;**30**:63-7.

77 Jalal S, Wretlind B. Mechanisms of quinolone resistance in clinical strains of *Pseudomonas aeruginosa*. *Microb Drug Resist* 1998; **4**:257-61. [9988043] [doi:10.1089/mdr.1998.4.257]

78 Livermore DM. beta-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995;**8**:557-84. [8665470]

79 Poole K. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. *J Mol Microbiol Biotechnol* 2001;**3**:255-64. [11321581]

80 Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXYoprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2000;**44**:3322-7. [11083635] [doi:10.1128/AAC.44.12.3322-3327.2000]

81 Woodruff WA, Hancock RE. Construction and characterization of *Pseudomonas aeruginosa* protein F-deficient mutants after in vitro and in vivo insertion mutagenesis of the cloned gene. *J Bacteriol* 1988;**170**:2592-8. [2836364]

82 Mayer KH. Review of epidemic aminoglycoside resistance world wide. *Am J Med* 1986;**80**:56-64. [3089005] [doi:10.1016/0002-9343(86)90480-8]

83 Studemeister AE, Quinn JP. Selective imipenem resistance in *Pseudomonas aeruginosa* associated with diminished outer membrane permeability. *Antimicrob Agents Chemother* 1988;**32**:1267-8. [2461164]

84 Ochs MM, McCusker MP, Bains M, Hancock RE. Negative regulation of the *Pseudomonas aeruginosa* outer membrane porin OprD selective for imipenem and basic amino acids. *Antimicrob Agents Chemother* 1999; **43**:1085-90. [10223918]

85 Lee A, Mao W, Warren MS, Mistry A, Hoshino K, Okumura R, Ishida H, Lomovskaya O. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. *J Bacteriol* 2000; **182**:3142-50. [10809693] [doi:10.1128/JB.182.11.3142-3150.2000]

86 Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 2000;**288**: 1251-4. [10818002] [doi:10.1126/science.288.5469.1251]

87 Vahaboglu H, Ozturk R, Aygun G, G, Coşkunkan F, Yaman A, Kay-gusuz A, Leblebicioglu H, Balik I, Aydin K, Otkun M. Widespread detection of PER-1–type extended-spectrum β-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. *Antimicrob Agents Chemother* 1997;**41**:2265-9. [9333059]

88 Naas T, Nordmann P. OXA-type β-lactamases. *Curr Pharm Des* 1999;**5**:865-79. [10539993]

89 Poirel L, Girlich D, Naas T, Nordmann P. OXA-28, an extended-spectrum variant of OXA-10 β-lactamase from *Pseudomonas aeruginosa* and its plasmid and integral in located gene. *Antimicrob Agents Chemother* 2001; **45**:447-53. [11158739] [doi:10.1128/AAC.45.2. 447-453.2001]

90 Danel F, Hall LM, Duke B, Gur D, Livermore DM. OXA-17, a further extended-spectrum variant of OXA-10 β-lactamase, isolated from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; **43**:1362-6. [10348753]

91 Livermore DM, Woodford N. Carbapenemases: a problem in waiting? *Curr Opin Microbiol* 2000;**3**: 489-95. [11050448] [doi:10.1016/S1369-5274(00)00128-4]

92 Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K, Kato N, Ohta M. Multifocal outbreaks of metallo-β-lactamase–producing *Pseudomonas aeruginosa* resistant to broad-spectrum β-lactams, including carbapenems. *Antimicrob Agents Chemother* 1996; **40**:349-53. [8834878]

93 Gibb AP, Tribuddharat C, Moore RC, Louie TJ, Krulicki W, Livermore DM, Palepou MF, Woodford N. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new *bla*IMP allele, *bla*IMP-7. *Antimicrob Agents Chemother* 2002; **46**:255-8. [11751148] [doi:10.1128/AAC.46.1.255-258.2002]

94 Lauretti L, Riccio ML, Mazzariol A, Lauretti L, Fontana R, Rossolini GM. Cloning and characterization of *bla*VIM, a new integrin-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. *Antimicrob Agents Chemother* 1999;**43**:1584-90. [10390207]

95 Arakawa Y, Murakami M, Suzuki K, Ito H, Wacharotayankun R, Ohsuka S, Kato N, Ohta M. A novel integron-like element carrying the metallo-β-lactamase gene *bla*IMP. *Antimicrob Agents Chemother* 1995; **39**:1612-5. [7492116]

96 Japoni A, Alborzi A, Kalani M, Nasiri J, Hayati M, Farshad S. Susceptibility patterns and cross-resistance of antibiotics against *Pseudomonas aeruginosa* isolated from burn patients in the South of Iran. *Burns* 2006;**32**:343-7. [16527415] [doi:10.1016/j.burns.2005.10.017]

97 Ekrami A, Kalantar E. Bacterial infections in burn patients at a burn hospital in Iran. *Indian J Med Res* 2007;**126**:541-4. [18219081]

98 Rastegar Lari A, Bahrami Honar H, Alaghehbandan R. *Pseudomonas* infections in Tohid Burn Center, Iran. *Burns* 1998;**24**:637-41. [9882062] [doi:10.1016/S0305-4179(98) 00090-4]

99 Estahbanati HK, Kashani PP, Ghanaatpisheh F. Frequency of *Pseudomonas aeruginosa* serotypes in burn wound infections and their resistance to antibiotics. *Burns* 2002;**28**:340-8. [12052372] [doi:10.1016/S0305-4179(02)00024-4]

100 Tsakris A, Pournaras S, Woodford N, Palepou MF, Babini GS, Douboyas J, Livermore DM. Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-1 carbapenemase in Greece. *J Clin Microbiol* 2000;**38**:1290-2. [10699045]

101 Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa:* comparison of risks associated with different antipseudomonal agents. *Antimicrob Agents Chemother* 1999;**43**:1379-82. [10348756]

102 Livermore DM. Of *Pseudomonas,* porins, pumps and carbapenems. *J Antimicrob Chemother* 2001;**47**:247-50. [11222556] [doi:10.1093/jac/47.3.247]

103 Levin AS, Barone AA, Penco J, MV, Marinho IS, Arruda EA, Manrique EI, Costa SF. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin Infect Dis* 1999;**28**:1008-11. [10452626] [doi:10.1086/514732]

104 Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, Blais J, Cho D, Chamberland S, Renau T, Leger R, Hecker S, Watkins W, Hoshino K, Ishida H, Lee VJ. Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa:* novel agents for combination therapy. *Antimicrob Agents Chemother* 2001;**45**:105-16. [11120952] [doi:10.1128/AAC.45.1.105-116.2001]

105 Hammond GG, Huber JL, Greenlee ML, Laub JB, Young K, Silver LL, Balkovec JM, Pryor KD, Wu JK, Leiting B, Pompliano DL, Toney JH. Inhibition of IMP-1 metallo-β-lactamase and sensitization of IMP-1–producing bacteria by thioester derivatives. *FEMS Microbiol Lett* 1999;**179**:289-96. [10518728]