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Properties and Prevention: A Review of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is responsible for most nosocomial infections in the United States. This number approximates 51,000 cases each year. Individuals at risk of infection by *P. aeruginosa* are those that are exposed to hospital equipment that has not undergone proper sterilization (i.e., catheters, mechanical ventilation, etc.) Furthermore, certain *P. aeruginosa* strains mutate or endogenously produce β-Lactamase that provides resistance to penicillins. β-Lactamase disrupts the center atomic structure of several antibiotics including Penicillins, Cephalosporins, Monobactams, Carbapenems. Other mechanisms that provide intrinsic and obtained resistance to Penicillins include the genetically encoded efflux pumps; studied and assumed, act as transmembrane proteins assisting in the secretion of toxic material. Mutations influencing gene expression of *P. aeruginosa* may provide enough support to provide immunity to antimicrobials. These include depression of specific genes resulting in the production of extended spectrum β-Lactamase. The increase in immunity to penicillin and other antibiotics is an important factor in the length of a patient's hospital stay and mortality rate. Bacterial conjugation leads to an increase in antibiotic resistance even to the point that some specific strains of *P. aeruginosa* are immune to all penicillins. The illumination of *P. aeruginosa* has led to the implementation of many preventative measures and pre planned steps to fight nosocomial outbreaks. These measures of control have been stated to work well by some and their efficacy tested by others. This review attempts to address these resistance mechanisms and discuss the effectiveness of the preventative measures used today.

Keywords: *Pseudomonas aeruginosa*; Nosocomial infections

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Introduction

P. aeruginosa, a proteobacteria is a member of the Pseudomonadaceae family. Discovered in 1882 by the French bacteriologist and chemist Carle Gessard, this gram-negative bacteria structure includes a .5-.8 µm by 1.5-3 µm rod shape utilizing one flagellum for mobilization. Individualizing itself from most gram-negative bacteria, *P. aeruginosa* is positive in an oxidase reaction. Moreover, *P. aeruginosa* is permanently unable to ferment lactose. *P. aeruginosa* is found in water, plants, soil, and on the epidermis of animals. In nature, it is commonly found as a plankton swimming through water or as a biofilm, clusters of bacteria sharing the same phenotype and common chemical properties [1]. Uniquely, *P. aeruginosa* can thrive and survive in a variety of temperature and infrequent nutrition. The bacterium has been observed in previous studies to grow in distilled water giving *P. aeruginosa* an advantage in adapting to changing environments [2]. Being an opportunistic pathogen, *P. aeruginosa* requires a lack of immunity to infect its host [3]. Moreover, this is the explanation as to why *P. aeruginosa* is such a sizeable nosocomial threat for patients with ventilation machines, cancers, and burns. Colonization of *P. aeruginosa* in the respiratory tract is associated with sepsis and death. Any patient that is immunosuppressed or has experienced significant amounts of trauma are at risk for the colonization of an infection. The mortality rate approximates 50% for said patients who obtain an infection [4]. *P. aeruginosa* has an acute ability to destroy human macrophages utilizing the production of exotoxin A to regress the uptake of 3H-Thymidine by cells, thus giving *P. aeruginosa* its high toxicity [5]. In the past infections of *P. aeruginosa* have been treated by a combination of β-Lactams (i.e., penicillin) a group of antibiotics classified by its four-membered ring. These bactericidal antibiotics actions

focus on the cell wall constructing enzymes, transpeptidase and carboxypeptidase. The actions of penicillin-binding proteins thus retard the synthesis of peptidoglycan, inhibiting the cell's ability to construct the cell wall [6]. Hydrophilic drugs like these β-lactams can pass through the complex outer membrane, so characteristic of *P. aeruginosa* and other gram-negative bacteria, by way of small pores in said membrane. These are referred to as porins and play an essential role in resistance to antimicrobials. The mechanisms highlighted in this review are intended to express the acute ability of *P. aeruginosa* to develop resistance to antibiotics. Therefore, stressing the importance of preventative measures and developing new treatments for this bacterium.

Literature Review

Mechanisms of defense

Membrane mediated defense: The membrane of the gram-negative opportunistic pathogen *P. aeruginosa* is quite complex. Due to the harsh environments it is so commonly found in, the bacterium has built a very protective membrane. Mutations and natural *P. aeruginosa* strains that have more permeable membranes become extremely susceptible to antibiotics; wild-type Z61 is a strain that is susceptible to β-lactams, aminoglycosides, rifampin, tetracycline, and chloramphenicol [7]. Moreover, studies have concluded that the removal of the outer membrane can lead to a higher susceptibility to antibiotics [8]. However, these studies have not provided enough evidence that this is noteworthy as the same properties have been documented in other bacteria [9]. It is of significance to note how extreme the selectivity of the outer membrane of *P. aeruginosa* in comparison to other gram-negative bacteria, due to the lack of open channels [10]. The membrane is filled with efflux pumps which can combat the influx of antimicrobial agents because of the highly selective membrane, this topic will be surpassed for now but touched on later. The removal of the porins in studies have shown a significant decrease in minimum inhibitory concentration [11]. A good portion of this high selectivity can be attributed to the porin acting proteins OprF. Opr Fis unique in that unlike other gram-negative bacteria, in that it is not like OmpF/OmpC trimeric porins. Instead, OprF is homologous to OprA in *Escherichia coli.* Opr F is used for structure, which is why it is not nearly as permeable. OprA and OprF phenotypically show similarities in their ability to form small porins and hydrophobic residues and aromatic residues in their structure. However, OprF has the ability also to form larger porins than OprA and the residues found in OprF are smaller, suggesting the ability of transporting ions [12,13]. OprD in *P. aeruginosa,* whose primary purpose is the diffusion of amino acids, is the entryway abused by carbapenems. Therefore, it is not surprising when the lack of OprD is a typical quality of multi-antibiotic resistant *P. aeruginosa* [14,15]. Myriad other proteins are expressed in the outer membrane of *P. aeruginosa,* but these proteins are not always associated with resistance to antibiotics [16].

Efflux pump mediated resistance: In addition, another resistance attribute to the mechanisms of *P. aeruginosa* are its efflux pumps, proteins found in nearly all bacteria that function to deport antimicrobials. Efflux pumps can be categorized into five families. Major facilitator (MF) multidrug and toxic efflux (MATE) resistance-nodulation-division (RND) small multidrug resistance (SMR) and ATP binding cassette (ABC). All these families have been studied and shown to be found within *P. aeruginosa.* However, of these pumps, RND is the most common in *P. aeruginosa* [17]. In a study conducted by Department of Microbiology, Kyoto Pharmaceutical University, researchers investigated the properties of three efflux pumps identified in *P. aeruginosa*. Mutants overexpressing *MexCD-OprJ* and *MexXY-OprM* were shown to expunge Penicillins, excluding carbenicillin and sulbenicillin as they have a negative charge that differentiates them from other penicillins [18]. More studies suggest that *MexAB-OprM* play a major role in intrinsic and acquired resistance for β-lactams including penicillins [19]. Overexpression of *MexAB-OprM* can be attributed to *mexR* mutations that terminate the repressor, as well as the repressor nalC or mutations to the gene *PA3721* [20,21]. The overexpression of *MexAB-OprM* as shown to lead to an increase in the expulsion of penicillins [21]. Moreover, the *MexAB-OprM* efflux pump has the largest range of substrates concerning β-lactams [21,22]. *MexCD-OprJ* is regulated by the *nfxB* gene. The *nfxB* gene negatively controls the expression of *MexCD-OprJ* [22]. Studies have been done on the RND *MexCD-OprJ* and have concluded that it does not contribute to the intrinsic resistance to antibiotics [23,24]. However, *nfxB* depression, increasing expression of *MexCD-OprJ*, will induce hypersensitivity to some β-lactams. Some studies do show that *MexCD-OprJ* will extrude most penicillins, apart from carbenicillin and sulbenicillin [19]. *MexCD-OprJ* and *MexAB-OprM* are only two of the twelve RND found within *P. aeruginosa*. The *MexXY* RND efflux pump is lacking a gene that encodes for an outer membrane protein. It, therefore, has been studied and found to interact with *OprM* and can function [25,26]. As stated above experiments have shown that overexpression of *MexXY-OprM* can extrude penicillins [18]. Typically, one can expect to observe the RND transporter in combination with two other complexes, periplasmic membrane fusion protein (MFP) and an outer membrane factor (OMF) [17]. The entire structure can cross the membrane. Thus, bestowing the pumps ability to extrude toxins. There are two genes in each operon that code for MFP and RND. Only half of the 12 operons code for the OMF [22]. These RND pumps explain why many strains of *P. aeruginosa* have such intrinsic resistance to antibiotics compared to other gram-negative bacteria.

Biofilm mediated resistance: It is not uncommon to encounter the bacteria *P. aeruginosa* in a colony or a biofilm. Biofilms consistently increase immunity to antibiotics when compared to their planktonic counterparts. For some bacteria, the resistance is increased a thousand-fold [27]. In a study conducted, colonies of *P. aeruginosa* were exposed to many drugs, including penicillins, in this study piperacillin was used. Results showed that no one colony was completely resistant to the penicillins tested. However, resistance was in fact increased throughout the sample. Planktonic *P. aeruginosa* was found to have developed approximately a 25 percent resistance to piperacillin, where *P. aeruginosa* that had developed a biofilm had acquired roughly a 50 percent resistance to piperacillin [28]. Thus, a positive correlation between biofilm formation and resistance was concluded. One complication does arise when examining the

effects of penicillins and other β-lactam antibiotics on colonies of *P. aeruginosa.* As the function of β-lactams is to inhibit the ability of growing cells to produce the peptidoglycan. The biofilm must be actively growing to measure the effects of the β-lactam on said biofilm [29]. Therefore, some studies conclude that there is no relationship between the presence of biofilms and the increase of antibiotic resistance. Some argue that other mechanics might be at play, just left undiscovered [30]. There is evidence that correlation exists between biofilms of *P. aeruginosa* and the increased expression of hypothetical efflux pumps and other proteins that resist antibiotics that are yet to be discovered [31-33]. Again, conclusions of causation are challenging to reach so that no definite statements can be made now. Biofilms of *P. aeruginosa* (BPA) related disease is an increasing issue as the dynamic properties of BPA are still left unknown [30]. It is an important matter to understand, as there have been links to increase in antibiotic-resistant biofilm colonies in patients with cystic fibrosis and chronic obstructive pulmonary disease. One of the explanations to *P. aeruginosa* high mortality rate in these patients [34]. Links of BPA and adaptations to enhance chances of success of infection in urinary tract conditions have been correlated. Explaining why BPA can cause complicated urinary tract infections [35]. Unrelated to the antibiotic resistance of *P. aeruginosa,* but relevant to note, biofilms are responsible for the spread of many nosocomial infections. These biofilms cluster on medical equipment used in patient care by mechanisms that are yet to be understood, adding to the lethality of *P. aeruginosa* [36].

OXA type: A major weapon in the arsenal of *P. aeruginosa* is β-lactamase [37]. These enzymes are recognized by their ability to hydrolyze the amides and esters located within and around the four-membered β-lactam ring of β-lactam antibiotics [38]. β-lactam antibiotics can stop the growth of prokaryotes that construct a peptidoglycan layer. The substrates of these antibiotics are the penicillin-binding proteins, DD-transpeptidase and DD-carboxypeptidase [39,40]. These antimicrobials include, but are not limited to, penicillins, cephalosporins, penems, carbapenems, and monobactams. These are commonly used against *P. aeruginosa* as well as other gram-negative bacteria. The most common category β-lactamase found in *P. aeruginosa* is the OXA-type, referred to as oxacillinase. Most of this family of enzymes share the characteristic of having chlorine inhibit its ordinary actions with β-lactams [41]. PER-type and extended spectrum β-lactamases and in rare cases, strains of *P. aeruginosa* have included some β-lactamase from the TEM type are encoded by *P. aeruginosa* [42-44]. The most elementary form of classification of these β-lactamase enzymes is by their primary structure of amino acids [45,46]. There are several groups including A, B, C, and D. It was originally thought that class D OXA β-lactamase (OTBL) has substrates limited to only penicillins, and contrary to their Class A counterparts, were able to hydrolyze these penicillins including oxacillin [47]. In later studies, OTBL was found to have become able to develop resistance to other antibiotics. Identifying a crucial testament to the antimicrobial resistance properties of *P. aeruginosa,* several extended spectrum ESBLs have been isolated within the bacteria. These include different derivatives of OXA 10 β-lactamase; OXA-13, OXA-14, OXA-16, OXA-17, OXA-19, and OXA-28 [48]. Note that the numbers identifying the strain of oxilliase reference the order in which they were discovered. The numbers do not distinguish similarities between oxacillinase. These provide defenses to a multitude of antibiotics specifically their profound resistance to all penicillins [49]. Epidemiology studies have shown that these ESBLs from OXA β-lactamase are primarily found only is *P. aeruginosa* [50]. OXA 40 and its derivatives OXA 25, OXA 24, have been found to have provided profound β-lactam resistance in *P. aeruginosa*, namely penicillins. Uniquely there seems to be a crystalline structure to OXA 40, explaining its substrate specificity to penicillins [51]. It has been found to act weakly against other β-lactams like cephalosporins.

TEM type: Another β-lactamase on the ESBL spectrum is TEM. This ESBL was first discovered in the 1960s in Greece. TEM was extracted from a patient named Temoniera, giving the ESBL its name TEM. TEM is the most common plasmid mediated strain of the ESBLs found is gram-negative bacteria [52,53]. Initially discovered in *E. Coli*, the plasmid-mediated properties of this ESBL have allowed it to spread to other species. Including *P. aeruginosa* and other strains of bacteria of the *Enterobacteriaceae* family. Despite the commonality of TEM in gram-negative bacteria, TEM is very rare in *P. aeruginosa.* Only the strain TEM-42, a type of TEM-2, has been isolated in *P. aeruginosa* [54-58]. TEM-2 isolates are extremely like TEM-1, differing by their native promoter and their isoelectric point [41,49]. Interestingly, in the isolate *PAe1100*, found to have TEM-42, is resistant to penicillins and other antipseudomonal β-lactam antimicrobials. This is despite the fact the patient that the sample was harvested from had not undergone extensive exposure to antibiotics [47]. For this review, it is interesting to note in a paper written by Poirel, and it states, "*P. aeruginosa* strains may become a hidden reservoir for ESBL genes." this is possible with mutational changes of *blaTEM-1* or acquired resistance via the absorption of *blaTEM-4*. Moreover, a similar concept has been demonstrated with OXA-type ESBLs in *P. aeruginosa* [55].

PER type: PER-1 strains were first detected in *P. aeruginosa,* and to date, PER-1 is the only PER type β-lactamase identified in *P. aeruginosa* [45,58]. These strains are prevalent in parts of Europe and have been attributed to many nosocomial infections. In a study conducted it was found that 11% of nosocomial infections of *P. aeruginosa* were encoding the *blaPER-1* gene [59]. In some locations, PER was found in approximately 20-25% of strains [60]. PER enzymes share a 25-26% homology with studied TEM and SHV enzymes [61]. PER enzymes are extremely effective at hydrolyzing the β-lactam ring of penicillins with the exclusion of oxacillin [62]. This property of TEM β-lactamase can be surpassed using clavulanic acid inhibition as that is their known inhibitor. This is a very useful method used is medicine, but recently there have been strains studied that have developed resistance to clavulanic acid [63]. For quite some time it was believed that PER-1 was located on the chromosome of *P. aeruginosa,* but a study in 1995 successfully discovered the transferability of this gene from one specimen to another [57]. Some suggestions have been made that conjugation of resistance plasmids between *P. aeruginosa* and *Acinetobacter* might be comparable to the conjugation of plasmids between PER-1 producing *S. typhimurium* [58]. This suggests that the gene for encoding PER-1 beta-lactamase is plasmid oriented. Epidemiologically, PER-1 producing *P.*

aeruginosa is very concerning to Europe and northern parts of Africa. Strains are being clinically isolated from surgery wards and intensive care units in France, Turkey, Poland, Italy, Egypt and more [59,64-66].

SHV type: SHV-1, the first discovered SHV type beta lactamase, provides antibiotic resistance to penicillins and other beta lactam antimicrobials. However, investigations have found 189 strains of SHV type enzymes. These enzymes are very common amongst gram-negative bacteria, yet only some strains are found in clinical strains of *P. aeruginosa.* Just a few specific strains have been isolated in nosocomial infections. Strains producing SHV-2a, SHV-12, and SHV-5 [67-69]. The study conducted of the SHV-5 strain. The patients that were given piperacillin and tazobactam were cured of the infection. The same strain when studied was shown to be susceptible to various penicillins; ticarcillin-clavulanate, piperacillin-tazobactam [70]. SHV-2a has a -35 region from the left inverted repeat of IS26 and the −10 region from the blaSHV-2a promoter, making up the composite promoter of SHV-2a. DNA surrounding the blaSHV-2a promoter of SHV-2a *is* interestingly homologous to the pMPA2a plasmid derived from *Klebsiella pneumoniae* KpZU-3. That and analyzing evidence of other genetic commonalities between sequences of DNA show signs that *K. pneumoniae* might be a potential origin of SHV-2a [69]. Concerning public health, the presence of ESBLs and plasmids create the possibility that ESBLs might spread through the environment via the food chain. Creating many risks for the population [70].

Prevention

As previously stated the CDC has reported that *P. aeruginosa* is the leading cause of nosocomial infections, the number of cases reported is approximately 51,000 each year. This number can be broken down in the following ways. Each year approximately 13 percent, 6,000, of these isolates found are described as multi-drug resistant. The definition of a bacteria being labeled as multidrug-resistant is vague and many definitions are being used today; however, the most common seems to be "resistance to three or more drugs" [71]. About 400 of these 6,000 cases have led to death. When juxtaposed, a much higher mortality rate is observed in multidrug-resistant strains ~30% compared to the ~17% mortality rate reported from non-resistant strains [72,73]. Exposure to *P. aeruginosa* outside of the hospital is widespread and rarely will lead to an infection. The only individuals at risk are newborns, anyone with constant exposure to water (i.e., whirlpools, heated pools) and individuals with extended wear contact lenses. Furthermore, these infections that do occur are extremely mild and are not of much concern, as opposed to nosocomial infections of *P. aeruginosa* that are labeled a threat level serious by the CDC. In hospital infections are still common even with modern-day understandings of pathogenesis. In the United States alone, the CDC estimates approximately 1.7 million nosocomial infections each year and roughly 99,000 deaths attributed to said infections. The primary cause of healthcare-acquired infections is due to urinary tract infections. Followed by surgical site infections. Many steps have been taken to minimize the number of infections via the use of a catheter, such as an external catheter for men, proper, catheter removal, only using catheters when necessary, antimicrobial catheters, and so on [74-76]. This awareness is not without a purpose. In 2008 a study was conducted measuring rates of catheter-related infections. The study shows a decline in rates from 7.0 cases/10,000 persons in 2003 to 5.1 cases/10,000 persons in 2008 [77]. The ability to diagnose these infections sooner every year is helping the fight against catheter-related infections.

Being able to spot pathogens before they become too invasive is preeminent to treating said pathogen. Therefore, utilizing contemporary methods of imaging is in the best interest of providers. This is a highlighted issue for *P. aeruginosa* related chronic sinusitis. In neutropenic patients with sinusitis, *P. aeruginosa* is one of the most common isolates [78]. Often recurrence of the pathogen is presented following successful antibiotic treatment or surgery. These concerns only add to the importance of high-quality imaging techniques to minimize cost to the patients and the spread of the pathogen preventing otherwise unnecessary costs. High quality magnetic resonance imaging (MRI) coupled with computed tomography (CT) would help distinguish a bacterial lesion from other lesions, thus, enabling providers to react quickly to prevent the severity of the infection [79]. State of the art techniques are essential already in the treatment planning of head and neck lesions [80].

Discussion

P. aeruginosa is a serious threat to patients with cystic fibrosis (CF) and other patients at risk for ventilator-associated pneumonia (VAP). *P. aeruginosa* was found to be the most common cause of VAP [81]. Patients with CF have a host of preventative measure to limit infection, as it can be fatal for the patient. Despite this, 95-80 percent of CF patients have respiratory failure from bacterial infection coupled with inflammation [82]. The primary cause of nosocomial pneumonia is through ventilation, 86%, and is responsible for the majority of intensive care unit mortality [83,84]. VAP is extremely difficult to diagnose; patients can be admitted into hospitals multiple times, explaining why VAP is so costly for hospitals and patients, a presented case by National Institutes of Health Clinical Center exemplifies this [85]. The CDC has set guidelines for sterilization and other techniques to fight the presence of nosocomial pneumonia and VAP. The list includes staff education and involvement in infection prevention, infection and microbiologic surveillance, sterilization or disinfection and maintenance of equipment and devices, prevention of person-to-person transmission of bacteria, increasing host defense against infection: administration of immune modulators, prevention of postoperative pneumonia and other prophylactic procedures for pneumonia [1]. Conversely, rates of VAP do not appear to be moving. New technology has risen, more light has been shed on the matter, but as shown in a study of comparing rates of VAP from 2005 to 2013. Rates seem to remain unchanged [86]. Current methods of prevention have a lack of research done on them and their effectiveness at preventing VAP. Furthermore, two studies have shown a decline in rates of VAP that follow modern day methods of prevention of VAP [87,88]. Some stay skeptic of the effectiveness of these "bundles" of practices and question the studies published [89,90]. Today, these bundles are the most modern techniques medicine has at fighting VAP. With increasing

education on the matter and as science proceeds, researchers hope to develop more effective antibiotic remedies as well as refine these bundles of care and decrease the rates of VAP and nosocomial infections of *P. aeruginosa*.

While the Medical community waits for new science to be conducted and conclusions to be made on new remedies, other communities can do their part to take preventative measures today. Increased support from hospital administration, and guests of the patients can hinder *P. aeruginosa* and another nosocomial pathogen's ability to spread. Administrators can hold continuing education courses for their hospital staff to demonstrate infection control programs and assure effectiveness and proper training for these programs. Incorporating well experienced infection control practitioners in their clinics and hospitals, coupled with determining the right physician/ nurse to patient ratio is vital to ensure every patient has the proper attention and care. Moreover, there might be an incentive to limit labor costs in a hospital. However, nosocomial infections cost hospitals billions of dollars annually and increase patient stay. These costs can soar between 28 billion to 42 Billion dollars [91]. Despite the thought that many of these nosocomial infections can be prevented. Guidelines are in place at hospitals for visitors to follow. Still, visitors are always running the risk of carrying infectious bacteria in with them when entering a hospital. *P. aeruginosa* is commonly found in ground soil, water, plumbing, and plants. Many hospitals do not ban visitors from bringing patients items of these sorts. This is when it is up to the visitors to inform themselves about nosocomial infections. The CDC has a clear list of steps for the public and administration to follow to help the spread of nosocomial infections in their CDC AR threat report. This includes knowing resistance trends in the surrounding area, coordinate local and regional infection tracking and control efforts, require facilities to alert each other when transferring patients with any infection. Ask everyone including doctors, nurses, other medical staff, and visitors, to wash their hands before touching the patient, take antibiotics only and exactly as prescribed.

Conclusion

P. aeruginosa is a lethal gram negative, opportunistic pathogen. The microbe has a host of defenses, intrinsic and acquired, that allows incredible resistance to our current spectrum of antibiotics. The medical community is fighting a war against *P. aeruginosa*, as mortality rates associated with the bacteria remain consistently high. Preventative measures are continually put in place and new antibiotics are becoming more effective, however, progress is slow and modern-day medicine has many trials ahead before it will resolve this issue. With the increase in awareness, greater understanding of this pathogen and research directed towards combating this bacterium, a breakthrough in fighting and containing this pathogen is hopeful in the future.

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