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REVIEW ARTICLE

Prevalence of Multidrug Resistance in *Pseudomonas Aeruginosa* in Healthcare Facilities

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ABSTRACT

Pseudomonas aeruginosa is on the list of Gram-negative pathogens that are increasingly being counted as significant causes of nosocomial infections leading to significantly raised levels of morbidity and mortality. Life-threatening infections become more debilitating for those having a compromised immunity. The importance of *Pseudomonas aeruginosa* as a disease-causing microbe is enhanced through its increasing resistance to antibiotic drugs, the virulence factors plus its strength to adapt to wider environmental conditions. *Pseudomonas aeruginosa* possesses multiple acquired and intrinsic mechanisms providing resistance, often with augmented rates of resistance to multiple antimicrobial drugs. In the last decade, the global dissemination of the presumed 'hazardous clones' of multiple drug-resistant and extensively drug-resistant *Pseudomonas aeruginosa* have emerged as a serious threat to communal healthcare requiring extensive study and should be managed with determination and urgency. From the list of infections that are due to Gram-negative bacteria, *Pseudomonas aeruginosa* counts as a leading microbe causative for health-care-related infections in hospitalized individuals. In accordance with the guidelines by WHO, certain measures adopted in healthcare settings can help prevent transmission of multidrug-resistant *Pseudomonas aeruginosa* including hand hygiene (using alcohol-based solutions), contact precautions, cleanliness of the environment, isolation of patient (cohort or single room), plus surveillance.

Keywords: Antibiotic resistance, Nosocomial, Infections, Resistance mechanisms, Pseudomonas aeruginosa

INTRODUCTION

Pseudomonas aeruginosa counts as a Gram-negative bacteria becoming important as a significant cause of infections in hospitalized patients.

This pathogen is amongst the most prevalent causes of infections in immunocompromised individuals especially neutropenic patients and those getting admission in intensive care unit (ICU). A majority of the strains of this pathogen have become resistant to many of the antibiotics that are in use currently. By virtue of multiple adaptative survival mechanisms and because of being resistant to multiple antimicrobials, infections due to *Pseudomonas aeruginosa* can be fatal and this is a global threat to healthcare[1].

The aim of current review is description of *Pseudomonas aeruginosa* in healthcare facilities alongwith associated infectious risk. Search has been carried out in PubMedas well as Scopus for reports published regarding pathogen *Pseudomonas aeruginosa*, infection, healthcare reservoir, ecology, control and prevention measures.

Bacteriology and ecology: *Pseudomonas aeruginosa* are found in either a straight or slightly curved morphological form. These are bacilli measuring approximately 1.5µm X0.5µm. *Pseudomonas aeruginosa* count as Gram-negative aerobic motile pathogens possessing a single or multiple polar flagella.

The bacteria has the salient characteristic of producing of water-soluble green-blue pigment pyocyanin, water-insoluble red-brown fluorescein pigment pyorubin as well as water soluble yellow-green pigment pyoverdin. More than 90% strains of *Pseudomonas aeruginosa* produce pyocyanin. An inverse relationship probably exists between pyocyanin production plus the bacterial rate of growth[2].

The pathogenicity account of *Pseudomonas aeruginosa* is linked to the genetic complexity alongwith the large and multiple array of multiple factors imparting virulence to the pathogen[3]. A mucoid layer of exopolysaccharide (D-mannuronic and L-guluronic acetylated acids polymer) is of essence in current isolates of this bacteria that has been isolated from sputum of patients having cystic fibrosis (CF); poundage of the mucoid content in these patients can considerably e greater than the total weight of entire bacteria. Subsequently, subculturing results in a more commonly found non-mucoid form.

The exopolysaccharide permits bacterial adherence to one another resulting in the formation of microcolonies in the respiratory system of patients leading to pneumonia due to *Pseudomonas aeruginosa.* The anionic matrix in the surrounding thus protects the significant bacterial clump from the phagocytes action, antibodies as well as the complement system. Presence of fimbriae in a majority of strains of *Pseudomonas aeruginosa* strains are basically colonisation and adhesion factors. Exotoxin A is an extracellular toxic protein produced by more than 90% of the isolates of *Pseudomonas aeruginosa*[4].

Pseudomonas aeruginosa is a microbe that possesses a high potency for adaptation in variable circumstances. It is frequently found in marine water and waste water, surfaces, soil, humid environment and generally in vegetation[2]. In natural environments, the non-parasitic amoebae belonging to Acanthamoeba genus feed on *Pseudomonas* spp.that are quite abundantly found in the natural environment[5]. Nevertheless, some species of the said bacteria have progressed to be highly resistant to predacity by the amoebae, as is shown by isolating the genus Acanthamoebae that are quite naturally infected with Pseudomonas species[6]. Thus, it is highly likely that the non-parasitic amoebae also act as a cenote for amoeba-resistant strains of *Pseudomonas* species[7,8].

Pseudomonas aeruginosa is occasionally found in the microflora of skin, the normal gut and the environment. Natural tendency of this pathogen to use simpler organic molecules as a source of energy hence carbon also promotes this bacteria to divide and multiply in aqueous solutions where normally the bacterial growth is hindered such as saline solutions, soaps and mild antiseptics[4]. Numerous mechanisms for surviving adverse conditions are present in *Pseudomonas aeruginosa*. These count as quorum sensing, formation of biofilm, viable but not culturable (VBNC) state as well as mechanisms for antibiotic resistance[9,10].

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Pseudomonas aeruginosa constitutes as one of the major microbe involved in the formation of biofilm. It has the ability of adhering to wet surfaces directly or surfaces that come in contact with certain fluids. [2] Moreover, the formation of biofilm is affected by the genetic makeover of environment and the bacteria. It also depends on the interaction between the two entities[3].

Shifting of the bacteria from planktonic growth mode to the formaton of biofilm depends on the generation of components of extracellular matrix and adhesins serving as a scaffold encasing bacteria in the biofilms. The matrix in the biofilm of this bacterium usually consists of polysaccharides, extracellular DNA, proteins as well as lipids. Its composition is dependent on the strain, biofilm age and growth conditions[11].

The extracellular matrix in the biofilm of *Pseudomonas aeruginosa* contains six times more extracellular DNA as compared to proteins and eighteen times more extracellular DNA in comparison to carbohydrates. This matrix is important for adherence of the bacteria. The origin of this extracellular matrix has been confirmed to be genomic in nature. The nucleic acids can appear from lysis of the old bacterial cell or be actively secreted by the living bacterial cell by unifying of membranous vesicles[12].

The biofilm matrix of *Pseudomonas aeruginosa* has been studied to be rich in nutrients and possesses the capability of protecting the bacteria from the disinfectants. It also constitutes as an active site for transferring of the virulence factors thus providing the bacteria resistance against the antibiotic agents[13]. This promotes the persistence power in bacteria leading ultimately to antimicrobial resistance[14].

QS is an intracellular cell density-based communication system that contributes noticeably in the regulation of bacterial virulence plus forming the biofilm. Quorum sensing network of this bacteria has been organized as a multilayered hierarchy that consists of minimum four interconnected mechanisms of signalling[15].

Another important mechanism for survival in *Pseudomonas aeruginosa* is the VBNC state. In stressful circumstances or sometimes as an integral part of the natural life cycle, this microbe adopts a certain VBNC state rendering the bacteria undetectable by the conventional methods of culturing making it increasingly resistant to antimicrobial treatment. Certain circumstances can reinstate these round VBNC cells of *Pseudomonas aeruginosa* returning the bacterial cells to the active and virulent rod-shape formation.[10] Ubiquitous presence and survival of *Pseudomonas aeruginosa* in healthare settings is based on multiple mechanisms providing intrinsic resistance to antibiotics leading to extraordinary capability of survival[1].

Antibiotic resistance in *Pseudomonas aeruginosa:* WHO has stated that *Pseudomonas aeruginosa* is amongst the list of highly resistance bacteria that is a threat to human health and healthcare[10]. This bacteria has numerous acquired as well as intrinsic mechanisms for resistance providing it with the ability to withstand multiple antibacterial agents[16].

Pseudomonas aeruginosa has been studied to be resistant intrinsically to a majority of antibiotics by virtue of its selective potency for preventing the permeation of multiple antibiotics through its outer membrane or extrusion if antibiotics reach the cell's interior. There are a number of antibiotic groups that are frequently prescribed. These include fluoroquinolones (e.g. levofloxacin and ciprofloxacin), β-lactams (e.g. piperacillin-tazobactam, cefepime), aminoglycosides (e.g. gentamicin, amikacin) as well as a few polymyxins. Resistance in *Pseudomonas aeruginosa* can nevertheless be due to multiple mechanisms including modification of the antimicrobial drugs, active efflux of the drugs, decreased permeability of the drugs as well as degradation of the antimicrobial agents[17].

The European Antimicrobial Resistance Surveillance Network of the European Centre for Disease Prevention and Control (ECDC) (EARS-Net) 2018 reports that 32.1% isolates of *Pseudomonas aeruginosa* in the European Union/European Economic Area were resistant towards at least one of the total antimicrobial groups under regular surveillance (fluoroquinolones, piperacillin-tazobactam, ceftazidime, carbapenems and aminoglycosides). Resistance against two or exceeding groups of antimicrobial drugs has been observed to be widespread and recorded in 19.2% of all the isolates tested. Quite a significant country wide variations were observed for all the antimicrobial groups. Quite increased percentages of resistance have been reported from eastern and southern Europe as compared to northern Europe[17].

During the last decade, the global dissemination of 'high-risk clones' of multiple drug resistant and extensively drug-resistant (MDR/XDR) *Pseudomonas aeruginosa* has emerged as a general health threat requiring research and therefore must be managed with determination and urgency[18,19]. The deficiency of alternate treatment regimes confers that infections because of antibiotic-resistant bacteria are nonetheless a significant commination with regards to mortality and morbidity[18].

An extensively conducted multicentre study based on isolates of *Pseudomonas aeruginosa* was conducted in 51 hospitals of Spain in 2017. This study revealed that 26.2% of the isolates of the bacteria under study were classified as MDR (Multidrug resistant), 17.3% as XDR (Extensively drug resistant) and only 0.1% as pandrug resistant. Carbapenemases/extended-spectrum beta-lactamases were also detected in 3.1% isolates. These included VIM, GES, IMP, OXA and PER enzymes. The clone been found in the highest frequency among XDR isolates was ST175 (40.9%), CC235 (10.7%), followed by ST308 (5.2%) as well as CC111 (4.0%). [20] The dissemination of Verona integron-encoded metallo-β-lactamase-producing carbapenem-resistant *Pseudomonas aeruginosa* (VIM-CRPA) is nevertheless alarming.
CRPA septicaemia is challenging to treat. This is due to the fact that effective and well tolerated treatment options are quite unavailable. Plus, the mortality associated with such infections is quite higher as compared to infections that are caused by carbapenem-susceptible strains of *Pseudomonas aeruginosa*[16,21].

Multiple focal points of VIM-CRPA have been ascertained linked to the medical management in European countries (Belgium, Germany, France, Italy, Greece, Hungary, Spain and Netherlands). A few might be associated with certain invasive medical procedures. For *Pseudomonas aeruginosa,* certain high-risk clones have been explained. These clones have been characterised by the hospital spread globally plus their potency for rapid acquisition of antimicrobial genes for resistance[16].
As per the ECDC's 'Surveillance of antimicrobial resistance in Europe 2018[17], the resistance to carbapenems in the EU/EEA has averaged to 17.2% in 2018, having wider variations among countries, from approximately 0% in Iceland to about 55.1% in Romania. In Italy alone, 15.8% of a total 3014 invasive isolates of this microbe were evaluated to be carbapenem resistant.

***Pseudomonas aeruginosa* reservoirs found in healthcare setting:** In a healthcare setting, the environmental reservoirs for *Pseudomonas aeruginosa* have been confirmed to be potable water, aerosols, faucets/taps, shower drains, sink, endoscopes, humidifiers, respiratory equipment, endoscope washers, water baths, bathing basins as well as hydrotherapy pools[22–27].

Another study has been conducted regarding the microbiological processing of duodenoscopes used in an endoscopy unit during a 3 years tenure[28]. This study consisted of 124 samples from these duodenoscopes having specifically 62 samples from the end part of the duodenoscopes and 62 collected from the instrument channel. *Pseudomonas aeruginosa* was identified in quite an increased concentration (10–2500 CFU/duodenoscope). Antibiogram showed 60% samples to be positive for *Pseudomonas aeruginosa* and had multidrug strains of the bacteria[28].

The ability of *Pseudomonas aeruginosa* to get transmitted through variable routes is remarkable. This includes environmental as well as person-to-person contamination[22,29,30]. By virtue of its augmented ability to withstand adverse consitions and a high adaptability, this pathogen can endure dry non-human surfaces in a healthcare setting from 6 h upto 6 months[31]. Yet another mode of dissemination of infection because of this pathogen are the hands of hospital personnel. This is quite a convenient way for transmission of *Pseudomonas aeruginosa* since hands of hospital personnel can easily get contaminated following contact with an infected/colonised patient or perhaps following use of a contaminated cream, water or soap.

Multiple reservoirs in a hospital setting have been enlisted for this microbe. Hospital water has been counted as a major source linked to hospital associated infections by this pathogen. Direct contact like surgical site, bathing, splashing from water or contact with the mucous membranes, medical equipment or devices that have been washed with contaminated water, coming in contact with surfaces that have been contaminated with water from contaminated equipment or indirect contact through contaminated hands can spread infections[14]. *Pseudomonas aeruginosa* has the potency to survive in hospital water for longer durations[32].

Spread of infection from a contaminated ICU sink has been reported by Hota *et al*[33]. Fluorescein injection into the sink drains showed splash-back measuring upto 1m from the sink with running water. It has been reported that contamination of the water system with *Pseudomonas aeruginosa* is usually limited to the water system's distal 2m end[34].

Pseudomonas aeruginosa frequently counts in the list of species that are found in dental unit waterlines[5,35,36,37]. Here, *Pseudomonas aeruginosa* gets the capability of freely forming the biofilms on the inner side of narrow-bore plastic tubings that carry water to high-speed handpiece, ultrasonic scaler and air/water syringe. Quite a small lumen size (0.5–2 mm), lesser throughput, a high surface area to volume ratio (6:1), the material of the tubing, water stagnation in DUWL when the units are not being used aggravate formation and growth of biofilms[36].

Another study reveals the level of contamination with this pathogen in 30 dental units[35]. The results showed that in water from the handpieces, the average concentration of *Pseudomonas aeruginosa* was 25.13±CFU/100 ml. Another study by Jensen *et al*[38] showed presence of *Pseudomonas aeruginosa* in water collected from dental sessions that were attended by patients suffering from Cystic Fibrosis. The samples of water were collected from triple function syringes, handpiece, turbines, contra-angles as well as ultrasonic scalers. The samples of sputum obtained from every patient having Cystic fibrosis were also evaluated for the presence of *Pseudomonas aeruginosa* before and after each dental appointment. Yet another study has shown genotypically identical (RFLP, pulsed-field gel electrophoresis) strains of this pathogen to be present in water samples collected from dental equipment and patient's sputum.

Infections caused by *Pseudomonas aeruginosa*: nfections caused by *Pseudomonas aeruginosa* are not quite as common in healthy individuals. Skin infections in healthy individuals due to this pathogen are temporary and self-lived.

Immunosupressed patients or individuals having chronic devitalizing diseases get severely infected with *Pseudomonas aeruginosa.* Therefore, the general health of the patient and the status of the immune system determines the consequence of infection[4]. The resistance to antibiotics, multiple virulence factors as well as the adaptability of this pathogen marks its significance[27]. Patients suffering with infection from drug resistant strains of this pathogen are nevertheless at a higher risk of prolonged hospitalisation and subsequently suffering from an increased risk of more antibiotic-resistant infections, a higher rate of morbidity as well as mortality[39].

Biofilm formation provides *Pseudomonas aeruginosa* with the added advantage of establishing a drug resistant infection inside the susceptible host[3,9]. Chronic infection with *Pseudomonas aeruginosa* settles in the respiratory airways of a patient suffering from Cystic fibrosis. Resultingly, 60–80% of such adults get a chronic infection with this bacteria[40]. *Pseudomonas aeruginosa* plays a pivotal role in the progression and worsening of respiratory disease in patients having CF[3].

Patients suffering from malignant blood cancers like leukaemias and neutropenia as a result of immunosuppressive therapy or perhaps pneumonia are at an increased risk. Quite similarly, extended venous or urinary catheterisation, critical burns and wounds and surgical procedures permit the microbes to cross the safeguarding layers of the skin colonizing multiple tissues thus leading to septicaemia[4].

Pseudomonas aeruginosa has the potency to cause multiple infections of different organ systems. It can infect the urinary tract, lead to the formation of infected burn wounds, can cause corneal ulcers as well as keratitis, septicaemia, abscesses, gastroenteritis in neonates, meningitis and bronchopneumonia. Much of its pathogenicity is by virtue of its invasive potency alongwith the active generation of extracellular substances like exotoxin A. [2] This bacteria can also cause increasingly devastating ocular infections following use of contaminated ophthalmological aqueous solutions or maybe due to severe facial burns[41].

From the list of debilitating infections caused by Gram-negative bacteria, *Pseudomonas aeruginosa* counts amongst the commonest pathogen that causes nosocomial along with healthcare related infections in patients admitted to the hospitals. Infections due to multidrug strains in a healthcare setting have been linked to poor treatment as well as high morbidity and mortality. The rising intensity of resistance in multidrug resistant *Pseudomonas aeruginosa* is most likely associated with in-patients transmission of the drug resistant strains. This can also be due to the newly gained resistance because of the exposure to antibiotics previously[41].

Another study by Kanayama *et al*[42] reports the upsurge of a multidrug resistant strain of *Pseudomonas aeruginosa* wherein a total of 23 MDRP cases have been studied. In this study, environmental samples collected from multiple wards were studied. Evaluation of the strains by multiplex PCR for carbapenemases showed expression of GES-type β-lactamase gene. The results of Pulsed-field gel electrophoresis showed that all tested environmental sample isolates and cases were quite similar (≥95%).

Another study by Bajolet *et al*[43] has described an eruption that took place in 2011 at a tertiary care hospital in Reims, France. This outbreak was linked to a sole endoscope that was found to be tarnished with extended-spectrum beta-lactamase generating strain of *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa is commonly associated with infections that occur in patients admitted to intensive care unit. This pathogen is amongst the list of five highly frequently linked pathogens in hospital related infections occurring in ICU. These infections include urinary tract infections, pneumonia, surgical site infections, soft tissue infections alongwith infections of the bloodstream[25].

Another study by Kikuchi *et al.* stated the outbreak of clonally related strains of CRPA in 20 patients that were admitted to an ICU. Patients experiencing positive respiratory specimens were mechanically ventilated with re-processed disinfected bite blocks while intubating. Swabs were obtained from oxygen masks, patient beds, bite block apparatus, body fluid aspiration tubes, humidified air inhalation tubes as well as tracheal endoscopes and these specimens were cultures. Even though the bite blocks were disinfected, nevertheless cultures were positive for *Pseudomonas aeruginosa*. Therefore, it was hypothesized that the minor cracks and crevices in the bite blocks could not be disinfected totally. [44] Water sources are more frequently contaminated with pathogens that are causative for healthcare related infections such as *Pseudomonas aeruginosa*[46,47,48]. This is possible due to the microbes surviving treatment regimens or perhaps through endpoint contamination[49].

Salm *et al*[50] has evaluated an upsurge of clonal MDR *Pseudomonas aeruginosa* in an intensive care unit of a tertiary care hospital. Evidence was found of a transferral route that was linked with the working procedures at hospital sinks. Yet another study[51] describes an upsurge of infection in the burn unit of a hospital located in Spain. This epidemic was caused by extensively drug-resistant (colistin resistant) strains of *Pseudomonas aeruginosa*.

DYNAPYO was yet another observational prospective multicentre study that was carried out in 10 intensive care units in France in a 5-month tenure[45]. Prevalence of *Pseudomonas aeruginosa* was calculated to be 15.3%. Various risk factors were found to be linked with colonisation bacteria such as usage of inactive antimicrobials against *Pseudomonas aeruginosa* (HR=1.60 [1.15–2.21], *P*<0.01), plus mechanical invasive ventilation (HR=4.70 [2.66–8.31], *P*<0.0001). The possible risk of colonisation increased by +66% (HR=1.66; 95% CI = [1.01 ± 2.75]) by polluted and contaminated tap water at the entrance point in patient room.

Control measures and Preventing infections by *Pseudomonas aeruginosa:* As per the guidelines by WHO, certain initiatives have to be adopted to reduce transmission of multidrug resistant *Pseudomonas aeruginosa* in healthcare settings. These include hand hygiene through proper utilization of alcohol-based solutions), patient isolation, precautions when in contact with a patient, environmental hygiene as well as surveillance.

The ECDC has recommended increasing and improving surveillance, protocols for the screening plus precautionary isolation in healthcare settings of individuals who have been transferred or been in direct contact with hospital environment or infected having an increased prevalence of MDR pathogens like *Pseudomonas aeruginosa*. Documentation related to any infection due to MDR *Pseudomonas aeruginosa* or being in a carrier state at the time of transferring would be of help in implementing effective measures for prevention of the pathogen's spread[16,52,53].

The intensive care units require stringent measures to contain infections especially with regards to the ventilator support and equipment. The Water safety plan by WHO entails the risks that can be associated with contaminated water supply. Thus the WSP is based on certain components such as active and routine infection monitoring, proper sterilization procedures, routine testing alongwith maintenance of water supply as well as timely examining water collected from most important points in water system of the hospital. Authentication procedures have to be undertaken that would ensure the effective working of WSP[54,55].

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