Hand swab contamination of medical personal by *pseudomonas aeruginosa* at MNCC clinics and wards

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Abstract

P.aeroginosa is one of the most serious pathogens of infections, it produces Metallo-β-lactamases which is a diverse set of enzymes that catalyze the hydrolysis of a broad range of β-lactam drugs including carbapenem. Contamination with this pathogen remain one of the most serious problems in Hospitals, especially with the intensive exposure to antibiotics, where many studies reported that *P.aeroginosa* has developed resistance thus cause high rate of mutations. This will complicate the situation and improve the maximum limit for possible antibiotic treatment (14; 29). Alternatively, diagnosis efficiency will lead to treatment failure and complicate the situation, therefore it is important to introduce new tools to the followed routine identification procedures of *P. aeruginosa* at Libyan hospitals.

I. INTRODUCTION

Hospital infections is known to be a major public health problem, vary frequency, severity, and of its overall medical and socio-economic impacts, most important being difficult to monitor and control them. According to the World Health Organization (WHO) records, about 8.7% of hospital patients will suffer from hospital infections, presenting a total of over 1.4 million people worldwide suffer from infectious complications during their hospitalization (1). Usually, hospital infections could be expressed either two possibilities, the first could be endogenous originate where the patient's own flora become the source of infection. While, the second could present exogenously where the pathogen comes from other patients, staff, or the hospital environment such water, air, or surfaces (2).

The contamination of surfaces depends on their characteristics, such as smoothness, porous, roughness as well as their statues like being dry, wet, new, or old. It also constitutes an ecological niche of bacteria capable of forming biofilms. These bacteria on many surfaces in a hospital last from a few days to long periods that can even go beyond 90 days (3). Numerous investigations made in hospitals have highlighted the possible place of the inert environment as a contamination reservoir of patients, namely, because of the presence of multidrug-resistant bacteria (MDR) (4).

One of these pathogens is *Pseudomonas aeruginosa* which has emerged as a 10-20% inflammatory in most hospitals, and is particularly prevalent in patients with some medical complications such as burns, fibrosis wounds, eye infections, patients with cystic fibrosis, which sometimes rise as a result of hospital contamination (Nosocomial infection).

As it is known, the main function of the skin is to control the microbial group that lives on the skin surface, thus prevents underlying tissues inflammation.

By the end of the twentieth century, it became clear that many bacterial species live in complex societies in their natural environment creating what is known as biofilms. These films provide many advantages to microorganisms specially the opportunistic bacterial pathogens (5). Many of those are considered as the leading cause of morbidity and mortality worldwide. However; due to the intensive use of antimicrobial agents in the infection treatment, many infections are lately characterized by strong bacterial growth and increased antibiotic resistance

The mechanism of resistance is still unknown, nonetheless biofilms are an organization of bacterial cells arranged in a self-produced polymer matrix consisting of polysaccharides, protein and DNA. These films cause chronic inflammation and tolerate antibiotics and disinfectant chemicals as well as resistance to phagocytosis and others.

There is a difference in physiological changes as well as a special nutritional representation of bacteria when they are in the form of biofilm (6). It has been found that the biofilm bacteria have about 1,000 times more antibiotic resistance than free bacteria (7).The microorganisms that make up the biological membranes vary and may include positive and gram-negative bacteria, yeasts and even filamentous fungi, including *Staphylococcus aureus* (including methycillin-resistant strains). Epidermidis, *Escherichia coli*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Vibrio cholera*, *Candida parapsilosis*, and *C. albicans*. Costerton et al. (1999) has provided a partial list of human infections where biofilms are involved.

Pseudomonas aeruginosa has emerged as a 10-20% inflammatory in most hospitals and is mainly prevalent in patients with burn and fibrosis wounds as well as sometimes as a result of hospital contamination and equipment (8).

Pseudomonas aeruginosa microorganisms are Gram-negative and aerobic and sporulation widely spread in soil and water moving through a single polar flagellum (9). These bacteria have PSL polysaccharides that have an important role in increasing adhesion between the surface of cells and between cells themselves to maintain the structure Biofilms, an important component of the biofilm matrix (10).

P. aeruginosa secretes a battery of extracellular products that are important for virulence; for example: exotoxin A, elastase, lasAprotease ,phospholipase C, alkaline protease rhamnolipid, pyocyanin, and alginate, many of these toxic exoproducts are involved in procuring nutrient and protecting the bacterium from the host immune system (11).

Unfortunately, during the last decades bacterial adaptation to antibiotics has increased dramatically causing health problems (12), and yet the rise of multiple bacterial strains resistant and possibly cross-resistance to several types of antibiotics in many wound infections has become difficult leading to treatment failure (13).

Therefore, it is very important to explore the field of contamination and expand the research area to cover resistance for the purpose of understand the mechanism of resistance as well as increase the chance of treatments or developed new drugs so as to reduce the severity of the problem.

In this project we are aiming to study the first phase of this problem and study contamination with *Pseudomonas aeruginosa* in hospitals. For this purpose random samples were collected from the hands of medical staff in the clinics and wards at Misurata National Cancer Center (MNCC) to investigate the possible contamination with *P. aeruginosa* using microbial tests and confirm the results using RT-PCR.

II. Pathogenicity

P. aeruginosa one of the main cause of hospitals contamination and infections, demonstrating high rate of morbidity and mortality. In the United States, *P. aeruginosa* is among the most

common hospital pathogens and is the second most common pathogen isolated from patients with ventilator-associated pneumonia. Given the severity of *P. aeruginosa* infections and the limitation of the used antimicrobial drugs in the treatment, finding alternative prevention and treatment strategies is an urgent priority. Table 1 summarizes the most common *P. aeruginosa* infections and risk factors (14).

Table I: The most common *P. aeruginosa* infections and risk factors

Infection	Major risk factors
Soft tissue	Burns, open wounds, post-surgery
Urinary tract	Use of urinary catheter
Bacteremia	Immunocompromised
Diabetic foot	Diabetes, impaired microvascular circulation
Respiratory/pneumonia	Old age, COPD, cystic fibrosis, mechanical ventilation
Otitis externa (ear's infections)	Tissue injury, water blockage in ear canal
Keratitis (corneal infection)	Extended contact lens wear, contaminated contact lens solution
Otitis media folliculitis (hot tub rash)	Improperly cleaned hot tubs

II. Genome of *P. aeruginosa*

The genome of *Pseudomonas aeruginosa* is considered as one of the largest and more complex compare to other microbial genomes. It has a large stable core genome. Whilst there is an abundance of accessory genes. (15). It varies greatly in size and ranging between 5.5 to 7 Mbp (16, 17). Such variation may refer due to the presence of a large accessory genome. Accessory genomes are strain specific blocks of DNA and can occupy up to 20% of the whole genome (18). They are composed of horizontally transferable elements which include prophages, transposons, insertion sequences (IS), genomic islands (GI) – (known also as pathogenicity Island SPIs) and plasmids (19). Accessory genomes are important for carrying virulence and acquired antibiotic resistance genes. The lateral transfer of those genes between strains contributes to the development of MDR virulent strains (20). Furthermore, mutational changes of chromosomal genes can also contribute to virulence and antibiotic resistance (21, 22).

Several reports have shown that the prevalence of such infections by multidrug-resistant (MDR) strains is increasing rapidly worldwide (23, 24, 25, 26), which makes this bacterium difficult to treat and hence there is a high risk of mortality associated with infection by *P. aeruginosa* (27). This pathogen has an exceptional capacity to develop resistance to antibiotics by the selection for genomic mutations and by exchange of transferable resistance determinants (28).

P. aeruginosa is one of the most serious pathogens of infections, it produces Metallo-β-lactamases which is a diverse set of enzymes that catalyze the hydrolysis of a broad range of β-lactam drugs including carbapenem. Contamination with this pathogen remain one of the most serious problems in Hospitals, especially with the intensive exposure to antibiotics, where many studies believe that *P. aeruginosa* has developed resistance and may be caused a high rate of mutation leading to a serious problem and represents the maximum limit for possible antibiotic treatment (14; 29).

Alternatively, inaccurate diagnosis will lead to treatment failure and of course will complicate the situation, therefore it is important to introduce new tools to the followed routine identification procedures of *P. aeruginosa* at Libyan hospitals.

III. Research methods & Materials

50 swabs were collected of medical staff hands in MNCC, using microbiology collecting swabs over 5 working days, then each swab were streaked on blood agar plates and incubated overnight at 37 ̊C. 16 samples reported bacterial growth. And were subjected to genomic DNA extraction using QIAamp DNA kit, (Qiagen, Hilden, Germany), according to the manufacture protocol. Followed by Real Time PCR Assay using Rotor-Gene Q- HRM (Qiagen, Hilden, Germany). For this purpose, a pseudomonas aeruginosa real-time PCR detection kit was designed and ordered from Bioeksen Company (Turkey), specific for *ecf*X gene that encodes the *ecf* sigma factor (extracytoplasmic function sigma factor).

The amplification mixture contains GoTaq Probe qPCR Master Mix (Biospeedy - Turkey), and p. aeruginosa- Oligo Mix, contains FAM-labeled p. aeruginosa targeted oligonucleotides (target) as well as HEX-labeled human RNase P gene as internal positive control (Biospeedy - Turkey). Sample derived inhibition control and kit reagent control were performed with the internal control. External Negative (NC) / Positive control (PC) were also included. Reaction program were run through 41 cycles: 1 initial denaturation at 95°C for 3 minutes, the 40 cycle amplification profile consisted of secondary denaturation at 95°C for 10 seconds, annealing at 55°C for 40 seconds, the reading will be through FAM and HEX channels.

IV. Results and discussion

The routine microbiology tests showed a bacterial growth in 16 samples out of 50 collected samples. These 16 samples were subjected to molecular investigation targeting *ecf*X gene assigned to *P. aeruginosa*. The RT-PCR results were interpreted using software provided by Qia Gen, and showed that 1 sample (assigned sample 13) confirmed positive for *p. aeruginosa* with a Ct value of 19.22 for the sample and 13.7 for the external positive control, and NC control reported no pea; confirming reaction sterility. The internal positive (IP) shoed clear signal for all 16 samples confirming that RT-PCR reaction worked well.

VI. Conclusion

The overall prevalence of bacterial contamination other than p. aeruginosa as seen on the microbial cultures used in primarily this study were quite clear in the different sources (Is not included in the results of this study). Nonetheless the contamination with p. aeruginosa were confirmed in 1 sample out of the 16 bacterial contaminated samples displaying a prevalence of (6.2%) out of all contaminants. The overall p. aeruginosa contamination prevalence of medical personal hands were reported to be at (2%). We can conclude from this quick and short study that p. aeruginosa is one of the contaminants in the hospitals and could be found in some places even on Human hands. This may complicate the situation and spread out the pathogen between patients and may worsen their condition especially with the increase rate of drug resistance and treatment failure.

VII. Recommendations

Based on the above findings, we strongly recommend that, Hospital contamination is a serious matter and defiantly has its negative impact on the health service in Libya. The aggressive pathogen p. *aeruginosa* is one of the contaminants, were found on hands of medical staff, this may help in spreading that pathogen amongst patients. Therefore, it is a must to obey the disinfection and sterilization rules in and out the clinics. Finally, further study and wider study is strongly recommended to screen for any contamination with this pathogen and others.

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