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Original Research

The Effect of *Pseudomonas* Infection with Burns Patients

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Abstract:

Pseudomonas aerginosa is Gram-negative, facultative aerobic rods, non- fermentative, non-sporulation, motile by polar flagellum one and the most important and opportunistic pathogens that cause a high rate of mortality and morbidity in hospitalized patients with compromised immune systems. It has the ability to infect almost all tissues of the body as a result of its possession of a large variety of virulence factors that contribute significantly to the pathogenicity of the host, so the rapid detection of these bacteria plays a crucial role in controlling the diseases that cause them, especially in burn injuries. More than 120 samples were collected, clinical samples for people with burns of both the second and third degree, were collected from the burn unit at Imam Al-Sadiq Hospital (peace be upon him) in Babylon, during the period from November 2022 to January 2023, to investigate the spread of *P. aeruginosa* bacteria that these bacteria have it with age from (1year to above 61year) , 57male, 63 female. The growing isolates were diagnosed after their cultivation on Blood agar and MacConkey agar by selective medium, Pseudomonas chromogenic agar, and the diagnosis was confirmed using the vitek2 compact system, aeruginosa isolates. The results of the current study, which included information about patients with burns such as gender, age, type of burn and degree of burn, showed that the number of diagnosed cases as burn injuries for males was 47.5%, while the percentage of females was 52.5%. The highest rate of infection was in the age group 1-10 years at 35%, which is the category of children, followed by the age group 11-20 with 23.3%, and the lowest infection rate was in the age group 51-60 and the group above 61 with 1.7%.Thus,there were a high significant difference ($P \leq 0001$). The results indicated that the diagnosed cases of injuries were distributed in varying proportions with regard to the type of burn, as the highest percentage of burn injuries was from liquid burns by 50%, followed by burns by fire at 46.7%, and the lowest percentage of injuries was for electric shock burns by 3.3%.Thus, there was a significant difference ($P \leq 0.002$). The results of the study showed that the highest percentage was for *P. aeruginosa*, 68(68%), *Klebsiella* 12(12%), *Eschreichia coli* 10(10%), *Proteus* 4(4%), and the least was *Acinetobacter* and *Citrobacter* 3(3%). This study concluded that the prevalence of *P. aeruginosa* bacteria is high among clinical samples.

Keywords: Gram-negative, *Pseudomonas aerginosa*, facultative, polar flagellum

Introduction

Pseudomonas aerginosa is Gram-negative, facultative anaerobic rods, non-fermentative, non-sporulation, motile by polar flagellum one and the most important and opportunistic pathogens that cause a high rate of mortality and morbidity in hospitalized patients with compromised immune systems (Driscoll et al., 2007). In recent years, infections caused by this bacterium are one of the major problems in hospitals and are related to high rates of mortality, which range from 18% to 61% (Moghaddam et al., 2012). Burn lesion is considered globally to be a one of major public health concern and is in high risk of nosocomial infections denatured and dead, moist tissue makes the burn wound sensitive to P. aeruginosa infection, breakdown of skin barriers, reduced immunity, and prolonged hospital stays significant factors leading to burn wound infected with these opportunistic pathogens particularly the "P. aeruginosa" (MDR), the multi-drug-resistant P. aeruginosa" induces 4-60% nosocomial inflammation of different countries as the cause of death and morbidity in burning-unit patients (Hasan et al., 2019). Pseudomonas aeruginosa possess a highly capacity to form biofilms that are cell communities enclosed in an extracellular self-produced matrix protects cells from antibiotics and host immune responses , biofilm can increase *P. aeruginosa* infection in comparison with planktonic bacterial cells and increase the degree of antibiotic resistance (Schaible et al., 2020). In most laboratories, the detection of P.aeruginosa is still accomplished by microbiological culture and biochemical tests.Thus early diagnosis and proper medical treatments are the best strategies for fighting against these infections (Riou et al., 2010). Although a comparative study has shown that these methods contain reliable detection results, they are time-consuming and require several days to be completed (Deschaght et al., 2011). Studies have shown that in appropriate initial antimicrobial therapies were associated with adverse outcomes for infection treatments. Conversely, false detection can result in the administration of ineffective antimicrobial therapies during the first 48 to 72 hrs. (Gerasimova & Kolpashchikov, 2013). Moreover, in some cases in which the bacterial count is low, especially in antibiotic-treated patients, false negative results can be achieved in routine laboratory tests. Thus access to rapid and specific methods that have a high sensitivity is of great importance. The automated systems promise shorter turnaround times to diagnostic results and are widely used in many clinical laboratories for identification of bacterial species and antimicrobial susceptibility testing (AST). The automated systems have many advantages, such as high degree of automation with a simple operating procedure, improved specimen handling, good reproducibility and accuracy, etc. Vitek are the common automated identification systems currently used. The Vitek is one of the earliest and most commonly used automated identification systems.(Hsieh et al.,2009). These automated systems not only identify Pseudomonas aeruginosa but are also capable of performing AST. Many scientists have used these instruments to analyze different sources of Pseudomonas aeruginosa (Bruins et al., 2004). This study aimed to detection the P. aeruginosa among burned victims and sewage water by using biocemical tests and by using VITEK2 technique This aim will be achieved through the following objectives: Isolation and identification of P. aeruginosa bacteria from first, second, and third degree of burn victims on Pseudomonas Chromogenic Agar. Isolation and identification of P. aeruginosa bacteria from sewage water on Pseudomonas Chromogenic Agar. Diagnosis of P. aeruginosa by biochemical characterization through the use of VITEK2 device.

Literatures Review:

Burns

A burn is an injury to the organic tissues resulting from a direct or an indirect effect of heat or by flame and hot liquids or contact with hot objects or exposure to corrosive chemicals, radiation and contact with electrical current. Burns lead to the destruction of the skin layer, which is an important tool against the invasion of microbial (Pereima et al., 2001; Siviero Do Vale, 2005). Burn injuries is an important health problem in

many countries in the world, as the risk of this injury is influenced by a number of factors, including the extent and depth of the burn, various host factors and virulence factors for bacterial colonies associated with burns (Church et al., 2006). Bacteria are among the most common pathogens of burns, and these bacteria form the biofilms of many types on burns within 48-72 hours of injury, these microorganisms travel from the patient's own skin (hair follicles, sweat glands, gastrointestinal tract, and respiratory system, as well as through contact with health care workers and the external environment). Heat injuries lead to the destruction of the skin layer, which usually prevents the invasion of microorganisms during the first weeks after the injury, With burning, studies indicate that 75% of deaths are caused by burn injuries, which are related to infections, on the other hand, the pattern of injury varies from patient to patient, so the various bacteria associated with burn cases may change dramatically through (Rajput et al., 2008). Burn wounds are a complex traumatic event of several systemic and local effects, affecting many organ systems after the skin. The pathology of the burns victim appears the high the complexity of inflammatory response reactions other hand (Çakir and Yeğen, 2004). Usually, the accidents of criminal burns happen due to failure to prevent them. This trauma has an element of many incidences and added to it in suspicion of a crime (Peranantham et al., 2014).

Type of burns

1. Thermal burns

They are caused by flashed light, flame, blazing, or contact with a hot surface and include:

2. Hot liquids and fire

The explosions of flammable liquids, natural gas, propane, gasoline results into flash burns.

3. Flame burns

Flame burns are usually caused by prolonged exposure to intense heat, frequently associated with clothing ignited by stoves and heaters, improper use of flammable liquids, automobile accidents and house fires.

4. Scald burns

Scalds involve burn hot liquids like water, oil, grease or tar. A deep burn can be caused by water at 140 degrees (F) in 3 seconds, but the same injury will be resulted in just one second at 156 degrees (F).

5. Contact burns

They are caused by hot coals, plastics, metals or glass. They may be painful and deep (Masood et al., 2016).

6. Chemical burns

They are caused by exposure to reactive chemical substances such as strong acids or alkalis (Gnaneswaran et al., 2015).

7. Electrical burns

Passage of electrical current from an electrical outlet or appliance through the body may result into the electrical burn (Buja et al., 2010).

8. Radiological burns

Alpha, beta or gamma radiations are responsible for radiological burn. To stop the injury process there is a need of decontamination procedure for the people exposed to these types of radiation (Masood et al., 2016).

Classification of burns degree

1. First degree burns

The epidermis is involved in first degree burns which are like sun burn, erythematous, sore, and coarse. The minor thermal injury or exposure to severe ultraviolet radiation may cause first degree burns. Their healing time is 5 to 10 days (Lloyd et al., 2012).

2. Second degree burns

It is further divided into two categories:

A superficial partial thickness burns

They usually invade into the superficial papillary dermis. They are characterized with reddish blisters. When pressure is applied, the blisters may shrink and their healing time is 2-3 weeks.

Deep partial thickness burns

They penetrate the reticular dermis and are yellow or white in color, rough in nature and are very

painful. They require more than 3 weeks for complete healing(Toussaint and Singer, 2014).

Third degree burns

They damage both inner and outer layers of the skin, that's why this is the most severe type of burns. They are white in color and usually non-achy. Just a few such burns are cured of themselves which is a long process (Shank et al., 2009).

Fourth degree burns

They invade into the harmed muscles, ligaments, tendons, nerves, blood vessels, and bones, through the skin. For this type of burns, severe medical emergency care is required. They're black and scorched (Vadukul, 2012).

Pseudomonas aeruginosa

The genus of Pseudomonas is ram-negative, aerobic, rod-formed and has unipolar pinion (Fariñas and Martínez-Martínez, 2013). Pseudomonas form with some members capable of producing pigments a positive oxidase reaction (Gellatly and Hancock, 2013). As well as P. aeruginosa is a high intrinsic antibiotic resistance, together with its rapid ability to gain new antimicrobial resistance this pathogen is an increasing problem for the pathology of infectious diseases, especially if the nosocomial originates, no medical trials exist to investigate the potential survival factors of hospitalized patients with P. aeruginosa urinary tract infections, the mortality of these patients except bacteremia is not understood (Horino et al., 2012). In critically diseased and weakened patients, particularly in ventilation related pneumonia (VAP) and

bloodstream infections, urinary tract, intra-abdominal wounds, skin soft tissue (Lynch etal., 2017). P. aeruginosa is one of six ESKAPE pathogens that is the main cause of infectious nosocomial and is a global menace, as it becomes increasingly immune to all antibiotics available (Tümmler, 2019) .The Pseudomonas genus consists of over 120 species, which are pathogenic to animals and humans and are widespread in moist environment such as water and soil ecosystems. P. aeruginosa is most often associated with human infections in the genus of Pseudomonas. The bacterium is considered an opportunistic pathogen, causing mainly nosocomial infections in patients affected by an immune problem. Existing knowledge of P. aeruginosa pathogenesis is obtained mainly by studying clinical isolates, particularly those that cause chronic pulmonary infection in patients with cyst fibrosis. Nosocomial infections most often linked to P. aeruginosa include ventilator-related pneumonia, catheter-related urinary-tract infections, serious burn patient wound infections, and multifactorial septicemia with pathogenesis. The bacterium is also able, via the type III secretions system, to produce many toxins. as well as Secretion of enzymes, proteins and elastases, phospholipase C and siderophores (Streeter and Katouli, 2016). The pathogenesis of these bacteria is challenging and is distinguished by the capacity for virulence and biofilm growth to lead to nosocomial infection (Silva et al., 2019). Pseudomonas aeruginosa demonstrates tolerance to a broad range of antimicrobials and expresses a variety of molecular epidemiology in different groups of antibiotic agents, such as β lactams, fluoroquinolones, tetracycline and aminoglycosides. Although the external membrane is poor in permeability, its hydrophilicity and unspecified behavior to small molecular transport. The mechanism for the resistance of P. aeruginosa to different chemical agents is due to the complex genes encoded chromosomally, different strains with inherent biofilm ability of P. aeruginosa further improve the resistance under different environmental factor (Mohanty et al., 2021).Moreover, the bacteria of Pseudomonas in the environment are normal. It is present in many ways artificially and environmentally friendly. You can get to it in soil, fresh water, sea and in many parts of the human world. Pseudomonasgoes from an enormous variety of bacteria to a smaller population of bacteria. This led to the transition of many bacteria to other genera, families and environmental types. In the past 100 years Pseudomonas has undergone many taxonomic changes with some characteristics that have become smarter and more orderly (Özen and

Ussery, 2012). This bacterium adapts greatly to its surroundings, which also choose to support the persistence of bacteria, The development of surface or cell adhesive bacterial biofilms associated with enhanced immune and antibiotic clearance are of a clinically important temporaladjustment, Extensive research has shown that bacterial flagella motility facilitates biofilm formation which is subsequently nonmobile in bacteria ,However recent evidence has shown that nonattached antibiotic resistant bacterial aggregates can develop and are documented in the context of lung infections, otitis media, non-healing wounds and soft tissue fillers, which do not comply with surface attachment (Demirdjian et al., 2019).

Taxonomy of Pseudomonas

Pseudomonas genus was described firstly in 1894, since that time, many species were isolated from this genus when the first trials for classification of Pseudomonas were made according to diagnostics characteristics (Peix et al., 2009). Gilardi put the first system to classify microorganisms related to the family of Pseudomonadaceae depended on phenotypic characteristics and divided it into the main seven groups: Pseudomallei, Alcaligenes, Stutzeri, Fluorescent, Diminuta, Facilisdelafieldii and acidovorans (Li et al., 2010). Study of Mac Aogáin et al.,(2012) mention that The best-characterized groups to classification of Pseudomonas genus are subdivided according to properties such as the presence of poly- hydroxyl butyrate (PHB), the production of fluorescent pigment pathogenicity; the presence of arginine dihydrolase, glucose utilization (Von Bodman et al., 2008) and production of Diaminoacetophenone as a group like odors. In recent days, a lot of well-educated techniques using for molecular

analysis come to be available; five groups were extensively refined from this genus. It was identification according to five individual rRNA groups within the genus. Those rRNA genes are highly preserved genes, and the 16S-rRNA sequences serve as the main genetic marker molecules in bacterial phylogeny with the extra information providing by the 23S-rRNA genes in addition to the sequences of genes coding for highly conserved proteins. Pseudomonads, based on their 16S-rRNA sequences, are classified as members of the group of □- Proteobacteria (Kung et al., 2010).

Table 2- Classification of P. aeruginosa (Kim et al., 2012)

Domain	**Bacteria**
Phylum	**Proteobacteria**
Class	**Gamma proteobacteria**
Order	**Pseudomonadales**
Family	**Pseudomonadaceae**
Genus	**Pseudomonas**
Species	**Pseudomonas aeruginosa**

2.5.1 Characteristic of Pseudomonas aeruginosa

A Gram-negative bacteria P. aeruginosa is nonfermenting bacillus, which belongs to genus Pseudomonas. It easily grows on regular media. This species creates several bacterial pigments such as pyocyanin. More than half of all clinical isolates produce the blue-green pigment pyocyanin(El Solh & Alhajhusain, 2009). P.aeruginosa has beensequestered from various environments such as soil, plants, and different aquatic environments. Human wastewater is one of the most common sources for the isolation of P.aeruginosa. Metabolically, a number of carbon sources- even aliphatic, halogenated and non-halogenated aromatic carbon compounds can be utilized by P. aeruginosa (Mah et al., 2003). This trait makes it an attractive bacterium for bioremediation and detoxification of contaminated soil and aquaticsystems. It is a major drawback for environmental applications (Kung etal., 2010). It can grow in distilled water", which is substantiation of its minimal nourishing needs. In the laboratory, the pretentious medium for growth of P.aeruginosa contains ammonium sulfate as a source of nitrogenand acetate as a source of carbon (Høiby, 2011). P. aeruginosa can endure the most challenging environments due to its low nutritional requirements and ability to exploit a range of natural and artificial compounds as a

carbon energy source. Consequently, it is no surprise that this ubiquitous bacterium can thrive in disinfectants and catheters (Williams et al., 2010). Pseudomonas spp. are bacteria in natural surroundings might be initiated in a planktonic form or an in biofilm, attached to some surface or substrate, as a unicellular organism, actively swimming using its flagellum (Wei & Ma, 2013). It can achieve anaerobic growth with nitrate as a terminal electron acceptor, and, in its absence, it is also able to hydrolysis arginine by substrate-level phosphorylation. Adaptation to anaerobic environments or microaerobic is essential for certain lives of P. aeruginosa, for example, through lung poison in cystic fibrosis patients, where thick layers of alginate adjacent bacterial mucoid cells can limit the diffusion of oxygen through its (Cooper et al., 2003). Pseudomonas aeruginosa stands a range of physical conditions, including temperature, even though the optimum temperature for its growth is 37°C. It is accomplished by growing at temperatures as high as 42°C (Ubonchonlakate et al., 2012). In liquid culture the cells occur singly, in solid culture the cells occur in pairs or occasionally in short chains. A slime substance surrounds the cells of some strains usually surrounds the alginate (Patrick & Baron, 2013).

Nosocomial infection

Hospital infection is one of the most important causes of deaths in burn units, as studies have indicated that a large proportion of deaths are related to this infection due to resistance of pathogens associated with burns to antibiotics (Tayh, 2013). The causes of hospital infection are of internal origin endogenous, which are caused by microorganisms that are part of the patient's natural flora, and of external origin exogenous that gain from the patient's exposure to the hospital environment (Samuel et al., 2010). The role of contaminated medical devices in the transmission of the healthcare-associated pathogen and environmental surfaces has been well reported (Otter et al., 2015). Previous studies recommend that microbial contamination of those devices and surfaces play the main role in the range of pathogens (Gebel et al., 2013). The ability of microorganisms to remain viable on dry surfaces effective on pathogen transmission, their resistance to disinfectants and the frequency that devices are in contact with patients and healthcare workers or contaminated surfaces (Weber et al., 2010).

The bacteria is one of the pollutants hospitals known, and its existence becomes one of the biggest problems experienced by personals who work in the hospitals such as doctors, nurses, workers (Krogulski, 2008). They are at risk of injury from clinical specimens or wounds exudate, which may be the source of contamination with the bacteria that moves to a group of not infected patients of others injured either by direct contact between patients and staff (Biccard and Rodseth, 2011). Another report showed that bacteria could transform from a variety wet sources in hospitals such as water cycles, faucets, wipers territory, clothing collection containers and soap savers (Vincent et al., 2000). Pseudomonas aeruginosa is one of the famous bacteria which could be found and caused pollution in the hospitals are isolated from plastic containers that used to carry bandages which were a way for the transfer of bacteria from one patient to another replaced with metal containers possibility sterilized after use (Schechner et al., 2009). These bacteria have the ability to live in some sterile solutions and polluted it, such as cleaning solvents to eyes and physiological fluids and sterile chloride solutions. The difference of contamination percentage between hospital depends on the type of patient treated and methods of supervision, control, and efficiency of medical staff (Irazoqui et al., 2010).

P. aeruginosa Associated wound and burn infection

Pseudomonas aeruginosa is one of the most common pathogens isolated from burn patients throughout the world (Sousa et al., 2018). P. aeruginosa is an opportunistic bacterium associated with healthcare infections in intensive care units (ICUs), ventilator-associated pneumonia (VAP), surgical site infections, and burns (López-Jácome et al., 2019). Burn wounds infection is a great problem because it may lead to death in 75% of patients with injuries (Santucci et

al., 2003). The undamaged human skin surface is vital to protect the homeostasis of bodily fluid, thermo-regulation and host infection control. As the first line of defense, the skin is equipped with arrange of immune mediators capable of engaging inflammatory cells to support neutralization and clearance of microbes, it is one of the most important pathogens involved in burn infections (Steinstraesser et al., 2004; Rafla and Tredget, 2011). Pseudomonas aeruginosa is a common nosocomial pathogen in burn patients, and acquires antibiotic resistance rapidly; thus the most successful method to fight infection is the efficient therapeutic approach (Ranjbar et al., 2019). The high prevalence and gradual increase of MDR, particularly in burn centers P. aeruginosa seriously threats the patients with severe burn injure (Dou et al., 2017; de Almeida Silva et al., 2017).

Burn wound infections are one of the most important complications that occur after burn injuries and may be associated with serious clinical complications and increased morbidity and mortality (Turner et al., 2014). Burn injury compromises the primary barrier of the host, the skin, which immediately places the host at risk for infection (Lopez et al., 2017). Burn wounds are major public health problems all over the world. Infection is one of the most complicated issues in burn patients, because the skin, a barrier against microbes, has been destroyed and the immunity agents cannot reach the sites of infection. There is a correlation between the severity of infection and the extent of the burn (Anvarinejad et al., 2014). This bacterium causes 75% mortality in burned patients as it can establish a persistent infection biofilm, express multiple virulence and antibiotic resistance mechanisms. Some of these virulence factors are proteases such as elastase and alkaline protease, or toxic metabolites such as pyocyanin which is one of the few microorganisms able to produce cyanide, which inhibits the cytochrome oxidase of host cells (López-Jácome et al., 2019). Multiple antibiotic resistant P. aeruginosa is a major cause of burn wound infections and inflammation of skin and soft tissue. Because of its resistance to commonly used antibiotics and antiseptics, there is a shortage of therapeutic options for effective treatment P. aeruginosa normally affects patients of infections with burn and wound where the primary condition can be more complicated and may also cause bacteremia (Inacio et al., 2014).

Epidemiology of P.aeruginosa

P. aeruginosa colonizes eukaryotic hosts including humans, animals, plants, worms and. P. aeruginosa's fondness for water extends, to moist objects in hospitals including respirational equipment, disinfectants, and hospital sinks. Thus it is a leading cause of nosocomial infections (Fujitani et al., 2011). Patient-to-patient transmission through contaminated medical devices and multi-vials drugs is a well-established mechanism of P. aeruginosa spreading in health care settings (HCS). Furthermore, the resistance of P.aeruginosa to a variety of chemical compounds, including antibiotics, hospital disinfectants facilitate, and detergents its long-term persistence in the HCS and the spreading among patients (Lanini et al., 2011). Pseudomonas aeruginosa seldom a member of the normal microbial flora in humans, representative colonization rates for specific sites in humans are 6.6% for the throat samples, 3.3% for the nasal mucosa and 2% for skin. Nevertheless, colonization rates may exceed 50% throughout hospitalization, mainly among patients who have experienced trauma or a breach in mucosal barriers or cutaneous by surgery, mechanical ventilation, catheters, tracheostomy, or severe burns, Patients with impaired immunity and disruption in the normal microbial flora as a result of antimicrobialtherapy have higher risks for colonization by this organism (Lister et al., 2009).

2.9. Pathogenesis

Most of Pseudomonas infections are both toxinogenic and invasive. The critical Pseudomonas infection may be seen as composed of three distinct stages bacterial attachment and colonization, local invasion and disseminated systemic disease. Conversely, the disease development may stop at any stage (Miyata et al., 2003). The pathogenicity of P.aeruginosa is

multifactorial depends on numerous virulence factors including cellassociated factors and secreted factors (Karatuna & Yagci, 2010). P.aeruginosa rarely infects healthy tissues, but when defenses are compromised, it can infect virtually all tissues (Morrison & Wenzel, 2015). These infections should be well-thought-out as severe, and even lifethreatening in specific situations, with the highest rate of mortality recorded for cases of bacteremia in neutropenic patients (Berthelot et al., 2005). P. aeruginosa is well-adapted to the respiratory tract environment, especially in patients with the chronic obstructive bronchopulmonary disease, who are hospitalized in intensive care units or immunocompromised, (Driscoll et al., 2007). It is a major cause of chronic respiratory infection (CRI). CRI by P. aeruginosa is the leading cause of morbidity and mortality in cystic fibrosis (CF) patients and a frequent complication of other respiratory diseases such as chronic obstructive pulmonary disease (COPD) or bronchiectasis (Mahar et al., 2010). According to Chastre & Fagon (2002), P. aeruginosa is the predominant cause of nosocomial pneumonia in ventilated patients. In neutropenic cancer patients undergoing chemotherapy is a common complication of Bacteraemia with P.aeruginosa (Krcmery et al., 2006). Bacteraemia and Septicemia can also occur in patients with immune deficiency-related to AIDS, diabetes mellitus or severe burns (Marra et al., 2006). Most of these contaminations are innate in hospitals and nursing homes. P.aeruginosa is also the third leading cause of hospital-acquired urinary tract infections. These infections can occur via descending or ascending routes and are usually secondary to urinary tract catheterization, surgery or instrumentation. „Swimmer‟s ear' (a form of external otitis) caused by P.aeruginosa is the predominant and malignant otitis in diabetic patients. Devastating ophthalmic infections, meningitis and brain abscesses can be caused by P. aeruginosa. Skin and bone infections can also occur by P.aeruginosa after puncture wounds, but it rarely causes infections of the digestive tract. Although, perirectal infections, typical gastroenteritis, and necrotizing enterocolitis (Lavery et al., 1994)

Virulence factors

Pseudomonas aeruginosa virulence is multifactorial and combinatorial, and it varies substantially depending on bacterial physiology as well as on the strain involved. This organism produces a broad array of toxins and other virulence factors that cause immune evasion, tissue damage, and haemorrhage. The virulence factors can be proteinaceous or chemical, and either cell-associated or secreted. Proteinaceous virulence factors are often secreted through one of the five protein secretion systems in P. aeruginosa: type I, II, III, V and the recently discovered type VI (Mikkelsen et al., 2009). These virulence factors can be summarized in **Table.**

Table (2) Virulence factors in P. aeruginosa (Brooks et al., 2007).

Cell-Associated Virulence Factors	
Virulence factor	**Functions**
Extracellular Slime layer Substance	capsular polysaccharide and associated with the outer membrane complex
Flagella	Motility, attachment of bacteria to host cells
Pili	Motility, epithelial interaction.
Lipopolysaccharide	Epithelial and TLR4 interaction.
Capsule(Alginate)	Epithelial interaction, bacterial protection.

Extracellular Secreted Virulence Factors	
Virulence factor	**Functions**
Pyocyanin	host-response , neutrophil apoptosis.
Pyoverdine	Iron chelation, regulation of exotoxin A.
Alkaline protease	Fibrin lysing protease, neutrophil function.
Protease IV	Degradation of host tissue and plasma proteins.
Elastase	Degrades tissue and plasma proteins, neutrophil function.
Phospholipase C	Surfactant inactivation, neutrophil function.
Exotoxin A	Inhibits elongation factor 2 (protein synthesis).
Neuraminidase	the enzyme acts to release sialic acid(N-acetyl neuraminic acid) from GM1-ganglioside receptors facilitates attachment of pili and increase adhesive with epithelial cells
Dnase	acts on DNA of host cells and inhibition of genetic machinery of phagocytic cells
Urease	Responsible for the production of renal stone. In gastrointestinal tract infections urease protect *P.aeruginosa* against stomach pH
Type III Secretion System	
Virulence factor	**Functions**
Exo S	Disrupts cytoskeleton interacts with TLR2
Exo T	Disrupts cytoskeleton.
Exo Y	Adenylate cyclase injected into host cytosol.
Exo U	Major cytotoxin, phospholipase activity.
Quorum sensing:	
las	*las*→AHL → transcriptional activation of virulence genes.
rhl	*rhl*→ HL → transcriptional activation of virulence genes.

Biofilm

Biofilms are highly organized microbial communities encased in a polysaccharide matrix and attached to a surface. In the recent years, a model has emerged regarding bacterial growth mode chronic infection and virulence during acute. Acute infection was understood to free-swimming cells that are highly virulent or involve fast- growing planktonic, while the chronic infection is believed to involve biofilms consisting of slower growing less virulent cells. Bring to mind that of planktonic cells in stationary phase has been showed by the physiology of biofilm cells, and this has been suggested to be a major factor in the resistance of biofilms to antibiotics. Additionally, less virulent strains appear to become more abundant over time in chronically infected cystic fibrosis CF patients, that in several cases are owing to mutations of the primary quorum sensing regulator lasR (Dtsch et al., 2012).

Resistance to Antimicrobial Agents

Pseudomonas aeruginosa signifies an unusual phenomenon of antimicrobial resistance among prokaryotes since practically all known mechanisms of resistance are found in this organism including decreased outer membrane permeability, increased expression of efflux pumps system, penicillin binding protein modification, alginate and enzymatic inactivation of antibiotics (Strateva & Yordanov, 2009). Intrinsic and acquired resistance make P.aeruginosa as one of the most difficult organisms to treat and eradicate. Even though its intrinsically sensitive to ßlactams, such as ceftazidime and imipenem, aminoglycosides like amikacin and tobramycin, and fluoroquinolones as ciprofloxacin and ofloxacin, resistance to these antibiotics has emerged (Sekiguchi et al., 2007). Pseudomonas aeruginosa has a unique ability for the development of antimicrobial surrender to almost all antipseudomonal agents over the selection of mutations in chromosomal genes leading to the hyperexpression of the chromosomal cephalosporinase ampC, cephalosporins, conferring resistance to penicillins and the inactivation of oprD the decisive resistance to carbapenems. The variation of the DNA topoisomerases, advising resistance to fluoroquinolones, the up-regulation of one of the several efflux pumps, potentially confers

resistance to multiple agents, such as β-lactams, fluoroquinolones, and aminoglycoside. These various mechanisms often lead to cross-resistance with other antimicrobial classes (Bulik et al., 2010).

Antibiotic Resistance P. aeruginosa

The nature of this organism's inherent resistance to several antibiotics (βlactam, penem group antibiotics) and its ability to develop further mechanism of resistance to various classes of antibiotics, including beta lactam, amino-glucoses and fluoro-quinolones, makes it difficult to treat infection caused by it. Microbes have implemented various mechanisms to preserve genome plasticity in their molecular evolution. Microbes are mainly used to shape biofilms, quorum sensing, horize gene and enzyme promiscuity for their survival (Pachori et al, 2019; Mohanty et al., 2021).As well as excessive use of antibiotics during treatment accelerates development of multidrug-resistant P. aeruginosa strains, leads to the inefficacy of empirical antibiotic treatment for this microorganism (Hirsch and Tam, 2010). Generally, the major mechanisms of P. aeruginosa used to counter antibiotic attack can be classified into intrinsic, acquired and adaptive resistance figure (1-1).

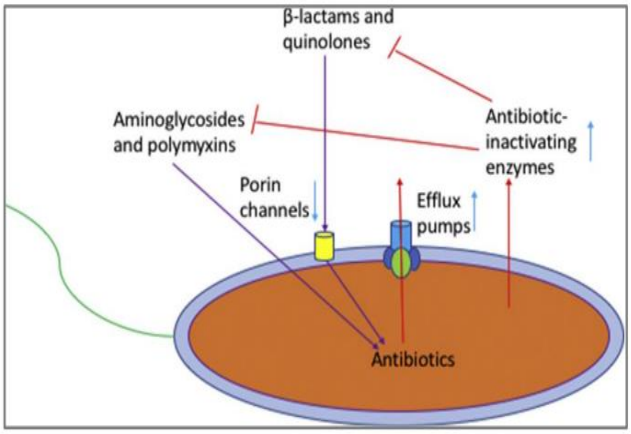

Figure (1-1). A schematic representation of the mechanisms of intrinsic antibiotic resistance in P. aeruginosa (Pang et al., 2019).

Intrinsic Resistance

The bacterial species' intrinsic antibiotic resistance refers to their innate capacity to reduce the effectiveness of a particular antibiotic by its own structural or functional properties (Pang et al., 2019). Pseudomonas aeruginosa has been shown to have a high degree of intrinsic antibiotic resistance due to restricted outer membrane permeability, efflux systems that pump antibiotics out of the cell, and alginate formation, as well as the transfer of resistance genes and the production of antibiotic-inactivating enzymes such as lactamases (Balasubramanian et al., 2013).

Outer membrane permeability

In order to meet intracellular goals, most antibiotics used in the treatment of infections of P. aeruginosa need to be able to enter the cell membrane (Lambert, 2002). For example, bacteria protein-binding synthesis with ribosomal 30S units is inhibited by the aminoglycoides family of antibiotics like tobramycin, gentamicin and amizacin (Mingeot-Leclercq et al., 1999). Quinolone antibiotics such as Ciprofloxacin and Levofloxacin interfere with DNA replication by inhibiting DNA gyrase and topoisomerase IV (Aldred et al., 2014). The β-lactam ring is present in the molecular structures of -lactam antibiotics such as penicillin, cephalosporin, carbapenem, and monobactam. This class of antibiotics blocks bacterial cell wall biosynthesis by targeting the penicillin binding proteins that are enzymes involved in peptidoglycan synthesis (Poole, 2004). Polymyxins are a class of polypeptide antibiotics that attach to the lipopolysaccharides (LPS) on Gram negative bacteria's outer membrane, causing increased permeability and antibiotic absorption. Polymyxin B and polymyxin E, also known as colstin, are the two polymyxins used in clinical practice, and they kill bacteria by induction of a hydroxyl radical-mediated cell death pathway (Zavascki et al., 2007). To enter the bacterial cell, β-lactams and quinolones penetrate cell membranes through porin channels, whereas aminoglycosides and polymyxin promote their own uptake by interacting with bacterial LPS on the outer membrane of Gramnegative bacteria (Lambert, 2002).

Antibiotic-inactivating enzymes

Bacterial cells produce enzymes that target antibiotics and render them inactive by chemical modifications such as the addition of specific

chemical moieties or complete destruction of the antibiotic molecule. Many antibiotics have chemical bonds such as amides and esters that are susceptible to hydrolysis (Wright, 2005; Munita and Arias, 2016; Arzanlou et al., 2017). By enzymes commonly produced by P. aeruginosa such as β-lactamases and aminoglycosidemodifying enzymes (Poole, 2005; J Wolter and D Lister, 2013). P. aeruginosa

has an amps gene that encodes the hydrolytic enzyme -lactamase, much like other Gram negative bacteria. This enzyme will sever the amide bond of the lactam ring, rendering -lactam antibiotics inactive (Wright, 2005). Furthermore, β- lactamases can be classified into four groups based on their amino acid sequences: A, B, C, and D. Via an active site serine, enzyme groups A, C, and D hydrolyze -lactams. Class B -lactamases, on the other hand, are metallo enzymes that require divalent zinc ions for -lactam hydrolysis (Bush and Jacoby, 2010). The class C β-lactamase produced by P. aeruginosa has been shown to inhibit anti pseudomonal cephalosporin's, a class of β-lactams (Berrazeg et al., 2015). Extended-spectrum-β-lactamases (ESBLs) have been discovered in some P. aeruginosa isolates, which confer a high level of resistance to the majority of lactam antibiotics, including penicillins, cephalosporins, and aztreonam. (Paterson and Bonomo, 2005; Rawat and Nair, 2010).

Adaptive Resistance

Pseudomonas aeruginosa's adaptive resistance includes biofilm formation in the lungs of infected patients, where the biofilm acts as a diffusion barrier to restrict antibiotic access to bacteria cells, In addition, multidrug-tolerant persister cells that are able to survive antibiotic attack can form in the biofilm; these cells are responsible for prolonged and recurrent infections in cystic fibrosis (CF) patients (Drenkard, 2003; Mulcahy et al., 2010).

Acquired Resistance

Bacteria can gain antibiotic resistance through mutational changes or acquisition of resistance genes via horizontal gene transfer (Munita and Arias, 2016). Moreover, in biofilms may form multidrug resistant cells capable of surviving antibiotic attacks, responsible for prolonged and recurring infections in CF patients (Hainrichson et al., 2007).

Resistance by mutations

Mutational changes are ready to cause reduced antibiotic uptake, modifications of antibiotic targets, and overexpression of efflux pumps andantibiotic-inactivating enzymes; all of which permit bacteria to survive within the presence of antimicrobial molecules (Munita and Arias, 2016). Porins form small water-filled channels within membranes that mediate the diffusion of hydrophilic antibiotics, up to a particular size exclusion limit (Welte et al., 1995). Spontaneous mutations can affect the expression or function of a selected porin, thereby reducing bacterial membrane permeability and increasing antibiotic resistance (Fernández and Hancock, 2012). As mentioned earlier, to stop the intracellular accumulation of toxic compounds, bacteria employ energydependent efflux systems to pump the toxic molecules out of the cells (Sun et al., 2014). As a result, clinical isolates of P. aeruginosa with overexpressed efflux pumps are less susceptible to antibiotics (Llanes et al., 2004; Cabot et al., 2011;Poonsuk et al., 2014 ; Cabot et al., 2016).

2.10.3.2.Acquisition of resistance genes

Antibiotic resistance genes can be carried on plasmids, transposons, integrons and prophages, and bacteria can acquire these genes via horizontal gene transfer from the same or different bacterial species (Breidenstein., 2011). Integrons are genetic elements that insert mobile gene cassettes into a specific genetic site via site-specific recombination (Hall and Collis, 1995). and they have been shown to play a critical role in dissemination of antibiotic resistance among P. aeruginosa strains (Chen et al., 2009; Odumosu et al., 2013; Khosravi et al., 2017). The main mechanisms of horizontal gene transfer involve transformation, transduction and conjugation (Arber, 2014). For example, six types of P. aeruginosa metallo-beta-lactamases (MBLs) have been identified, including imipenemase (IMP),

Verona integron-encoded metallo-lactamase (VIM), and Sao Paulo metallo-lactamase (SPM), which belong to the class B -lactamases that hydrolyze most - lactam-based antibiotics (Hong et al., 2015). The genes for these P. aeruginosa MBLs have been detected being carried by genetic elements, including integrons and plasmids (Castanheira et al., 2004; Bonomo and Szabo, 2006; Khajuria et al., 2013 ; Cavalcanti et al., 2015).

Results and Discussion

Data distribution

A total of 120 samples were collected from (Imam AlSadiq Hospital).

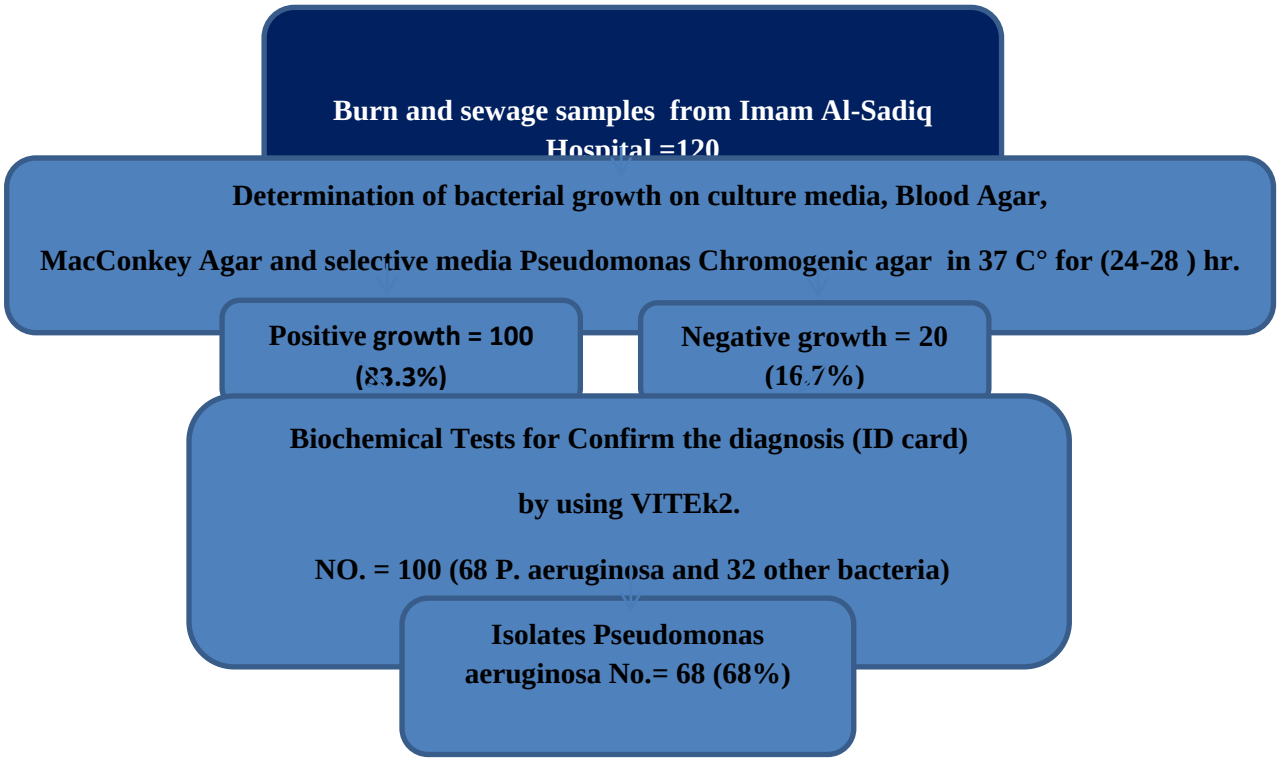

Figure show Isolation and identification of Pseudomonas aeruginosa isolates.

Distribution of burn patients according to the age

All samples were taken from burned patients with age from 1- 80 years for both sexes male and female (table 4-1).

Table (4). The distribution of burn patients according to the age.

Age group (years)	No. of patient (%)	p-value
1-10	**42 (35%)**	0.0001**
11-20	**28(23.3%)**	
21-30	**15(12.5%)**	
31-40	**19(15.8%)**	
41-50	**10(8.3%)**	
51-60	**4(3.3%)**	
Above 61	**2(1.7%)**	
Total	**120**	

Table (4) showed the distribution of burn patients according to the age the most prevalent age group (1-10 years), followed by (11-20 years), (21-30 years), (31-40 years), (41-50 years), (51-60 years) and the least prevalence age group is those over (61 years) it represented by the following percentages (35%, 23.3%, 12.5%, 15.8%, 8.3%, 3.3%, 1.7%) respectively of the collected burned patients included in this study. There was high significant differences ($P \leq 0.0001$) this increase in child (1-10 years) linked to several reasons like lots of movement of children a lack of awareness and lack of knowledge of the nature of fire-causing materials and how to deal. This result is agreement with of study that conducted by Agbenorku et al. (2011), which reported that scalds were seen commonly in children. And with the study from Palestine published by Tayh (2013) which showed that burn injuries in children (72%) were much more than burn injuries in adult (28%),

this may be due to the fact that children have more mobility inside houses and have less sense and awareness of dangers. Also, this study was consistent with Peck (2011) which found that the highest rate of burn injuries was in the children's group in the age group between (0 - 9) years, at a rate of 58 54.2% where deaths caused by burns occur among children at a rate of ten times in developing countries about it in the developed world. Burns are one of the top fifteen major causes of child deaths, and the reason may be due to the neglect that children are exposed to while they are inside their parents' homes. Slovis (2011) found that the highest incidence of scald burn occurs among children under the age of five. Tekin et al. (2013) showed that 65% of cases have scalding burn and high rate of scalding burns was observed in the 0-5 year age group. Elsous et al. (2015) revealed that self-effect burns injuries carry significant morbidity and mortality between then appropriate younger persons, this search measured the outcome and epidemiologic pattern of this injury in a burns spread in Pakistan.

Distribution of burn patients according to the sources, gender

Table (5). The distribution of burn patients according to the sources, gender.

Gender		Imam Al-Sadiq Hospital %	Total
Male	Children	**35 (61.4 %)**	**57(47.5%)**
	Adult	**22 (38.6 %)**	
Female	Children	**22 (34.9 %)**	**63(52.5%)**
	Adult	**41 (65.1 %)**	
Total		**120**	

Table (5) showed the distribution of burn patients burn injuries according to the sources, gender, most commonly observed the highest percentage of burns in females was 63(52.5%) comparison to that of males which was 57(47.5%). This wasconsistent with the results of Farhood and Chelab (2017) which found that the number of diagnosed cases of burn injuries for female were 61 cases, with a percentage of 57% while there was 46 cases of burns for males with a percentage of 42.9%. Church et al. (2006) found that the rate of isolation of 63% for females and 36% for males. This is related with nature of women's work at home and their preoccupation with household chores, especially with regard to cooking or near sources of fire, liquids and hot fumes. Also, other studies from developing countries such as Zambia, South Africa, Malawi, Peru, Turkey and many countries (Mukerji et al., 2001; Peck et al., 2008; Agbenorku et al., 2011; Aliosmanoğlu, 2011; Samuel et al., 2011). Big majority of females were housewife and scalding

burns were frequently encountered in females. It also agrees with the study conducted by Panjeshahin et al. (2001) in Iran in which females were the victims of burns more frequently than males. They attributed the high number in females to the following reasons: First, most of females were housewives with low level of literacy, as these people mainly work at kitchen. Second, traditionally the style of females' clothes which has a higher volume compared to European females' clothes. Third, the material of females' clothes is mostly synthetic type comparing to the males' clothes suggesting that the females' clothes are more easily flammable.This is in contradiction to a study conducted in Morocco by Essayagh et al.(2019) which reported a higher incidence of burn injuries in male (64%) than in female (36%). Also, Gayathri et al. (2015) found that the incidence of males is greater than that of females, as the percentage of males was 54%, while the percentage of females was 46%. It requires a great deal of risk or because of doing some free business that is not related to the state's departments. Furthermore, Sharmeen et al. (2012) found that largest death from burns in new married women in India and most middle East its conceder a big problems. The reason is back open fire on bread and cooking by ovens called tandoor

were traditional habits ofwomen in were living in rural areas and some areas city We thought that it was responsible for high rates of burns in women and children. On the other hand It can be concluded that children, female homemakers, and workers in the Iraq society are at a higher risk of burns.

Gender distribution of burned patients according to the cause of burn

Table (6). Gender distribution of burned patients according to the cause of burn.

Gender		**Cause of burn (%)**			**Total cases (%)**		**p-value**
		Fire	Liquid	Electricity			
Male	Children	**13 (37.1%)**	**21 (60 %)**	**1(2.9%)**	**35 (29.2 %)**	**57(47. 5%)**	
	Adult	**12(54.5 %)**	**8(36.4%)**	**2(9.1%)**	**22(18. 3%)**		
Female	Children	**4(18.2 %)**	**18(81.2%)**	**0**	**22(18. 3%)**	**63(52. 5%)**	0.04*
	Adult	**27(65.9 %0**	**13(31.7%)**	**1(2.4%)**	**41(34. 2%)**		
Total		**56(46.7 %)**	**60(50%)**	**4(3.3%)**	**120**		
p-value	0.002*						

Table (6) showed the gender distribution of burned patients according to the cause of burn. The results of the current study showed that the most common cause of burns in burn patients was liquid material burns 60(50%) and included (hot water, milk, tea, petrol, oil, and gasoline), followed by fire burns 56(46.7%) and electricity burns 4(3.3%). The results showed that the percentages of liquids burns in men [children 21(60%) , adult 8(36.4%)] and the percentages of liquids burns in women [children18(81.2%), adult 13(31.7%)] and this shows that women are more exposed to liquid burns (scalding) than men, followed by fire burns (flame), where the percentages in men were [children 13(37.1%), adult 12(54.5%)] and in women [children 4(18.2%), adult 27(65.9%)] This is also prevalent in women compared to men, followed by electrical burns in men [children1(2.9%), adult 2(9.1%)] and in women 0(0%) which showed that they are more prevalent in men than in women.($p \leq 0.05$). The results of the current study are almost in agreement with a study conducted in Palestine by Tayh (2013) which showed that the accidents of hot liquids (scalds) (66.1%), followed by fire (33.9%) were the main reasons for burn accidents. This may be explained based on the fact that hot liquids are of high importance at our homes (where women and children usually exist) and most frequently used in many life aspects. Farhood and Chelab (2017) found that the highest rate of burn injury was burning with boiling water by 38.3%, followed by burning by gas flame with a rate of 28.9%, and the lowest percentage of burn injury was electrical burns by 4.67% . Yousefi-Mashouf and Hashemi (2006) mentioned that the highest burning rate was with boiling water by 23.4%, then burning by gas flames by 14.5%, and the lowest burning rate was by electric burns by 4%. Özkurt *et al*. (2012) found that the highest insulation rate was for boiled water, with an isolation rate of 65.5%, then gas flame burns, with an isolation rate of 13.6%. Jithendra *et al*. (2015) found that the highest incidence of infection was with hot water, followed by flame burns. Also, this finding compatible with other studies in Egypt Nasser et al. (2009) , Haik et al. (2007), Alaghehbandan et al. (2001), and Ho and Ying (2001). Additionally, the current study was consistent with the results of two Iranian studies that reporting the most prevalent causes of burns in men as oil and its products, in children and in women as scalds (Ahmadi et al., 2006; Goodarzi et al., 2014). Essayagh et al. (2019) reported that

the predominant burn agent to be gas flame, followed by scalding liquid and contact with an electrical source. American Burn Association (2019) reported that, overall, flame burns are still the majority of injuries in the USA (41%), with scalds second at (31%), Chemical (3.5%) and electrical burn injuries (3.6%) occur much less commonly. The results of the current study did not agree with study of Samuel et al. (2011) Which stated that the highest rate of injury was by burning by gas flames, followed by burning by boiling water burns rate was 29%, followed by electric burns by 18%. Also, did not agree with the study conducted by him Irfan et al. (2014) in which it was stated that the highest incidence of gas burns was 29%, followed by electrical burns by 18%. According to gender, the present study revealed that burns are more prevalent in female homemakers workers and children, were more likely to get burns due to hot liquids (scalds). Samimi et al. (2011) reported that most the burns to be more in children, and Stampolidis et al. (2012) reported burns to be more prevalent in female homemakers and workers. With regard to the findings of the present study and other studies, it can be concluded that although the causes of burns are known and several, the people are not cautious and ignore the safety instructions and standards in the use of flammable and explosive materials.

Bacterial growth and percentage in burns

The prevalence of bacterial growth in collected swab sample was 83.3% while growth negative was 16.7%,

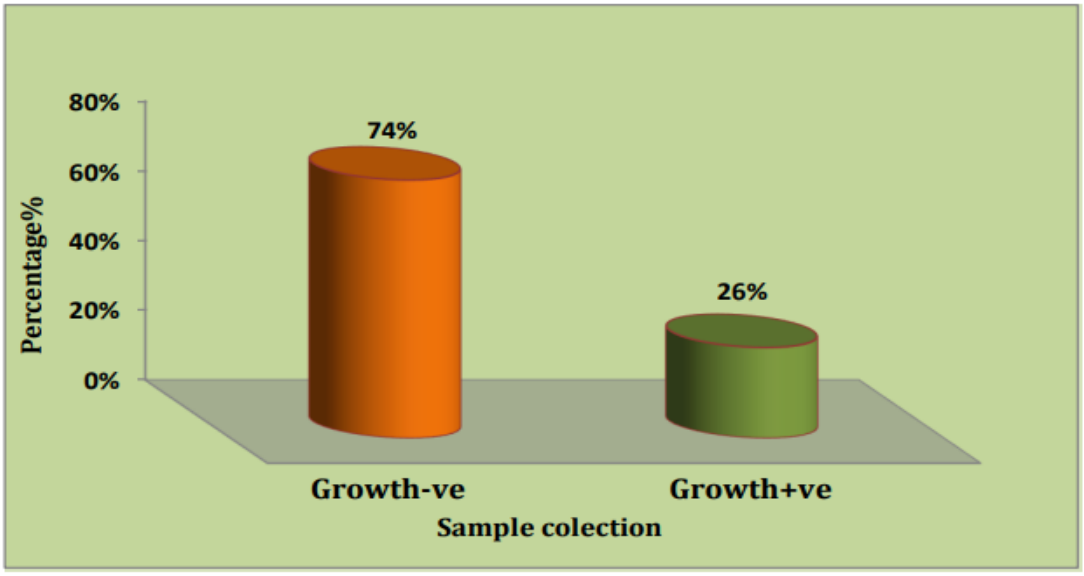

Figure. Distribution of growth positive bacteria in collected samples.

Severe burns are one the serious forms of trauma, including loss of the skin barrier and tissue destruction. Indeed, tissue injury at burn sites results in the production of biological fluids which defined as burn wound exudates (BWEs) (Oncul et al., 2009; Gonzalez et al., 2016). The immunosuppression state and burntissue microenvironment are favorable features for burn wound pathogens colonization and proliferation which lead to the spread and growth of different type of bacteria in the burn area according to the degree of burning of the body (Gonzalez et al., 2016).

Bacterial culture

The growth and types of bacteria vary over time in the burn area from one person to another, where when victims arrive at the hospital after a criminal or accidental accident. An initial culture was done to diagnose whether they had a bacterial infection or not. This is related to the patient's delay in arriving at the hospital or not coming directly. Most of the culture results confirmed that most of the patients from whom samples were collected showed bacterial infection after the third day of admission. This supports the findings of Al-Musawi and AlGarawi (2015) who found that

66% of skin swabs gave positive growth culture for three days in the hospital, while the result was 88% after seven days of hospitalization. On the other hand, the results of the current study proved that there was a positive relationship between a longer stay in hospital and the high prevalence of pathogenic bacteria causing burn infections. Contaminated burning wards and duration of patients stay in hospital, in addition to the size of surfacearea of burned skin are the most important reasons to increase of persistent and multiplication of pathogenic bacteria in the burned areas (Al-Aali, 2016).

Table (7). Gram negative bacterial isolates obtained from burned patients explain adult and children.

Isolated bacteria	**Male**		**Female**		**Total Number**
	Adult	Children	Adult	Children	
Pseudomonas aeruginosa	6(8.8%)	25(36.8%)	28(41.2%)	9(13.2%)	68(68%)
Klebsiella	1(8.3%)	3(25%)	2(16.7%)	6(50%)	12 (12%)
E. coli	2(20%)	3(30%)	2(20%)	3(30%)	10 (10%)
Proteus	1(25%)	0	3(75%)	0	4 (4%)
Acinetobacter	0	1(33.3%)	2(66.7%)	0	3 (3%)
Citrobacter	1(33.3%)	0	2(66.7%)	0	3 (3%)
Total summation	43(43%)		57(57%)		100(100%)
p-value	0.026*				

Table (7) showed the gram-negative bacterial Isolates obtained from burned patients.

The results showed the numbers and percentages that were obtained from clinical samples of burn patients of both sexes, males and females, which showed high levels of p. aeruginosa bacteria, followed by Klebsiella, E. coli, Proteus, Acinetobacter and Citrobacter they represent the following percentages 68 (68%), 12(12%), 10(10%), 4(4%), 3(3%) and 3(3%) respectively as shown in the figure (3-3) which shows the distribution of these percentages.

According to gender the results obtained showed that the prevalence of P.aeruginosa bacterial isolates was more in women [adult 28(41.2%) , children 9(13.2%)] than in men [adult 6(8.8%) ,children 25(36.8%)] ($p \leq 0.05$) This result agrees with the other result obtained by Singh et al. (2017), they found that the P. aeruginosa is the most common source of burn wound infection. And also agree with Nikokar et al. (2013) they mentioned that the high frequency rate of P.aeruginosa found in burn units might be due to the prolonged hospital stay and intensive use of antibiotics. The studies of Kirketerp-Møller et al. (2011) and Mhada et al. (2012), revealed the predominant organisms isolated from burns wounds were Pseudomonas aeruginosa [35.84%], Klebsiella species [27.30%], Acinetobacter species [20.13%], Escherichia coli [2.38%], Staphylococcus aurous [8.87%]. Farhood and Chelab (2017) showed that the highest isolate rate was for P. aeruginosa bacteria with an isolate rate of 38 (32.47%), followed by K.pneumonia bacteria with an isolated percentage of 25(21.36) and the lowest percentage of infection with

S.epidermis bacteria With an isolate rate of 2(1.7), while the percentages of the following bacterial species A.baumenii, E.coli,E.cloacae, S.auraus, B.cepesa, P.miribilles, P.agglomer were 15 (12.82%), 13(11.1%), 6(%5.12), 6(5.12%), 5(4.27%), 3(2.56%) respectively. These bacteria are considered opportunistic pathogens and rarely cause disease in healthy people, but they are highly virulence in patients with weak defensive mechanisms causes bacteremia, and therefore the contamination in hospitals with these pathogens have a pathological effect to deteriorate the condition of those sleeping there (Brown et al., 2012). Boyer et al. (2011) found that the isolates of P. aeruginosa bacteria were with an rate of 43 (41.3%). Also, nearly similar to the study of Kanagapriya et al. (2015) where he found the percentage of isolates of P. aeroginosa, K.pneumonia, E.coli, and P.miribila bacteria was 28%,20%,8%,4%, respectively. Tayh et al. (2016) mentioned that the percentage of isolates of P. aeruginosa, K.pneumonia, E.claocae, and A.baumannii bacteria were 37.50%, 25%, 10%, and 5%, respectively.Whereas Jithendra et al. (2015) that found, an increase in the rate of isolation of S.aureus bacteria in the first place, with an isolation rate of 39.8%, then followed by P. aeruginosa bacteria with an isolation rate of 35.3%. The reason may be due to the number of samples Which were included in the study or according to the geographical location, it varies from one location to another and from one hospital to another, as these bacteria were not found in this percentage in another hospital in the same city. George et al. (2015) found that the isolationpercentage of S.aureus, P.aeroginosa, K.pneumonia, E.coli, A.baumenii was 39.4%, 14.2, 13.4, 8.7, 7.9 respectively.According to gender, the results of the current study confirmed the prevalence of Pseudomonas aeruginosa bacteria in women more than men. And this result is agree with a study conducted in Iraq, Karbala city by Alkateeb et al. (2016), andwith the results of Kireçci and Kareem (2014) in the city of Sulaymaniyah, Iraqand Shewatatek et al. (2014) in Ethiopia. The results indicated a higher incidence of the bacterium in female and elderly patients. Many of the virulence factors formed by P. aeruginosa are ordered with diverse systems (Aljebory, 2019).Whereas, Chand et al. (2020) found the prevalence of P. aeruginosa isolates was 4.29%, in which the distribution in male patients 56 (64.36%) was higher than in female patients 31 (35.63%). The possible reasons might be types of studied populations, different geographical locations, type of hospitals. In addition, other reasons may be the male have a routine outdoor work and they are frequently in the risk of infection from the infected environments (Manandhar et al., 2018). It also contradicts Mokhtari and Amini (2019) which indicated that the percentage of P. aeruginosa in males was (53%) and in female patients (47%) and the highest percentage (28%) ranged between 24-29 years compared with the elderly. The current study showed a clear predominance of P. aeruginosa bacteria, and the prevalence of this bacteria may be due to its resistance to antibiotics and antiseptics, and the transformation of the burn area into a suitable medium for the growth of these bacteria due to the weak resistance of the skin tissues subject to burning and damage, in addition to the presence of this bacteria in abundance in the environment surrounding the patient in the burn unit or the nursing staff In hospitals, in addition to their presence in abundance on the number and medical supplies, in addition to the severity of overcrowding in the burn unit at times(Mooij et al., 2007). It was observed when comparing the current results with the local and global results that there is a convergence and difference in the rates ofisolation from different samples, and this is due to many reasons, including the variation in the number of samples collected by the researcher, as well as the degree of cleanliness and the type of sterilizers and disinfectants used in hospitals, as well as the difference in hygiene habits in each country. The study showed that P. aeruginosa was the most common bacterium from burned isolates and that its resistance to antibiotics was high, which requires careful monitoring of these microbes through continuous programs and activation of infection control committees in hospitals and the need to rethink the way to deal with infections according to health regulations applicable.

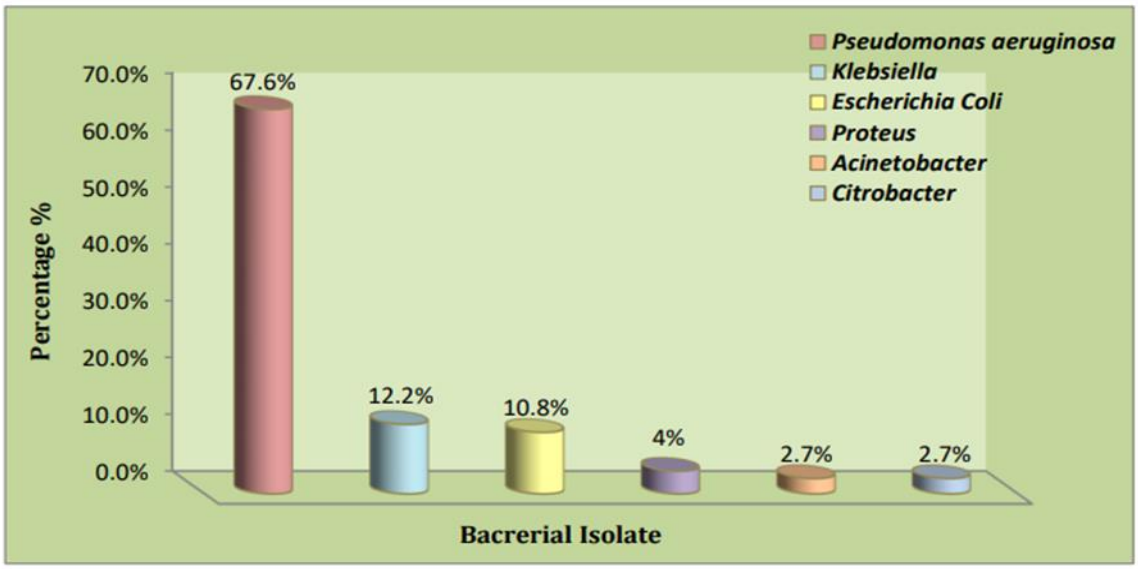

Figure .Distribution percentage of gram-negative bacterial isolates.

Isolation and identification of pseudomonas aeruginosa

The diagnosis was made based on the phenotypic characteristics of the bacterial isolates on each of the culture media that were used in the diagnosis, which is represented by the medium of MaCconkey agar, as the bacterial colonies were appeared as pale in color and not fermented the sugar lactose (lactose nonfermentation) (Forbes et al., 2002; Baron et al ., 2007). Either on blood agarmedium, the β-hemolysis bacterial colonies appeared, evidence of the production of hemolysin enzyme. (Selim et al., 2015; Procop et al., 2020). And onPseudomonas chromogenic agar medium which is a selective medium for P.aeruginosa incubated at 37 ºC during 24-48 hours, the bacterial colonies were appeared as magenta in color and the color of the medium that change from green to blue-green . Many studies found that the chromogenic agar for P. aeruginosa is promising medium for direct isolation and identification with high sensitivity and specificity (Laine et al., 2009; Momin et al., 2017). In addition to, Pseudomonas chromogenic agar will not only aid routine to detect P. aeruginosarapidly using only one media, but it will also provide Corresponding author the opportunity to conduct such procedures in a cost-effective and reliable manner(Sivri et al., 2014). As well as Pseudomonas chromogenic agar is a promising medium allowing for the isolation and simultaneous identification of P. aeruginosa from in burn infection (Al-Dahmoshi et al., 2018).

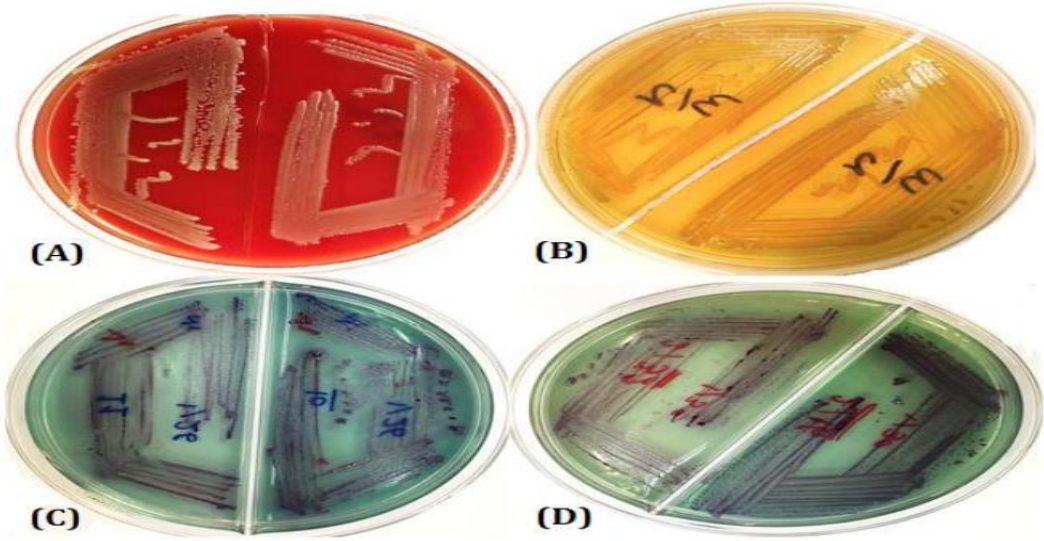

Figure . pseudomonas aeruginosa colonies on Culture Media. (A): pseudomonas aeruginosa isolate on Blood agar medium. (B): pseudomonas aeruginosa isolate on MacConkey agar medium. (C) and (D): pseudomonas aeruginosa isolates on Pseudomonas Chromogenic agar medium.

Conformational identification by VITEK2 GN ID card System

Diagnosis of pseudomonas aeruginosa isolates depends on the colonial morphology, biochemical tests, VITEK2 GN/ID card system. After the colonies of P. aeruginosa were grown on culture media (MacConkey agar / Pseudomonas Chromogenic agar) as shown in the figure (3-4B,C,D), diagnosis confirmed by using VITEK2 system, It is one of the best systems and devices to identify bacterial species in a short period and very accurately and was developed by the French company Biomerieux, characterized by fast detection of bacteria without the need for many of culture media as well as reduce cultural contamination, through the using GN-ID cards which contained 64 biochemical tests. Table 3-6 demonstrated that pseudomonas aeruginosa were confirmed with level excellent.

Table (8). Identification results pseudomonas aeruginosa by VITEK2 GN- ID card System

bioMérieux Customer: Microbiology Chart Report Printed May 3, 2023 9:55:44 AM AST

Patient Name: A3. Patient ID: 32107
Location: Physician:
Lab ID: 32107 Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	6.43 hours	**Status:**	Final
Selected Organism	96% Probability	**Pseudomonas aeruginosa**		
	Bionumber:	0043053343500210		
ID Analysis Messages				

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

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