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Molecular Characterization and Antibiogram of *Pseudomonas Aeruginosa* Isolated from Postsurgical Infections

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Molecular Characterization and Antibiogram of *Pseudomonas Aeruginosa* Isolated from Postsurgical Infection

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Jerusalem-Palestine

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Dedication:

To my Father, my mothers, my Husband, my brothers, and to my sisters.

A great thank for your support.

Hiba Najeh Yassen Milhem

Declaration:

I Certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted to any for a higher degree to any other university or institution.

Signed: Hiba ……………

Hiba Najeh Yassen Milhem

Date: 22/12/2016

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Abstract:

Background: Surgical site infection (SSI) is one of the most common problem facing patients who undergo operative procedures. It remains a common and widespread problem contributing to morbidity, mortality, and high cost to the health care system, partly attributed to increase in infections due to antimicrobial resistant bacterial pathogens. In Palestine there has been very limited data regarding the magnitude of SSIs due to antimicrobial resistant pathogens as well as the resistant pattern to antibiotics commonly used in the treatment of these infections. *P. aeruginosa* is an aerobic Gram negative, which is considered as one of the most problematic nosocomial pathogens especially among postoperative patients. : This study aimed to investigate the incidence level of *P. aeruginosa* in postoperative infection, its antibiotic susceptibility pattern and their relatedness using molecular typing technique.

Methodology: A total of 29 clinical isolates were collected from wounds of postoperative patients of different wards at Alia and Alahli hospital. The isolates were identified as *P. aeruginosa* using selective media and API20. The susceptibility pattern to different antibiotics was done using disk diffusion method. Molecular characterization of the strains were carried out using RAPD. Furthermore a retrospective study of a hospital records of the same period of other pathogens causing postsurgical wound infection were investigated.

Results:

The results showed that the incidence of *P. aeruginosa* in surgical site infection was 19.0%. The sensitivity pattern of *P. aeruginosa* isolated from patients in postoperative was mostly sensitive to meropenem, and Imipenem (93%), to amikacin and ceftazidem (76.0%), azetroneame 56.6%, piperacillin and cefipem 46.6%. *P. aeruginosa* was

mostly resistant to gentamycin and ciprofloxacin 50 % and 46.6% of isolates were resistant to levofloxacin.

Three RAPD clusters were obtained and were designated as C1 to C7 groups. C1, C3, C4 and C6 had identical strains while the rest of the groups had 70% or above similarity but not identical.

The most prevalent pathogen of the retrospective study was *S. aureus* and the least was Enterobacter/*Citrobacter freundii*.

In comparison with other postsurgical infection pathogens, *P. aeruginosa* was ranking third.

Age group and gender had significant correlation with infection, age group in the range of 41-50 had higher incidence and males were higher than females.

Conclusions:

This study shows the incidence of *P. aeruginosa* in post-operative wound infections. The most causative agents of post operation infections were *S. aureus*, followed *by E. coli* then *P. aeruginosa*.

Molecular techniques used in this study was random amplified polymorphic DNA"RAPD-PCR".

Key words: SSI, *P. aeruginosa*, RAPD, susceptibility to antibiotic.

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Abbreviations and Definitions:

SSI	Surgical Site Infection.
RAPD	Random Amplification of Polymorphic DNA.
PCR	Polymerase chain Reaction.
MRSA	Methicillin-Resistant *Staphylococcus aureus*.
CDC	Centers for Disease Control and Prevention.
LPS	Lipopolysaccharide.
MDR	Multi Drug Resistant.
MIC	Minimum Inhibitory Concentration.
CLSI	Clinical Laboratory Standards Institute.
VRE	Vancomycin Resistant Enterococcus.

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Chapter One:

Introduction

Surgical site infection (SSI) means wound infection that occurs somewhere in the operative site by both pathogens and body commensals, the infection that happen during the operative called a primary wound infection whereas the after operative called secondary wound infection which may be due to subsequent complications (Masaadeh, Hani A, Jaran, & S., 2009). surgical site infection classified into two types, the first one is incisional SSI which include both superficial incision and deep incisional and the second one is organ SSI (Musmar, Ba'ba, & Owais, 2014).

Surgical site infection is a serious problem despite the advance development in technology of surgery and other medical fields (Akinkunmi, Adesunkanmi, & Lamikanra, 2014), The incidence of SSI according to CDC was 15.45% in USA, and it is considered the third most common cause of hospital associated infection and account for about 25% of all nosocomial infection in USA (Ramesh, Sumathi, Anuradha, Venkatesh, & Krishna, 2013), It has been shown that postoperative infection leads to increase in the mortality and morbidity rates (Afifi & Baghagho, 2010). Mortality rates are 2-3 times higher in patients in whom SSI develops contrasted with un-infected patients (Bratzler, Houck, & Richards, 2005). About 77% of the death of surgical patients were related to surgical site infection , It also contributes to prolonged hospitalization and therapy that ranges from 1 to 16 days (Akinkunmi et al., 2014), the cost of therapy increased, according to a report in Denmark showed that the cost of care for surgical site infections expend 0.5% of the annual hospital budget (Fry, 2002), and in USA the estimated average cost of each SSI is 2,739$ (Abdulsalam et al., 2013).

Surgical site infection usually appear from fifth to tenth post operative day, but they may appear as early as first post operative day or even years later, according to the (CDC); SSI may occur in the first 30 days after surgery or up to one year if implant is in place and the infection appears to be related to the operation (Silva & Barbosa, 2012). The contamination of surgical site can happen during perioperative period or transoperative period while manipulating the tissue in which the entry of microorganisms is easy. (Silva & Barbosa, 2012).

Bacteriological studies have shown that surgical site infection is global problem and that the bacteria types existing vary with geographical site whether it is resident on the skin, clothing at the site of wound, time between wound and examination (Oguntibeju & Rau, 2004).

The identification of risk factors that lead to SSI, can provide information for preventing, controlling, monitoring, and improving the condition to decrease the occurring of SSI, and increase the principles of patients safety (Silva & Barbosa, 2012).

P. aeruginosa Gram-negative, aerobic rod, free-living bacterium, commonly found in soil and water. However, Its adaptability and high intrinsic antibiotic resistance enable it to survive in a wide range of other natural and artificial settings, including surfaces in medical facilities (LaBauve & Wargo, 2012).

P. aeruginosa is primarily a nosocomial pathogen. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week, and it is a frequent cause of nosocomial infections. According to the CDC, the overall incidence of *P. aeruginosa* infections in U.S. hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for about 10 percent of all hospital-acquired infections, Furthermore, the infection is complicated and can be life threatening (Gales, Jones, Turnidge, Rennie, & Ramphal, 2001).

The major virulence factors of *P. aeruginosa* is: Flagella and pili, these proteinaceous appendages function both as motility, adhesions and initiate the inflammatory response of the host. Other virulence factors are also secreted by *P. aeruginosa* and have varying effects on the host. Proteases can degrade host complement factors, mucins, and disrupt tight junctions between epithelial cells leading to dissemination of the bacteria. Lipases and phospholipases can target lipids in the surfactant as well as host cell membranes. Pyocyanin, a blue-green pigment, can interfere with host cell electron transport pathways and redox cycling (Gellatly & Hancock, 2013), also biofilms formation make higher antibiotic tolerance (Lambert, 2002).

The widespread use of antimicrobial drugs leads to the rise of multidrug resistance (MDR), Methicillin resistant *Staphylococcus aureus* (MRSA), extended-spectrum-β-lactamases (ESBL). vancomycin resistant enterococci (VRE). The antibacterial susceptibility testing of bacterial isolates are effective to determine the appropriate therapy for patients (Bhatt et al., 2014).

P. aeruginosa is an extremely difficult organism to control with antibiotics, all of the major classes of antibiotics used to treat *P. aeruginosa* infections need to cross the cell wall to reach their targets, but low permeability of the cell wall makes it resistant to antimicrobial agents, Furthermore, *P. aeruginosa* causes inactivation and modification of antibiotics, another possible mechanism of resistance is induced genetic mutations in certain enzymes that are vital to the cell metabolism (Lambert, 2002).

One of the molecular techniques widely used in strain typing include random amplified polymorphic DNA"RAPD-PCR", a method based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence. The main advantage of

RAPD-PCR over other molecular genotyping techniques is its simplicity, low cost and it does not require any specific sequence information for the target genome (Ho, Phang, & Pang, 1995).

The primer is short (e.g. 10-mer), there is a high probability that the genome contains several priming sites close to one another that are in an inverted orientation. The technique essentially scans a genome for these small inverted repeats and amplifies intervening DNA segments of variable length (Hadrys, Balick, & Schierwater, 1992).

Aims and Objectives:

1. To determine the incidence of *P. aeruginosa* from postoperative wound infections.
2. To investigate the antimicrobial susceptibility pattern of the *P. aeruginosa* isolates.
3. To identify the relatedness of the *P. aeruginosa* isolates using RAPD molecular typing technique.

Chapter Two

Literature Review:

1.2 Surgical site infections

Studies on the SSI are complicated because of heterogeneous nature of these infections. The prevalence and incidence rates of postoperative wound infections vary widely according to procedures used, hospitals, surgeons, patients and geographical locations (Chahoud, Kanafani, & Kanj, 2014).

The incidence of SSIs is determined by both pre-operative and intra-operative risk factors. Several factors have been leading to increase SSIs risk, such as: Diabetes, smoking, obesity, steroid use, alcohol abuse, and old ages (Chahoud et al., 2014).

Limited studies in Palestine investigated the postsurgical infection. In a study carried out by Adwan *et al*. about the surgical site infections, found out that 63.3% of isolates in postoperative wound infections were *E. coli* which was susceptible to Meropenem and less resistant to Ceftazidime and Amikacin, also showed multi-drug resistant to the commonly prescribed antibiotics such as Nalidixic acid, Trimethoprim/Sulfamethoxazole, Tetracycline, Norfloxacin, Ciprofloxacin and Kanamycin (Adwan. et al., 2016).

Another study carried out in Egypt on 121 patients undergoing surgery, showed that the incidence rate of SSI was 8.264%, which was remarkably higher compared to two previous studies in Egypt that showed 2.7% and 4.02%incidence rate among patients. *Staphylococcus, Pseudomonas* spp. and *E. coli* were the predominant causative agents (Afifi & Baghagho, 2010).

The incidence of *P. aeruginosa* in postoperative wound infection was determined at four Jordanian hospitals from February to December, 2005; 115 specimens were collected, the results showed that 27.8% of isolates were *P. aeruginosa*, followed by

E. coli (15.6%), *S. aureus* (14.7%), *Acinetobacter calcoaceticus* (13.0%), *Klebsiella species* (12.1 %), *Proteus species* (6.0 %), *Citrobacter freundii* (3.4%), *Streptococcus pyogenes* (2.6 %), *Enterococcus faecalis* (2.6%). In the same study, it was noticed that the occurrence of *P. aeruginosa* was higher among young groups compared to other groups. The lowest causative agents of postoperation infections were *Streptococcus pyogenes*, and *Enterococcus faecalis* (Masaadeh et al., 2009).

The incidence of postoperative wound infection in developing countries is becoming a more serious problem. Previous studies in different countries such as Pakistan, India, Mali and Ethiopia reported that the infections rates ranges from 11-13%, 9-12%, 10.2% and 10.9%, respectively (Abdulsalam et al., 2013).

Assessment of SSIs from Signs & Symptoms of the wound and associated factors in public hospitals at Yemen, during the period of July- September 2012 using random sampling method, showed that 34 % suffered from SSIs. In this study, it was concluded that the rate of infection among males was 26.47% and among females 73.53% and it showed that age above 50 years was a risk factor for SSI. Several factors, such as diabetes, obesity, hypertension, heart diseases were also considered to be a risk factors (Abdulsalam et al., 2013).

Prophylactic antibiotic reduces the incidence of wound infection. The appropriate choice of antibiotic for prophylaxis should depend on its activity against expected bacteria at the specific surgical site. Properly timed accurate dose of preoperative antibiotics reduces the incidence of SSI (Elbur, Yousif, Sayed, & Abdel-Rahman, 2014).

In 2011, a Palestinian study carried out to investigate the level of adherence to guidelines of antibiotic prophylactic use in surgery. The prospective cohort study included 400 abdominal, orthopedic, and gynecological operations, which were performed during the study period. The result showed that none of the hospitals is following guidelines for perioperative prophylaxis, with high rate of broad spectrum antibiotic use, long duration and inappropriate time of first dose (Musmar et al., 2014). In a Nigerian study at 2011, they found that obstetrics ward had the highest number of surgical wound infections (41.7%), followed by both male orthopaedic and male surgical ward (16.7%) while the female surgical ward had the lowest (8.3%) (Ekom & Edem, 2012).

2.2 Microbiology

In most postoperative SSIs, the causative pathogens originate from endogenous flora of the patient's skin, mucous membranes or hollow viscera (Reichman & Greenberg, 2009).

When mucous membranes or skin is incised the organisms usually involved are aerobic gram-positive cocci such as staphylococci, whereas a gastrointestinal organ is incised during an operation, the source of pathogens is usually gram negative bacilli such as *E. coli,* and gram positive organisms such as enterococci (Reichman & Greenberg, 2009). Several studies investigated the postsurgical infection caused by various pathogens and showed that *Staphylococcus* was the most common isolated pathogen in addition to several other pathogens such as *E. coli*, *Pseudomonas* spp., *Klebsiella* spp., etc. (Bowler, Duerden, & Armstrong, 2001).

Among the Staphylococci, *Staphylococcus aureus* is considered the most important pathogen and is usually found as a normal flora in the nasopharynx of up to 15 % of the population. It can cause exogenous suppuration in wounds. Strains resistant to antibiotics such as MRSA can cause epidemics and more severe infection.

Staphylococcus epidermidis, was regarded as a commensal but is now recognized as a major threat in prosthetic surgery and in indwelling vascular catheters (Mangram, Horan, Pearson, Silver, & Jarvis, 1999).

A study was done at a university hospital in Iran, on a surgical patients reported that *S. aureus* to be the commonest bacterial pathogen (43%), followed by *Escherichia coli* (21%), *Klebsiella* spp. (13%), *Pseudomonas* spp. (10%) and coagulase negative *staphylococcus* (5%). In the same study, MRSA accounted for a high rate of 78.9% of all *S. aureus* isolates (Khorvash et al., 2008).

Escherichia coli is another important nosocomial pathogen involved in several postsurgical infections (Williams, 2008). In a study carried out by Schnuriger *et al.* , they found that *E. coli* was the commonest cause of surgical site infection of colonic injury accounting for 64.7% (Schnuriger et al., 2010).

P. aeruginosa is an opportunistic pathogen which is considered as one of the leading causes of hospital-acquired infections especially lung, urinary tract and surgical wound infection (Iglewski, 1996).

Several studies have reported the occurrence of *P. aeruginosa* in postoperative wound infections. A study investigated the a postoperative infection of 80 samples found that *P. aeruginosa* was major agent, accounting for 33.3% followed by *Staphylococcus aureus* (21.7%), *Klebsiella* spp. (16.7%), *Escherichia coli* (11.7%), coliform (6.7%), *Proteus* spp. (6.7%), *Streptococcus pyogenes* (1.7%) and *Enterococcus faecalis* (1.7%) (Oguntibeju & Rau, 2004). Another study carried out in Jordan 2003, on caesarean surgeries, *P. aeruginosa* accounted for 5.3% (Kaplan, Smadi, Al-Taani, & El-Qudah, 2003). A Saudi Arabian study conducted at 2016 on postsurgical infected patients found that *P. aeuoginosa* was the most common isolate accounting for 31.6%, followed by Methicillin–Resistant *Staphylococcus aureus* (21%) and *Acinetobacter baumannii* (17.5%) (El-Ageery & Otibi, 2016).

2.3 Pathogenesis

The risk of development of SSIs depends on several factors such as the dose and virulence of the pathogens, and host defense mechanisms.

Virulence of pathogens depends on their ability to produce toxins and other substances that rise their ability to invade the host and causing tissue damage.

Lipopolysaccharide triggers the release of precursor of coagulant factors and inflammatory mediators such as cytokine, which may initiate inflammatory response and cause multiple systemic organ failure. Some bacteria produce polysaccharide capsule, which inhibits phagocytosis which is a critical host immune response following bacterial infection (Gellatly & Hancock, 2013).

2.4 Risk factors for surgical site infections

A number of preoperative, perioperative and postoperative factors found to be increase the risk of postoperative SSIs.

Preoperative factors such as patient age, diabetes mellitus, education status, socio-economic, obesity, hypertension, heart diseases were significantly increased the risk of SSI (Afifi & Baghagho, 2010),(Abdulsalam et al., 2013).

In a study carried out in USA during the period of February 1991 to July 2002 at 11 hospitals on 144,485 patients underwent surgery showed that the rate of SSIs was 1.2%. Age was a significant risk factor for SSIs. (Kaye et al., 2005).

Perioperative transfusion of blood or its components is a risk factor for the development of postoperative bacterial infection including SSI. According to Amenu et al. the women with intra-operative blood loss of more than 1000 ml were more likely to have perioperative blood transfusion and had significant association with increased risk of SSIs (Amenu, Belachew, & Araya, 2011).

Postoperative care including the use of aseptic non-touch technique for changing or removing surgical wound dressings and the using of antibiotic decrease the risk of SSIs. (Press, 2008).

2.5 Antimicrobial chemotherapy

Prophylactic Antibiotics

It has been shown that postoperative infections can be prevented by using appropriate prophylactic antibiotics given before surgery (Kirk R.M., 2004), several studies showed that's if antibiotic treatment was not administered it will lead to increase incidence and severity of postoperative SSIs (Amenu et al., 2011). Inappropriate use of antibiotics for surgical prophylaxis increases both cost and emergence of resistant bacteria, and makes the choice of empirical antimicrobial agents more difficult (Goswami et al., 2011). Antibiotic prophylaxis should be used only when wound contamination is expected or when operations on a contaminated site may lead to bacteremia, also when an implant or vascular graft has been inserted, and in valvular heart disease to prevent infective endocarditis. Normally in a clean operation, one dose is sufficient. In contaminated operations three doses are usually given (Kirk R.M., 2004).

Antimicrobial susceptibility pattern

The widespread uses of antimicrobial drugs lead to cause MDR, which is a condition causing organism to resist distinct drugs or chemicals of a variety of structure and function targeted , so that Multi-drug resistant (MDR) bacteria are resistant to two or more classes of antibiotics, such as, Methicillin resistant *Staphylococcus aureus* (MRSA), extended-spectrum-β-lactamases (ESBL) producing enterobacteria and vancomycin resistant enterococci (VRE). These resistant bacteria have become most hospital problems and leaving physician with few therapeutic options, therefor when the first choice is not effective they tend to use the other choice of antibiotics which are usually less effective toxic, and more expensive (Bhatt et al., 2014). Antibiotic resistance can be controlled by suitable antimicrobial prescribing, new therapy alternatives, and continued surveillance (Goswami et al., 2011).

P. aeruginosa is an example of opportunistic nosocomial pathogen, which cause a wide spectrum of infections and leads to morbidity in immunocompromised patients and develops resistance to many antibiotics which results in difficult selection of appropriate treatment.

The study of antibacterial susceptibility patterns of *P. aeruginosa* are effective to determine appropriate therapy for infected patients (Bhatt et al., 2014). The sensitivity pattern of *P. aeruginosa* isolated from patients in postoperative wound infections was studied in four hospitals in Jordan, *P. aeruginosa* was sensitive to amikacin, gentamicin, tobramycin, ciprofloxacin and aztreonam with amikacin showing the highest percentage sensitivity (Masaadeh et al., 2009).

Recently, a study in Palestine showed that the prevalence of pathogens among surgical site infections was 56.7%, 30%, 6.7%, 3.3% and 3.3% for *E. coli*, *S. aureus*, *Klebsiella* sp., *Enterobacter* sp., and *Acinetobacter* sp., respectively. *E. coli* isolates showed high resistance against Nalidixic acid (88.2%), Trimethoprim/ Sulfamethoxazole (76.5%), Tetracycline (70.6%), Norfloxacin (64.7%), Ciprofloxacin (58.5%). *S. aureus* showed high resistance against Nalidixic acid (88.9%), Norfloxacin (77.8%), Amoxycillin/ clavulanic acid (77.8%), Kanamycin (66.7%) and Ciprofloxacin (55.6%). Methicillin resistant *S. aureus* accounted for 33.3% of all *S. aureus* isolates. Resistant to 3 or more antibiotics were detected in 94.1% and 77.8% of *E. coli* and *S. aureus* isolates, respectively (Adwan. et al., 2016).

In a study conducted by Garba et al., the sensitivity pattern of *P. aeruginosa* to various antibiotics were as follows: ofloxacin (72%), ceftriazone (54%), cefuroxime (54%) and gentamycin (54%), strong resistance to cotrimoxazole, amoxicillin tetracycline and augmentin (Garba et al., 2012).

Another study carried out in two main tertiary care hospitals in Saudi Arabia, showed that the antibiotic susceptibility rates among *P. aeruginosa* for amikacin, ciprofloxacin and cefotaxim were 83.3 %, 75.9% and 70.4% respectively and the resistant rates for piperacillin/tazobactum, cefepime and ceftazidime were 38.5 %, 32.4% and 29.6% respectively. Approximately 43% of the isolates were found to be sensitive to all antibiotics, while 36.1% of them were found to be resistant to more than three antibiotics. Only 3.7 % of the isolates were found to be resistant to one antibiotic, while 8.3% and 9.3% of isolates were found to be sensitive to 2 and 3 of antibiotics, respectively (Ahmed, 2016).

Various literature have documented several risk factors associated with isolation of antimicrobial resistant pathogens from patients. In a case control study conducted in Denmark to identify possible risk factors for MRSA and methicillin susceptible *Staphylococcus aureus* (MSSA), hospitalization for more than 7 days tended to be associated with MRSA (Bocher et al., 2008).

According to Etok et al., extended spectrum beta-lactamase (ESBL) production was seen in 50 out of the 100 Gram-negative isolates. All *E. coli* isolates and 66.7% of the *Proteus* isolates were ESBL producing. All *S. aureus* isolates were methicillin resistant (Ekom & Edem, 2012).

2.6 Prevention of surgical site infections

Protection of surgical patients from infection is an essential consideration during the preoperative, perioperative and postoperative phases of care. Bacterial infection of surgical incisions may results from small stitch abscess to massive tissue necrosis, septicemia and even death. Some of the factors that determine surgical site infection and its effect are beyond the control of surgeons, but others can be controlled (Oluwatosin, 2005).

prior the surgery, all the materials must be sterilized; this include any device, needles, sutures, dressings, gloves and solutions that may come into touch with the wound and exposed tissues. In addition, the surgical team must prepare themselves by scrubbing the hands with soap and water and using the antiseptic as required. Head and hair must be covered and mask should be worn during the operation. Only the person involved in the surgery should contact the sterile equipment (Nichols, 2001).

After the surgery, the wound is protected from potential contamination by sterile dressings. The most effective method is administering antibiotic prophylaxis at the appropriate time, (20 min before surgery), if it is administered too late or too early then it will reduces the efficacy of the antibiotic and may increase the risk of SSI. The administering of antimicrobial prophylaxis before surgery has been proved to decrease greatly the incidence of postoperative infection (Scottish Intercollegiate Guidelines Network, 2008).

A study of 3,836 patients undergoing abdominal, vascular or trauma surgery given a single dose of cefuroxime (plus metronidazole for colorectal cases) compared the rate of SSI for time intervals between 0 and 2 hours prior to the procedure. The overall SSI rate was 4.7% and administration of antibiotic prophylaxis 30–60 minutes pre-incision resulted in the lowest rates of SSI (2.42% for 45–59 minutes and 3.33% for 30–44 minutes) (Scottish Intercollegiate Guidelines Network, 2008).

Skin and Hand Hygiene

When it comes to preventing SSI, skin antisepsis is the cheapest and simplest way to minimize SSI. There are about 100-1000 microbes on the skin and are mostly located on corneal layer, if the hands are not disinfected properly after patient contact, the microbes will be transferred to the next patient. Antiseptics commonly used for hand washing are 4% Chlorhexidine gluconate , hexachlorophane or Povidone-iodine (Larson, 1999).

Aseptic in operation room:

Operation room should be located away from the inpatient area and located on the top floor, big enough for free circulation, temperature between 18 and 24^{o} C and humidity of about 50 to 55%.

Cleaning, disinfection and sterilization are the fundamental in ensuring operation room asepsis. Formaldehyde fumigation procedure commonly used to sterilize the operating room, also daily ultra violet irradiation for 12 -16 hours is a useful procedure (Eredie, 2008)

Chapter Three

Materials and Methods

3.1 Study Design

A cross sectional study was designed to find out the prevalence of *P. aeruginosa* bacteria, which is isolated from patients undergoing surgical operations with postoperative wound infection, also to determine the antimicrobial susceptibility and molecular characterization of the *P. aeruginosa* isolates.

3.2 Microbiological identification of *P. aeruginosa*

Fifty *P. aeruginosa* obtained from surgical wounds of patients during Oct. 2015 to May 2016 from two hospitals in Hebron (Queen Alia hospital and AL-Ahli hospital), Palestine. The isolates were identified at the hospitals laboratories, stocked, and stored at Al-Quds University Laboratory at -20 ^{0}C, in 50%v/v sterile glycerol/LB. Only 29 of the isolates could be revived, purified and subjected to further identification. The epidemiological data for the patients in whom *P. aeruginosa* isolates are listed in table 1.

Table 1. Epidemiological data of patients in whom *P. aeruginosa* isolates obtained from Queen Alia and Al Ahli hospitals.

Patient number	Gender	Age	Hospital ward	Hospital name	Enter date
1	Male	66	Medical ward	Queen Alia	22/5/2016
2	Male	57	Urology ward	Queen Alia	7/4/2016
3	Male	74	Urology ward	Queen Alia	15/5/2016
4	Male	81	ICU ward	Queen Alia	17/5/2016
5	Male	29	Surgical ward	Queen Alia	5/4/2016
6	Female	52	Surgical ward	Queen Alia	10/5/2016

7	Female	62	Emergency ward	Queen Alia	14/4/2016
8	Female	54	Surgical ward	Queen Alia	1/5/2016
9	Female	60	Kidney dialysis ward	Queen Alia	11/4/2016
10	Male	49	Emergency ward	Queen Alia	11/5/2016
11	Male	36	Medical ward	Queen Alia	8/3/2016
12	Male	41	Surgical ward	Queen Alia	11/3/2016
13	Female	45	Urology ward	Queen Alia	7/4/2016
14	Male	49	Surgical ward	Queen Alia	26/4/2016
15	Male	21	E.N.T ward	Queen Alia	3/2/2016
16	Female	17	Surgical ward	Queen Alia	1/10/2015
17	Male	36	Kidney dialysis ward	Queen Alia	17/2/2016
18	Male	50	E.N.T ward	Queen Alia	14/3/2016
19	Male	71	Urology ward	Queen Alia	22/3/2016
20	Male	13	Surgical ward	Queen Alia	21/3/2016
21	Female	57	Medical ward	Queen Alia	30/5/2016
22	Male	68	Surgical ward	Al-Ahli	26/4/2016
23	Female	64	Surgical ward	Al-Ahli	7/10/2015
24	Male	63	Surgical ward	Al-Ahli	20/1/2016
25	Male	47	Medical ward	Al-Ahli	5/5/2016
26	Male	42	Orthopedic ward	Al-Ahli	15/102015
27	Male	22	Surgical ward	Al-Ahli	20/3/2016
28	Male	97	Medical ward	Al-Ahli	8/5/2016
29	Male	13	Surgical ward	Al-Ahli	1/3/2015

3.3 Gram Stain and Culturing

The specimens were subjected to gram staining and cultured at blood agar, MacConkey agar and chocolate agar, then the plates were incubated at 37°C for 24 hours. The plates were read in the following day but extended to 48 hours if there was no bacterial growth within 24 hours. The primary identification of *P. aeruginosa* isolates were made based on colony appearance, pigmentation, and API 20E, (Noble & Michael, 2002).

3.4 Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed at Al-Quds University Laboratory on Muller Hinton agar (Hi Media, Mumbai) media using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) +guidelines (Jean B. Patel et al., 2016).

Antibiotics used in our study were piperacillin (100 µg), ceftazidime (30µg), cefepime (30µg), imipenem (10µg), meropenem (10µg), gentamicin (10µg), amikacin (30 µg), Levofloxacin (5µg), aztreonam and ciprofloxacin (5 µg) (Biomaxima, Polish).

Antibiotic discs were placed on HMA media inoculated with test *P. aeruginosa* isolates media and incubated at 37°C for 24 hrs. After 24 hours each plate was examined and growth zones were measured to the nearest millimeter.

3.5 Extraction of DNA from *P. aeruginosa* isolates

Boiling method was used for DNA extraction, one colony placed into 200µl of ultra-pure water then boiling for 10 minute on 95℃, finally it was centrifuged at 14.000 rpm for 5 minute (Clarke, Millar, & Moore, 2003).

3.6 **RAPD-PCR:**

3.6.1. Selection of Primers

Two Decamer oligonucleotides were used in this study 5' TGCGCGCGGG 3′ - 5'GCCCGAGCGG 3′ and 5' ACGGCCGACC 3' – 5' GCTGGGCCGA 3' (Mahenthiralingam, Campbell, Foster, Lam, & Speert, 1996). After preliminary experiments the 5' TGCGCGCGGG 3′ - 5' GCCCGAGCGG 3′ gave better discriminatory power and larger number of bands.

3.6.2. PCR Amplification

PCR reaction was carried out in a total volume of 25 µl as following20 µl AccuPower PCR PreMix (Bioneer, Korea), 1µl of template DNA, 1µl primers (1µM) and 3 µl ultra-pure water. PCR was performed using the following protocol: 94°C for 5min, followed by 35 cycles of denaturation 94°C for 1min, then Annealing is carried out at 35 °C for 2 min, and Extension at 72°C for 2min, followed by 72°C for 10 min.

3.6.3. Agarose Gel Electrophoresis

Electrophoresis of PCR products was carried out on 1.5% (w/v) agarose gel in 1.0 × TAE buffer at 100 V for 90 minute. The gel was stained with ethidium bromide. A 100bp ladder (Genedirex, Taiwan) was used as a molecular weight standard. Negative control and positive control (*P. aeruginosa* CCUG 17619) was included in the PCR reaction. Gels were photographed under UV light and comparison of band patterns were analyzed based on UPGMA method using Ward's Method/ Squared Euclidean Distance by SPSS software version 20.

Chapter Four

Results

Of the 264 post-surgical infections collected from the two hospitals 50 (19%) were *P. aeruginosa* and the remaining pathogens were distributed as following: 23.0%, 20%, 8.7%, 6.8%, 5.7%, for *S. aureus*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., *Acinatobacter* spp., respectively. The rest 16% were miscellaneous as shown in table 2. The majority of *P. aeruginosa* isolates were obtained from Queen Alia Hospital 30 (60.0%) while 20 (40.0%) from AL- Ahli Hospital.

Table 2: The frequency and percentage of microorganisms isolated from patients with postoperative wound infection in from Queen Alia and AL- Ahli Hospitals.

Microorganism	Number of isolates		Percentage %
	Queen Alia	Al Ahli	
S. aureus	49	12	23
E. coli	33	20	20
P. aeruginosa	30	20	19
***Klebsiella* spp.**	9	14	8.7
***Enterococcus* spp.**	12	6	6.8
***Acinetobacter* spp.**	4	11	5.7
Proteus	8	4	4.5
MRSA	12	0	4.5
Staphylococcus epidermidis	11	0	4.1
α-hemolytic *Streptococcus*	6	0	2.3
***Enterobacter* spp.**	2	0	0.7
Citrobacter freundii.	2	0	0.7

There was a significant correlation between age and rate of infection since the age group 41-50 had higher incidence of infection in comparison to other groups with a P value 0.020 as shown in table 3.

Gender also showed a strong correlation since males had higher rate of infection than females with a P value of .024 as shown in Fig.1

Table 3: Percent of postoperative wound infections caused by *P. aeruginosa* according to age group.

Age group	frequency	Percent
0-10	2	4.0
11-20	2	4.0
21-30	5	10.0
31-40	7	14.0
41-50	14	28.0
51-60	8	16.0
61-70	7	14.0
71-above	5	10.0
Total	50	100.0

The sensitivity pattern of *P. aeruginosa* isolated from patients in postoperative were mostly sensitive to Meropenem, and Imipenem (93%), followed by Amikacin and Ceftazidem (76.0%), Azetroneame (56.6%), and piperacillin and Cefipem (46.6%). *P. aeruginosa* was resistant to Gentamycin and ciprofloxacin (50%), 46.6% of isolates were resistant to Levofloxacinas shown in table 4, Fig. 2 and 3.

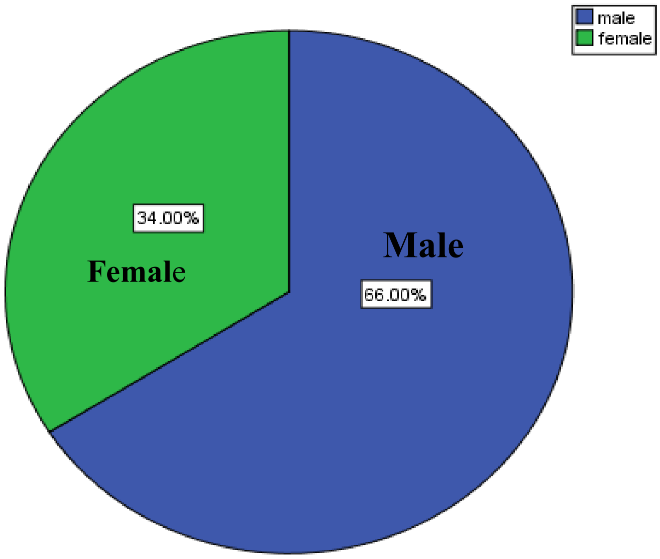

Figure 1. Percent of postoperative wound infections caused by *P. aeruginosa* according to gender.

Table 4: Antibiotics sensitivity pattern of *P. aeruginosa* isolates recovered from patients

Antibiotics	Number & % Resistant	Number & % Sensitive	Number & % Intermediate
Meropenem (MEM)	2 (6%)	28 (93%)	0
Impenem (IMP)	2 (6%)	28 (93%)	0
Amikacin (AK)	5 (16%)	23 (76%)	2 (6%)
Ceftazedim (CAZ)	2 (6%)	23 (76%)	5 (16%)
Azetroname (ATM)	2 (6%)	17 (56.6%)	11 (36.6%)
Levofloxacin (LEV)	14 (46.6%)	15 (50%)	1 (3.3%)
Cefipem (FEP)	10 (33.3%)	14 (46.6%)	6 (20%)
Piperacllin (PRL)	10 (33.3%)	14 (46.6%)	6 (20%)
Ciprofloxacin (CIP)	15 (50%)	14 (46.6%)	1 (3.3%)
Gentamycin (CN)	15 (50%)	8 (26.6%)	7 (23.3%)

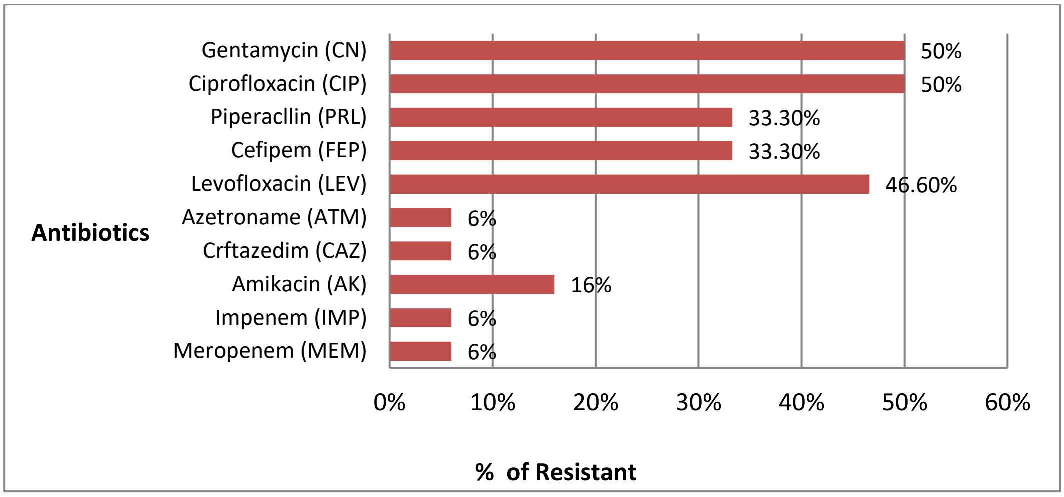

Figure 2. Antibiotic resistant pattern of *P. aeruginosa*.

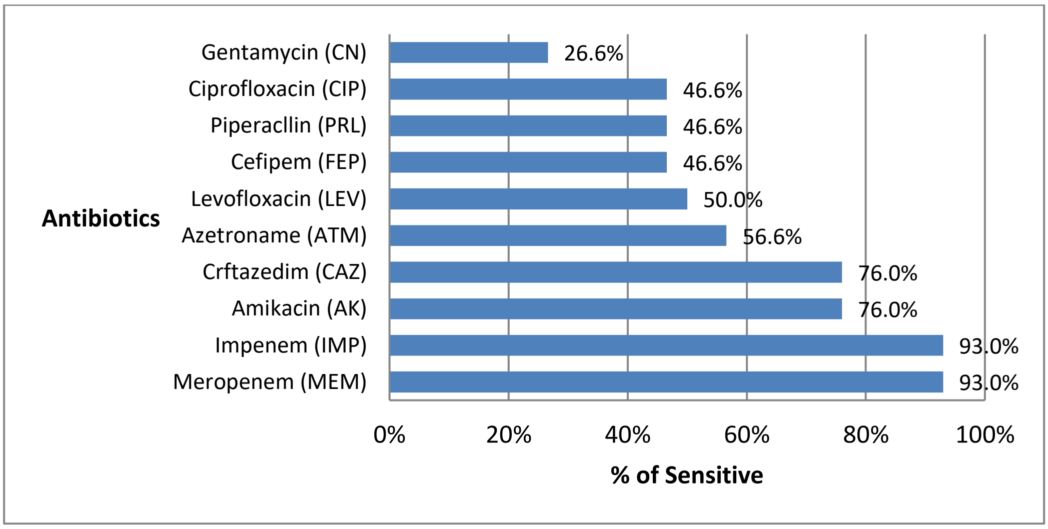

Figure 3. Antibiotic sensitivity pattern of *P. aeruginosa*.

RAPD typing.

Out of the 29 isolates subjected to RAPD, a total of 7 RAPD-PCR clusters (C1-C7) at a 70% similarity level. Results of RAPD-PCR profiles are presented in Figures 4, 5, 6, 7 and 8. These results also showed that *P. aeruginosa* isolates numbered S16, S19 and S2 in C1 are identical, while isolates S13, S14 and S15 in C3 are identical, S3 and S11 in C4 are identical S6, S1, S10 and S8 in cluster C6 are identical while C2, C5, and C7 groups were clustered but not identical as shown in Fig. 8.

The size of the amplified DNA fragments ranged from 90 to 2000 bp. All the isolates were run at the same PCR reaction to avoid the difficulties in reproducibility of the technique.

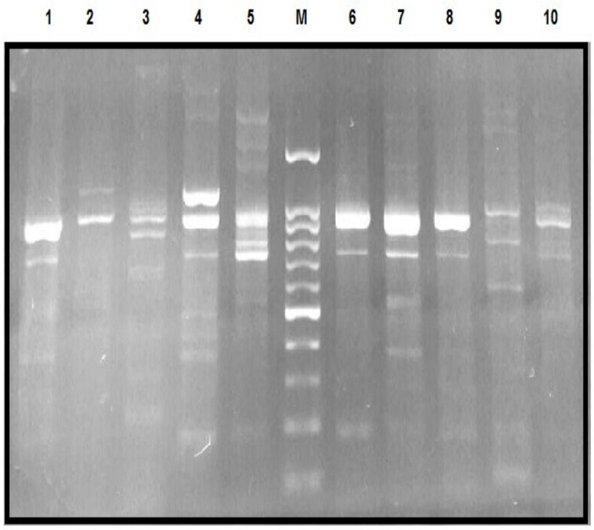

Figure 4 - RAPD profile analysis of 10 *P. aeruginosa* isolates recovered from patients with postoperative wound infection from Queen Alia hospital.

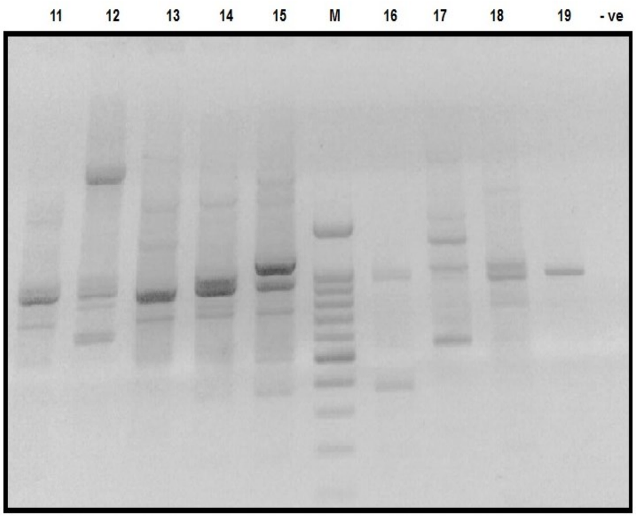

Figure 5 RAPD profile analysis of 9 *P. aeruginosa* isolates recovered from patients with postoperative wound infection from Queen Alia hospital.

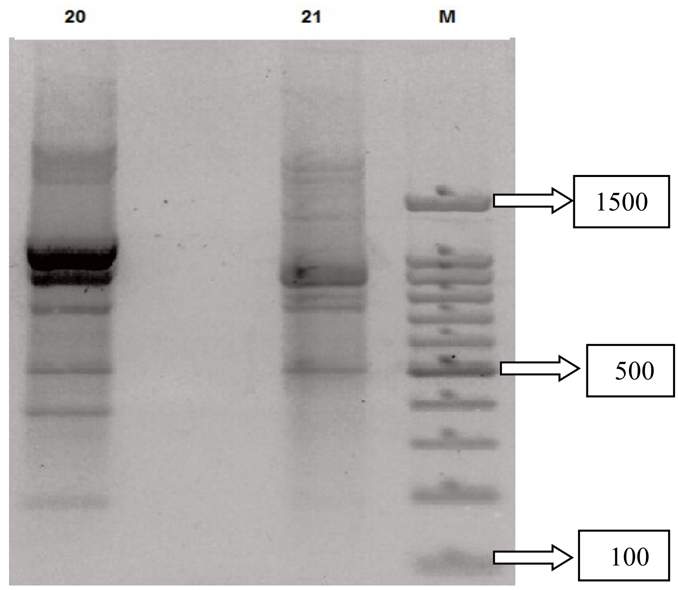

Figure 6- RAPD profiles of 2 *P. aeruginosa* isolates from Queen Alia hospital
M: 100 bp DNA molecular weight marker.

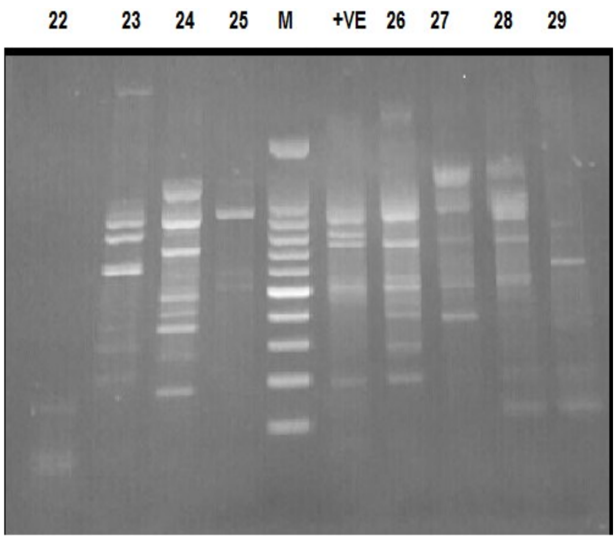

Figure 7- RAPD profile of 8 *P. aeruginosa* isolates recovered from patients with postoperative wound infection from Al-Ahli hospital
M: 100 bp DNA molecular weight marker.
+VE: *P. aeruginosa* positive control (CCUG 17619).

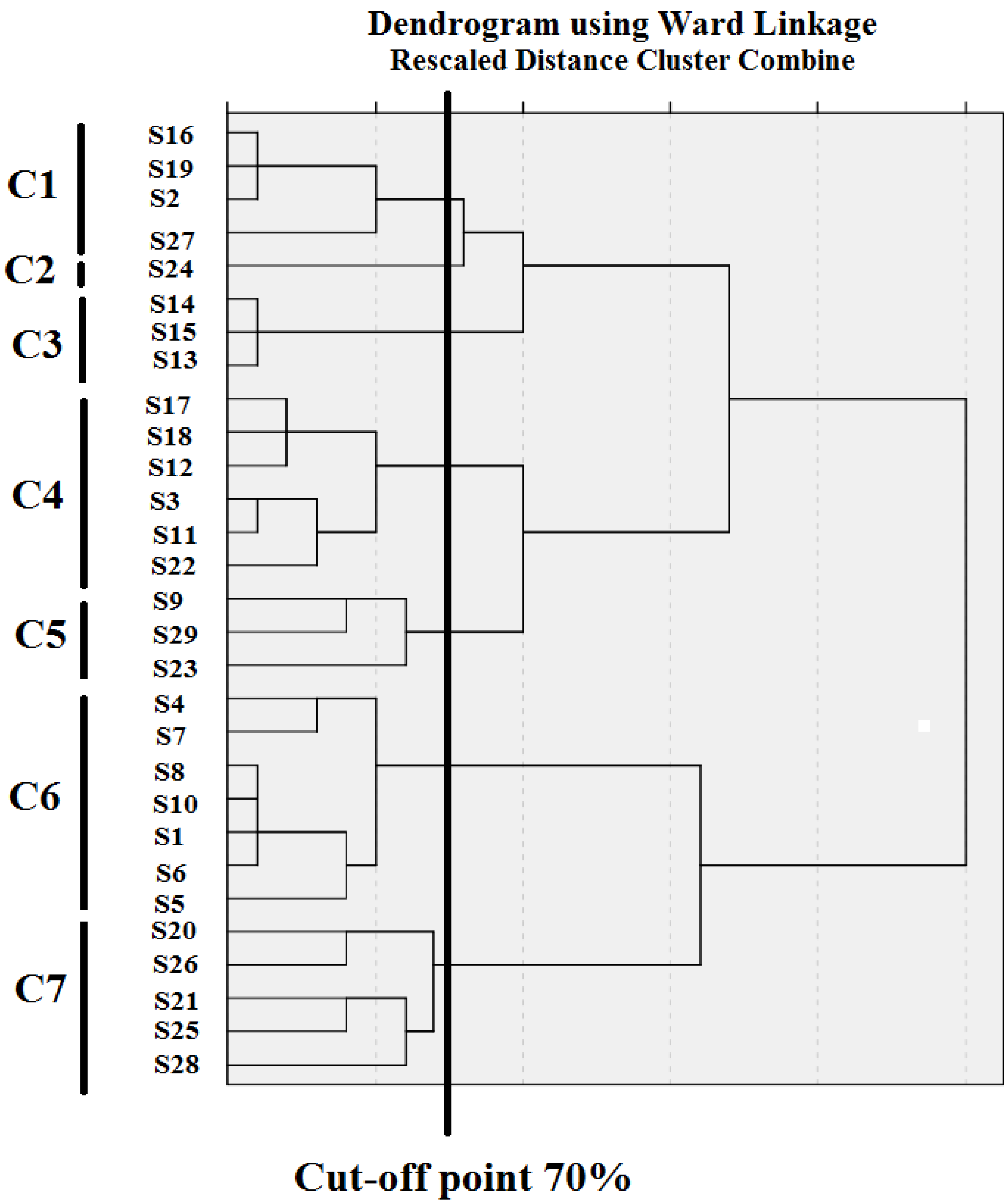

Figure 8: Dendrogram of 29 clinical *P. aeruginosa* isolates recovered from surgical site infections based on the UPGMA method using Ward's Method/ Squared Euclidean Distance by SPSS software version 20, derived from analysis of the RAPD-PCR-profiles at a 70% similarity.

Chapter 5

DISCUSSION

A surgical site infection is a postoperative complication that brings about trouble to the surgeon, financial burden, extreme discomfort to the patient, and sometimes death. The effective management of patients suffering from infection depends upon the identification of the types of organisms that cause the diseases and the selection of an effective antibiotic against the organism. The wound of surgical site is considered one of the major health problems in the world (Garba et al., 2012).

Most of the SSIs are hospital acquired and vary from hospital to hospital. The incidence of *P. aeruginosa* in surgical site infection is becoming more serious in developing countries because of relaxation in general hygienic measures, low quality antiseptic and medicinal solutions for treatment (Bertrand, Thouverez, Patry, Balvay, & Talon, 2001). Our study showed that the prevalence rate of *P. aeruginosa* was 19%, among all the pathogens isolated from the surgical wound during the study period, ranking third after *S. aureus* and *E. coli* with prevalence rate of 23% and 20% respectively. Our finding was in concordance with a recent study carried out by Murphy et al., which showed that *P. aeruginosa* was isolated from 16% of isolates ranking second and *S. aureus* was the first with 34% (Murphy et al., 2016).

Other studies have reported *P. aeruginosa* to be the most prevalent with 29.6%, and 32% carried out by Ranjan et al., and Anupurba et al. respectively (Ranjan, Ranjan, Bansal, & Arora, 2010),(Anupurba, Bhattacharjee, Garg, & Sen, 2006).

More studies from nearby countries have also reported similar or even higher rate of prevalence. In a study done by Masaadeh *et al.*, from Jordan who reported 27.8% (Masaadeh et al., 2009), Raafat et al. from Egypt have reported 17.8% (Marwa M.

Raafat, 2016), and in a Saudi Arabian study *P. aeruginosa* was reported to be 31.6% (El-Ageery & Otibi, 2016).

When factors such as age and sex were considered, it was found the *P. aeruginosa* was higher in males (66%) and in patients with age group of 41 – 50 years 28.0% followed by 51 – 60 years age group of 16.0%. This was in agreement with several other studies. Jamshaid et al., also reported that *P. aeruginosa* infections were more common in males, and Stephen et al., also reported in their study that male patients had higher isolation rates. Regarding age group Stephen et al., found that *P. aeruginosa* was more commonly isolated from patients in the age group 21–30 years (Stephen S. S et al., 1990). This may be related to males risk-taking activities at work, In addition, it has been suggested that hair growth and shaving interfere with wound dressing adherence, which could lead to a higher risk of infection among men who have thicker, coarser hair (Cohen et al., 2013). In regard to age group it has been suggested that as people advance in age, is considered as important factor for the development of SSIs as an old age patients there is low healing rate, low immunity, and presence of underlying diseases such as diabetes, hypertension, etc (Khan, P, Rashed, & Banu, 2013)

P. aeruginosa isolates recovered from patients with postoperative wound infections showed higher susceptible to meropenem and impenem, (93.0%), which are usually used empirically to treat *Pseudomonas* infections followed by ceftazidem and amikacin (76.0%). Navaneeth et al., in their study, reported 88% susceptibility against both imipenem and meropenem, among *P. aeruginosa* isolates (Navaneeth, Sridaran, Sahay, & Belwadi, 2002). Bonfiglio et al., in their study, concluded that meropenem was the most active compound against *P. aeruginosa* isolates, followed by amikacin (Bonfiglio et al., 1998).

Other study have reported the excellent activity of amikacin against *P. aerugonosa* (Ahmed, 2016).

On the other hand, *P. aeruginosa* was resistant to levofloxacin 46%, ciprofloxacin 50%, and gentamicin 50%. Gentamicin is a cheap and easily available drug that is used extensively in hospitals, this may be the main reason for the development of resistance in bacteria against this drug**.**

Bacterial resistance to beta-lactam antibiotics such as piperacillin and azetroneame is primarily due to the production of beta-lactamase of the antibiotics rendering them inactive.

RAPD-PCR generated seven cluster designated as (C1-C7). *P. aeruginosa* isolates numbered S16, S19 and S2 in C1 are identical, while isolates S13, S14 and S15 in C3 are identical S3 and S11 in C4 are identical S6, S1, S10 and S8 in cluster C6 are identical.

Patterns were considered different when they differed by more than one band (Betancor et al., 2004), and the intensity of the band was not consider as polymorphic (Sayed et al., 2009).

There was generally relationship between RAPD-PCR and antibiotic susceptibility of the isolates. Strains number S6 and S8 of cluster "C6", RAPD had similar antibiotic profile, when looking at the epidemiological data both isolates were from the same ward (surgical word), at the same time. In addition, strains number S16 and S19 of pattern "C1" share a relationship with the antibiotic susceptibility profile suggesting cross-infection between patients in the same wards and even between different wards of Queen Alia Hospital.

CONCLUSIONS

In this study, the incidence of *P. aeruginosa* ranked third after *S. aureus* and *E. coli*. in SSIs. The males had higher rate of infection in comparison to females. *P. aeruginosa* isolates recovered from patients with postoperative wound infections showed higher susceptible maximum to meropenem and impenem (93%). The rates of resistance of *P. aeruginosa* isolates was 46% for levofloxacin and 50% for each ciprofloxacin and gentamicin. *P. aeruginosa* from surgical wound infections have multi drug resistance to a many of the antibiotics used in this study. RAPD-PCR is a useful method for fast and inexpensive for investigation of strain relatedness. The results of RAPD and antibiotic susceptibility suggest a cross contamination between patients.

Appendices

Bacterial culture media

Table1: Bacterial culture media used during this study

Media	**Manufacture**
Blood agar	Himedia (India)
Muller Hinton agar	Himedia (India)
Macconkey agar	Himedia (India)
Nutrient agar	Himedia (India)
Cetrimide agar	Himedia (India)

Reagents

Table2: Reagents and materials employed in the study

Reagent	**Manufacture**
Gram stain reagents	Sigma (USA)
API20e	BioMerieux
DNA molecular weight marker (100)bp ladder	Promega (USA)
Master Mix	Promega (USA)
Primers	TIB MOLBIOL (Germany)
Antibiotic disks	Himedia(India), Oxoid (UK
Ethidium bromide	Sigma (USA)

Equipment

Table3: Apparatus and special equipment that were used in the study

Thermal cycler	Eppendorf
Research pipettes	Eppendorf
PCR microfuge tube, 0.2 ml	Eppendorf
Microwave oven	LG
Hoefer Shortwave UV light Table, (Trans illuminator)	Hoefer (USA)
Micro-Centrifuge	Sanyo (UK)
Electrophoresis set-up	BioRad (USA)

REFERENCES

Abdulsalam, M. A., Nasser, Xinping Zhang, Lianping Yang, Fares J. Sawafta, & Salah, B. (2013). Assessment of Surgical Site Infections from Signs & Symptoms of the Wound and Associated Factors in Public Hospitals of Hodeidah City, Yemen. *International Journal of Applied Science & Technology*.

Adwan., G., Hasan., N. A., Sabra., I., Sabra., D., Al-butmah., S., Odeh., S.,. Badran., H. (2016). Detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile. *International Journal of Medical Research & Health Sciences*, 75-82.

Afifi, I. K., & Baghagho, A. E. (2010). Three months study of orthopaedic surgical site infections in an Egyptian University hospital. *International Journal of Infection Control, Vol 6, No 1*.

Ahmed, O. B. (2016). Incidence and Antibiotic Susceptibility Pattern of Pseudomonas aeruginosa Isolated from Inpatients in Two Tertiary Hospitals. *Clinical Microbiology, 5*(2). doi: 10.4172/2327-5073.1000248

Akinkunmi, E. O., Adesunkanmi, A. R., & Lamikanra, A. (2014). Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles. *Afr Health Sci, 14*(4), 802-809. doi: 10.4314/ahs.v14i4.5

Amenu, D., Belachew, T., & Araya, F. (2011). Surgical site infection rate and risk factors among obstetric cases of jimma university specialized hospital, southwest ethiopia. *Ethiop J Health Sci, 21*(2), 91-100.

Anupurba, S., Bhattacharjee, A., Garg, A., & Sen, M. (2006). Antimicrobial susceptibility of <i>Pseudomonas aeruginosa</i> isolated from wound infections. *Indian Journal of Dermatology, 51*(4), 286-288. doi: 10.4103/0019-5154.30298

Bertrand, X., Thouverez, M., Patry, C., Balvay, P., & Talon, D. (2001). Pseudomonas aeruginosa: antibiotic susceptibility and genotypic characterization of strains isolated in the intensive care unit. *Clin Microbiol Infect, 7*(12), 706-708.

Betancor, L., Schelotto, F., Martinez, A., Pereira, M., Algorta, G., Rodriguez, M. A., . . . Chabalgoity, J. A. (2004). Random amplified polymorphic DNA and phenotyping analysis of Salmonella enterica serovar enteritidis isolates collected from humans and poultry in Uruguay from 1995 to 2002. *J Clin Microbiol, 42*(3), 1155-1162.

Bhatt, C. P., Rina Baidya, Prakash Karki, Rikesh Kumar Shah, Rashiak Miya, Pratima Mahashate, & Mishra, K. K. (2014). Multi Drug Resistance Bacterial Isolates of Surgical Site Infection. *4*. doi: 10.4236/ojmm.2014.44022

Bocher, S., Gervelmeyer, A., Monnet, D. L., Molbak, K., Skov, R. L., & Danish, C. A. M. S. G. (2008). Methicillin-resistant Staphylococcus aureus: risk factors associated with community-onset infections in Denmark. *Clin Microbiol Infect, 14*(10), 942-948. doi: 10.1111/j.1469-0691.2008.02055.x

Bonfiglio, G., Carciotto, V., Russo, G., Stefani, S., Schito, G. C., Debbia, E., & Nicoletti, G. (1998). Antibiotic resistance in Pseudomonas aeruginosa: an Italian survey. *J Antimicrob Chemother, 41*(2), 307-310.

Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev, 14*(2), 244-269. doi: 10.1128/CMR.14.2.244-269.2001

Bratzler, D. W., Houck, P. M., & Richards, C. (2005). Use of antimicrobial prophylaxis for major surgery: Baseline results from the national surgical infection prevention project. *Archives of Surgery, 140*(2), 174-182. doi: 10.1001/archsurg.140.2.174

Chahoud, J., Kanafani, Z., & Kanj, S. S. (2014). Surgical site infections following spine surgery: eliminating the controversies in the diagnosis. *Front Med (Lausanne), 1*, 7. doi: 10.3389/fmed.2014.00007

Clarke, L., Millar, B. C., & Moore, J. E. (2003). Extraction of genomic DNA from Pseudomonas aeruginosa: a comparison of three methods. *Br J Biomed Sci, 60*(1), 34-35.

Cohen, B., Choi, Y. J., Hyman, S., Furuya, E. Y., Neidell, M., & Larson, E. (2013). Gender differences in risk of bloodstream and surgical site infections. *J Gen Intern Med, 28*(10), 1318-1325. doi: 10.1007/s11606-013-2421-5

Ekom, N., & Edem. (2012). Aetiology and antimicrobial studies of surgical wound infections in University of Uyo Teaching Hospital (UUTH) Uyo, Akwa Ibom State, Nigeria.

El-Ageery, S. M., & Otibi, A. A. M. A. (2016). Phenotypic and Genotypic Characterization of *Pseudomonas Aeruginosa* Isolates from Burn Patients. *Egyptian Journal of Medical Microbiology, 25*.

Elbur, A. I., Yousif, M. A., Sayed, A. S. A. E., & Abdel-Rahman, M. E. (2014). Misuse of prophylactic antibiotics and prevalence of postoperative wound infection in obstetrics and gynecology department in a Sudanese hospital. *Health, Vol.06No.02*, 7. doi: 10.4236/health.2014.62025

Eredie, A. (2008). Operating Room Technique.

Fry, D. E. (2002). The economic costs of surgical site infection. *Surg Infect (Larchmt), 3 Suppl 1*, S37-43. doi: 10.1089/sur.2002.3.s1-37

Gales, A. C., Jones, R. N., Turnidge, J., Rennie, R., & Ramphal, R. (2001). Characterization of Pseudomonas aeruginosa isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis, 32 Suppl 2*, S146-155. doi: 10.1086/320186

Garba, Y. H., Lusa, E., Bawa, M. B., Tijjani, M. S., Aliyu , U. U., Zango, & Raji, M. I. O. (2012). Antibiotics Susceptibility Pattern of Pseudomonas aeruginosa Isolated from Wounds in Patients Attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Nigerian Journal of Basic and Applied Sciences, 20*, 32-34.

Gellatly, S. L., & Hancock, R. E. (2013). Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. *Pathog Dis, 67*(3), 159-173. doi: 10.1111/2049-632X.12033

Goswami, N. N., Trivedi, H. R., Goswami, A. P., Patel, T. K., & Tripathi, C. B. (2011). Antibiotic sensitivity profile of bacterial pathogens in postoperative wound infections at a tertiary care hospital in Gujarat, India. *J Pharmacol Pharmacother, 2*(3), 158-164. doi: 10.4103/0976-500X.83279

Hadrys, H., Balick, M., & Schierwater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol Ecol, 1*(1), 55-63.

Ho, C.-L., Phang, S.-M., & Pang, T. (1995). Application of polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD) primers in the molecular identification of selected Sargassum species (Phaeophyta, Fucales). *European Journal of Phycology, 30*(4), 273-280. doi: 10.1080/09670269500651051

Iglewski, B. H. (1996). Pseudomonas. In S. Baron (Ed.), *Medical Microbiology* (4th ed.). Galveston (TX).

Jean B. Patel, Cockerill, F. R., George M. Eliopoulos, Stephen G. Jenkins, Lewis, J. S., Brandi Limbago, . . . Barbara L. Zimmer. (2016). Performance Standards for Antimicrobial Susceptibility Testing *Clinical and Laboratory Standards Institute (CLSI), 26*.

Kaplan, N. M., Smadi, A. A., Al-Taani, M. I., & El-Qudah, M. A. (2003). Microbiology of wound infection after caesarean section in a Jordanian hospital. *East Mediterr Health J, 9*(5-6), 1068-1074.

Kaye, K. S., Schmit, K., Pieper, C., Sloane, R., Caughlan, K. F., Sexton, D. J., & Schmader, K. E. (2005). The effect of increasing age on the risk of surgical site infection. *J Infect Dis, 191*(7), 1056-1062. doi: 10.1086/428626

Khan, A. K. A., P, V. M., Rashed, M. R., & Banu, G. (2013). A Study on the Usage Pattern of Antimicrobial Agents for the Prevention of Surgical Site Infections (SSIs) in a Tertiary Care Teaching Hospital. *J Clin Diagn Res, 7*(4), 671-674. doi: 10.7860/JCDR/2013/5323.2878

Khorvash, F., Mostafavizadeh, K., Mobasherizadeh, S., Behjati, M., Naeini, A. E., Rostami, S., . . . Khorvash, F. A. (2008). Antimicrobial susceptibility pattern of microorganisms involved in the pathogenesis of surgical site infection (SSI); A 1 year of surveillance. *Pak J Biol Sci, 11*(15), 1940-1944.

Kirk R.M., R., W.J. (2004). *Clinical surgery in general (RCS course manual), 4th ed. Churchill livingstone, London, UK*.

LaBauve, A. E., & Wargo, M. J. (2012). Growth and laboratory maintenance of Pseudomonas aeruginosa. *Curr Protoc Microbiol, Chapter 6*, Unit 6E 1. doi: 10.1002/9780471729259.mc06e01s25

Lambert, P. A. (2002). Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. *J R Soc Med, 95 Suppl 41*, 22-26.

Larson, E. (1999). Skin hygiene and infection prevention: more of the same or different approaches? *Clin Infect Dis, 29*(5), 1287-1294. doi: 10.1086/313468

Mahenthiralingam, E., Campbell, M. E., Foster, J., Lam, J. S., & Speert, D. P. (1996). Random amplified polymorphic DNA typing of Pseudomonas aeruginosa isolates recovered from patients with cystic fibrosis. *J Clin Microbiol, 34*(5), 1129-1135.

Mangram, A. J., Horan, T. C., Pearson, M. L., Silver, L. C., & Jarvis, W. R. (1999). Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. *Am J Infect Control, 27*(2), 97-132; quiz 133-134; discussion 196.

Marwa M. Raafat, M. A.-T. A. E. A. (2016). Phenotypic and genotypic characterization of Pseudomonas aeruginosa isolates from Egyptian hospitals. *African Journal of Microbiology Research, 10(39)*. doi: 10.5897/AJMR2016.8254

Masaadeh, Hani A, Jaran, & S., A. (2009). Incident of Pseudomonas aeruginosa in Post-Operative Wound Infection. *American Journal of Infectious Diseases, 5*.

Murphy, R. A., Okoli, O., Essien, I., Teicher, C., Elder, G., Pena, J., Bernabe, K. J. (2016). Multidrug-resistant surgical site infections in a humanitarian surgery project. *Epidemiol Infect*, 1-7. doi: 10.1017/S0950268816001758

Musmar, S. M., Ba'ba, H., & Owais, A. (2014). Adherence to guidelines of antibiotic prophylactic use in surgery: a prospective cohort study in North West Bank, Palestine. *BMC Surg, 14*, 69. doi: 10.1186/1471-2482-14-69

Navaneeth, B. V., Sridaran, D., Sahay, D., & Belwadi, M. R. (2002). A preliminary study on metallo-beta-lactamase producing Pseudomonas aeruginosa in hospitalized patients. *Indian J Med Res, 116*, 264-267.

Nichols, R. L. (2001). Preventing surgical site infections: a surgeon's perspective. *Emerg Infect Dis, 7*(2), 220-224. doi: 10.3201/eid0702.700220

Noble, & Michael, A. (2002). Bailey and Scott's Diagnostic Microbiology, Eleventh Edition. Betty Forbes, Daniel F. Sahm, and Alice S. Weissfeld. St. Louis, MO: Mosby, 2002, 1069 pp. ISBN 0-323-01678-2. *Clinical Chemistry, 48*(10), 1816-1816.

Oguntibeju, O., & Rau, N. (2004). Occurrence of Pseudomonas aeruginosa in post-operative wound infection. *Pakistan Journal of Medical Sciences, 20*, 187-192.

Oluwatosin, O. M. (2005). Surgical Wound Infection. *Annals of Ibadan Postgraduate Medicine, 3 No 2*.

Press, R. (2008). Surgical site infection prevention and treatment of surgical site infection. *National Collaborating Centre for Women's and Children's Health*.

Ramesh, Sumathi, Anuradha, Venkatesh, & Krishna. (2013). Bacteriology of postoperative wound infections. *International Journal Of Pharmaceutical And Biomedical Research*(0976-0350).

Ranjan, K. P., Ranjan, N., Bansal, S. K., & Arora, D. R. (2010). Prevalence of Pseudomonas aeruginosa in post-operative wound infection in a referral hospital in Haryana, India. *J Lab Physicians, 2*(2), 74-77. doi: 10.4103/0974-2727.72153

Reichman, D. E., & Greenberg, J. A. (2009). Reducing surgical site infections: a review. *Rev Obstet Gynecol, 2*(4), 212-221.

Sayed, M., Zain Hasan, B., M. S., and, S., M., q. R., & Shah. (2009). Analysis of Random Amplified Polymorphic DNA (RAPD) of Artemisia capillaris (Wormwood capillary) in East Coast of Peninsular Malaysia. *World Applied Sciences Journal*.

Schnuriger, B., Inaba, K., Eberle, B. M., Wu, T., Talving, P., Bukur, M., . . . Demetriades, D. (2010). Microbiological profile and antimicrobial susceptibility in surgical site infections following hollow viscus injury. *J Gastrointest Surg, 14*(8), 1304-1310. doi: 10.1007/s11605-010-1231-x

Scottish Intercollegiate Guidelines Network. (2008). Antibiotic prophylaxis in surgery. *A national clinical guideline.*

Silva, Q. C. G. d., & Barbosa, M. H. (2012). Risk factors for surgical site infection in cardiac surgery. *Acta Paulista de Enfermagem, 25*, 89-95.

Stephen S. S , S., B., Ang , M., I., Pala , M., R., & Baclig. (1990). Aerobic Surgical Infection: A Surveillance on Microbiological Etiology and Antimicrobial Sensitivity Pattern of Commonly Used Antibiotics. 27-33.

Williams, N. S., Bulstrode, C.J.K., O'conel, P.R.,. (2008). Bailey and Love's Short Practice of Surgery.

التوصيف الجزيئي والمضادات الحيوية لبكتيريا الزائفة الزنجارية (عصية القيح الأزرق) المعزولة من العدوى بعد العمليات الجراحية .

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الملخص:

التهاب العمليات الجراحية هو من المشاكل الأكثر شيوعا للمرضى الذين خضعوا لإجراء العمليات. تعد مشكلة واسعة النطاق تساهم في الممراضة والوفيات، وارتفاع تكلفة الرعاية الصحية، ويعزى ذلك جزئيا إلى زيادة في عدد الإصابات بسبب مسببات الأمراض البكتيرية المقاومة للمضادات الحيوية. في فلسطين هناك بيانات محدودة للغاية فيما يتعلق حجم الاصابة بسبب الجراثيم المقاومة المضادة للميكروبات فضلا عن نمط مقاومة للمضادات الحيوية المستخدمة عادة في علاج هذه الالتهابات. الزائفة الزنجارية هي سلبية غرام الهوائية، والتي تعتبر واحدة من مسببات الأمراض المكتسبة داخل المستشفى والأكثر إشكالية خاصة بين المرضى بعد العملية الجراحية. وكان الهدف من هذه الدراسة إلى معرفة مستوى الإصابة بالزائفة الزنجارية بعد العملية الجراحية، نمطها عند التعرض للمضادات الحيوية والقرابة وذلك باستخدام تقنية الوصف الجزيئي.

المنهجية: تم جمع ما مجموعه 30 العزلات السريرية من جروح المرضى بعد العملية الجراحية من اقسام مختلفة. وقد تم التعرف على العزلات كما الزائفة الزنجارية باستخدام اوساط غذائية خاصة لنموها و API20 وفحص نمط الحساسية للمضادات الحيوية المختلفة. تم ايضا ايجاد العلاقة الجزيئية المسببة للاصابة باستخدام RAPD وبالإضافة إلى ذلك تم الرجوع لسجلات المستشفى من نفس فترة جمع عينات الزائفة الزنجارية لمعرفة الانواع الاخرى من البكتيريا التي تسبب عدوى بعد الجراحة.

أظهرت النتائج أن معدل الإصابة الزنجارية الزائفة في عدوى موضع الجراحة كان 19.0٪ وكشف أيضا أن المزيد من العمر هم أكثر أهمية أن يكون للعدوى في الفئة العمرية 41-50 مع قيمة

احتمالية كان 0.020، وكان الذكور أعلى اصابة من الإناث إلى الزائفة الزنجارية وكانت احتمالية الإصابة 0.024.

كان النمط حساسية الزائفة الزنجارية المعزولة من المرضى في ما بعد الجراحة في الغالب حساسية للكل من مضاد ميروبينيم ، وإيميبينيم (93٪) ، وأيضا لأميكاسين وسيفتازيديم (76.0 ٪) ، وازيترونيم 56.6 ٪، بيبيراسيلين و سيفيبيم 46.6 ٪ . والزائفة الزنجارية معظمها مقاومة للجنتاميسين وسيبروفلوكساسين 50٪، أيضا (46.6٪) من الليفوفلوكساسين كان مقاومة.

وقد تم الحصول على ثلاث مجموعات RAPD وكانت. المجموعة (أ) 4، المجموعة (ب) (3) ومجموعة(ج) 4 سلالات. وكانت بقية 19 سلالة ليس لها صلة.

نتيجة دراسة مسببات الأمراض الأخرى التي تسبب عدوى جروح بعد اجراء العمليات من عزل 265بكتيريا 23.0٪، 20٪، 8.7٪، 6.8٪، 5.7٪، 4.5٪، 4.5٪، 4.1٪، 2.3٪، 0.7٪، و 0.7٪ للمكورات العنقودية الذهبية ، الإشيريشية القولونية، الكلبسيلة، الراكدة، على التوالي, والباقي 16% كانت النتاءج متنوعة.

بالمقارنة مع غيرها من مسببات الأمراض العدوى بعد الجراحة، تعتبر الزائفة الزنجارية في المرتبة الثالثة.