

## 1.1. Introduction

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram negative, aerobic, non-fermenting with unipolar motility (Carmeliet *al.*, 2010). It produces different pigments including pyocyanin which is blue green, pyoverdine which is yellow green and fluorescent and pyorubin which is red brown pigment (Hidronet *al.*, 2008). The organism can cause diseases in animals and humans and an opportunistic pathogen in humans and plants (Jones and Masterton, 2001). It is also the cause of infections associated with contaminated contact lens solutions. It is found in soil, water and considered part of the normal flora (Streitet *al.*, 2004). *Invitro*, *P. aeruginosa* is identified by its pearlscent look and grape- like or tortilla like odor. It has the ability to grow best at 42°C.

Pathogenic *P. aeruginosa* infects damaged tissues of people with reduced immunity. Its infections have variable symptoms such as generalized inflammation and sepsis (Streitet *al.*, 2004). It can be fatal if such colonization took place in vital organs such as the lungs, kidneys and urinary tract (Hidronet *al.*, 2008). *P. aeruginosa* flourishes in a wet a surface that's why it is widely spread in medical equipment such as catheters, causing cross infections in clinics and hospitals (Jones and Masterton, 2001). The organism is the most serious pathogen causing ventilator-associated pneumonia (VAP) (Carmeliet *al.*, 2010).

It is known that *P. aeruginosa* develop resistance to antimicrobial agents continuously (Bonomo and Szabo, 2006). Several researches that conducted by Paganiet *al.*, (2005), Hidronet *al.*, (2008) and Streitet *al.*, (2004) showed that *P. aeruginosa* has high resistance to fluoroquinolones, ciprofloxacin and levofloxacin respectably. In addition to that,

these isolates that are attained reflected the highest rates compared to different hospital patients. *P. aeruginosa* isolates from ICU patients reflected higher rates of  $\beta$ -lactam resistance followed by aminoglycosides especially to gentamicin (Bonomo and Szabo, 2006). More researches that carried out by Jones and Masterton, (2001) and Paganiet al., (2005) showed lower rates of *P. aeruginosa* resistance to amikacin and tobramycin.

According to Paganiet al., (2005) resistance to individual drugs is not a concern but it is the multidrug resistance is the serious treatment challenge. Multidrug resistance is defined by a resistance to three or more drug classes (Jones and Masterton, 2001).

## **1.2. Rationale**

Worldwide the prevalence of *P. aeruginosa* has been investigated in several studies such as Magiorakos et al., (2011), Gaynes and Edwards, (2005) and Hidron et al., (2008) that

considered the microorganism as one of the serious pathogens. The National Nosocomial Infections Surveillance System reports *P. aeruginosa* to be the second most common organism isolated in nosocomial pneumonia after *K. pneumonia*, the third most common organism isolated in both urinary tract infection and surgical site infection, and the fifth most common organism isolated from all sites of nosocomial infection (Tam *et al.*, 2010). Furthermore, there are gaps in literature when comes to investigate the presence of MDR *P. aeruginosa* and how is that related to patients' types and samples in Tawam Hospital in United Arab Emirates. Consequently, the need for the current study rose to fill all the mentioned gaps.

### **1.3. Objectives**

#### **1.3.1. General objective**

To detect multidrug resistant (MDR) *P. aeruginosa* in Tawam Hospital, UAE.

#### **1.3.2. Specific objectives**

A) To re-identify *P. aeruginosa* isolated from patients attending Tawam Hospital, UAE.

B) To perform antibiotic susceptibility test for deferent specimens of *P. aeruginosa*.

C) To detect Multi Drug Resistant *P. aeruginosa*.

## CHAPTER TWO

### LITERATURE REVIEW

#### **2.1. *Pseudomonas aeruginosa***

The *P. aeruginosa* is a Gram negative, aerobic, non-fermenting bacillus bacterium with unipolar motility (Jones and Masterton, 2001). *P. aeruginosa* is a ubiquitous microorganism that's can be found in soil, water, humans, animals, plants, sewage, hospital and considered part of the normal flora (Siegel, *et al.*, 2007). It is very difficult to eliminate since it shows intrinsic resistance too many types of chemotherapeutic antibiotics and drugs (Gould, 2008). *P. aeruginosa* is the main occupant and the most abundant organism on earth (Bonomo and Szabo, 2006).

*P. aeruginosa* it has an outer membrane which contains Protein F (OprF). OprF functions as a porin, allowing certain molecules and ions to come into the cells and maintaining the bacterial cell shape (Carmeliet *al.*, 2010). It also lowers the permeability of the outer membrane to decrease the intake of harmful substances into the cell and give the pathogen a high resistance to antibiotics (Goossens, 2003).

*P. aeruginosa* uses its single and polar flagellum to move around and to display chemotaxis to useful molecules, like sugars (Jones and Masterton, 2001). Its strains either have a-type or b-type of flagella, the flagellum is very important during the early stages of infection, for it can attach to and invade tissues of the hosts (Jones and Masterton,

2001). Similarly to its flagellum, *P. aeruginosa* pili contribute greatly to its ability to adhere to mucosal surfaces and epithelial cells (Gould, 2008).

## **2.2. Clinical significance**

It is a powerful pathogen that usually attacks about two thirds of the critically- ill hospitalized individuals and this indicates more aggressive diseases (Jones and Masterton, 2001). In addition, *P. aeruginosa* is a leading Gram negative bacteria that is responsible for 40- 60% mortality rate (Jones and Masterton, 2001). The risk is higher when it comes to cystic fibrosis 90%. It is considered one of the top three most frequent worst visual diseases (Pagani *et al.*, 2005).

*P. aeruginosa* rarely infects healthy people that is why it is considered opportunistic. It can cause nosocomial pathogen of immunocompromised individuals such as AIDS, cancer, cystic fibrosis or traumatic patients (Pagani *et al.*, 2005). *P. aeruginosa* typically infects the airway, urinary tract, burns, wounds and also causes other blood infections (Siegele *et al.*, 2007). It is also the cause of infections associated with hot tubs and contaminated contact lens solutions (Carmeli *et al.*, 2010).

*P. aeruginosa* can cause serious infections that are associated with high risk groups such as neutropenic patients in an infection of septic shock (Hidron *et al.*, 2008). Premature infants and neutropenic cancer patients can be at high risk if they get gastrointestinal infections (Jones and Masterton, 2001). Moreover, pneumonia can be critical with cystic fibrosis patients and so is a skin and soft tissue infection in case of burns victims and patients with wound infections (Siegele *et al.*, 2007).

It is the most common cause of infections of the outer ear (otitis externa), and is the most frequent colonizer of medical devices (e.g., catheters)(Gould, 2008). *Pseudomonas* can be spread by equipment that gets contaminated and is not properly cleaned or on the hands of healthcare workers(Hidronet *al.*, 2008).*Pseudomonas* can, in rare circumstances, cause community acquired pneumonias, as well as ventilator associated pneumonias, being one of the most common agents isolated in several studies(Carmeliet *al.*, 2010). Pyocyanin is virulence factor of the bacteria and has been known to cause death in *C. elegans* by oxidative stress (Jones and Masterton, 2001). However, research indicates salicylic acid can inhibit pyocyanin production. One in ten hospital-acquired infections is from *Pseudomonas*(Bonomo and Szabo, 2006). *Cystic fibrosis patients* are also predisposed to *P. aeruginosa* infection of the lungs. *P. aeruginosa* may also be a common cause of "hot-tub rash" (dermatitis), caused by lack of proper, periodic attention to water quality (Pagani *et al.*, 2005). Since these bacteria like moist environments, such as hot tubs and swimming pools, they can cause skin rash or swimmer's ear(Gould, 2008).The most common cause of burn infections is *P. aeruginosa*. The bacterium is frequently associated with osteomyelitis involving puncture wounds of the foot(Siegelet *al.*, 2007).

This gives them the ability to resist many defenses, including anti-*Pseudomonas* antibiotics such as ticarcillin, ceftazidime, tobramycin, and ciprofloxacin, because once the bacteria sense that their outer layer of biofilm is being destroyed, the inner layers will grow stronger to reestablish the community (Hidronet *al.*, 2008). *P. aeruginosa* is also

resistant to many antibiotics and chemotherapeutic agents due to their intrinsic resistance (Carmeli *et al.*, 2010). This is caused by the low permeability to antibiotics of the outer membrane and by the production of  $\beta$ -lactamases against multidrug efflux pumps and  $\beta$ -lactam antibiotics (Siegelet *et al.*, 2007).

*P. aeruginosa* can be transmitted to a host via fomites, vectors, and hospital workers who are potential carriers for multiply-antibiotic-resistant strains of the pathogen (Hidronet *et al.*, 2008). The pili and flagella of *P. aeruginosa* play a vital role in the infection of burns and wounds (Goossens, 2003). Controlled infection of burn wounds on animal and plant models with *P. aeruginosa* strains devoid of pili and flagella demonstrate a trend of decreased virulence (Hidronet *et al.*, 2008). Without these morphological virulence factors, the bacteria exhibit a substantially decreased survival rate at the wound site and a decreased ability to disseminate within the host organism (Carmeli *et al.*, 2010). The spread of *P. aeruginosa* within host organisms is also dependent on the microorganism's elastase production and other protease mechanisms (Hidronet *et al.*, 2008). Bacterial elastase and other bacterial proteases degrade the host's proteins, including the structural proteins within membranes, disrupting the host's physical barriers against the spread of infection (Bonomo and Szabo, 2006).

### **2.3. Resistant *Pseudomonas aeruginosa***

Antibiotics that have activity against *P. aeruginosa* may include (Hidronet *et al.*, 2008):

- a. aminoglycosides (gentamicin, amikacin, tobramycin, but *not* kanamycin)
- b. quinolones (ciprofloxacin, levofloxacin, but not moxifloxacin)



- c. Cephalosporins (ceftazidime, cefepime, cefoperazone, cefpirome, ceftobiprole, but *not* cefuroxime, cefotaxime)
- d. Antipseudomonal penicillins: carboxypenicillins (carbenicillin and ticarcillin), and ureidopenicillins (mezlocillin, azlocillin, and piperacillin). *P. aeruginosa* is intrinsically resistant to all other penicillins.
- e. Carbapenems (meropenem, imipenem, doripenem, but *not* ertapenem)
- f. Polymyxins (polymyxin B and colistin)
- g. Monobactams (aztreonam).

These antibiotics must all be given by injection, with the exceptions of fluoroquinolones, aerosolized tobramycin and aerosolized aztreonam (Bonomo and Szabo, 2006). For this reason, in some hospitals, fluoroquinolone use is severely restricted to avoid the development of resistant strains of *P. aeruginosa* (Gould, 2008). In the rare occasions where infection is superficial and limited (for example, ear infections or nail infections), topical gentamicin or colistin may be used (Hidron *et al.*, 2008).

## 2.4. Antibiotic resistance

One of the most worrisome characteristics of *P. aeruginosa* is its low antibiotic susceptibility, which is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes (Bonomo and Szabo, 2006). In addition to this intrinsic resistance, *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfers of

antibiotic resistance determinants (Andrade *et al.*, 2003). Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events, including acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes (Goossens, 2003). Hyper-mutation favors the selection of mutation-driven antibiotic resistance in *P. aeruginosa* strains producing chronic infections, whereas the clustering of several different antibiotic resistance genes in integrons favors the concerted acquisition of antibiotic resistance determinants (Falagas *et al.*, 2006). Some recent studies have shown phenotypic resistance associated to biofilm formation or to the emergence of small-colony variants may be important in the response of *P. aeruginosa* populations to antibiotics treatment (Hidronet *et al.*, 2008).

## **2.5. Treatment**

*P. aeruginosa* has immense potential to develop resistance against antibiotic as is evident from the fact that its genome contains the largest resistance island with more than 50 resistance genes (Goossens, 2003). Mechanisms underlying antibiotic resistance have been found to include production of antibiotic-degrading or antibiotic-inactivating enzymes, outer membrane proteins to evict the antibiotics and mutations to change antibiotic targets (Jones and Masterton, 2001). Presence of antibiotic-degrading enzymes such as extended-spectrum  $\beta$ -lactamases like PER-1, PER-2, VEB-1, AmpC cephalosporinases, carbapenemases like serine oxacillinases, metallo- $\beta$ -lactamases, OXA-type carbapenemases, aminoglycoside-modifying enzymes, among others have been reported (Hidronet *et al.*, 2008). Use of  $\beta$ -lactamase inhibitors such as sulbactam is

being advised in combination with antibiotics to enhance antimicrobial action even in the presence of certain level of resistance Andrade *et al.*, (2003). Combination therapy after rigorous antimicrobial susceptibility testing has been found to be the best course of action in the treatment of multidrug-resistant *P. aeruginosa* (Paterson, 2006). Some next-generation antibiotics that are reported as being active against *P. aeruginosa* include doripenem, ceftobiprole and ceftaroline (Andrade *et al.*, 2003). However, these require more clinical trials for standardization (Gould, 2008). Therefore, research for the discovery of new antibiotics and drugs against *P. aeruginosa* is very much needed (Hidron *et al.*, 2008).

## **2.6. Multidrug resistance**

While the prevalence of *P. aeruginosa* in the last two decades has remained stable, the prevalence of resistant strains has increased dramatically (Magiorakos *et al.*, 2011).

MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories. XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories). PDR is defined as non-susceptibility to all agents in all antimicrobial categories (i.e. no agents tested as susceptible for that organism). Thus, a bacterial isolate that is characterized as XDR will also be characterized as MDR. Similarly, a bacterial isolate would have to be XDR in order for it to be further defined as PDR (Magiorakos *et al.*, 2011).

Moreover, the use of standard terminology will optimize epidemiological surveillance systems, facilitating the exchange of information between the medical community, public health authorities and policy makers in order to promote the prudent use of antimicrobials and other public health measures (Paterson, 2006).

Resistant *P. aeruginosa* infections are associated with high mortality, morbidity, and increased resource utilization and costs (Falagas *et al.*, 2006). Further, the acquisition of resistance during anti-pseudomonal therapy among initially susceptible isolates and the emergence of MDR isolates make treatment even more challenging (Paterson, 2006).

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1. Study design**

##### **3.1.1. Type of study**

This is a laboratory-based study.

##### **3.1.2. Study area**

The study was carried out in Microbiology Laboratory, Tawam Hospital, UAE.

##### **3.1.3. Study duration**

The study was conducted during the period January to April 2015.

#### **3.2. Source of isolates**

The isolates of 70 *Pseudomonas aeruginosa* were obtained from the Research Laboratory, Tawam Hospital, UAE, which isolated from urine, blood, wound swabs and sputum.

#### **3.3. Re-identification of the isolates**

##### **3.3.1. Checking purity of the isolates**

The isolates were cultured on nutrient agar and incubated at 37°C for 18-24 hrs. Purity of each isolate was checked microscopically following simple stain.

### **3.3.2. Gram Stain**

Gram stain was essential technique for initial identification of bacterial isolates. The procedure was carried out according to Falagaset *al.*, (2006) as follows; smear was prepared from overnight culture on a clean and dry slide. The smear was left to air dry. Fixation was done by rapid pass of the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30–60 seconds, and then washed by tap water. Lugol's iodine was added for 30-60 minutes then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. Finally, the smear was covered with saffranin stain for 2 minutes and washed by tap water. The back of slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X.

### **3.3.3. Identification and antibiotic susceptibility**

The identification and antibiotic susceptibility test were done by VITEK Machine (BioMerieux, France) which is automated and semi-automated technology in microbiology(Paterson, 2006).

#### **3.3.3.1. Principle of the VITEK**

The VITEK is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. All three systems

accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

#### **3.3.3.2. Preparation of bacterial suspension**

The suspension was prepared by emulsifying 2-3 colonies from an overnight culture in 5ml normal saline. The obtained suspension was adjusted to 0.5 McFarland using the Densichek. This suspension was used for both identification and antibiotic sensitivity tests for the VITEK 2 system (CLSI, 2009).

#### **3.3.3.3. Inoculation of VITEK cards**

The cards intended for identification and antimicrobial susceptibility testing were inserted in the VITEK tubes and then were put in VITEK machine. In the second day the identification and the susceptibility was read from the VITEK screen.

#### **3.3.4. Confirmation of Susceptibility of bacterial isolates to antibiotics**

Antibiotic susceptibility testing of *P. aeruginosa* was carried out by the disk diffusion technique. Eleven (n=11) antibiotics commercially available discs (MAST Diagnostic Ltd, USA) were tested. The antibiotics used were, Cefepime (FEP, 10 g), Aztreonam (ATM, 30 g), ceftazidime (CAZ, 30 g), Colistin (CT, 30 g), ciprofloxacin (CIP, 5 g), Meropenem (MEM, 30 g), Piperacillin (PIP, 10 g), amikacin (AK, 30 g), gentamicin (GEN, 10 g), imipenem (IPM, 10 g), Piperacillin/Tazobactam (TZP, 30 g).

The test was performed using Kirby-Bauer disc diffusion method according to CLSI (2009) as follows:

#### **3.3.4.1. Culture medium**

Sterilized molten Muller-Hinton agar (PH 7.4±2) was prepared, cooled to 45-50<sup>0</sup>C and poured in sterile dry Petri plates on a level surface, to a depth of 4mm.

#### **3.3.4.2. Quality control**

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *E. coli* ATCC **25922** obtained from the Central Public Health Laboratory.

#### **3.3.4.3. Preparation of inoculums**

The inoculum was prepared by transfer of 3-5 well isolated colonies of same appearance with sterile wire loop to 2.0 ml of sterile physiological saline. The turbidity of this suspension was adjusted to a 0.5 McFarland standard. This suspension was used within 15 minutes of preparation.

#### **3.3.4.4. Seeding of plates**

A sterile non- toxic cotton swab was dipped into the inoculums tube and then the swab was rotated against the side of the tube above the level of the suspension to remove excess fluid. The plate of Muller-Hinton agar was inoculated by streaking the swab evenly over the surface of the medium in three directions. The surface of agar was allowed 3-5 minutes to dry.



#### **3.3.4.5. Antibiotic disc application**

The selected antibiotics were applied on the surface of agar by using sterile forceps which evenly distributed in the inoculated plate. Each disc was pressed down to ensure its contact with the agar.

#### **3.3.4.6. Incubation**

The inverted plates were incubated aerobically at 35°C for 16-18 hours.

#### **3.3.4.7. Reading zones of inhibition**

Following overnight incubation, by using a ruler on the underside of the plate, the diameter of each zone of the inhibition was measured in millimeters.

#### **3.3.4.8. Interpretation of the results**

Zone of inhibition for each antibiotic was compared to their standard inhibition zone on the chart provided by manufacture. The results were interpreted as sensitive (S) or resistance (R). MDR *P. aeruginosa* was judged according to Tawam Hospital criteria (Appendix 3).

## CHAPTER FOUR

### RESULTS

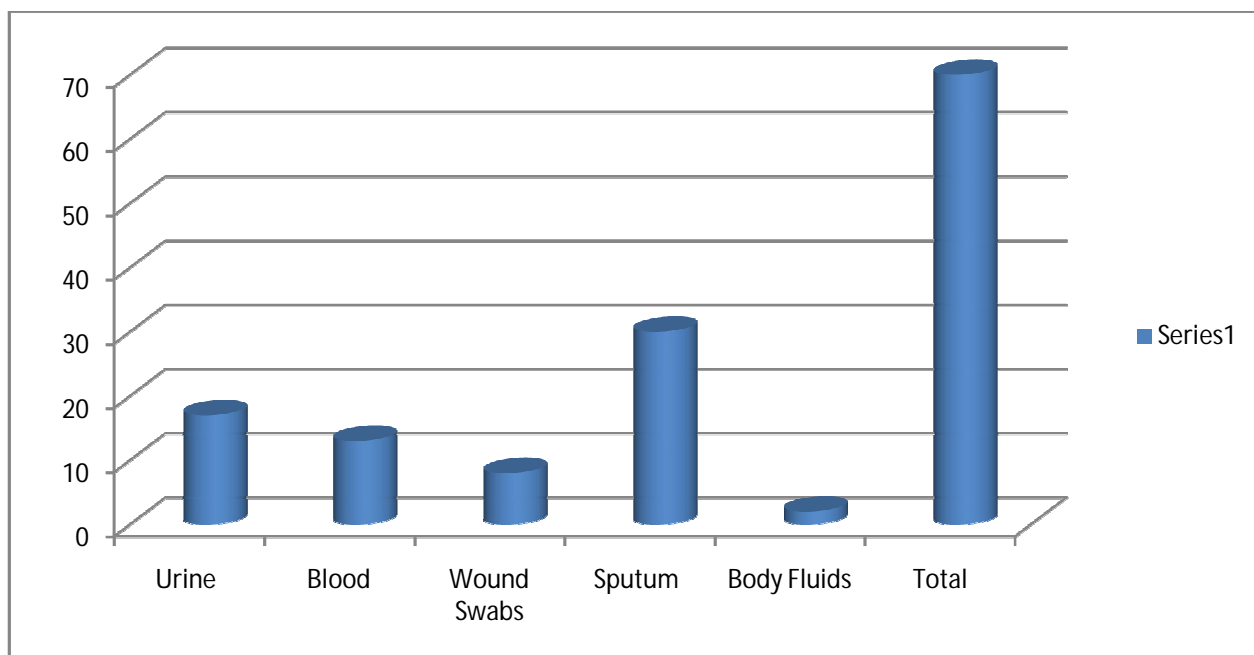
A total of seventy *P. aeruginosa* isolates were obtained from Microbiology Laboratory, Tawam Hospital, UAE. Data registered in the log book of the laboratory indicated that the isolates were recovered from different clinical specimens: urine, blood, wound swabs, sputum and body fluids (Fig 1). Re-identification of the isolates confirmed that all isolates were *P. aeruginosa*. Results presented in Fig (2) showed that the majority of isolates recovered from patients in ICU, followed by medical unit.

Study on antibiotic susceptibility of *P. aeruginosa* in Table (1) that includes eleven antibiotics; revealed that their resistance ranged from 2.8% to 78.6%. Sensitivity from 1.4 % to 94.3 % and Intermediate is from 2.8 % to 44.3 %.

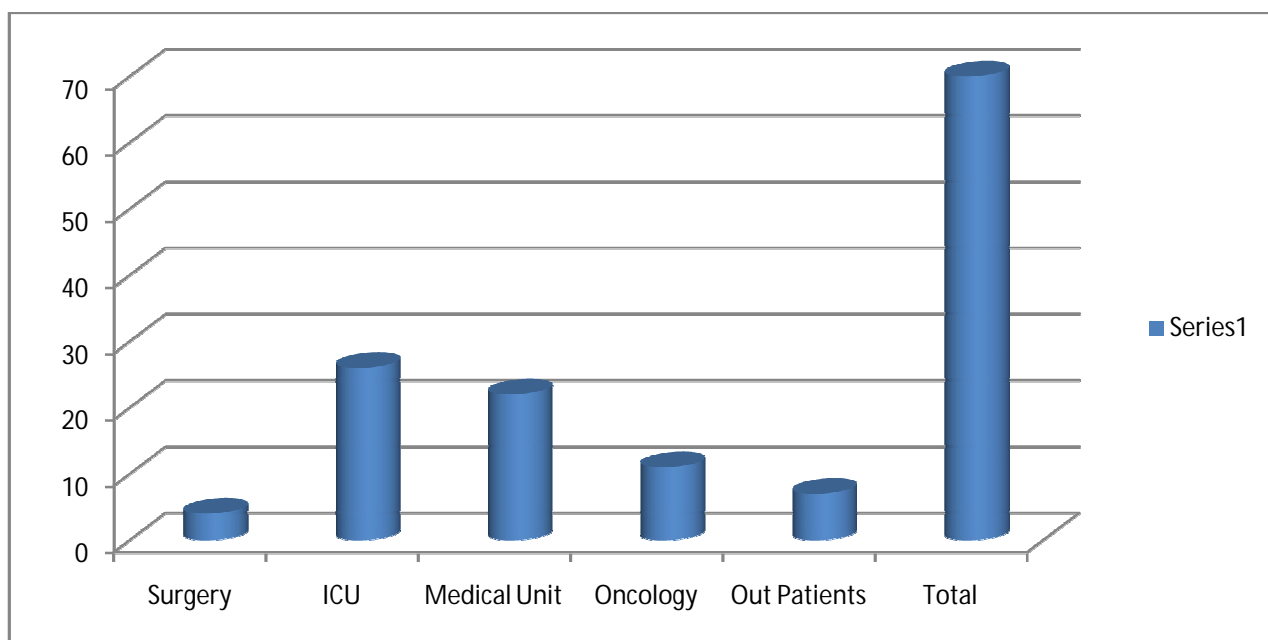
The World Health Organization (WHO) and TwamaHospital policies agree that the human age categories are children 1- 18 years old, adults >18- 60 and elderly >60. So, in this study children were found to be (5.7%), adults (42.9%) and old age (51%) (Table 2).

In Fig (3) the distribution of positive MDR *P. aeruginosa* according to the word is found the most in diabetic patients, followed by oncology and renal transplantation.

The specimens were collected from both males 43 (60.4%) and females 27 (38.6%).



**Fig 1. Types and frequency of specimens**



**Fig 2. Distribution of positive *P. aeruginosa* MDR according to ward**

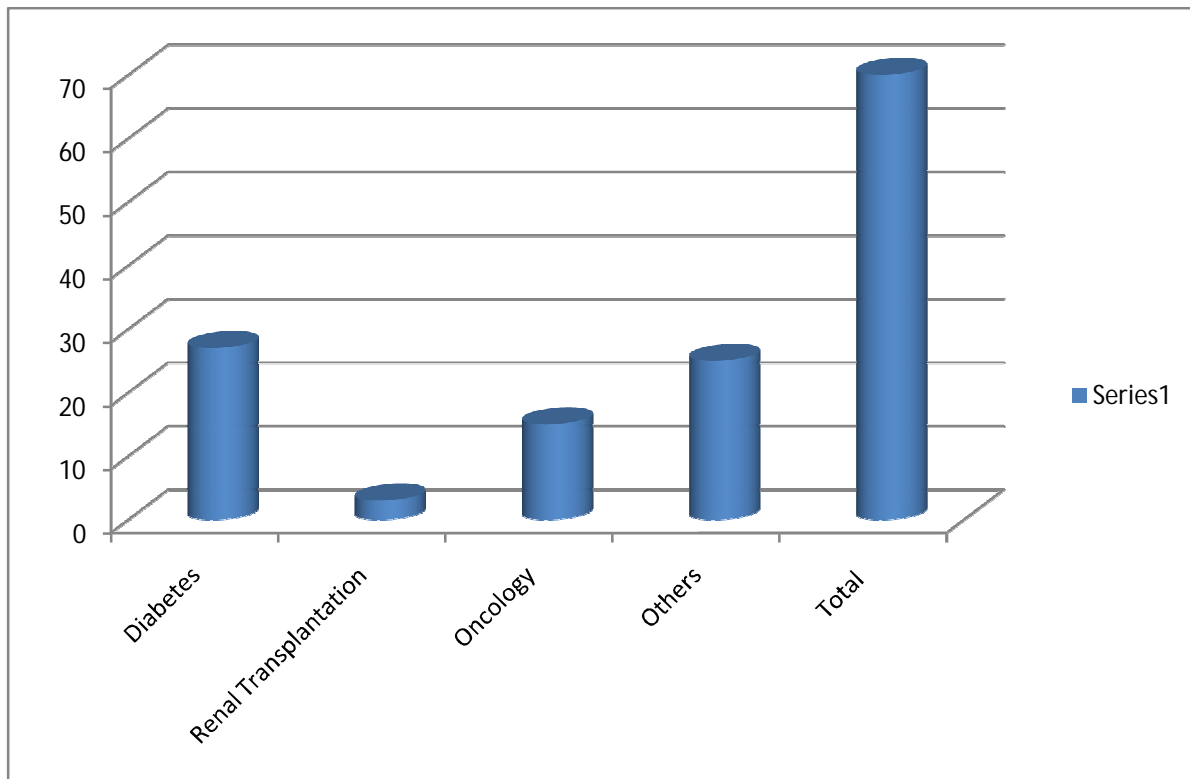
**Table 1. Susceptibility of *P.aeruginosa* (n=70) to different antibiotics**

<b>Antibiotic</b>	<b>Susceptibility to antibiotics</b>					
	<b>R</b>	<b>R (%)</b>	<b>I</b>	<b>I (%)</b>	<b>S</b>	<b>S (%)</b>
Amikacin	42	60 %	-	-	28	40%
Cefepime	38	54.2 %	31	44.3 %	1	1.4 %
Aztreonam	44	62.8 %	21	30%	5	7.1 %
Ceftazidime	38	54.3 %	26	37.1%	6	8.6%
Ciprofloxacin	40	57.1 %	8	11.4%	22	31.4 %
Colistin	2	2.8 %	2	2.8%	66	94.3 %
Gentamicin	29	41.4 %	6	8.6 %	35	50 %
Imipenem	54	77.1 %	4	5.7 %	12	17.1%
Meropenem	55	78.6 %	5	7.1 %	10	14.3%
Piperacillin	44	62.8%	18	25.7 %	8	11.4 %
Piperacillin/Tazobactam	40	57.1 %	12	17.1 %	18	25.7%

Key: S=Sensitive; R=Resistant; I=Intermediate

**Table 2. Frequency of MDR *P. aeruginosa* according to age group category.**

Age Group	Frequency	%
Children (1- 18 Years old)	4	5.7
Adult (>18- 60 Years old)	30	42.9
Elderly (> 60 Years old)	36	51.4
Total	70	100



**Fig 3. Distribution of positive *P. aeruginosa* MDR according to type of disease**

## CHAPTER FIVE

### DISCUSSION

#### 5.1. Discussion

Resistant *P. aeruginosa* can cause community acquired pneumonia, as well as ventilator associated pneumonia, being one of the most common agents isolated in several studies (Falagas *et al.*, 2006), (Paterson, 2006) and (Gould, 2008). This microorganism is naturally resistant to a vast majority of antibiotics that may demonstrate additional resistance after unsuccessful treatment (Andrade *et al.*, 2003). In addition, the bacterium is the cause of infection in burns and immunocompromised individuals (Siegel *et al.*, 2007). The increasing appearance of multidrug resistance among *P. aeruginosa* isolates has confined the suitable therapeutic choices for the treatment of the infections (Magiorakos *et al.*, 2011).

In the present study, (n=70) *P. aeruginosa* were investigated for multidrug resistance. The isolates were recovered from patients in ICU, diabetic, oncology out patients and surgery wards which agrees with the studies of Gould (2008) and Pagani *et al.*, (2005).

In this study Gram stain and VITEK system confirmed the identity of the seventy isolates as *P. aeruginosa* which indicated the accuracy of the primary recovery of these isolates. The isolates were recovered from various specimens. The majority were recovered from sputum, followed by urine and blood.

Study on susceptibility of the isolates showed high resistance to Meropenem (78.6 %) compared to other antibiotics tested. The lowest rate of resistance was reported in Colistin (2.8 %). These results are in agreement with that reported by Paterson (2006). The high resistance to the antibiotics detected during this study may be attributed to abuse of antibiotics.

According to the definition of MDR *P. aeruginosa* by (Magiorakos *et al.*, 2011); all the seventy isolates were MDR. The number of MDR *P. aeruginosa* was found to be higher in males than in females. This result is in agreement with several researches (Andrade *et al.*, 2003), (Falagas *et al.*, 2006), (Siegele *et al.*, 2007) and (Paterson, 2006). Concerning, in the age factors numerous studies showed that MDR *P. aeruginosa* is much common in elderly patients followed by mid-age individuals then children (Magiorakos, *et al.*, 2011). Elderly patients are the most susceptible individuals to MDR *P. aeruginosa* due to the age factor and the long exposure to different antibiotics (Andrade *et al.*, 2003). Also, researches such as Paterson, (2006) and Andrade *et al.*, (2003) focused on ICU patients, medical device and immune-compromised as the most common patients susceptible to be MDR *P. aeruginosa* (Falagas *et al.*, 2006).

## **5.2. Conclusion**

The study concluded that MDR *P. aeruginosa* is highly prevalent among patients attending Tawam Hospital. Presence of multi-drug resistant *P. aeruginosa* necessitates the need for efficient and rapid method for detection of MDR *P. aeruginosa* routinely to avoid further spread among patients and deeper complications.

### **5.3. RECOMMENDATIONS**

1. Antibiotic susceptibility test is highly recommended for each isolate.
2. Continuous surveillance to detect MDR among isolates is highly recommended.
3. Further studies with large number of isolates and more sophisticated methods such as PCR are required to validate the results of the present study.

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## 7. APPENDICES

### Appendix 1: VITEK principle

<p><b>OBJECTIVE</b> This chapter describes the VITEK 2 automated microbiology system and its application in the identification of microorganisms.</p>	<p><b>PRINCIPLES</b> The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. Figure 1 shows the VITEK 2 compact system. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.</p>
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### Appendix 2: *Pseudomonas aeruginosa*; antimicrobial categories and agents used to define MDR, XDR and PDR.

Antimicrobial category	Antimicrobial agent	Results of antimicrobial susceptibility testing (S or NS)
Aminoglycosides	Gentamicin	

	Tobramycin	
	Amikacin	
	Netilmicin	
Antipseudomonal carbapenems	Imipenem	
	Meropenem	
	Doripenem	
Antipseudomonal cephalosporins	Ceftazidime	
	Cefepime	
Antipseudomonal fluoroquinolones	Ciprofloxacin	
	Levofloxacin	
Antipseudomonal penicillins + $\beta$ -lactamase inhibitors	Ticarcillin-clavulanic acid	
	Piperacillin- tazobactam	
Monobactams	Aztreonam	
Phosphonic acids	Fosfomycin	
Polymyxins	Colistin	
	Polymyxin B	

### Appendix 3: Antibiotics used in the susceptibility test and their families

Antibiotics of choice to detect MDR *P. aeruginosa* according to TawamHospital policies are written elaborated with respect to their families as: Amikacin and Gentamicin; Aminoglycoside, Cefepime; Cephalosporin, Aztreonam; Monobactam, Ceftazidine; Third generation cephalosporin , Ciprofloxacin; Fluoroquinolones, Colistin; Polymyxin, Imipenem and Meropenem; Carbapenems, Piperacillin;  $\beta$ -lactam and Piperacillin/Tazobactam; Tazocin. They are all listed in the table below.

Antibiotic	Family
Amikacin and Gentamicin	Aminoglycoside
Cefepime	Cephalosporin,
Aztreonam	Monobactam
Ceftazidine	Third generation cephalosporin
Ciprofloxacin	Fluoroquinolones
Colistin	Polymyxin
Imipenem and Meropenem	Carbapenems
Piperacillin	$\beta$ -lactam
Piperacillin/Tazobactam	Tazocin

**Appendix 4: Tawam Hospital Policies and Procedures regarding *Enterobacteriaceae* family and *Pseudomonas* species MDR (Multi Drug Resistant).**

**1. Purpose**

1.1 Define the criterion to classify the tested isolates of the *Enterobacteriaceae* family and *Pseudomonas* species for antimicrobial susceptibility, as MDR (Multi Drug Resistant)

**2. Policy/Principle**

- 2.1 Multi Drug Resistance isolates are considered critical results
- 2.2 The infection control team is informed for quick intervention.
- 2.3 Prevent nosocomial infections with MDR (Multi Drug Resistant).
- 2.4 For epidemiological and infection control purpose

**3. Sample**

3.1 Sample includes:

<b>Organism Identification</b>	<b>Source of culture</b>	<b>Clinical Significance of culture</b>	<b>Antibiotic Susceptibility Expert Analysis</b>	<b>Antibiotic Susceptibility Kirby Bauer method</b>
<i>Enterobacteriaceae</i> family	Any Body Site	Pathogen Requires antibiotic susceptibility	The expert analysis is consistent with the organism Identification	Proper confluent Growth of the Isolate ( i.e. proper
<i>Pseudomonas</i> species				

		testing	( Green circle seen)	inoculum used)
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### 3.2 Criteria for rejection:

3.2.1 Organism identification other than *Enterobacteriaceae* family, and  
*Pseudomonas species*

3.2.2 The expert analysis is not consistent with the organism Identification  
(Yellow or red square)

3.2.3 Antibiotic Susceptibility by Kirby Bauer method shows heavy or  
light Growth of the Isolate (i.e. improper inoculum used)

## 4. Reagents /Media and Supplies

N/A

## 5. Equipment Calibration and Maintenance

## 6. Special Safety Precautions

6.1 Refer to TOL: LAB-MIC-TOP-SAF-009

6.2 Refer to TOL: LAB-GEN-SOP-LAB-09

6.3 Refer to MSDS sheets

## 7. Quality Control

Refer to Microbiology LAB-MIC- TOP-QUA- 004

6.1 Refer to Microbiology LAB-MIC- TOP- ANT- 013

## 9. Procedure Instructions

### 9.1 Procedure

9.1.1 MDR (Multi Drug Resistant) Definition for :



**Gram negative bacilli: from the *Enterobacteriaceae* family**

**(Not *Pseudomonas* species)**

9.1.1.1 The isolate of Gram negative bacilli from the *Enterobacteriaceae* family is considered MDR if it is resistant or intermediate to **3 different Antibiotic Groups at the same time**

9.1.1.2 Each antibiotic group includes different antibiotics within the group.

**9.1.1.3 HOW TO COUNT:**

9.1.1.3.1 The isolate is considered resistant to an antibiotic group, if it is resistant or intermediate to any one antibiotic within a certain antibiotic group.

9.1.1.3.2 The isolate could be resistant or Intermediate to one, two or more antibiotics within the Antibiotic Group , but you count that specific group as one

9.1.1.3.3 So, regardless of how many resistant or Intermediate antibiotics are within the same Antibiotic Group, you count that Antibiotic Group as ONE.

9.1.1.3.4 Gram negative bacilli from the *Enterobacteriaceae* family are considered as MDR(Multi Drug Resistant) IF Resistant or Intermediate to 3 different antibiotic groups Listed in the following table

9.1.1.3.5 Table of the antibiotic groups to be considered for the

**MDR Criteria for *Enterobacteriaceae* family (NOT *Pseudomonas* species):**

<b><i>Antibiotic Group</i></b>	<b><i>Antibiotic Name</i></b>
<b><i>Quinolones</i></b>	Ciprofloxacin
<b><i>Aminoglycosides</i></b>	Amikacin

	Gentamicin
<b><i>B-lactam /<math>\beta</math>-lactamase inhibitor combinations</i></b>	Piperacillin/Tazobactam (Tazocin)
<b><i>Carbapenems</i></b>	Imipenem
	Meropenem
	Ertapenem
<b><i>Cephems</i> (3<sup>rd</sup> generation <i>Cephalosporins</i>)</b>	Cefepime
	Cefatazidime
	Ceftriaxone or Cefotaxime

#### 9.1.2 MDR (Multi Drug Resistant) Definition FOR :***Pseudomonas species***

9.2.1 The isolate **OF *Pseudomonas species*** is considered MDR if it is resistant or intermediate to **3 different Antibiotic Groups at the same time.**

9.2.2 Each antibiotic group includes different antibiotics within the group.

9.2.3 HOW TO COUNT:

9.2.3.1 The isolate is considered resistant to an antibiotic group, if it is resistant or intermediate to any one antibiotic within a certain antibiotic group.

9.2.3.2 The isolate could be resistant or Intermediate to one, two or more antibiotics within the Antibiotic Group , but you count that specific group as one

9.2.3.3 So, regardless of how many resistant or Intermediate antibiotics are within the same Antibiotic Group, you count that Antibiotic Group as ONE.

9.2.3.4 Gram negative bacilli of *Pseudomonas* species MDR (Multi Drug Resistant) IF Resistant or Intermediate to 3 different antibiotic groups Listed in the following table

9.2.3.5 Table of the antibiotic groups to be considered for *Pseudomonas* species **ONLY**

**MDR Criteria for *Pseudomonas* species:**

<i>Antibiotic Group</i>	<i>Antibiotic Name</i>
<i>Aminoglycosides</i>	Amikacin
	Gentamicin
<i>B-lactam /β-lactamase inhibitor combinations</i>	Piperacillin/Tazobactam (Tazocin)
	Piperacillin
<i>Carbapenems</i>	Imipenem
	Meropenem
<i>Cephems</i> (3 <sup>rd</sup> generation <i>Cephalosporins</i> )	Cefepime
	Cefatazidime
<i>Monobactams</i>	Aztreonam
<i>Quinolones</i>	Ciprofloxacin

### 8.3 Reporting MDR results

8.3.1 On Cerner, verify the VITEK 2 MIC or Kirby-Bauer results.

8.3.2 On Cerner, add contact comment, phone the result to the ward and report and the time and name of the nurse who was notified. Finalize the result as MDR isolated.

8.3.3 Save the isolate and write the information in the Saving Isolates Log book.

## **10. Method Performance Specifications**

N/A

## **11. Calculations**

N/A

## **12. Results/Interpretation/Alert Values**

12.1 The decision to adopt the above mentioned criteria for defining an isolate as MDR was taken by a committee of the Microbiology Consultant, Microbiology

Section Chief and the TAWAM Hospital infection Control Committee.

12.2 Defining MDR varies among different hospitals in different countries.

12.3 We define MDR to prevent the organism from establishing itself in the Patient and also to prevent its spread.

12.4 Always consult the Clinical Microbiologist and Senior medical technologists for technical or results interpretation advice, or when in trouble.

## **13. References**

13.1 JCIA, Accreditation Standards for Hospitals, 4<sup>th</sup> edition, 2011, AOP.5

13.2 Manual of Clinical Microbiology, 9<sup>th</sup> Edition

13.3 TOP: LAB-MIC-TOP-QUA-004

13.4 ANTIBIOTIC SENSITIVITY TESTING LAB-MIC- TOP-ANT-013.5