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ISOLATION, IDENTIFICATION AND ANTIBIOGRAM OF *PSEUDOMONAS AERUGINOSA* FROM NOSOCOMIAL WOUND INFECTION IN QUETTA DISTRICT

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ABSTRACT

The study was conducted to isolate Nosocomial pathogen *Pseudomonas aeruginosa* from clinical specimen of patients admitted in two government hospitals in Quetta district of Pakistan and to determine the antimicrobial susceptibility of isolates. Pus samples (*n=100*) were collected from infected patients of age groups from 20-60 years and belonged to both sexes. Two public sector hospitals of Quetta district were targeted. Identification of *Pseudomonas aeruginosa* isolates was performed by the combination of staining and biochemical testing. Antimicrobial susceptibility of *Pseudomonas aeruginosa* against different antibiotics was performed by Kirby-Baur Method. *Pseudomonas aeruginosa* was found in 45% of clinical samples. Bacterial isolate was relatively high in male patients. Frequency of bacterial isolate was highest in patients of age group 40-49 years. In susceptibility assay, 7 different antibiotics were tested. *Pseudomonas aeruginosa* isolates showed highest susceptibility against Ciprofloxacin (100%) followed by Amikacin (88%), Gentamicin (42%), Chloramphenicol (25%) and low susceptibility to Doxycycline (7%). *Pseudomonas aeruginosa* isolates were completely resistant against Tetracycline and Oxacillin. Study revealed the high frequency of existence of nosocomial pathogen *Pseudomonas aeruginosa* in wound infection of hospitalized patients in district Quetta,

Pakistan and the Pathogen is found resistant to multiple antibiotics used clinically. Ciprofloxacin and Amikacin are found effective against these isolates.

Keywords: *Pseudomonas aeruginosa*, Nosocomial Susceptibility, Pathogen, Kirby-Baur

INTRODUCTION

Nosocomial infection (NI) or hospital acquired infection are those infections which do not exist at the time of patient's admission but they get these infections after they have been admitted. NI infection may be localize or become systemic [1] these infection developed after 48-72 hour after admission in the hospital [2] Nosocomial infection are major cause of mortality and morbidity cause complication in the treatment of patients, increase the cost and cause prolong hospital stay of the patient [3]. Major sites of nosocomial infections are respiratory tract, blood stream, urinary tract and surgical sites [4].

Nosocomial wound infection starts with the invasion of microorganisms into the tissues, damage them by interfering their defense mechanism, discharge pus so cause serious complication in healing of the wounds. Post-surgical wound infection starts after surgery and cause many problems in the treatment of patient [5] Frequency of Nosocomial infection can increased in immunosuppressed patient during therapy, and prolong hospital stay [6]. In diabetic patients, smokers, old aged and mal nourished person the risk of nosocomial infection is increased [7]. The nosocomial infection can be controlled by proper hand hygiene, quality of instruments and adequate medical services [8].

Most common types of nosocomial infections are Urinary tract, skin, lower respiratory and surgical wound [9] virus, bacteria and fungi are involved in nosocomial infection [10] viral and fungal nosocomial infections are less common than bacterial infection [11] Different types of bacteria involved in nosocomial infection. *Staphylococcus aureus, streptococcus pneumonia* and pseudomonas are most common bacteria that involved in this infection [12].

Surgical site or surgical wound infection is the nosocomial infection that occurs after operation, induce complications in treatment even can cause death of operative patient [13]. Microorganisms that are responsible for surgical wound infection cause activation of immune system results tissue damage and inflammation. All these damage occurs due to bacterial toxins, super antigen and uncontrolled proliferation of T cells [14*] Pseudomonas aeruginosa, staphylococcus aureus and Escharichia coli* are most common bacteria involve in surgical site infection [5].

Pseudomonas aeruginosa is Gram negative rod, facultative anaerobe, motile. It is found everywhere including soil, water, plants, animal and human. It is nosocomial infection causing bacteria spread from contaminated material in the hospital. It also spread from medical staff [15]. Pseudomonas aeruginosa is an opportunistic pathogen and it invade in tissues, cause different infections like urinary tract infection, endocarditis, gastrointestinal infection, meningitis specially in immunocompromised patients [16]. The media used for pseudomonas aeruginosa are Cetrimide Agar, MacConkey Agar [17], blood agar [18]. Pseudomonas aeruginosa is biochemically identify by catalase test, oxidase test, citrate utilization test, indole test, triple sugar iron test, urease test, methyl red test, voges proskauer test and gel liquification [19]. *Pseudomonas aeruginosa* is resistant to quinolones, tetracycline, chloramphenicol [20]. It is sensitive to gentamicin [21].

Nosocomial infection found world widely. There prevalence is different in various countries. In united state 1.7 million hospital associated infection are found annually, in France it is found 6.7%, in Italy 4.9%, in United Kingdom 10%, in Switzerland it is 2 to 14% and in finland its prevalence rate is 8.5% [22].

Aim of this study was the isolation, biochemical characterization and antibiogram of Pseudomonas aeruginosa from surgical wounds patients in Quetta district.

MATERIALS AND METHODS

Materials

Brain Heart Infusion Broth (Oxoid, United kingdom)**,** Brain Heart Infusion Agar (Oxoid, United kingdom), Cetrimide Agar (Oxoid, United kingdom), MacConkey Agar (Oxoid, United kingdom),Blood Agar Base (Oxoid, United kingdom), SIM Medium (Oxoid, United kingdom), Simmon Citrate Agar (Oxoid, United kingdom), Triple Sugar Iron Agar (Oxoid, United kingdom)and Mueller-Hinton agar (Oxoid, United kingdom)For antibiotic susceptibility the antibiotic discs Amikacin (30 µg), Chloramphenicol (30 µg),Doxycycline (30 µg), Gentamicin (10µg), Tetracycline (30 µg) and Ciprofloxacin (5 µg) were used [23]. The antibiotic susceptibility test was performed on Mueller-Hinton agar by disc diffusion method, after incubation the zones of inhibition were measured.

Methods

Study Design

Total of 100 patients from 2 different government hospitals (60 from Bolan medical Complex and 40 from Provisional Sandeman Hospital) of Quetta District were

selected in this study **Table 1**. All patients were selected from age groups range from 20 years to 60 years **Table 2**. Pus sample were taken from surgical site wounds for primary isolation of bacteria [24]. Apparatus and glass wares were sterilized using standard hot air oven sterilization method and autoclave.

Isolation and Biochemical Characterization of bacteria

Samples were taken by sterile cotton swabs and shifted to CAVAB in cool chain conditions [25]. All these samples were inoculated in brain heart infusion broth for activation. After incubation for 24 hours at 37°C, the inoculum was streaked on BHI agar for isolation and reincubated at same temperature and time [26]. After that Gram's staining of selected colonies from BHI agar were performed and these colonies were triple cloned for further purification. After purification, these colonies were streaked on cetrimide agar for selective growth of pseudomonas aeruginosa [27]. After that these colonies were streaked on MacConkey agar to check lactose fermentation and on blood agar to check the hemolysis. All these media were incubated at 37°C for 24 hours after streaking [28]. For confirmation of these isolated colonies the biochemical tests like catalase, oxidase, indole, methyl red-voges proskauer [29] citrate utilization, triple sugar iron test, motility, urease and gel liquification tests were performed [30].

Antibiotic susceptibility test

Antibiotic susceptibility was done by disc diffusion Kirby-Bauer method the results were compared with controlled strain of Pseudomonas aeruginosa ATCC 27853 against Amikacin 30, ciprofloxacin 5 mg, chloramphenicol 30mg [31]. Gentamicin 10 µg, Tetracycline 30 µg [32] Oxacillin 1µg [33] Doxycycline 30 µg and dilution of sample was made by 0.5 McFarland's standard [34].

RESULTS AND DISSCUSSION

Results

Isolation and Identification of Pseudomonas aeruginosa from Pus samples of Patients

100 pus samples from surgical wounds were taken from Bolan Medical complex hospital and provisional Sandman hospital (CIVIL) of Quetta district from which 45 were found positive (30 males and 15 female). After incubation colonies shows beta hemolysis on blood agar and Non lactose fermented pale clear colonies were obtained on MacConkey agar. After purification *Pseudomonas aeruginosa* was confirmed by biochemical test like Catalase, oxidase, Simon citrate, urea, Indol, motility, methyl red Voges-Proskauer and Gel liquification. Sugar test like glucose, lactose and sucrose with acid

and H2S production were also performed, the result of these test were given in a (**Table 3.1)** and represented in figures **(Fig 1 to Fig 8).**

Cetrimide Agar Media Test

Yellow green pigments were produced on cetrimideagar as shown in figure 1.

Growth on Macconkey Agar

Non lactose fermented pale clear colonies were produced after 24 hours at 37°Cincubation on macConkeyagar media as shown in fig 2.

Growth On Blood Agar Media

The hemolytic positive strains of *Pseudomonas* aeruginosa produced a clear zone of hemolysis (beta hemolysis) at 37°C for 24 hours incubation on blood agar media as shown in fig 3.

Gram Staining

Organisms were Gram negative, rods under oil immersion (100X) lens as shown in fig 4.

Motility Test

Following overnight incubation all the test tubes inoculated with *pseudomonas aeruginosa* observed for hazy appearance which was the indication of motile organism as shown in fig 5.

Indole Test

No ring formation showed the pseudomonas aeruginosa negative for indole production activity as shown in fig 6.

Catalase Test

The presence of bubble formation on the glass slide was indicated a positive reaction.

Citrate Utilization Test

The green color of the medium was turned into blue indicated a positive result as shown in fig 8.

Triple Sugar Iron Test

The negative results were indicated by alkaline production (change in color from red to pink) indicated no sugar production and the color of medium was not turning into black; indicate the absence of H2S production. *Pseudomonas aeruginosa* as non fermentative as shown in fig 9.

UREASE TEST

When inoculated the culture that fail to produce deep pink color indicate negative reaction as shown in fig 10.

Antibiotic susceptibility test

Pseudomonas aeruginosa shows resistance against chloramphenicol 75%, gentamicin 58%, doxycycline 93%, tetracycline 100%, and oxacillin 100% and shows sensitivity to amikacin 88%, and ciprofloxacin 100% as given in Table 4.

DISSCUSSION

Hospital acquired infections, also known as Nosocomial infections are life threatening infections acquired by patient during his or her stay in hospital. Almost 90% of these infections are of bacterial in origin but can

be caused Viral, Fungal, Protozoal invasion. Among bacteria, noteworthy are *Staphylococcus aureus*, *Proteus mirablis, E. cloi, Streptococcus* Spp., *Klebsiella pneumonia,* enterococci and *Pseudomonas aeruginosa* [35]. *Pseudomonas aeruginosa,* a Gram'-ve bacterium, the second most common bacterial pathogen that causes Nosocomial infections. It can enter in wounds of host, colonizes there and cause serious complications. It contains several virulence factors like lipopolysaccharides, exotoxin A, leukocidin, proteases and manymany more reported by [36,19]. Emergence of Multidrug resistance (MDR) *Pseudomonas aeruginosa* in Nosocomial infections is an alarming situation as leading to high mortality and morbidity rate [37]. Nosocomial Infections caused by *Pseudomonas aeruginosa* are very difficult to treat because intrinsic resistance of this bacterium against major classes of antibiotics (β-lactam, quinolones, aminoglycosides). Major resistance mechanisms possess by Pseudomonas bacteria are poor membrane permeability to antibiotics, over expression of efflux pump mechanism, production of β-lactamase enzymes, production of aminoglycoside modifying enzymes and alteration in topoisomerase II and IV which determine resistance against quinolones. Unfortunately, all these mechanisms exist simultaneously give rise MRD strains of *Pseudomonas aeruginosa* [38]. It is imperative to perform antibiotic susceptibility testing in order to make wise able decision for selecting an appropriate antibiotic for treating Nosocomial infections caused by *Pseudomonas aeruginosa.*

In the present study attempt was made to isolate *Pseudomonas aeruginosa* from surgical wounds of patients admitted in two public sector hospitals of district Quetta of Pakistan, followed by determination of their susceptibility to 7 different types of antibiotics. Total of 100 different pus samples were collected from 100 different patients admitted in two public sector hospitals (Bolan Medical Complex 60 Patients and Civil hospital 40 Patients). Patients were both male and female with an age groups of range 20-69 years from surgical wounds. About 45% of patients from each of both hospitals were found +ve (**Table 3.2).** The percentage of male patients +ve for *Pseudomonas aeruginosa* was high in case of Bolan Medical Complex (BMC) as confirmed through different biochemical testing (**Table 1**). *Pseudomonas aeruginosa* isolates were higher in patients of age group (40-49 years). Our study clearly shows high percentage (45%) of the presence of *Pseudomonas aeruginosa* in surgical

wound and the most vulnerable age group is 40-49 years **(Table. 2).** These findings are highly consistent with a closely related study conducted by Ranjan and colleagues in India where samples were taken from Postoperative wound infections and amoung other bacteria, *Pseudomonas aeruginosa* was the most frequently existing bacteria (29%) of total samples taken and mostly in age group 20-41years with high prevalence in Male patients [19]. High frequency of *Pseudomonas aeruginosa* (23.33%) of total isolates was also reported in another study [23].

Highest susceptibility of our *Pseudomonas aeruginosa* isolates *was* against Ciprofloxacin (100%) followed by Amikacin (88%). *Pseudomonas aeruginosa* isolates were totally resistant against Tetracycline and Oxacilline **(Table 3)**. A study conducted in Indian reported high susceptibility (83%) of *Pseudomonas aeruginosa* isolates of surgical wound infection against Ciprofloxacin [39] which is very close to our finding (100%). Pourshafie et al. in their study reported 98% susceptibility of Nosocomial *Pseudomonas aeruginosa* against Amikacin which is very consistent to our results [40]. A study was conducted to evaluate the relative susceptibility of *Pseudomonas aeruginosa* isolates against Gentamicin and Amikacin. Isolates were obtained from patients of a tertiary care hospital in Karachi district of Pakistan [41]. Study showed 69% susceptibility of Gentamicin which was slightly greater than our finding (42%). Tetracycline is the antibiotic which was found totally ineffective against our *Pseudomonas aeruginosa* isolates. These findings are consistent with a study conducted in Jamaica in which all *Pseudomonas aeruginsa* isolates from nosocomial infection victims were found resistant against Tetracycline antibiotics [42]. Oxacillin was another antibiotic in our study against which isolates showed 100% resistance. Carbapenems producing Pseudomonas aeruginosa isolates were obatined from Patients admitted in Hospital Asfahan, Iran to get their antimicrobial resistant profile against clinically used antibiotics. Results showed 100% resistance of isolates against Oxacillin antibiotic which is in strong agreement with our findings [43].

CONCLUSION

This study was a screening of wound infections for frequency of existance of nosocomial pathogen *Pseudomonas aeruginosa* as well as their susceptibility to major classes of clinically useable antibiotics in hospitals of local area of Pakistan. This indicates the alarming sign for heath care personnel dealing nosocomial infections. Emergence of these

MDR *Pseudomonas aeruginosa* isolates in our study is indicating the unjustifiable usage of antibiotics for treating common infections. Quinolone like Ciprofloxacin and Aminoglycosides, Amikacin were the most effective antibiotics against our isolates. Tetracyclines and Oxacillin showed 0% response against our Isolates. The health professionals are suggested to perform antibiotic susceptibility testing of patient's specimens before reaching to the final selection of antimicrobial for treating infections. These attempt may not only reduce the cost of the therapy but will also help to overcome the possible risk of therapeutic failure.

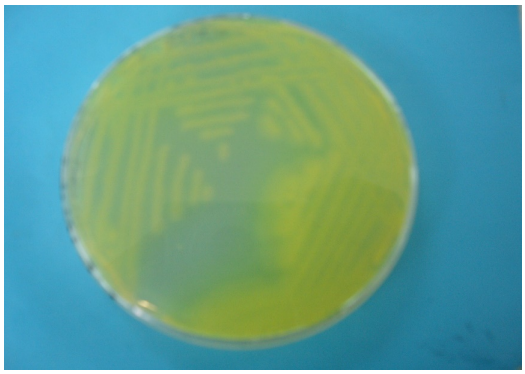

Fig 1: Colonies formation of *pseudomonas aeruginosa*on Cetrimideagar

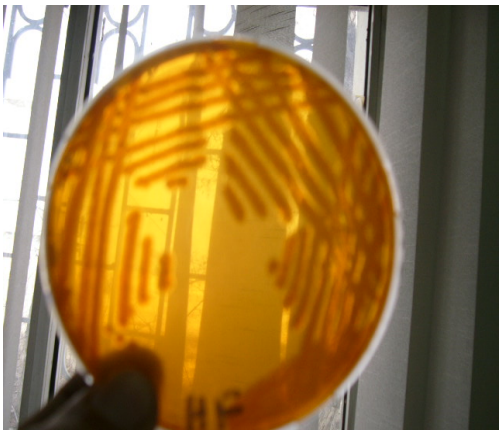

Fig 2: Colonies of *pseudomonas aeruginosa* on MacConkey agar plate

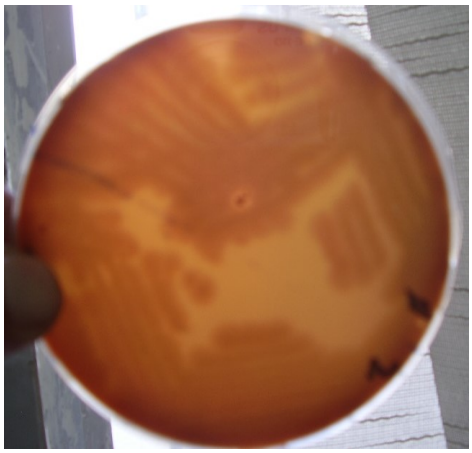

Fig 3: β hemolytic colonies of *pseudomonas aeruginosa*on Blood agar plate

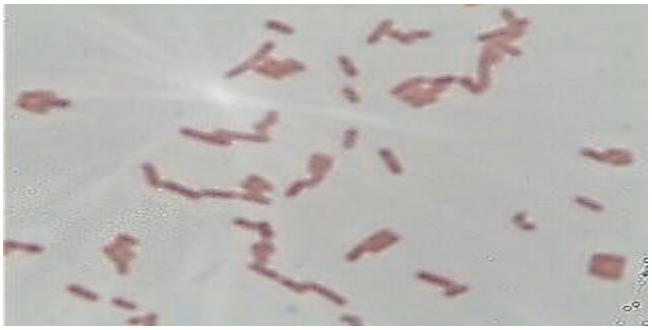

Fig 4: Microscopic structure and staining characteristics of *Pseudomonas aeruginosa.*

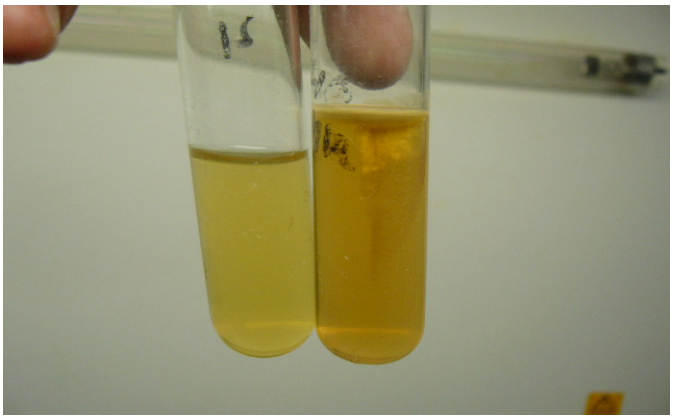

Fig 5: Motility test of *Pseudomonas aeruginosa* showing Control and growth on SIM media.

Fig 6: Indole test of *pseudomonas aeruginosa* showing negative outcomes

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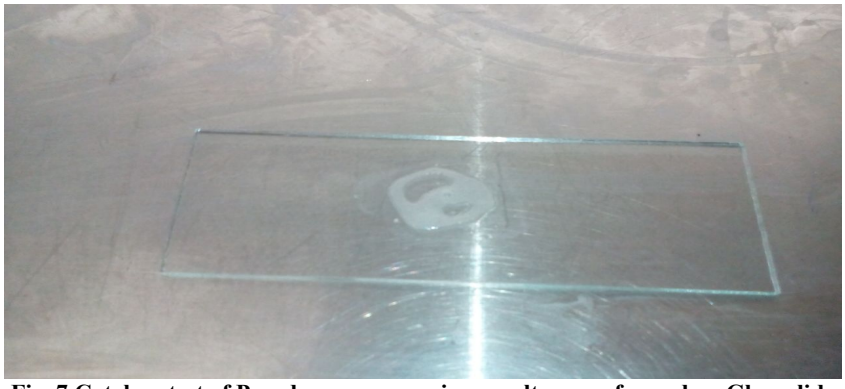

Fig. 7 Catalase test of Pseudomonas aeruginosa culture performed on Glass slide

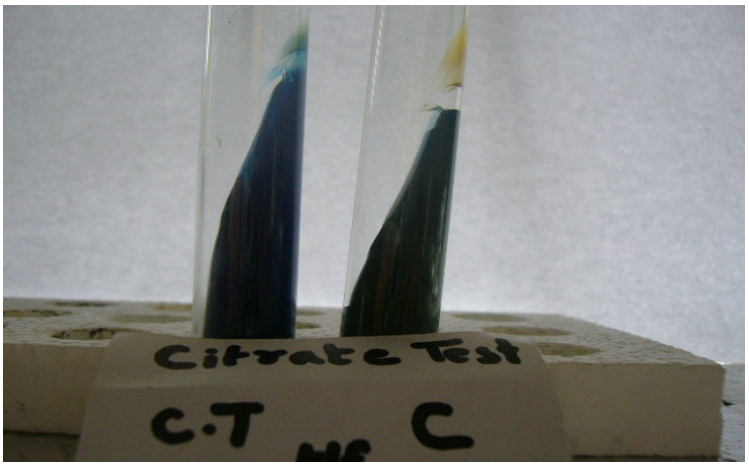

Fig 8: Positive outcomes of citrate utilization by Simon Citrate test comparing with control

Fig 9: Triple Sugars Iron Reaction. Left tube is anun inoculated negative control.The tube on right side indicating the color change with alkaline reaction in a slant without sugar and H2S production

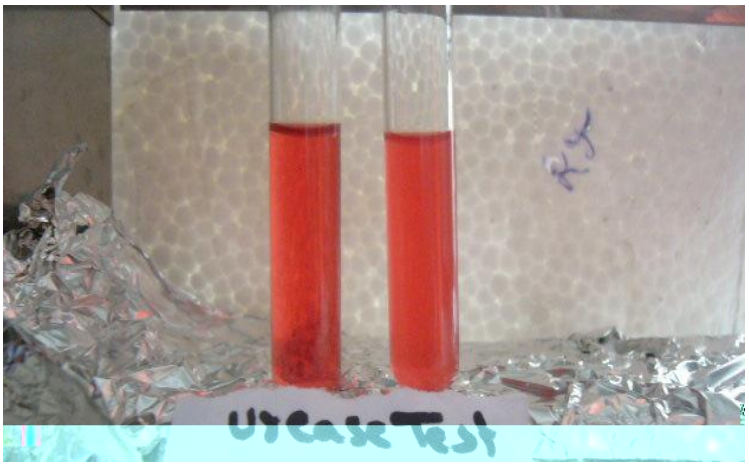

Fig 10: Negative outcomes of urease test

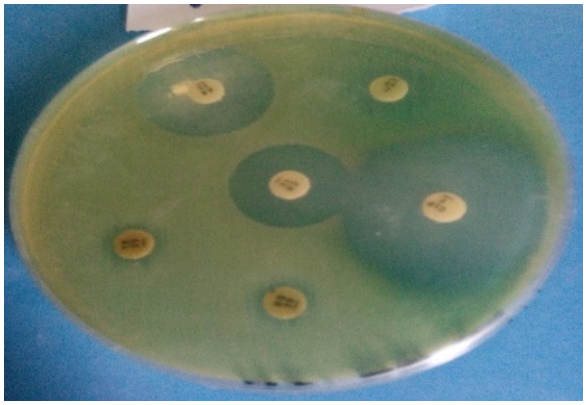

Fig 11: Zone of inhibition against certain antibiotics

Table 1: Patients Selected from Two Different Hospitals of Quetta District

Total Patients	Male	Female	+ve Male	+ve Female	Total Positive	Total Negative
100	65	35	30	15	45	55
60 BMC	40	20	19	8	27	33
40 CIVIL	25	15	11	7	18	22

BMC=Bolan Medical Complex;

Table 2: Distribution of Specimen with Positive *Pseudomonas aeruginosa* Isolates.

Groups Years	Total isolates	Male	Female	BMC Male	BMC Female	Civil Male	Civil Female
20-29	9	7	2	3	1	4	1
30-39	7	5	2	3	2	2	0
40-49	16	10	6	7	4	3	2
50-59	12	8	4	6	1	2	3
60-69	1	0	1	0	0	0	1

BMC= Bolan Medical Complex.

Table 3: Results of Different biochemical tests performed to confirm *Pseudomonas aeruginosa*

Biochemical Tests for *Ps. aeruginosa*	RESULT
Catalase	+
Oxidase	+
Simmon citrate,	+
Urease	-
Indol	-
Motility	+
Methyl red	-
Voges-proskauer	-
Gel liquification	+
Glucose	-
Lactose	-
Sucrose	-
Acid	-
H2S	-

Table 4: Antibiotic Susceptibility Pattern against *Pseudomonas aeruginosa*

Antibiotics	Codes of antibiotics	Antibiotics concentration	Resistance %	Sensitive %
Amikacin	AK 30	30 µg	11	88
Chloramphenicol	C-30	30 µg	75	25
Gentamicin	CN 10	10 µg	58	42
Doxycycline (30 µg)	DO 30	30 µg	93	7
Tetracycline	TE 30	30 µg	100	0
Ciprofloxacin	CIP 5	5 µg	0	100
Oxacillin	OX	1 µg	100	0

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