IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 9 Ver. IV (Sep. 2017), PP 62-69
www.iosrjournals.org

Prevalence of Pseudomonas Aeruginosa in Khartoum Teaching Dental Hospital.

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Abstract: *The present study was carried out to estimate the prevalence of Pseudomonas aeruginosa in Khartoum teaching dental hospital. Swab samples were collected from the water pipe lines of the dental chairs in the hospital (104 sample) and cultured; then treated with some antibiotics; Ciprofloxacin, Ceftazidime, Meropenem and Tetracycline to determine antibiotic sensitivity. Pseudomonas aeruginosa was isolated from swabs cultured from dental chair water pipes. Four swabs (3.8%) grew on the cultured media while the other 100 swab samples (96.2%) showed no growth. Drugs that showed maximum effect against Pseudomonas aeruginosa bacteria were Meropenem, Ciprofloxacin. Tetracycline was moderately active but Ceftazidime showed no effect on the bacteria cultured. Pseudomonas aeruginosa was detected only in areas cleaned by Chloroxylenol product (antiseptic Dettol), while other areas in the hospital which were disinfected and shocked by Sodium Hypochlorite were free.*

Keywords*: prevalence, Pseudomonas aeruginosa, Khartoum Dental Hospital, antibiotic resistant*

Date of Submission: 28 -08-2017 Date of acceptance: 13-09-2017

I. Introduction

Health of human being, patient's safety and defeating disease, is the main concern of health workers. Associated hospital acquired disease has always been a challenge; nosocomial infection is an infection acquired in hospitals by patient who are admitted for more than 48 hours. The highest prevalence of nosocomial infection occurs in surgical operating rooms and wards. Dental clinics are a critical area in which infection and disease can easily be transmitted [(1)]. Contaminated instruments, equipment surfaces and water in dental clinics represent infectious areas that my cause cross- infection between patients and health workers and result in transmission of infectious pathogens within the clinical environment. [(2, 3)] Pathogenic bacteria can be easily transmitted by direct or indirect contact. [(3)] Pseudomonas aeruginosa is a pathogenic bacterium that became a big concern to health workers especially in dental clinics. It has the ability to form biofilms in waterlines and acquired multidrug resistance. [(2)] It is a Gram-negative, aerobic and opportunistic bacterium that lives in this environment. It prefers the moist habitat. It belongs to the genus Pseudomonas; the species of which has characteristics of being non spore forming, motile and positive to catalase and oxidase test. [(1)] It is an opportunistic human pathogen that is considered worldwide the fourth most common cause of hospital acquired infections(urinary ,gastrointestinal and respiratory tract infections) in immune compromised patients that lead to death[(2)] .It is a major cause of wound infections, blood stream infections, and nosocomial pneumonia .[(4,5)]Pseudomonas aeruginosa strains have the ability to develop resistance toward first line antimicrobial drugs and cause persistent infections in wound and blood stream that may lead to death .[(1,2,4)]

II. Literature Review

It is known that patients usually get infected when the immune system is weekend; [(6, 8)] Pseudomonas aeruginosa strains gain the pathogenicity from mutation of gens; this gives Pseudomonas aeruginosa the ability to make biofilms which are resistant to antimicrobial treatment. Mutation gens also can promote virulence factors production and secretion, however other extrinsic factors such as site of infection, type of immune response and infection phase play a role too.[(2)]Pseudomonas aeruginosa has always been considered a problem in dentistry as many studies showed that strains such streptococcus and legionella and pseudomonas species were detected in dental water systems. These strains may cause cross infection especially in a medically compromised patient. Another study proved that Pseudomonas aeruginosa can be transmitted through the hands of health workers and patients can be easily infected from incubated waterlines in the hospital. [(7)]Studies

approved that in order to keep this pathogen under control; appropriate shocking and purring in the Dental Unit Water Lines should be done. [9, 20]Three major classes of antibiotics are used commonly against Pseudomonas aeruginosa; which are amino-glycosides (Tobramycin), β-lactams (Ceftazidime), and Quinolones (ciprofloxacin). Quinolones and β-lactams inhibit DNA gyrase and cell wall Peptidoglycan-assemble trans-peptidases of the bacteria, while amino glycosides inhibit protein synthesis by binding to the 16S rRNA within the 30S ribosomal subunit. P.aeruginosa exhibits intrinsic resistance to many antibacterial agents and tends to acquire additional resistance during therapy[1,11,17] .It is well known that pseudomonas aeruginosa has the ability to develop resistance toward antimicrobial treatment.[10,12,14,16] .The virulence factors that help Pseudomonas aeruginosa to gain the pathogenicity are encoded chromosome genes , the low permeability of the outer membrane, the ability to form a constitutive expression of membrane efflux (Mix) pumps, and the natural occurrence of an inducible chromosomal b-lactamases.[1]A study conducted in Afghanistan used 230 specimens of Pseudomonas aeruginosa in which specimens were collected from burn ward and ICU. Out of 230 strains of P. aeruginosa, 49.5% were Imipenem resistant(an antibiotic that has the same mechanism of action of Meropenem) because of the difference between the Metallo-beta-lactamases produced by the other strains in the study.[19] It is confirmed that early eradication of Pseudomonas aeruginosa by antibiotics approach is very effective in delaying chronic infections such as cystic fibrosis.

[20].Appropriate cleaning and disinfection procedures should be performed in the dental office. Disinfection is a process that kills most pathogenic microorganisms, but rarely kills all bacterial spores. Disinfection is achieved through using pasteurization or by use of chemical agents i.e. disinfectants. Two levels of disinfection are usually performed in dental clinics, high-level disinfection (HLD) is a process capable of killing vegetative bacteria, mycobacteria, fungi, and enveloped and non-enveloped viruses, as well as some bacterial spores. Chemical products used usually include 2% glutaraldehyde, 6% hydrogen peroxide, 0.2% Peracetic acid, 7% accelerated hydrogen peroxide and 0.55% ortho-phthalaldehyde. HLD is used for semi-critical instruments like dental mirror and probes. These items are usually cleaned before being disinfected. Low-level disinfection (LLD) is a process capable of killing most vegetative bacteria, as well as some fungi and enveloped viruses. [22]Low-level disinfection is needed for non-critical patient care items and some environmental surfaces are cleaned. The chemical products used for LLD include chlorine-based products (e.g. diluted household bleach), 0.5% accelerated hydrogen peroxide, 3% hydrogen peroxide, 60-95% alcohols, iodophors, Phenolic and quaternary ammonium compounds. [22]

Justifications: The presence of Pseudomonas aeruginosa in water systems is documented in the literature, but the link between contamination of the water system and hospital-acquired infections (HAI) is not clear. The water collected from the outflow of Dental Unit Water Lines (DUWL) is densely populated with bacterial counts ranging from a few thousand to as high as 106 colony forming units (CFU)/ml, which reflects the colonization of waterlines by biofilms.

2.1 General objective: The aim of this study is to determine the prevalence of Pseudomonas aeruginosa in Khartoum dental teaching hospital.

2.1 Specific objectives:

1. To assess the culture of Pseudomonas aeruginosa from samples collected from certain dental units in the hospital.
2. To evaluate the cause of presence of Pseudomonas aeruginosa in the hospital.
3. To determine the most effective antibiotics against Pseudomonas aeruginosa.

Study Design: Cross sectional hospital based study.
Study area: Khartoum teaching dental hospital- Khartoum state Sudan.
Working area: Research Laboratories of the University of Medical Sciences and Technology.
Study population: All suspected and critical areas in Khartoum teaching dental hospital including dental spittoons, dental lights, hand pieces, the light handles, suctions apparatuses, trolleys and, floor of the theatre rooms and wards.
Sample Size: 104 samples were collected from different areas in the hospital.
Ethical consideration: It was taken from SUMASRI Institutional Review Board (SIRB) (see Appendices).

III. Materials And Methods

Swab samples (104) were taken from suspected areas in the hospital and maintained in a safe container and cultured within two hours. Swabs were inoculated on Blood agar and MacConkey agar plates by the use of striking method and incubated aerobically at 37 C^0 overnight. [23]Blood agar (Enriched medium) is used in this study because it is the best medium for growing fastidious bacteria [23] while MacConkey agar is used for

isolation and differentiation of the cultured bacteria based on its ability to ferment lactose. Bile salt (inhibitor of the growth of Gram-positive bacteria) is used together with neutral red; (a Ph indicator) that is colourless above a Ph of 6.8 and red at a Ph below 6.8. [(24)] Modified Kirby-Bauer method is used for antimicrobial susceptibility test. After that a semi solid medium is used to provide motility medium; the isolated organism is incubated by a straight wire in the semi solid medium and the wire is removed before reaching the bottom of tube, and incubated at $37C^0$ over night. [(24)] The isolated strains were tested for their antibiotic susceptibility using Kirby-Bauer disk diffusion test. This was done by disc diffusion method using certain type of antimicrobial discs; these include Ceftazidime (30mcg), Tetracycline (30mcg), Ciprofloxacin (5mcg) and Meropenem (10mcg).

IV. Results

Out of the 104 swabs cultured, four (3.8%) were positive for Pseudomonas aeruginosa after performance of biochemical test; while 100 (96.2%) showed no growth. Table 1

Table 1: Show the Results of isolation P. aeruginosa recovered from different surface in the hospital.

Site of collection	No. of sample examined	No. of positive isolates	site of positive
Intensive care unit	3	_	_
Theatre room (1,2,3)	12	2	Operating table (TR1) Suction (TR1)
Ward Room	24	2	Ward bed (wr2) Tap (wR9)
Outpatient	37	_	_
Conservation clinic	20	_	_
Periodontology clinic	15	_	_
Pediatric clinic	13	_	_
Orthodontic clinic	3	_	_
Prosthodontics clinic	4	_	
Septic clinic	6	_	

Table 1: biochemical test to identify Gram negatives Bacilli.

Oxidase test	Citrate utilizing test	Urease test	Indole test	Motility test	KIA				Suggested organisms
					Slope	Butt	H_2S	Gas	
+ve	+ve	+ve	-ve	motile	R	R	-ve	-ve	Pseudomonas aeruginosa

Key: KIA: Kligler's Iron Agar, +ve: positive, -ve: negative, R: red – pink (alkaline reaction), Y: yellow (acid reaction), *: origin bacteria cannot ferment the lactose but, can become lactose fermenting, H2S: Hydrogen Sulphide (black).

5.2 Antibiotic susceptibility test: (100%):

According to clinical and laboratory standard institute ,the results showed that the most active drugs against Pseudomonas aeruginosa bacteria were Meropenem (100%), Ciprofloxacin, while Tetracycline (100%) was found to be intermediate but it is resistant to Ceftazidime (Table 2).

Table 2: show the sensitivity of bacterial isolate to certain Antimicrobial disc.

Antibiotics / Bacterial isolate	Ciprofloxacin (5mg),	Meropenem (10mg)	Ceftazidime (30mcg)	Tetracycline (30mcg)
Pseudomonas aeruginosa	4 (100%)	4 (100%)	0 (00%)	4 (100%)

Table 3: show the Zone size interpretative chart standard break point extracted from the Clinical and Laboratory Standards Institute(CLSI) . [(25)]

Antibiotics	**Resistant**	**Intermediate**	**Sensitive**
Ciprofloxacin(5mcg)	Less than 24	25-33	More than 34
Ceftazidime (30mcg)	Less than 21	22-29	More than 30
Meropenem (10 mcg)	Less than 26	27-33	More than 34
Tetracycline (30 mcg)	Less than 17	18-25	More than 26

V. Discussion

Pseudomonas aeruginosa is considered a major pathogen as it may cause cross infection to people seeking dental treatment. In this study, the prevalence of Pseudomonas aeruginosa in Khartoum Teaching Dental hospital was found to be 3.8%. The strains were collected from the suction, operating table, a tap, and a ward bed. Pseudomonas aeruginosa was detected in areas cleaned by Chloroxylenol product (antiseptic Dettol)

but not in other areas in the hospital which were disinfected and shocked by Sodium Hypochlorite. The strains were cultured and tested to determine the susceptibility to a number of antibiotics; all were found sensitive to Meropenem and Ciprofloxacin, moderately sensitive to Tetracycline and resistant to Ceftazidime. Studies conducted to test the use of solvents other than Sodium Hypochlorite and Chloroxylenol products. This study agrees with previous studies conducted to determine the effect of Sodium Bicarbonate (SB), Sodium Metaperiodate (SMP) and Sodium Dodecyl Sulfate (SDS) combination, on biofilm formation and dispersal in dental unit waterline on associated bacteria and yeast; the results of which proved that the combination was very effective on viability of both gram positive and gram negative bacteria but there is only minimum reduction in the numbers of Pseudomonas aeruginosa biofilms. The composition of Sodium Hypochlorite and Chloroxylenol products gave a very potent effect as the composition is environmentally friendly, biologically safe and retards the formation of biofilm. [(18)]A study conducted in Afghanistan did not agree with the results of our current study because they used a ready 230 spacemen of Pseudomonas aeruginosa strains from specimens taken from burn ward, ICU , Out of the 230 strains of P. aeruginosa, 49.5% were Imipenem resistant,(an antibiotic that has the same mechanism of action of Meropenem) , the cause of this major difference between results is due to Metallo-beta-lactamases which was produced by the other strains in their study.[(19)]

VI. Conclusions

The nosocomial infection is considered a major health issue to health workers in hospital and community due to pathogenic commensal that grow and form biofilms. Pseudomonas aeruginosa is opportunistic pathogen that has the ability to cause cross infections especially in patients with medically compromised conditions. The strains of Pseudomonas aeruginosa that colonize the dental water units, has the ability to develop antimicrobial resistance. It colonizes the waterlines and moist habitat like tabs and tools used in operations such suctions apparatuses. Pseudomonas aeruginosa has the ability to develop antimicrobial resistance toward antimicrobial drugs commonly used.

Recommendations

- Hospitals should ensure the availability of enough appropriate disinfectants for theatre rooms and clinics to eradicate opportunistic microorganisms and protect patients and health workers from cross infection.
- Increase the awareness of dentists and dental assistants towards the issue of infection control.

Conflict of interest: the authors declared none.

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Appendex:

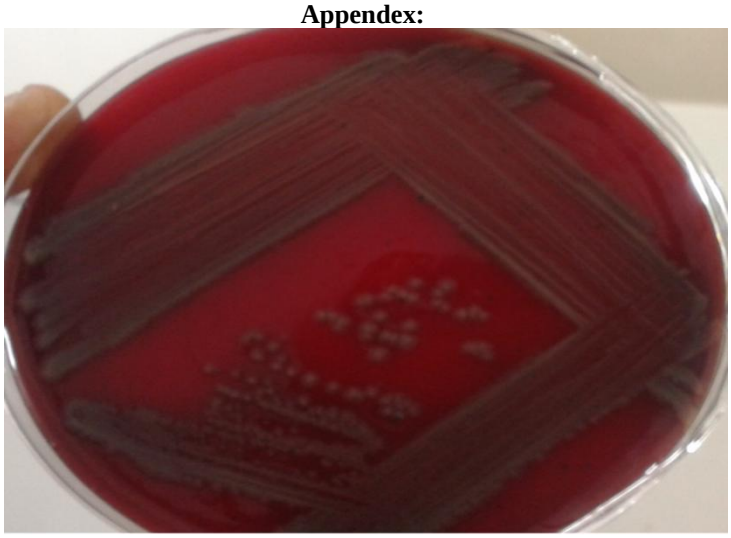


Figure (1) show thePseudomonas aeruginosa on Blood agar, non-haemolysis, and green pigment.

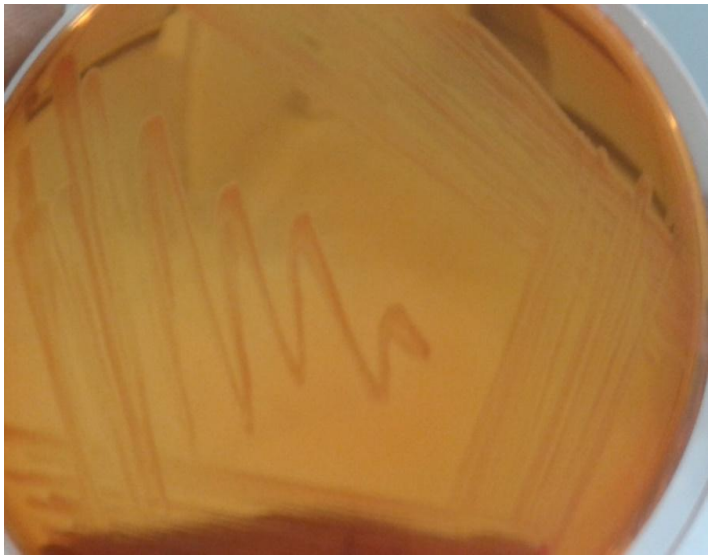


Figure (2) show thePseudomonas aeruginosa on MacConkey agar, non-Glucose non-lactose fermenting and pale yellow colour.

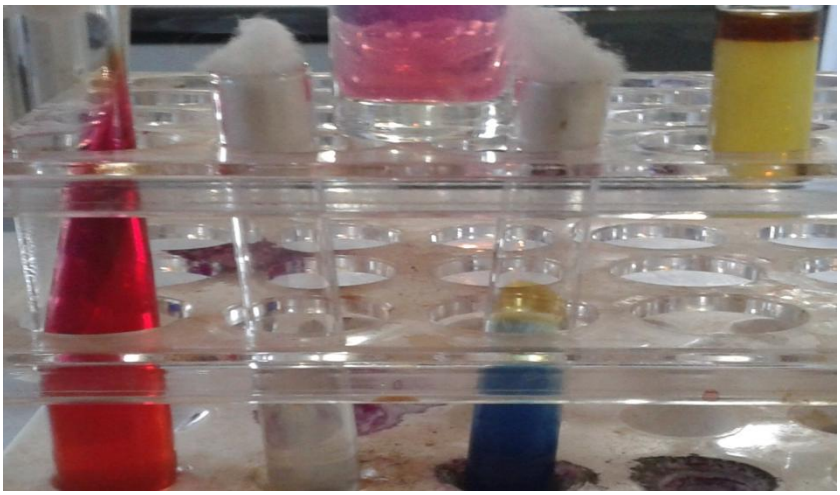


Figure (3) show the biochemical Test for identification of Pseudomonas aeruginosa.

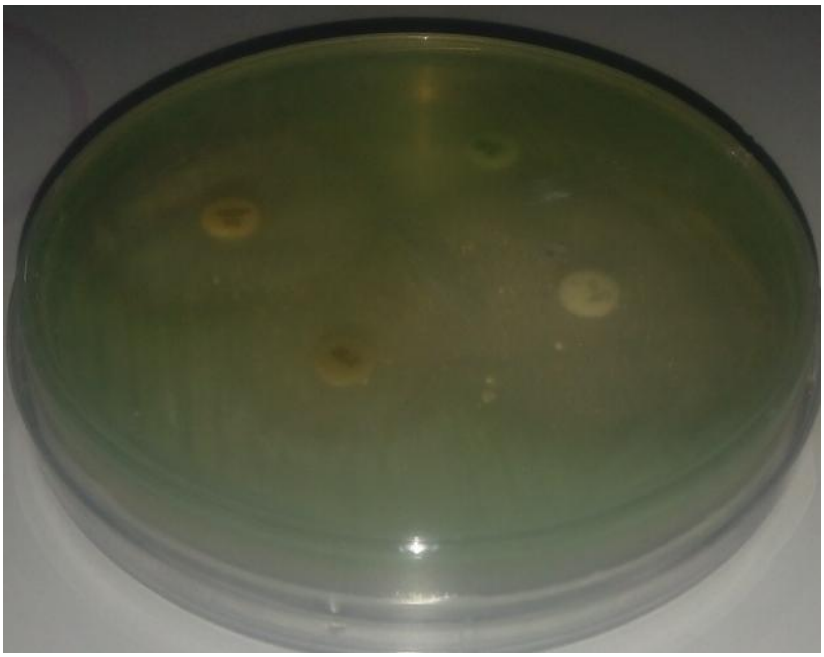


Figure (4) show the green pigment of Pseudomonas aeruginosa on Mueller and Hinton agar.

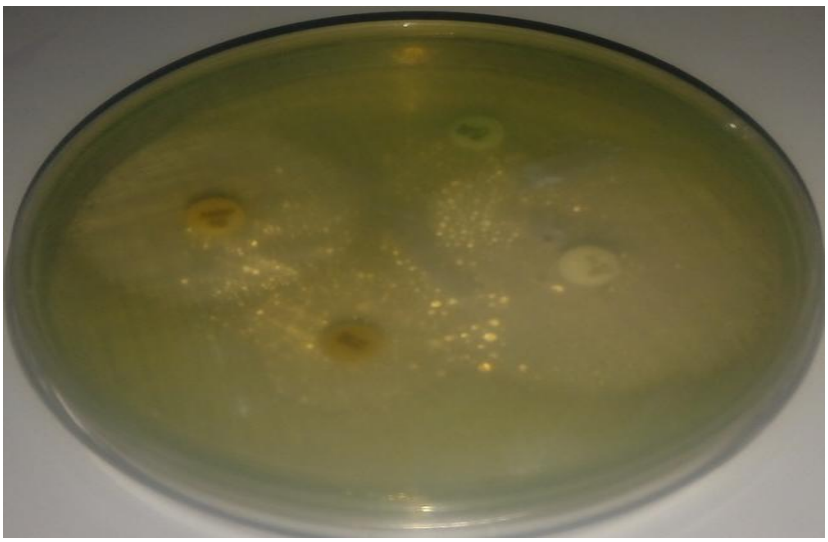
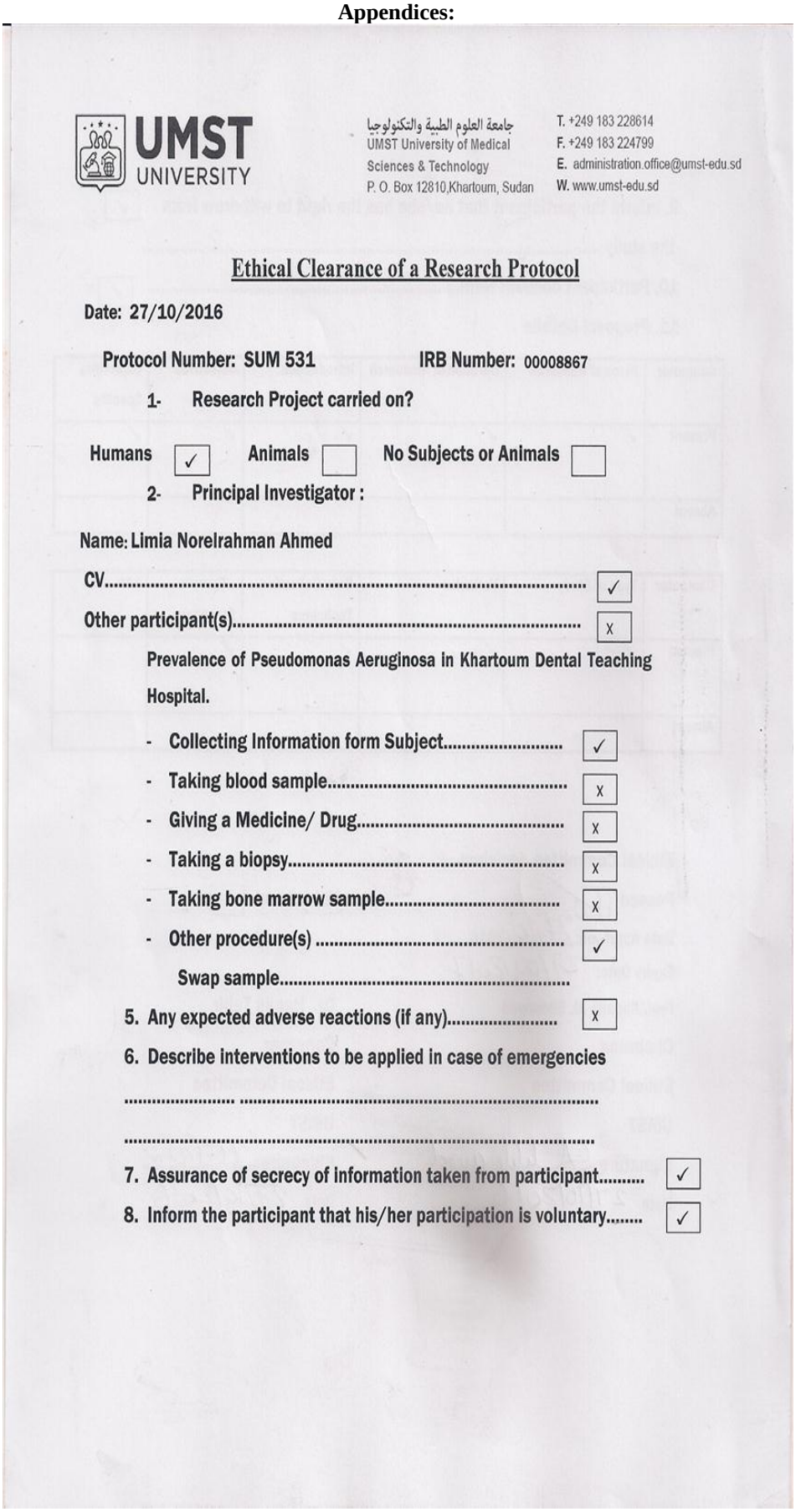


Figure (5) Show the antimicrobial Susceptibility test.

Appendices:



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Ethical Clearance of a Research Protocol

Date: 27/10/2016

Protocol Number: SUM 531 IRB Number: 00008867

1- Research Project carried on?

Humans [✓] Animals [ ] No Subjects or Animals [ ]

2- Principal Investigator :

Name: Limia Norelrahman Ahmed

CV..... [✓]

Other participant(s)..... [X]

Prevalence of Pseudomonas Aeruginosa in Khartoum Dental Teaching Hospital.

- Collecting Information form Subject..... [✓]
- Taking blood sample..... [X]
- Giving a Medicine/ Drug..... [X]
- Taking a biopsy..... [X]
- Taking bone marrow sample..... [X]
- Other procedure(s) ..... [✓]

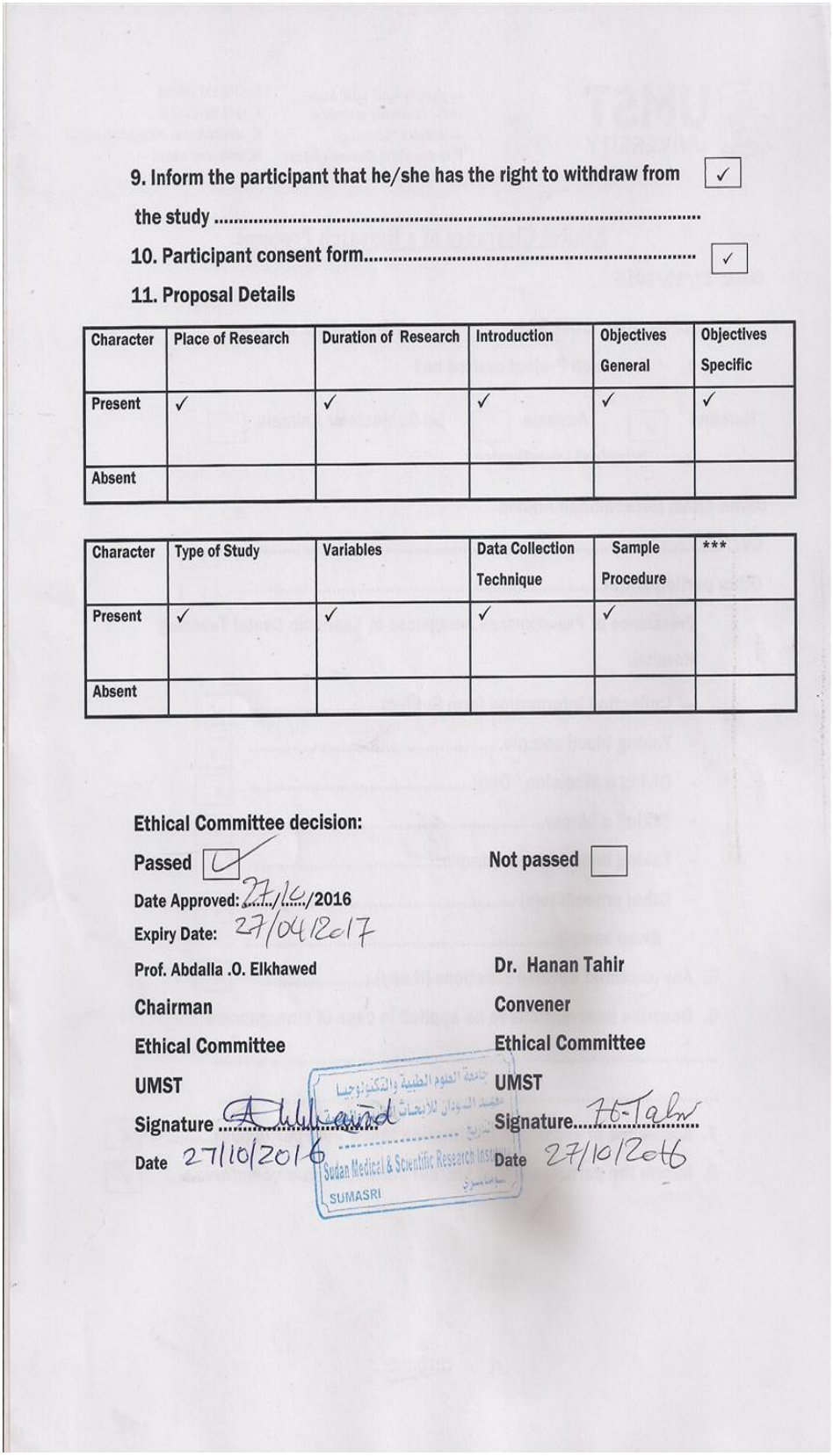
Swap sample.....

5. Any expected adverse reactions (if any)..... [X]
6. Describe interventions to be applied in case of emergencies

....

.....

7. Assurance of secrecy of information taken from participant..... [✓]
8. Inform the participant that his/her participation is voluntary..... [✓]



9. Inform the participant that he/she has the right to withdraw from the study ..... ✓

10. Participant consent form..... ✓

11. Proposal Details

Character	Place of Research	Duration of Research	Introduction	Objectives General	Objectives Specific
Present	✓	✓	✓	✓	✓
Absent					

Character	Type of Study	Variables	Data Collection Technique	Sample Procedure	***
Present	✓	✓	✓	✓	
Absent					

Ethical Committee decision:

Passed ☑ Not passed ☐

Date Approved: 27/10/2016

Expiry Date: 27/04/2017

Prof. Abdalla .O. Elkhawed
Chairman
Ethical Committee
UMST
Signature .....
Date 27/10/2016

Dr. Hanan Tahir
Convener
Ethical Committee
UMST
Signature.....
Date 27/10/2016

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