CHAPTER ONE

1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

Infection transmission in the hospital enviroment remain significant hazard for hospitalized patients and health care workers , as the result of many pathogens transmitted by hands and by stethoscopes (Patent Storm, 2004*).*

The stethoscopes is commonly instrument used by physicians and other health professionals to hear the sounds made by the heart, lungs and various other body organs. Stethoscopes used in hospitals by medical doctors, medical students and other health practitioners for assessing patient health have been reported as apotential vector for transmitting infections in the hospital enviroment in various part of the world (Cohen *et al*., 1997; Zuliani - Maluf *et al*., 2002).

Several studies in medical literature have demonstrated that many physicians' stethoscopes are contaminated with pathogenic bacteria and could serve as a mode for transmission of infection (Jones *et al*., 1995; Smith *et al*., 1996).

The universal use of the stethoscope and it is direct contact with multiple patients makes it an important potential factor in the dissemination of microorganisms from one patient to another (Breathnach *et al*., 1992; Wright *et al*., 1995; Gerken *et al*., 1972).

Hospital acquired infections are frequently caused by microorganisms in the hospital environment and are a significant cause of morbidity and mortality. They also result in increased health care costs. About one third of all nosocomial infection are preventable (Hughes, 1988).

This is the rationale for the time honoured advice for all to wash their hands before and after seeing each patient. However, transmission of infection though contaminated medical devices is also a possibility. Outbreaks of nosocomial infections have already been linked to devices like electronic thermometers, blood pressure cuffs, stethoscopes and latex gloves (Patent Storm, 2004*).*

For planning preventive actions, it is essential to identify the reservoirs of microorganisms that cause nosocomial infections. Hands of the hospital staff, medical equipment such as catheters, surgical instruments, implants, ventilators, endoscopes is hardly ever undertaken (Smith *et al*., 1996; Breathnach *et al*., 1992). Isopropyl alcohol is most effective one in cleaning stethoscopes (Jones et al., 1995).

Bacteria display a unique ability to adapt to changes in their environment and to develop mechanisms to protect themselves against toxic compounds. Their ability to develop resistance mechanisms to antimicrobial drugs has assumed catastrophic proportions, rendering more and more infections difficult or impossible to treat (Cohen, 1992).

1.2. Rationale

There are increasing reports of tremendous risk of transmitting antibiotic resistant bacteria from one patient to another from stethoscopes. Because most hospital - acquired infections are primarily nosocomial and not autoinfections (Hoogkamp-Korstanje *et al*., 1982), their acquisition in the hospital enviroment add to morbidity, mortality, and economic costs (Parmar *et al*., 2004).

1.3.1. General objectives

To assess of multi drug resistant bacteria isolated from stethoscopes.

1.3.2. Specific objective

1. To collect bacteria recovered from stethoscopes.

2. To check purity of the isolates.

3. To perform susceptibility test.

4. To perform Minimum Inhibitory Concentration.

5. To determine multi drug resistant bacteria.

CHAPTER TWO

2. Litreture review

2.1. Stethoscope

Stethoscopes are essential tools of the medical profession and because of their universal use might be a source of microorganisms that cause nosocomial infections. Stethoscopes come in direct contact with numerous patients daily and their disinfection after each use is not an established practice. Several studies in medical literature have demonstrated that many physicians' stethoscopes are contaminated with pathogenic bacteria and could serve as a mode for transmission of infection. This phenomenon may be a particular problem in areas where the outbreak of multidrug resistant bacteria, such as, methicillin-resistant *Staphylococcus aureus* (MRSA), occurs or where patients with increased susceptibility to infection are to be found (Jones *et al.*, 1995 ; Smith *et al.*, 1996).

Healthcare workers' stethoscopes are potential vectors for transmission of pathogens because they frequently come in contact with the skin of patients and are not routinely cleaned between examinations (Fenelon *et al*., 2009; Alleyne *et al.*, 2009).

Stethoscopes get contaminated by the organisms colonised on the patients' skin, or those resident on the hands or outfits of the health care providers, or when they come in contact with blood and other biological secretions. The universal use of the stethoscope and its direct contact with multiple patients makes it an

important potential factor in the dissemination of microorganisms from one patient to another. In hospitalised patients, this means an exposure of an already susceptible host to a higher microbial overload and for the patients attending Out Patient Department, an exposure to the ominous antibiotic-resistant hospital-flora (Gerken *et al*., 1972).

Hospital environment is a reservoir of wide varieties of microorganisms. Several strains of pathogenic bacteria have been frequently reported colonizing medical equipments (like Stethoscopes) (Schabrun *et al.*, 2006).

These pathogens include superbugs like Vancomycin Resistant *Enterococcus* spp., Methicillin Resistant and Sensitive *Staphylococcus* species and Multidrug resistant, *P. aeruginosa, E. coli, Klebsiella* spp. and *Streptococcus* species (Bernard *et al.*, 1999; Wood *et al.*, 2007).

During auscultation stethoscope contamination is common; if the same stethoscope is used for the next patient without disinfection, it might bring risk of infection to the patient and may continuously impose the risk serially to all patients (Whittington et al., 2009).

MRSA and *P. aeruginosa* have been isolated from hospital surfaces including stethoscopes, catheters, and even disinfectant soap dispensers (Brooks *et al.*, 2002; Guinto *et al.*, 2002; Stickler, 2002).

Draping of stethoscopes around the neck is still a commonly seen practice, resulting in the risk of recontamination of the diaphragm of the stethoscope from the unclean earpieces, with normal flora and pathogenic bacterial strains

harboring the ears of the health care workers. A single stethoscope often used for all inpatients and outpatients (Parmar *et al*., 2004; Hayden *et al.*, 2006).

The universal and unavoidable use of the stethoscope and its direct contact with multiple patients makes it an important potential factor in the dissemination of microorganisms from one patient to another. Exposure of the already susceptible hospitalized patient to resident flora of the hospital environment (in most cases are multidrug resistant pathogens unless proved) may worsen the clinical condition of the patient. Infection prevention protocols are effective in reducing the health care associated infections (Knox *et al.*, 2010). The present study demonstrated that the bacterial contamination of the stethoscopes was directly related to the area of the stethoscope which was in contact with the patient's skin or clothes, and that it was inversely related to the procedure and the frequency of cleaning of the stethoscopes. The period of contact between a patient's skin and the stethoscope can result in the transfer of bacteria. Our study demonstrated that stethoscopes (mainly the diaphragms) get contaminated with pathogenic as well as nonpathogenic bacteria. If these are not cleaned properly with a suitable disinfectant at regular intervals, this can transfer bacteria from the skin of one patient to another. Our study demonstrated the importance of cleaning the stethoscopes with a disinfectant. Comparatively fewer bacterial colonies were obtained from the stethoscopes of the individuals who cleaned their stethoscopes with alcohol. This is similar to the findings of Marinella and others (Patent Storm , 2004).

In this study, most of the stethoscopes showed presence of contamination. The implication of the finding is that stethoscopes could be a vector, playing an important role in the transmission of potential pathogenic microorganisms as well as antibiotic-resistant strains. Cleaning of the heads of stethoscopes between patients could be a means of stopping this. The diaphragm design of many stethoscopes involves a rim that can only be thoroughly cleaned by disassembling the diaphragm which is impractical for regular cleaning. However, simple cleaning would reduce the risk of transmission of microorganisms and prevent the outbreak of infection (Whittington *et al*., 2009).

The agents most frequently found on stethoscopes are *Staphylococcus* species, among which are included strains resistant to antibiotics (Jones *et al*.,1995; Smith *et al*., 1996 ; Wright *et al*., 1995).

Transmission of microorganisms through contaminated medical devices such as electronic thermometers, blood pressure cuffs, stethoscopes, respiratory equipment/devices, gloves, gowns, masks, and white coats, or the skin and nasopharynx of hospital personnel is always a possibility because of their contact with patient bodies (Mangi *et al*., 1972; Leontsini *et al*., 2013; Bhatta et *al*., 2011; Kilic *et al*., 2011; Jadhav *et al*., 2013).

The contamination of stethoscope particularly the diaphragm is reported mainly due to lack of regular disinfection (before and after examining each patient (Schabrun *et al*., 2006).

The stethoscope is a tool in constant use among health professionals. It is often passed from one professional to another and is always in direct contact with patients. Disinfection of stethoscopes is an issue that has been neglected (Jones *et al*., 1995; Leão *et al*., 1999).

Regular disinfection of stethoscopes or disposable cover should be used to minimize the possibility of spreading infectious agents in hospitalized patients. This is especially important today, since hospitals now care for more immunocompromised patients than in previous times and also there is increased resistance of bacteria to available antibiotics (Bernard *et al.*, 1990).

Isopropyl alcohol has been shown to reduce bacterial colony counts when applied to the stethoscope diaphragm (Marinella *et al*., 1997).

The use of 70% propyl alcohol found to be effective in reducing contamination of stethoscopes and other medical equipments than other agents like detergents (Knox *et al*., 2010; Nelson *et al*., 2006).

2.2. Multi-drug Resistant

Multidrug-resistant organisms (MDROs) are bacteria that are not able be treated with certain types of antibiotics and require treatment with other medicines that may be less effective, more toxic, and more expensive. There are several types of MDROs may be found in healthcare facilities, including methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae* (CRE) vancomycin-intermediate or vancomycin resistant

Staphylococcus aureus (VISA/VRSA), and vancomycin-resistant *Enterococci* (VRE) (Gorwitz , 2008)**.**

2.1.3. Examples of Multi drug resistant bacteria:

Methicillin-resistant *Staphylococcus aureus* **(MRSA)** is a type of *Staphylococcus* (staph) bacteria that is resistant to certain antibiotics called beta-lactams. These antibiotics include methicillin and other more common antibiotics such as oxacillin, penicillin, and amoxicillin. In the community, most MRSA infections are skin infections and may appear as red boils or pimples. More severe or potentially life-threatening MRSA infections occur most frequently among patients in healthcare settings and may initially present as symptoms such as fever and pain at the site of infection. While 25% to 30% of people are colonized in the nose with staph, less than 2% are colonized with MRSA (Magill *et al.*, 2014).

Like MRSA, **Vancomycin-intermediate** *Staphylococcus aureus* **(VISA)** and **Vancomycin-resistant** *Staphylococcus aureus* **(VRSA)** are types of staph bacteria that are resistant (or have intermediate resistance) to certain antibiotics. Because of their resistance to antibiotics, VISA/VRSA infections can be more difficult to treat. VISA/VRSA infections may affect the skin or may get into the bloodstream, causing a more serious type of infection. Enterococci are a type of bacteria found naturally in the environment, as well as in the human intestines and the female genital tract. When these bacteria develop resistance to vancomycin, they become **Vancomycin-resistant**

Enterococci **(VRE)**. Most VRE infections occur in people who are hospitalized (Magill *et al.*, 2014).

The emergence of resistance of bacteria to antibiotics is a common phenomenon. Emergence of resistance often reflects evolutionary processes that take place during antibiotic therapy. The antibiotic treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of antibiotics. Under certain conditions, it may result in preferential growth of resistant bacteria, while growth of susceptible bacteria is inhibited by the drug (Levy *et al.*, 1994).

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance. As this problem continues to grow, harmonized definitions with which to describe and classify bacteria that are resistant to multiple antimicrobial agents are needed, so that epidemiological surveillance data can be reliably collected and compared across healthcare settings and countries. In the strictest sense, multidrug-resistant organisms (MDROs) are labelled as such because of their *in vitro* resistance to more than one antimicrobial agent. Infections with MDROs can lead to inadequate or delayed antimicrobial therapy, and are associated with poorer patient outcomes (anderson *et al.*, 2006; Ibrahim *et al.*, 2000).

Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs. This review discusses our current knowledge on the molecular mechanisms involved in both types of resistance (Nikaido , 2009).

Antimicrobial agents or categories, there are bacterial species within certain organism groups (i.e. the Enterococcus spp. and the Enterobacteriaceae) that are intrinsically resistant to one or more antimicrobial agents within acategory or to all agents within acategory (EUCAST, 2008).

CHAPTER THREE

3. Materials and Methods

3.1.Study design

3.1.1. Type of study

This is cross sectional study

3.1.2. Study population

The studied population was 200 swabs sample collected from stethoscopes from different hospitals.

3.1.3. Study area

This study was done on sample collected from omdourman,ibrahum malik,bahry and mialatary hospital.

3.1.4. Sample size

200 samples were collected from different stethoscopes.

3.1.5. Study duration

From June to July 2014

3.1.6. Study location

Sudan University of Science and Technology, college of medical laboratory science.

3.2. Source of isolates

The isolated bacteria obtained from the research lab which isolated from omdourman hospital, mialatary hospital, bahry hospital and ibrahim malik hospital and reidentified as follow :

sub culture on macckay agar medium

3.2.1. Gram stain (Sod. Fine Chem. Ltd,China)

Requirements

Crystal violet	Stain
Lugols iodine	Mordant
Acetone alcohol	Decolarizer
Saffranin	Stain background

Methods

By using sterile wire loop (Laboratories Pvt,Ltd,an India) small colony was taken into clean and dry slide contain drop of normal saline (Scharlau,Spain) and spread, we let to dry and fixed by passing the slide over the flame 3 times. Crystal violet was added to smear for 1 minute then washed by tap water, luglos iodin was added for 1 minute , then washed by tap water, aceto alcohol added for seconds and washed by tap water. Fainally, the smear covered by saffranin for 2 mins., and washed by tap water, we let the smear to dry by air , then we added drop of oil and examined under light microscop (Carl Zeiss, Germany) by oil lens x100 (Laboratories Pvt,Ltd,India).

All done according to Cheesbough, 2006

3.2.2.1. Indole test

By using sterile wire loop small portion of the organism was inoculated into trypton water (appendix1) and mixed. Then incubated for 24 hrs in the incubater. In the second day kovacs reagent (BDH Chemical, Japan) was added.

Positive result : red ring

Negative result : yellow ring

3.2.2.2. Urease test (Himendia laboratory)

By using sterile straight loop small portion of the organism was added to urea agar (appendix 3)and incubated for 24hrs.

Positive result : pink colour

Negitive result : orange colour

3.2.2.3. Citrate test (Himendia laboratory)

By using sterile wire loop small portion of the organism was inoculated into kossers citrate media (appendix 3) under aseptic condition and incubated over night.

Positive result : blue colour

Negative result : green colour

3.2.2.4. KIA test (Himendia laboratory)

Kligler Iron Agar (appendix4) test is done by using sterile straight loop (Laboratories Pvt,Ltd,India) by toughing the colony under aseptic condition and inoculated into stap in staight line and butt in zigzag form, then incubated for over night.

	Slope	Butt	Gas	H2S
L.F.	Yellow	Yellow	+ crack	+ black
N.L.F.	Pink	Yellow	_ no crack	_ no black

3.2.2.5. Catalase test

In sterile tube contain 2ml of 3% H2O2 (Supplies Angles Burry, UK) small portion of the colony was taken by wooden stick (Nimbgo Yudia) and transfferedinto tube.

Positive result : active air bubbles

Negative result : no air bubbles

3.2.2.6. Manitol Salt Agar (Himendia laboratory)

The organism inoculated into MSA (appendix5) by streaking under aseptic condition, then incubated for over night.

Manitol fermenter : yellow colonies

Non manitol fermenter : pink colonies

3.2.2.7. DNase test (Himendia laboratory)

The organism under test was inoculated into DNA agar (appendix6) by making heavy spots under aseptic condition and incubated for over night, in the second day the culture was covered by Hcl (Scharlau, Spain).

Positive result : clear zone around the colony

Negative result : no zone around the colony

3.3. Sensitivity tests

3.2.1. Name of method

Modified Kirby Bauer Disc Diffusion.

3.2.2. Preparation of suspention

3-5 colony of similar appearance were suspend into sterile normal saline under aseptic condition, then the turbidity of suspension were matched to turbidity of 0.5 % Mc Farland standard.

3.2.3. 0.5% Mc Farland standard

1% v/v solution of sulphuric acid prepared by adding 1ml of concentrated sulphuric

acid to distilled water mixed well and prepared 1.17%w/v solution of barium chloride 100ml distilled water also was prepared. The turbidity standard 0.5ml

of 1.17%w/v barium chloride solution was added to 99.5ml of 1% sulphuric acid solution and mixed 0.5ml Mc Farland standard. Transferred asmall volume of the turbid solution to ascrew-cap bottle of the same types as used for preparing the test and control inoculum . Stored in awell seal scaled bottle in adark tempreture (20-28c) standard. This standard has the turbidity of asuspension of approximately 1.5*108 bacterial /ml.

3.2.4. Seeding of plate and application of antibiotics

Under aseptic condition we take inoculum from the suspention and inoculated into plate contain Mullor Hinton agar by seeding the plate. The antibiotics were added by using sterile forceps in even distribution, then incubated aerobically at 37c over night.

3.2.5. Reading and interpretation of the results

The plate was inverted and the diameter of each zone of inhibition was measured by ruler, then each zone size was interpreted by using interpretive chart.

CHAPTER FOUR

4. RESULT

During the period betweem june and july 2014, sensetivity testing methods were done on 200 samples collected by research laboratory to detect multi drug resistant bacteria, and the result of isolated bacteria was listed in Table (1)

Bacteria	Number	Percentage
Escherichia coli	10	18.9%
Klebsiella pneumoniae	11	20.8%
Pseudomonas aeroginosa	12	22.6%
Proteus spp	6	11.3%
Staphylococcus aureus	14	26.4%
Staphylococcus epidermidis	38	19%
Staphylococcus haemolyticus	11	5.5%
Staphylococcus hominis	7	3.5%
Staphylococcus warneri	16	8%
Staphylococcus lugdunensis	6	3%
Staphylococcus saprophyticus	6	2.5%
Bacillus	43	21.5%
No growth	21	10.5%

Definition for multi drug resistant (MDR) bacteria.

Table (2)

Bacteria	MDR	
Staphlococus spp.	The isolate is non-susceptible to at least 1 agent in more than or equal 3 antimicrobial categories as listed	Gentamycin,Oxacillin Ciprofloxaci,Vancomycin Clindamycin, Erythromycin,Chloramphenicol Tetracyclin
Enterobacteriaceae	The isolate is non-susceptible to at least 1 agent in more than or equal 3 antimicrobial categories as listed	Aminoglycosides (gentamycin-tobramycin-amikacin). Ceftazidime, Ampicillin Ciprofloxacin, Amoxicillin Chloramphenicol, Tetracyclin
Pseudomonas aeroginosa	The isolate is non-susceptible to at least 1 agent in more than or equal 3 antimicrobial categories as listed	Aminoglycosides (gentamycin-tobramycin-amikacin). Imipenem, Meropenem Ceftazidime,Pipracillin Ciprofloxacin

The result of antimicrobial susceptibility listed in table (3) ,(4)and table(5) as follow : Table (3)

0rganism	Antibiotics								
	Tetracyclin		Ampicillin		Ceftazidime		Gentamycin		Total
	S	R	S	R	S	R	S	R	
E.coli	2	8	1	9	1	9	10	0	10
K.pneumoniae	3	8	1	10	0	11	10	1	11
Proteus spp	6	0	0	6	1	5	5	1	6

continue

Organism	Antibiotics								
	Ciprofloxacin		Tobramycin		Chloramphenicole		Amoxicillin		Total
	S	R	S	R	S	R	S	R	
E.coli	10	0	10	0	10	0	1	9	10
K.pneumoniae	7	4	10	1	7	4	1	10	11
Proteus spp	6	0	6	0	5	1	0	6	6

Table (4)

Organism	Antibiotics								Total
	Ciprofloxacin		Tobramycin		Ceftazidime		Gentamycin		
Pseudomonas aeroginosa	S	R	S	R	S	R	S	R	
	12	0	12	0	0	12	12	0	12

Continue

Pseudomonas aeroginosa	Pipracillin		Amikacin		Meropenem		Imepenem		Total
	S	R	S	R	S	R	S	R	12
	8	4	11	1	1	11	12	0	

Table (5)

Organism	Antibiotics								
	Oxacillin		Vancomycin		Erythromycin		Clindamycin		Total
	S	R	S	R	S	R	S	R	
S.aureus	0	14	4	10	10	4	2	12	14
S.epidermidis	1	37	5	33	30	8	12	26	38
S.haemolyticus	1	10	8	3	10	1	9	2	11
S.hominis	1	6	0	7	6	1	1	6	7
S.warneri	0	16	12	4	10	6	3	13	16
S.lugdunensis	0	6	5	1	4	2	5	1	6

S.saprophyticus	0	5	4	1	4	1	1	4	5

Contiue

Organism	Antibiotics								
	Chloramphenicol		Gentamycin		Ceftazidime		Ciprofloxaci		Total
	S	R	S	R	S	R	S	R	
S.aureus	8	6	8	6	4	10	11	3	14
S.epidermidis	30	8	22	16	6	32	20	18	38
S.haemolyticus	8	3	9	2	1	10	11	0	11
S.hominis	5	2	6	1	5	2	7	0	7
S.warneri	11	5	14	2	6	10	11	5	16
S.lugdunensis	4	2	6	0	0	6	6	0	6
S.saprophyticus	4	1	3	2	0	5	3	2	5

Escherichia.coli and *Klebsiella.pneumoniae* are multi drug resistant bacteria, because it is resistant to amoxicillin, tetracyclin, ampicillin and ceftazidime.

- *Proteus spp*. are multi drug resistant bacteria for amoxicillin, ampicillin and ceftazidime.

- *Staphylococcus aureus* is multi drug resistant bacteria for oxacillin, vancomycin and clindamycin.
- *Staphylococcus epidermidis* is multi drug resistant bacteria for oxacillin, clindamycin and ceftazidime.
- *Staphylococcus hominis* multi drug resistant bacteria for oxacillin, vancomycin and clindamycin.

- *Staphylococcus warneri* is multi drug resistant bacteria for oxacillin, clindamycin and ceftazidime.
- *Staphylococcus saprophyticus* is multi drug resistant bacteria for oxacillin, clindamycin and ceftazidime.

CHAPTER SEX

6.APPENDIXES

Appendix 1

Macconkeys agar medium

differential and selective medium used to enhance the growth of pathogen and members of Enterobacteriaceae . It contains pepton, bile salt to inhibit non-intestinal bacteria and lactose with neutral red (indicator) to distinguish the lactose fermenting coliforms from the lactose non- fermenting bacterial group.

Appendix 2

Pepton water

Ingredient

Peptic digest of animal tissue	10g
sodium chloride	5g

Preparation

15g of. powder dissolve in 1L of D.W. then sterilize by autoclave (Gritten and George ltd, England) at 121c for 15 minutes, cool and pour in tube. Final PH 7.2.2

Appendix 3

Urea agar base (Christensen)

Ingredient

Peptic digest of animal tissue	1g
Dextrose	1g
sodium chloride	5g
Monopotassium phosphate	.80g
Phenol red	.012g
Agar	15g

Preparation

24g of powder dissolve in 1L of D.W. and sterilize by autoclave (Gritten and George ltd, England) at 121c for 15 minutes then cool and add aseptically 50 ml of 40 %urea, mix and pour in tube in vertical position. Fainal PH 6.8.2

Appendix 4

Kossers citrate medium

Ingredient

Magnesium sulfate	.2g

Potassium dihydrogen sulfate	1g
sodium ammonium sulfate	1.5g
Trisodium citrate	2.5g
Bromothymol blue	.016g

Preparation

5.2g dissolve in 1L of D.W., then sterilize by autoclave (Gritten and George ltd, England) at 121c for 15 minutes and pour in tube.

Appendix 5

Kligler Iron Agar (KIA)

Ingredient	15g
Peptic digest of animal tissue	3g
Beef extract	3g
Yeast extract	10g
Protease pepton	10g
Lactose	1g
Ferrous sulfate	.20g
Sodium chloride	5g

Sodium thiosulfate	.3g
Phenol red	.022g
Agar	15g

Preparation

57.5g dissolve in 1L of D.W., then sterilize by autoclave (Gritten and George ltd, England) at 121c for 15 minutes then cool and pour in tube in slope slant position.

PH 7.4.2.

Appendix 6

Mannitol Salt Agar

Ingredient

Meat extract	1g
Casein pepton	5g
Sodium chloride	75g
D.manitol	10g
Agar	15g

Preparation

111g of powder dissolve in 1L of D.W. and sterilize by autoclave (Gritten and George ltd, England) at 121c for 15 minutes then cool and pour in petridishes.

Fainal PH7.4.2

Appendix 7

DNase agar

Ingredient

Casien enzymic hydrolysate	15g
Papic digest of soya bean meal	5g
Deoxy ribonuclric acid	2g
Sodium chloride	5g
Agar	15g

Preparation

42g of powder dissolve in 1L of D.W. and sterilize by autoclave (Gritten and George ltd, England) at 121c for 15 minutes then cool and pour in petridishes.

Fainal PH 7.3.2

Appendix 8

Mullor Hinton Agar

Ingredient

Beef infusion	300g
Casien and hydrolysate	17.50g
Starch	1.5g
Agar	17g

Preparation

38g was dissolved in 1L of D.W. and sterilize by autoclave (Gritten and George ltd, England) at 121c for 15 minutes then cool and pour in petridishes.

Fainal PH 7.3.2

Diameters/mm of zones of inhibition of antibiotics against *Escherichia coli*

code	Antibiotics															
	AMP		GEN		TE		CAZ		TOB		CIP		AMX		C	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
121	0	R	29	S	10	R	15	R	20	S	40	S	0	R	30	S
58	0	R	30	S	18	R	15	R	25	S	40	S	0	R	3 5	S
69	0	R	23	S	18	R	15	R	24	S	40	S	0	R	35	S
114	0	R	28	S	9	R	0	R	20	S	35	S	0	R	30	S
35	0	R	30	S	15	R	15	R	25	S	40	S	0	R	30	S
71	0	R	28	S	18	R	13	R	15	S	40	S	0	R	35	S
80	8	R	35	S	28	S	19	S	25	S	40	S	22	S	35	S
139	0	R	15	S	18	R	0	R	20	S	28	S	0	R	30	S
79	0	R	30	S	20	S	15	R	24	S	40	S	0	R	30	S
140	0	R	29	S	18	R	15	R	24	S	35	S	0	R	30	S

Diameters/mm of zones of inhibition of antibiotics against *Klebsiella pneumoniae*

code	Antibiotics															
	AMX		GEN		TE		CAZ		TOB		CIP		C		AMP	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
133	0	R	15	R	0	R	0	R	15	S	0	R	14	R	10	R
132	0	R	22	S	0	R	0	R	23	S	30	S	30	S	0	R
94	0	R	25	S	20	S	0	R	23	S	34	S	24	S	0	R
67	0	R	15	R	0	R	0	R	15	S	0	R	14	R	0	R
38	0	R	20	S	13	R	0	R	23	S	34	S	14	R	0	R
88	10	R	25	S	13	R	0	R	24	S	30	S	19	S	0	R
157	23	S	45	S	25	S	0	R	28	S	40	S	30	S	12	R
158	20	S	40	SQ	23	S	0	R	30	S	40	S	30	S	0	R
175	0	R	12	R	0	R	0	R	15	S	8	R	30	S	0	R
178	0	R	10	R	0	R	0	R	0	R	0	R	25	S	0	R
7	0	R	25	S	0	R	0	R	15	S	30	S	0	R	0	R

Diameters/mm of zones of inhibition of antibiotics against *Pseudomonas aeroginosa*

code	Antibiotics															
	GEN		CAZ		TOB		CIP		PI		MEM		AK		IMP	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
8	18	S	0	R	20	S	27	S	19	R	0	R	23	S	32	S
44	20	S	0	R	25	S	35	S	24	S	0	R	24	S	45	S
66	26	S	0	R	26	S	40	S	25	S	20	S	30	S	50	S
77	25	S	0	R	23	S	30	S	25	S	8	R	20	S	45	S
78	30	S	0	R	21	S	26	S	20	S	8	R	16	R	33	S
92	23	S	0	R	24	S	32	S	23	S	0	R	24	S	25	S
137	32	S	0	R	19	S	29	S	17	R	2	R	22	S	26	S
146	22	S	0	R	30	S	40	S	23	S	0	R	30	S	33	S
148	25	S	0	R	20	S	30	S	16	R	2	R	25	S	40	S
156	30	S	10	R	22	S	32	S	25	S	0	R	30	S	37	S
159	23	S	0	R	22	S	44	S	22	S	12	R	33	S	40	S
180	23	S	0	R	22	S	40	S	21	S	10	R	30	S	45	S

Diameters/mm of zones of inhibition of antibiotics against *Proteus spp.*

code	Antibiotics															
	CAZ		AMX		AMP		C		CIP		GEN		TE		TOB	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
63	0	R	10	R	0	R	18	S	40	S	29	S	25	S	25	S
55	20	S	0	R	0	R	15	R	35	S	35	S	24	S	21	S
64	0	R	15	R	0	R	18	S	33	S	26	S	20	S	28	S
89	0	R	10	R	0	R	20	S	32	S	25	S	20	S	24	S
181	0	R	0	R	0	R	23	S	40	S	30	S	18	S	30	S
182	0	R	0	R	0	R	20	S	30	S	25	S	19	S	28	S

Diameters/mm of zones of inhibition of antibiotics against *Staphylococcus aureus*

code	Antibiotics															
	OX		VA		E		CD		CIP		CAZ		GEN		C	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
1	0	R	20	S	22	S	25	S	35	S	18	S	26	S	29	S
3	0	R	0	R	20	S	0	R	0	R	0	R	0	R	0	R
27	0	R	0	R	22	S	0	R	29	S	11	R	17	S	20	S
39	0	R	40	S	30	S	10	R	22	S	21	S	28	S	33	S
42	0	R	20	S	22	S	0	R	32	S	18	S	25	S	25	S
56	0	R	0	R	22	S	10	R	30	S	10	R	17	S	25	S
47	0	R	20	S	20	S	20	S	22	S	11	R	20	S	30	S
57	0	R	0	R	20	S	0	R	20	S	0	R	26	S	20	S
96	0	R	0	R	30	S	0	R	20	S	0	R	0	R	15	R
104	0	R	0	R	0	R	0	R	23	S	0	R	0	R	10	R
107	0	R	0	R	0	R	0	R	25	S	0	R	12	R	10	R
108	0	R	0	R	0	R	0	R	0	R	0	R	0	R	12	R
149	0	R	10	R	22	S	0	R	30	S	18	S	20	S	20	S
169	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R

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Diameters/mm of zones of inhibition of antibiotics against *Staphylococcus epidermidis*

Antibiotics																
	OX		VA		E		CD		CIP		CAZ		GEN		C	
code	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
2	0	R	0	R	30	S	10	R	20	S	10	R	30	S	30	S
10	0	R	0	R	25	S	0	R	24	S	0	R	25	S	23	S
12	10	R	20	S	20	S	12	R	10	R	20	S	23	S	33	S
16	12	R	45	S	12	R	22	S	30	S	22	S	33	S	13	R
18	0	R	30	S	20	S	25	S	12	R	23	S	22	S	25	S
19	0	R	0	R	22	S	12	R	16	R	16	R	20	S	33	S
21	0	R	0	R	10	R	0	R	22	S	30	S	20	S	23	S
23	0	R	0	R	20	S	0	R	23	S	12	R	30	S	42	S
24	0	R	0	R	0	R	0	R	25	S	15	R	22	S	10	R
25	0	R	0	R	10	R	12	R	33	S	0	R	30	S	0	R
31	0	R	22	S	25	S	25	S	14	R	34	S	25	S	0	R
34	0	R	0	R	22	S	30	S	15	R	23	S	23	S	0	R
36	0	R	0	R	23	S	13	R	0	R	33	S	27	S	19	R
41	0	R	0	R	24	S	12	R	0	R	30	S	30	S	23	S
45	0	R	35	S	30	S	10	R	14	R	27	S	30	S	25	S
52	0	R	10	R	12	R	0	R	37	S	28	S	32	S	42	S
59	0	R	10	R	25	S	0	R	25	S	40	S	20	S	30	S
61	0	R	0	R	23	S	30	S	25	S	26	S	20	S	26	S
62	0	R	0	R	30	S	23	S	26	S	29	S	12	R	27	S
81	0	R	0	R	35	S	33	S	20	S	32	S	0	R	24	S
82	0	R	0	R	12	R	26	S	30	S	30	S	13	R	32	S
83	0	R	0	R	23	S	12	R	0	R	22	S	0	R	30	S
85	0	R	0	R	26	S	13	R	0	R	22	S	14	R	26	S
97	0	R	0	R	32	S	0	R	14	R	24	S	12	R	22	S
100	0	R	0	R	32	S	16	R	19	R	39	S	11	R	20	S
101	0	R	10	R	22	S	0	R	12	R	22	S	0	R	25	S
111	0	R	12	R	13	R	0	R	16	R	24	S	32	S	37	S
126	0	R	12	R	15	R	0	R	10	R	25	S	25	S	30	S
134	0	R	0	R	24	S	23	S	15	R	25	S	14	R	0	R
142	0	R	0	R	26	S	13	R	22	S	26	S	13	R	25	S
145	0	R	12	R	30	S	16	R	28	S	27	S	0	R	27	S
147	0	R	15	R	25	S	0	R	20	S	32	S	0	R	22	S
164	0	R	10	R	24	S	0	R	25	S	34	S	32	S	22	S
165	0	R	10	R	28	S	20	S	25	S	30	S	23	S	25	S
167	0	R	12	R	22	S	12	R	10	R	34	S	12	R	27	S
172	0	R	12	R	30	S	13	R	30	S	29	S	16	R	33	S
173	0	R	14	R	30	S	25	S	12	R	20	S	17	R	30	S
189	0	R	15	R	33	S	28	S	22	S	22	S	10	R	10	R

Diameters/mm of zones of inhibition of antibiotics against *Staphylococcus haemolyticus*

code	Antibiotics															
	OX		VA		E		CD		CIP		CAZ		GEN		C	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
5	0	E	20	S	0	R	10	R	33	S	0	R	20	S	0	R
15	0	R	0	R	22	S	22	S	23	S	0	R	22	S	0	R
46	0	R	30	S	20	S	24	S	24	S	0	R	10	R	0	R
51	0	R	22	S	24	S	27	S	20	S	12	R	0	R	30	S
87	20	S	12	R	25	S	30	S	32	S	10	R	23	S	20	S
93	0	R	0	R	29	S	12	R	34	S	0	R	33	S	20	S
110	0	R	22	S	33	S	22	S	33	S	20	S	43	S	20	S
112	0	R	25	S	20	S	20	S	23	S	15	R	24	S	25	S
113	0	R	29	S	24	S	23	S	27	S	15	R	22	S	22	S
129	10	R	20	S	30	S	33	S	25	S	10	R	27	S	20	S
198	0	R	30	S	23	S	25	S	28	S	0	R	30	S	23	S

Diameters/mm of zones of inhibition of antibiotics against *Staphylococcus hominis*

code	Antibiotics															
	OX		VA		E		CD		CIP		CAZ		GEN		C	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
4	20	S	10	R	20	S	0	R	22	S	26	S	22	S	26	S
43	0	R	0	R	22	S	9	R	28	S	26	S	30	S	23	S
99	0	R	9	R	0	R	0	R	30	S	23	S	34	S	33	S
109	0	R	12	R	23	S	20	S	24	S	10	R	23	S	12	R
144	0	R	0	R	30	S	12	R	27	S	9	R	33	S	20	S
161	0	R	0	R	22	S	10	R	24	S	20	S	24	S	10	R
188	0	R	10	R	20	S	0	R	22	S	22	S	12	R	23	S

Diameters/mm of zones of inhibition of antibiotics against *Staphylococcus warneri*

code	Antibiotics															
	OX		VA		E		CD		CIP		CAZ		GEN		C	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
26	0	R	20	S	10	R	12	R	12	R	23	S	12	R	12	R
30	0	R	23	S	34	S	0	R	17	R	23	S	0	R	15	R
33	0	R	22	S	29	S	0	R	20	S	33	S	22	S	14	R
37	0	R	15	R	22	S	13	R	30	S	10	R	22	S	0	R
40	0	R	10	R	12	R	10	R	23	S	12	R	25	S	0	R
50	0	R	22	S	16	R	33	S	33	S	0	R	26	S	22	S
74	0	R	26	S	20	S	23	S	26	S	0	R	20	S	25	S
115	0	R	23	S	22	S	16	R	20	S	22	S	30	S	30	S
119	0	R	30	S	20	S	10	R	26	S	20	S	23	S	27	S
168	0	R	0	R	0	R	0	R	20	S	0	R	22	S	26	S
174	0	R	0	R	12	R	0	R	0	R	27	S	23	S	24	S
177	0	R	22	S	28	S	0	R	20	S	0	R	26	S	20	S
186	0	R	24	S	33	S	12	R	20	S	10	R	23	S	22	S
187	0	R	28	S	23	S	23	S	0	R	12	R	25	S	27	S
193	0	R	32	S	22	S	12	R	30	S	13	R	29	S	30	S
194	0	R	20	S	10	R	12	R	0	R	15	R	26	S	33	S

Diameters/mm of zones of inhibition of antibiotics against *Staphylococcus lugdunensis*

code	Antibiotics															
	OX		VA		E		CD		CIP		CAZ		GEN		C	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
49	0	R	10	R	10	R	10	R	23	S	10	R	22	S	12	R
75	0	R	22	S	22	S	22	S	24	S	0	R	24	S	10	R
90	0	R	23	S	25	S	24	S	22	S	0	R	26	S	22	S
91	9	R	26	S	20	S	23	S	26	S	12	R	27	S	22	S
124	0	R	24	S	10	R	27	S	23	S	0	R	33	S	24	S
153	0	R	22	S	23	S	23	S	27	S	12	R	25	S	20	S

Diameters/mm of zones of inhibition of antibiotics against *Staphylococcus saprophyticus*

code	Antibiotics															
	OX		VA		E		CD		CIP		CAZ		GEN		C	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
95	0	R	20	S	0	R	0	R	12	R	12	R	12	R	0	R
117	0	R	20	S	20	S	10	R	10	R	13	R	0	R	22	S
122	5	R	22	S	22	S	0	R	22	S	12	R	23	S	27	S
127	0	R	0	R	23	S	12	R	23	S	10	R	23	S	28	S
162	0	R	20	S	20	S	22	S	24	S	10	R	20	S	27	S

Antimicrobial code :

OX : Oxacillin

VA : Vancomycin

E : Erythromycin

CD :Clindamycin

CIP : Ciprofloxacin

CAZ : Ceftazidime

GEN : Gentamycin

C : Chloramphenicole

TE : Tetracyclin

MEM : Meropenem

PI : Pipracillin

IMP : Imepenem

AMP : Ampicillin

AMX : Amoxicillin

TOB : Tobramycin

AK : Amikacin

CHAPTER SEVEN

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