CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

The white coat is one of the more established symbols of the medical profession and is probably the item of clothing worn most by physicians,the white coat was worn initially for the purpose of protection against cross-contamination but also because it connotes life, purity, innocence and goodness There has been growing concern, however, that these coats may actually play a role in transmitting pathogenic bacteria in a hospital setting *(*Uneke andIjeoma*,* 2010*)*.

The most common bacteria that contaminate white laboratory coat are usuallyenvironmental organism and skin commensals. They include *Staphylococcus aureus*, *Diphtherodis, Enterococci*, *Pseudomonas aeruginosa*etc.(Nester *et al.,* 2004*)*.

The high rates of the bacterial contamination of white coats may be associated with the following 2 facts: Firstly, patients continuously shed infectious bacteria in the hospital environment and the health care providers are in constant contact with these patients. Secondly, it has been demonstrated that pathogens can survive between 10 and 98 days on fabrics which are used to make white coats (Chacko*et al*., 2003).

Beta –lactamase enzyme responsible for of resistance of bacteria to beta lactam antibiotic like Penicillin, Cephalosporin, Monobactams and Carbapenems. These

groups of antibiotics are typically used to treat both Gram positive and Gram negative. The β- lactamase enzyme breaks the beta lactam ring (Greenwood *et al.,* 2012).

1.2. Rationale

Thecontaminationof the lab coat by bacteria from the hospital environment can be potentially infectious, and can cause nosocomial infection for both patientS and doctors.Patient-to-patient transmission of nosocomial pathogens has been linked to transient colonization of health care workersand studies have suggested that contamination of health care workers' clothing, including white coats, may be a vector for this transmission (Treakle *et al.,* 2008).

1.3. Objectives

1.3.1. General objective

To detect β- lactamase in bacteria isolated from lab coats.

1.3.2. Specific objectives

1. To re-identify bacteria isolated from lab coat.

2. To detect β- lactamases produced by bacteria isolated from lab coats.

CHAPTER TWO

LITERATURE REVIEW

1.2. White coat

White coats are worn primarily for identification, but there has always been some concern that white coats, may play a part in transmitting pathogenic bacteria in a hospital setting, as white coats are known to be potentially contaminated with pathogenic drug resistant bacteria. White coats are known to be potential transmitting agents of multi-drug resistant organisms.

(Banu*et al.*, 2012).

One critical factor for the transmission of pathogens from person to person or from the environment to a person (patient or health care worker) is the ability of the pathogen to survive on an environmental surface(Neely, 2000).

White coats are considered to be a sign of protection for our patients as wall as a type of uniform for those who are medical professions(Assad andkhan,2015).

Healthcare workers may be potential vectors of disease, disseminating virulent bacteria among their patients (Saloojee and Steenhoff, 2001).

Because patients can shed infectious pathogens into the healthcare environment, by the virtue of their constant contact with patients, healthcare workers are also at risk of transmitting pathogens. Thus, both patients and healthcare workers can transmit infection through direct contact with patients, as well as through indirect contact with inanimate objects. Items such as stethoscopes, masks, neckties, pens,

badges and lanyards and white coats all have the potential to transmit diseases(Uneke and Ijeoma, 2010).

Of these items the white coat is one of the more established symbols of the medical profession and is probably the item of clothing worn most by physicians(Kazory, 2008).

The white coat was worn initially for the purpose of protection against cross-contamination but also because it connotes life, purity, innocence and goodness (Van Der Weyden, 2001).However,there has been growing concern, that these coats may actually play a role in transmitting pathogenic bacteria in a hospital setting (Wilson *et al.*,2007).

2.2. Beta- lactam agents

Penicillin, Cephalosporin and other compounds they feature a β-lactam ring in their structure fall into β-lactam group. All of these compounds bind to protein situated at the cell wall. Other β-lactam agent, various agents with diverse properties share the structural feature of β-lactam ring with penicillin and cephalosporin include carbapeneme, monobactam, oxa-cephen, clavam and sulphones. The opening of β-lactam ring by hydrolytic enzymes, collectively called β-lactamase. Many such enzyme found in bacteria. Those elaborated by Gram negative enteric bacilli are particularly diverse in their activity and properties. Gram negative bacteria able to produce enzyme that inactivate many

different β-lactam antibiotic so called extended spectrum β-lactamase. Sometime become endemic in hospitals, causing serious problems (Warren, 2012).

2.3. Beta- lactamase

This enzyme responsible for resistance of bacteria to β- lactam antibiotic like penicillin, cephalosporin, monobactam, and carbapenemthese groups of antibiotics are typically used to treat both of Gram positive and Gram negative bacteria. The β-lactamase enzyme breaks β-lactam ring (Greenwood *et al.,* 2012).Hydrolysis of β-lactam antibiotics by β-lactamases is the most common mechanism of resistance for this class of antibacterial agents in clinically important Gram-negative bacteria. Because Penicillins, Cephalosporins, and Carbapenems are included in the preferred treatment regimens for many infectious diseases, the presence and characteristics of these enzymes play a critical role in the selection of appropriate therapy (Bush *et al.,* 1995).

2.3.1. Classification of β-lactamase

Classification of β-lactamases has traditionally been based on either the functional characteristics of the enzymes or their primary structure. The simplest classification is by protein sequence, whereby the β-lactamases are classified into four molecular classes, A, B, C, and D, based on conserved and distinguishing amino acid motifs Classes A, C, and D include enzymes that hydrolyze their substrates by forming an acyl enzyme through an active site serine, whereas class

B β-lactamases are metalloenzymes that utilize at least one active-site zinc ion to facilitate β-lactam hydrolysis (Bush *et al.,* 1995).

2.3.2. Updated functional classification

A. Group 1 Cephalosporinases

Group 1 enzymes are cephalosporinases belonging to molecular class C that are encoded on the chromosomes of many Enterobacteriaceae and a few other organisms. They are more active on cephalosporins than benzylpenicillin and are usually resistant to inhibition by clavulanic acid and active on cephamycins, such as cefoxitin (Jacoby*et al.,* 2004).

B. Group 2 serine β-lactamases

Functional group 2 β-lactamases, including molecular classes A and D, represent the largest group of β-lactamases, due primarily to the increasing identification of ESBLs during the past 20 years. Subgroup 2*a* penicillinases represent a small group of β-lactamases with a relatively limited spectrum of hydrolytic activity and are the predominant β-lactamases in Gram-positive cocci, including the staphylococci and occasionally enterococci. These enzymes preferentially hydrolyze benzylpenicillin and many Penicillin derivatives, with poor hydrolysis of Cephalosporins, carbapenems, or monobactams. Subgroup 2*a* β-lactamases are inhibited by clavulanic acid and tazobactam(Zscheckand Murray, 1991).

Subgroup 2b β-lactamases readily hydrolyze penicillins and early cephalosporins, such as cephaloridine and cephalothin, and are strongly inhibited by clavulanic acid and tazobactam enzymes (Queenan *et al*., 2004).

C. Group 3 MBLs

Metallo-β-lactamases (MBLs), a unique group of β-lactamases both structurally and functionally, are usually produced in combination with a second or third β-lactamase in clinical isolates. They differ structurally from the other β-lactamases by their requirement for a zinc ion at the active site. Functionally, they were once distinguished primarily by their ability to hydrolyze carbapenems, but some serine β-lactamases now have also acquired that ability. In contrast to the serine β-lactamases, the MBLs have poor affinity or hydrolytic capability for monobactams and are not inhibited by clavulanic acid or tazobactam. Instead, they are inhibited by metal ion chelators such as EDTA, dipicolinic acid, or 1, 10-*o*-phenanthroline (Marchiaro *et al*., 2008).

2.3.3. Beta- lactamase detection

ß-Lactamases are the main cause of bacterial resistance to penicillins and cephalosporins. Definitive identification of this enzyme is only possible by gene or protein sequencing (Livermore and Williams1996).

Nevertheless, simple ß-lactamase detection and typing tests can be valuable in the clinical laboratory. These include: (i) direct tests for ß-lactamase activity in fastidious Gram-negative species; (ii) tests for extended ß-Lactamases (ESBLs); and (iii) tests for inducibility of chromosomal ß-lactamases. Tests for metallo-

carbapenemases are being developed and may become increasingly useful if these enzymes spread in the future. In the longer term, gene chip technology may allow precise routine identification of ß-lactamases (Felmingham and Brown, 2001).

Numerous β-lactamase detection tests have been devisedbut few are convenient for routine use (Livermore and Williams, 1996).

Most use chromogenic cephalosporins, or link the hydrolysis of penicillin to a color change mediated by iodine or a pH indicator. Chromogenic cephalosporins are very specific, whereas acidification and the reduction of iodine can occur for reasons other than β-lactamase action, potentially giving false-positive results. Positive and negative controls should be run in parallel with all tests but, because of the risk of false-positive results, are especially critical for the acidimetric and iodometric methods (David *et al.,* 2001).

i.Nitrocefin test

Nitrocefin is a chromogenic cephalosporin that changes from yellow to red on hydrolysis. It provides the most sensitive test for most β-lactamases, exceptions being staphylococcal penicillinase and ROB-1, an uncommon plasmid-mediated enzyme of haemophili (David *et al.,* 2001).

ii. Iodometric tests

Hydrolysis of penicillin yields penicilloic acid, which reduces iodine, decolorizing starch-iodine complex. This reaction can be exploited to detect β-lactamase activity in tubes or on paper strips. These tests are particularly sensitive for

staphylococcal penicillinase, but are less sensitive than nitrocefin for most of the β-lactamases from Gram-negative bacteria.

iii. Acidimetric tests

Hydrolysis of the β-lactam ring generates a carboxyl group, acidifying un-buffered systems. The resulting acidity can be tested in tubes or on filter papers. The method is useful for tests on *Hemophliusinfluenzae* and *Neisseria gonorrhoeae*(David *et al.,*2001).

iv. Microbiological tests of ß-lactamase activity

β-Lactamase activity can be detected biologically by demonstrating the loss of activity of a β-lactam agent against a susceptible indicator organism. There are several variations, including the cloverleaf (Hodge) method, which is highly sensitive for *Staphylococci* and the Masuda double disc method, which can be used with whole cells or cell extracts of test strains. While the use of such methods has declined, they remain very sensitive (Livermore*et al*., 1996).

Microbiological analysis of swabs taken from the cuffs and pocket mouths of physicians white coats in an acute care hospital showed that 91.3% of the coats had bacterial contamination. Specifically *Diphtheroids*, *Staphylococcus aureus* and Gram-negative bacilli were isolated (Uneke and Ijeoma, 2010).

Loveday and colleagues attempted to gauge the public's perception of health care worker uniforms in relationship to infections and concluded that "the general public's perception is that uniforms pose an infection risk when worn inside and outside clinical settings"(Loveday *et al.,* 2007).

Study on tertiary care hospitals show that *Staphylococcus aureus* was the most common isolate followed by coagulase negative *Staphylococci* and Gram negative non fermenters (Banu *et al.,* 2012).

A study in Israel isolated pathogenic bacteria on the uniforms of 85 of 135 physicians and nurses (63%) (Wiener-Well*et al.,* 2011).

Study in University Teaching Hospital (UTH) in Lusaka show that total Of 107 white coats screened, 94 (72.8 %) were contaminated with bacteria.(Mwamungule, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a Lab-based study conducted to detect theβ-lactamases produced bacteria.

3.1.2. Study area

The experimental work of presented study was carried out in the Research laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

This study was conductedduring the period from May to June 2015.

3.1.4. Bacterial isolate

Bacterial isolates were obtained from the Research Laboratory, SUST. The isolate were checked for purity and then re-identified by conventional bacteriological methods.

3.2. Culture media

3.2.1. Nutrient agar

Basic media was used to support the growth of bacteria that had not special nutritional requirements. It contains peptone, lab-lemco powdered, yeast extract, sodium chloride and agar (Cheesbrough, 2006).

3.2.2. MacConkey's agar

MacConkey's agar is differential and low selectively media was used to distinguish lactose fermenting from non-lactose fermenting bacteria. It contains peptone, lactose, bile salt, sodium chloride, neutral red and agar (Cheesbrough, 2006).

3.2.3. Mannitol salt agar

Mannitol salt agar was used to differentiate *Staphylococcus aureus* from afecal specimen in the investigation of *Staphylococcal* food- poisoning. It contains peptone, manitol, sodium chloride, phenol red and agar (Cheesbrough, 2006).

3.3. Method

3.3.1. Purification of isolates

The isolates were streaked on nutrient agar and incubated overnight at 37°C, then after the incubation, a discrete colony was picked up and checked for purity under microscope.

3.3.2. Bacterialre-identification

3.3.2.1. Gram stain

The Gram stain reaction was used to help identify pathogen in specimen and cultured by their Gram reaction (Grampositive or Gramnegative)and morphology. Gram positive bacteria stain dark purple with crystal violate and not decolorized by alcohol and Gram negative bacteria stain red because after stain with crystal violate decolorized by alcohol. The smear were fixed by dry heat and then covered with crystal violate for 30-60 seconds. The stain was rapidly washed by tap water and tipped off the slide. The stained smear was then covered with iodine for 30-60 seconds. Iodine washed off and the smear was decolorized with alcohol and immediately washed with clean water. Safranin was added to the smear for 30-60 seconds. The red stain was then washed off with tap water and smear was subsequently air dried and microscopically examined using high resolution objective power (Cheesbrough, 2006).

3.3.2.2. Biochemical tests

3.3.2.2.1. Oxidase test

This test depends on the presence of certain oxidase that will catalyze the transport of electrons between electron donors in the bacteria and redox dye- tetramethyl-*p*-phenylene-diamine. The dye is reduced to a deep purple color.A strip of filter paper was soaked with little freshly made 1% solution of the reagent and then at once used by rubbing a speck of culture on it with wooden stick. A positive reaction was indicated by an intense deep purple hue, appeared within 5-10

second, a negative reaction by absence of coloration or coloration later than 60 second (Cheesbrough, 2006).

3.3.2.2.2.Indole test

In this test the tested organism produce tryptophanase which breakdown tryptophan and produce indole, which react with kovac's reagent and give pink ring. The tested organism was inoculated into peptone water and incubated at 37°C for overnight, the kovac's reagent was added. If there is pink ring the result was indicated as positive. If there is no pink ring in surface the result was indicate as negative (Cheesbrough, 2006).

3.3.2.2.3.Citrate utilization test

In this test organism has ability to use citrate as only source of carbon. By straight wire a part of tested colony was emulsified in Kosser citrate medium and incubated overnight at 37°C. Positive give blue color and negative give no change (Cheesbrough, 2006).

3.3.2.2.4.Urease test

The testorganisms inoculated heavily in test tube contain Christensen's modified urea. Incubated at 37°C for overnight. Pink color: positive urease test, no pink color: negative urease test(Cheesbrough, 2006).

3.3.2.2.5.Sugar fermentation, Gas and H_2S production

The tested organisms were inoculated in the medium by stabbing the butt and streaking the surface of the tube. Lactose fermenting organisms totally acidify the medium resulting in yellow color. The bacteria which do not ferment the lactose acidify only the bottom of the tube. Formation of hydrogen sulfide blackens the medium. (Cheesbrough, 2006).

3.3.2.2.6.Catalase test

Two to three ml of hydrogen peroxide was poured into a test tube. Using a sterile wooden stick, a portion of a good growth of tested organism was transferred, and then immersed in the hydrogen peroxide solution. Immediate bubbling is positive result (Cheesbrough, 2006).

3.3.2.2.7.Coagulase test

Coagulase is an enzyme that causes plasma to clot. The test used to differentiate *S. aureus*, which produce coagulase enzyme from other *staphylococc*i. 0.5ml of diluted plasma was placed in small test tube. 5 drops of bacterial suspension was added and then mixed gently, incubated at 37°C for up to 4 hours, and then examined for clot formation (Cheesbrough, 2006).

3.3.2.2.8. Mannitol fermentation test

The organism under test was inoculated on mannitol salt agar (MSA). Mannitol fermenter produced yellow color, while non-fermenter gave pink color (Cheesbrough, 2006).

3.3.2.2.9.DNase test

This test was used to identify *S. aureus*, which produce deoxyribonuclease enzyme. DNase hydrolyses deoxyribonucleic acid (DNA). The test organism was cultured on a medium which contain DNA. After overnight incubation at 37°C, the colonies were tested for DNase production by flooding plate with a weak hydrochloric acid solution (1mol). DNase producing colonies were surrounded by clear area due to DNA hydrolyses (Cheesbrough, 2006).

3.3.3. Detection of beta lactamase enzyme

The isolated bacteria were tested for β-lactamase enzyme by using iodometric test. Benzylpenicillin, 6g/L in 0.1 M phosphate buffer pH 6.0, was distributed in 0.1 ml quantities in tubes. Bacterial growth from agar was suspended in this solution until they are heavily turbid. The suspension were held at room temperature for 30-60 minutes, then 20µL volume of 1% (w/v) soluble starch in distilled water were added, followed by 20µL of 2% (w/v) iodine in53% (w/v) aqueous potassium iodide. β –lactamase activity is indicated by decolourisation ofthe iodine within 5 minutes (David*et al.,* 2001).

CHAPTER FOUR

RESULTS

The re-identified bacteria(n=14) were as followed*Staphylococcus epidermidis*(*S. epidermidis*) (35.7%) 5, *Staphylococcus aureus* (*S. aureus*) (28.5%) 4, *Pseudomonas aeruginosa*(*P. aeruginosa*) (21.4%) 3 and *Staphylococcus intermidius*(*S. intermidius*) (14.2%) 2. Biochemical tests and their result were tabulated in Table (1) and Table (2).

Studies on the detection of β-lactamases of the isolates by using iodometric method revealed that 2 of the isolates were positive for the test (14%) these result in Table (3).

The Frequency and percentage of isolated bacteria is presented in Table (4).

Table1.Identification 0f Gram negative bacteria

Isolate code	Biochemical test							Suggested organism
	KIA				Urease	Indole	Citrate	
	S	B	G	H$_2$S				
C1	R	R	_	_	negative	negative	positive	*Pseudomonas aeruginosa*
C2	R	R	_	_	negative	negative	positive	*Pseudomonas aeruginosa*
C3	R	R	_	_	negative	negative	positive	*Pseudomonas aeruginosa*

Key

KIA= kilgler Iron Agar

S= Slope

B= Butte

G= Gas

R= Red

Table2.Identification of Gram positive bacteria

Isolate code	Biochemical test			Suggested organism
	Catalase	Mannitol fermentation	DNase	
C4	Positive	positive	positive	*S. aureus*
C5	Positive	negative	positive	*S. intermidius*
C6	Positive	negative	negative	*S. epidermidis*
C7	Positive	positive	positive	*S. aureus*
C8	Positive	negative	negative	*S. epidermidis*
C9	Positive	positive	positive	*S. aureus*
C10	Positive	positive	positive	*S. aureus*
C11	Positive	negative	negative	*S. epidermidis*
C12	Positive	negative	positive	*S. intermidius*
C13	Positive	negative	negative	*S. epidermidis*
C14	Positive	negative	negative	*S. epidermidis*

Table3. Detection of beta lactamase enzyme

NO.	Organism	color	result
1	*S. aureus*	colorless	positive
2	*Ps. earuginosa*	Iodine color	negative
3	*S. aureus*	Iodine color	negative
4	*S. epidermidis*	Iodine color	negative
5	*S. epidermidis*	Iodine color	negative
6	*Ps. earuginosa*	Iodine color	negative
7	*Ps. earuginosa*	Iodine color	negative
8	*S. aureus*	colorless	positive
9	*S. intermidius*	Iodine color	negative
10	*S. intermidius*	Iodine color	negative
11	*S. epidermidis*	Iodine color	negative
12	*S. epidermidis*	Iodine color	negative
13	*S. aureus*	Iodine color	negative
14	*S. epidermidis*	Iodine color	negative

Table4. Frequency and percentage of the result

Isolated bacteria	Result	Frequency	Percentage
S. aureus	Positive	2	50%
	Negative	2	50%
S. epidermidis	Positive	0	0%
	Negative	5	100%
S. intermidius	Positive	0	0%
	Negative	2	100%
Ps. aeruginosa	Positive	0	0%
	Negative	3	100%

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Emergence of resistance to β-lactam antibiotics began even before the first β-lactam, Penicillin was developed. The first β-lactamase was identified in *Escherichia coli* prior to the release of penicillin for use in medical practice. The age of penicillin saw the rapid emergence of resistance in *Staphylococcus aureus* due to a plasmid-encoded penicillinase. This β-lactamase quickly spread to most clinical isolates of *S. aureus* as well as other species of *staphylococci*. On the other hand, isolation of these organisms from white coats may consider potentially pathogenic bacteria (Pydi *et al*., 2015).

This study was conducted to detect the beta-lactamases in bacteria isolated from physicians' white coats. The bacterial isolates were *S. aureus* (4), *S. epidermidis* (5), *S. intermedius* (2) and *P. aeruginosa* (3). Isolation of these bacteria from white coats was reported by many authors, Uneke and Ijeoma (2010) isolated diphtheroids, *S. aureus* and Gram-negative bacilli, Banu *et al*., (2012) isolated *S. aureus*, coagulase-negative staphylococci and Gram-negative non-fermenters, Treakle*et al*., (2009) isolated *S. aureus* and Wong*et al*., (1991) isolated *S. aureus.*

In the present study fourteen (n=14) isolates were investigated for presence of β-lactamases using iodometric method. Many methods have been proposed for the detection of β-lactamase in clinical isolates. The method was used by Lee and

Komarmy (1981) for detection of β-lactamases in clinical isolates. The results of this study revealed that only two (14.2%) *S. aureus* were beta-lactamases producers. The rest (n=12) (85.8%) were non-beta-lactamases producers. This result is less than that by Devapriya *etal*., (2013) who reported 64.5% of *S. aureus* were β-lactamases producers.

5.2. Conclusion

The study concluded that the detection of β-lactamase among clinical isolates indicates the need for treatment of bacterial infection by β-lactamase-susceptible antibiotics.

5.3. Recommendations

1. All medical staff should be encouraged to wash there lab coats by using disinfectant to avoid bacterial contamination which cause infectious diseases.

2. Further studies with large sample size are highly recommended to validate these findings.

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Appendix (1)

Culture media

2.2. Nutrient Agar

Approximate formula* per liter

Beef Extract……………………………………………………………………….3.0g

Peptone……………………………………………………………….……………5.0g

Agar…………………………………………………………………...……………15.0g

2.3. MacConkey's Agar

Approximate formula * per liter

Peptone…………………………………………………………………………...20.0g

Lactose………………………………………………………………...……………10.0g

Bile Salt……………………………………………………………………………5.0g

Sodium Chloride………………………………………………...………………...5.0g

Agar…………………………………………………………………...……………12.0g

Nutrient Red…………………………………………………….…………………0.05g

2.4. Mannitol Salt Agar

Approximate formula* per liter

Protease Peptone no. 3…………………………..……………………………….10.0g

Beef Extract……………………………………..……………………………...1.0g

D- Mannitol……………………………………………………………………10.0g

Sodium Chloride…………………………………………………………….75.0g

Agar…………………………………………………..………………………15.0g

Phenol Red………………………………..……..……………………………25.0g

Final PH: 7.3± 0.1 at 25°C

2.6. Kliglar Iron Agar (KIA)

Approximate formula* per liter

Lab-Lemco Powder ……………………………………………………….17.5g/L

Yeast Extract ………………………………………………………….…...3.0g/L

Peptone ……………………………………………………………………..20.0g/L

Sodium chloride …………………..………………………………………5.0g/L

Lactose …………………………………..……………………………...10.0g/L

Dextrose (Glucose) …………………………………………………………1.0g/L

Ferric citrate ………………………………………..…………………….0.3g/L

Sodium thiosulphate ………………………………………………………0.3g/L

Phenol red ………………………………………………………………0.05g/L

Agar …………………..………………………………………………………12.0g/L

2.7. DNAse Agar

Approximate formula* per liter

Tryptose…………………………………………………………………20g/L

Deoxyribonucleic acid ……………………………………………………2g/L

Sodium chloride …………………………………..…………………………5g/L

Agar …………………………………………………..…………………………12g/L

2.8. Christensen's Urea agar

Approximate formula* per liter

Glucose ……………..……………………………………………………5g

Sodium chloride …………………………………..……………………..5g

Potassium dihydrogen phosphate ……………………………………..…2g

Peptone ………………………………………………………………………..1g

Agar ……………………………………………………………..………..20g

Distilledwater …………………………………………………………..1Liter

2.9. Simmons citrate medium

Approximate formula* per liter

Koser's medium …………………………………………………………1Liter

Agar ……………………………..………………………………………..20g

Bromothymole blue, 0.2%………………………………………………….40ml

Preparation of reagents

1. Gram stain reagents

Crystal violet

Approximate formula* per liter

Crystal violet …………………….…………………………………………20.0g

Ammonium oxalate ………………..………………………………………..9.0g

Ethanol, absolute ……………………….…………………………………95ml

Distilled water ……………………………………………………….to 1Liter

Lugols Iodine

Approximate formula* per liter

Potassium iodine …………………………………………………………..20.0g

Iodine ………………………………………………………………………10.0g

Distilled water ……………………………………………………………to 1Liter

Acetone- alcohol decolorizer

Approximate formula* per liter

Acetone …………………………...……………………………………….500ml

Ethanol, absolute ………………………….…………………………………475ml

Distilled water …………………………………………………………………25ml

Saffranin

Approximate formula* per liter

Saffranin …………………………………………………………………….2.5g

95% ethanol ………………………………………………………………..10ml

Distilled water ……………………………………………………………….to100ml

2. Physiological saline

Sodium chloride …………………….………………………………8.5g

Distilled water …………………………………………………..…...to 1Liter

3. Kovac's reagent

Approximate formula* per liter

Amyle or isoamyle alcohol ……………………………………………15ml

p- dimethyl- aminobenzaldhyde ……..…………………………………10g

Hydrochloric acid concentrated ……………………………….…….…..50ml

5. Hydrogen peroxide

H_2O_2 Solution …………………………………………..……………10vol

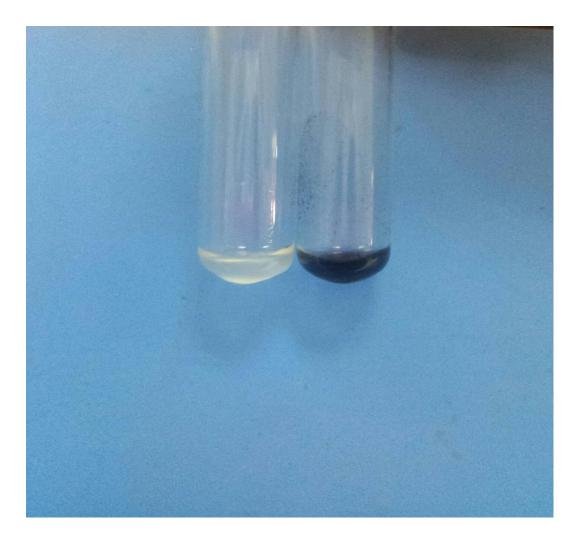**Appendix (2) color plate**

Fig.1. Beta lactamase detection by iodometric method: left positive right negative