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Sudan University of Science and Technology

College of Graduate Studies

Molecular detection of mecA gene in methicillin resistant Staphylococcus aureus isolated from food samples in Khartoum State

الكشف الجزيئي عن جين mecA في المكورات العنقودية الذهبية المقاومة للميثيسيلين المعزولة من عينات الأغذية في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in Medical Laboratory Science (Microbiology)

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الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ)

صدق الله العظيم

سورة المجادلة (11)

DEDICATION

****

..To my father

To my mother

… To my family …

Friends

…. Teachers ….

ACKNOWLEDGEMENT

First of all, I thank God **Allah** for all his endless blessings and giving us the power and the intention to accomplish this work in this final shape.

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ABSTRACT

This study was conducted in Khartoum State from August to November 2018. This study aimed to detect *Staphylococcus aureus* resistance genes isolated from food samples obtained from different retailers in Khartoum State, by conventional PCR assay. A total of four hundred samples were collected in this study. 84 (21%) of 400 of isolates were identified as *S. aureus*, 110 (27.5%) of 400 were identified as other than *S. aureus* and 206 (51.5%) of 400 yielded no growth. Oxacillin sensitivity test showed 31(36.9%) of 84 were Oxacillin resistant and 53 (63.1%) of 84 were Oxacillin sensitive. Finally PCR was done for the detection of resistace gene *mecA* in *Staphylococcus aureus*.

The result of PCR assay for mecA gene was 15 of 84 (17%) positive and 69 (83%) negative.

The distribution of *mecA* in types of samples was 17% in milk, 15% in fish, 20% in meat and 20% in cheese.

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ملخص الاطروحة

أجريت هذه الدراسة في ولاية الخرطوم من أغسطس إلى نوفمبر 2018. هدفت هذه الدراسة إلى اكتشاف جينات المقاومة للمكورات العنقودية الذهبية المعزولة من عينات الأغذية التي تم الحصول عليها من مختلف تجار التجزئة في ولاية الخرطوم، بواسطة اختبار PCR التقليدي. تم جمع ما مجموعه 400 عينة في هذه الدراسة. تم التعرف على 84 (21٪) من 400 عزلة على أنها S. aureus، 110 (27.5٪) من 400 تم تحديدها على أنها غير S. aureus و206 (51.5٪) من 400 لم تسفر عن أي نمو. أظهر اختبار حساسية أوكساسيلين 31 (36.9 ٪) من 84 كانت مقاومة أوكساسيلين و53 (63.1 ٪) من 84 كانت حساسة أوكساسيلين. أخيرًا تم إجراء PCR للكشف عن جينات resistace mecA في المكورات العنقودية الذهبية.

كانت نتيجة فحص PCR لجين mecA 15 من 84 (17 ٪) إيجابية و69 (83 ٪) سلبية.

كان توزيع mecA في أنواع العينات 17 ٪ في الحليب و15 ٪ في الأسماك و20 ٪ في اللحوم و20 ٪ في الجبن.

List of Contents

No.	Title	Page
	الاية	I
	Dedication	Ii
	Acknowledgement	iii
	Abstract	Iv
	Table of content	V
	List of tables	vi
	List of figures	vii
CHAPTER ONE **INTRODUCTION**		
1.1.	Background	
1.2.	Rationale	
1.3.	Study objectives	
1.3.1.	General objective	
1.3.2.	Specific objectives	
CHAPTERTWO **LITERATUREREVIEW**		
2.1	Staphylococci	
2.2	*Staphylococcus aureus*	
2.2.1.	Description	
2.2.2.	Transmission	
2.2.3.	Pathogenesis	
2.2.4.	Virulence factors	
2.3.	Methicillin-resistant *S. aureus* (MRSA)	
2.4.	Laboratory diagnosis	
2.5.	mecA gene	
2.6.	Detection of mecA gene	
2.7	Treatment	
2.8	Control and prevention	

CHAPTER THREE MATERIALS AND METHODS		
3.1.	Study design	
3.2.	Study area	
3.3.	Study duration	
3.4.	Ethical considerations	
3.5.	Sample size	
3.6.	Sample collection	
3.7.	Samples processing	
3.7.1.	Cultivation	
3.7.2.	Identification of organisms	
3.7.3.	Colonial morphology	
3.7.4.	Indirect Gram's stain	
3.7.5.	Biochemical tests	
3.7.5.1.	Catalase test	
3.7.5.2.	Coagulase test	
3.7.5.3.	DNase test	
3.7.6.	Antimicrobial susceptibility test	
3.7.6.1.	Preparation of the bacterial suspension	
3.7.6.2.	Seeding of the plate and application of discs	
3.7.6.3	Reading and interpretation	
3.8.	Genotyping of *S. aureus* mecA gene	
3.8.1.	DNA Extraction	
3.8.2.	Amplification of DNA	
3.9.	Visualization of the DNA product	
3.9.1.	Preparation of 10 X and 1X TBE buffer	
3.9.2.	Preparation of ethidium bromide	
3.9.3.	Preparation of agarose gel	
3.9.4.	Electrophoresis	

CHAPTER FOUR RESULTS		
4.1.	Results	
CHAPTER FIVE DISCUSSION		
5.1.	Discussion	
5.2.	Conclusion	
5.3.	Recommendation	

LIST OF TABLES

No.	Name
Table 1	Frequencies of samples
Table 2	Distribution of samples among collection areas
Table 3	Frequencies of isolates
Table 4	Frequencies of mecA gene

List of figures

No.	Name
Figure 1	Growth of bacteria
Figure 2	Antimicrobial sensitivity
Figure 3	Gel electrophoresis of DNA product

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

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1.1. introduction

Staphylococcus aureus is both a commensal bacterium and a human pathogen. Approximately 30% of the human population is colonized with *S. aureus*. Simultaneously, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections (Tong *et al.*, 2015).

Shortly after the introduction of penicillin in the 1940s, the first penicillinase-producing *S. aureus* strains were detected, leading to the development of the penicillinase-resistant semi-synthetic penicillins such as methicillin, oxacillin and the first/second generation cephalosporins. Within a year after the introduction of these drugs, methicillin-resistant *S. aureus* (MRSA) were reported in the United Kingdom (UK). Resistance is due to a modified penicillin binding protein (PBP2' or PBP2a) encoded by the *mecA* gene. (Monecke *et al.*, 2011).

Over the last several years, there have been dramatic enhancements to *S. aureus* strains to confer resistance against the antibiotic methicillin. The number of Methicillin-resistant *S. aureus* (MRSA) infections is a worldwide concern, particularly in nosocomial settings. MRSA accounts for 10 to 40% of the overall *S. aureus* isolates in the United States and European countries. MRSA infections are serious and difficult to treat, and only a few antimicrobial agents are available for treating MRSA (Soo *et al.*, 2016).

The occurrence of *S. aureus* and MRSA in foods of animal origin, pose a serious threat to the well-being of humans due to innumerable clinical implications. And there is a potential risk of transmission of *S. aureus* and

MRSA transmission to humans through food if consumed without maintaining adequate hygienic standards (Monecke *et al.*, 2011).

1.2. Rationale

S. aureus causes many infections in human. Sensitive methodologies for detection of resistance gene is important because the resistance of antibiotics is increasing and research status in this area in Sudan emphases more enforcement. Moreover detection of resistance genes of *S. aureus* is important for epidemiological purposes.

Food may play role in transmission of MRSA to food handlers.

Previous studies conducted in Sudan to detect mecA gene in *S. aureus* was done in milk product and food handlers this study focus in many types of food.

1.3. Study objectives

1.3.1. General objective

Molecular detection of *S. aureus* mecA resistance gene isolated from different food samples.

1.3.2. Specific objectives

1- To isolate *S. aureus* from food samples by conventional methods.

2- To Identify isolates by primary and secondary bacterial techniques including biochemical tests.

3- To determine sensitivity of the isolates to certain antibiotics.

4- To detect mecA gene by Polymerase chain reaction.

5- to determine the frequency of MRSA on food samples.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2.1 Staphylococci

The genus *Staphylococcus* was included with the genus *Micrococcus* in the family *Micrococcaceae.* However, molecular phylogenetic and chemical analysis has indicated that these two genera are not closely related. The *Staphylococcus* spp. has now been combined with the *Bacillaceae, Planococcaceae,* and *Listeriaceae* into the order Bacillales. There are approximately 45 species and 21 subspecies within the genus *Staphylococcus* (Patricia, 2017).

The species most commonly associated with human diseases are *S. aureus* (the most virulent and best-known member of the genus), *S. epidermidis*, *S.haemolyticus, S.lugdunensis,* and *S.saprophyticus* (Murray *et al.,* 2015).

2.2 *Staphylococcus aureus*

2.2.1. Description

S. aureus is a Gram-positive coccus about 1 µm in diameter. The cocci are usually arranged in grape-like clusters. The organisms are non-sporing, non-motile and usually non-capsulate. When grown on many types of agar for 24 h at 37 $^{\circ}$C, individual colonies are circular, 2–3 mm in diameter, with a smooth, shiny surface; colonies appear opaque and are often pigmented (golden-yellow, hence the ‘aureus’). The main distinctive diagnostic features of *S. aureus* are production of an extracellular enzyme, coagulase, which converts plasma fibrinogen into fibrin, aided by an activator present in plasma, production of thermostable nucleases that break down DNA and

Production of a surface-associated protein known as clumping factor or bound coagulase that reacts with fibrinogen (Greenwood *et al*., 2012).

2.2.2. Transmission

Humans are the reservoir for staphylococci. The nose is the main site of colonization of *S. aureus*, and approximately 30% of people are colonized at any one time. People who are chronic carriers of *S. aureus* in their nose have an increased risk of skin infections caused by *S. aureus*.The skin, especially of hospital personnel and patients, is also a common site of *S. aureus* colonization. Hand contact is an important mode of transmission, and hand washing decreases transmission. *S.aureus* is also found in the vagina of approximately 5% of women, which predisposes them to toxic shock syndrome. Additional sources of staphylococcal infection are shedding from human lesions and fomites such as towels and clothing contaminated by these lesions (Levinson, 2014).

2.2.3. Pathogenesis

Localized skin or soft tissue infections (SSTIs) may involve hair follicles (i.e., folliculitis) and spread into the tissue causing boils (i.e., furuncles). More serious, deeper infections result when the furuncles coalesce to form carbuncles. Impetigo, the *S. aureus* skin infection involving the epidermis, is typified by the production of vesicles that rupture and crust over. Regardless of the initial site of infection, the invasive nature of this organism always presents a threat for deeper tissue invasion, bacteremia, and spread to one or more internal organs, including the respiratory tract. Furthermore, these serious infections have emerged more frequently among the general population and are associated with strains that produce the Panton-Valentine leukocidin (PVL) toxin. PVL is toxic to white blood cells, preventing clearance of the organism by the immune system. These serious soft tissue

“community-associated” infections are frequently mediated by methicillin-resistant *S. aureus* (community acquired MRSA or CA-MRSA) (Patricia, 2017).

S. aureus also produces toxin-mediated diseases, such as scalded skin syndrome and toxic shock syndrome. In these cases, the organisms may remain relatively localized, but production of potent toxins causes systemic or widespread effects. With scalded skin syndrome (Ritter disease), which usually afflicts neonates, the exfoliative toxin is a serine protease that splits the intracellular bridges of the epidermidis, resulting in extensive sloughing of epidermis to produce a burn like effect on the patient. The toxic shock syndrome toxin (TSST-1), also referred to as pyrogenic exotoxin C, has several systemic effects, including fever, desquamation, and hypotension potentially leading to shock and death (Patricia, 2017).

2.2.4. Virulence factors

Recent years have seen a greater understanding of the pathogenic interaction between the host and *S. aureus*. Most strains possess a large number of cell-associated and extracellular factors, some of which contribute to the ability of the organism to overcome the body’s defenses and to invade, survive in and colonize the tissues. Although the role of each factor is not fully understood individually, it is likely that they are responsible for the establishment of infection, enabling the organism to bind to connective tissue, opposing destruction by the bactericidal activities of humoral factors such as complement, and overcoming uptake and intracellular killing by phagocytes (Greenwood *et al*., 2012).

2.3. Methicillin-resistant *S. aureus* (MRSA)

MRSA produces a penicillin binding protein 2a (mediated through the mecA gene), which is carried on the staphylococcal cassette chromosome mec

(SCCmec) of which there are at least six different types recognized, and this results in resistance to all beta-lactam antibiotics. There is much debate on whether strains of MRSA are intrinsically more virulent than methicillin-susceptible isolates but it is agreed that MRSA causes the same range of infections resulting in excess healthcare costs, prolonged hospital stay and significant mortality. MRSA is endemic in hospitals globally except in Scandinavia and in the Netherlands although declining rates of MRSA Blood stream Infections (BSI) have been seen in recent years in the UK, France and other European countries (Greenwood *et al.*, 2012).

Vulnerable patients particularly at risk are those who have undergone major surgery and patients in the intensive care unit. Although 50–60% of patients with MRSA are merely colonized, i.e. representing asymptomatic carriage, serious infections occur such as BSI, respiratory tract and bone/joint infections. These infections are then more difficult to treat than infections caused by methicillin-susceptible isolates, and MRSA can spread easily among patients in hospital. Community-acquired MRSA is increasing, especially in the USA, where 50% or more of *S. aureus* infections presenting to the Emergency Department may be methicillin resistant (Greenwood *et al.*, 2012).

2.4. Laboratory diagnosis

Smears from staphylococcal lesions reveal Gram-positive cocci in grape like clusters. Cultures of *S. aureus* typically yield golden-yellow colonies that are usually β-hemolytic. *S. aureus* is coagulase-positive. Mannitol-salt agar is a commonly used screening device for *S. aureus*. Cultures of coagulase negative staphylococci typically yield white colonies that are non-hemolytic (Levinson, 2014).

There are no serologic or skin tests used for the diagnosis of any acute staphylococcal infection. In toxic shock syndrome, isolation of *S. aureus* is not required to make a diagnosis as long as the clinical criteria are met. Laboratory findings that support a diagnosis of toxic shock syndrome include the isolation of a TSST-producing strain of *S. aureus* and development of antibodies to the toxin during convalescence, although the latter is not useful for diagnosis during the acute disease (Levinson, 2014).

For epidemiologic purposes, *S. aureus* can be subdivided into subgroups based on the susceptibility of the clinical isolate to lysis by a variety of bacteriophages. A person carrying *S. aureus* of the same phage group as that which caused the outbreak may be the source of the infections (Levinson, 2014).

Several nucleic acid amplification tests have been developed and approved by the U.S. Food and Drug Administration (FDA) for staphylococci detection, most of which use single-locus polymerase chain reaction (PCR) amplification methods. Most of these tests are designed to detect methicillin-resistant staphylococci, most of which target *S. aureus* specifically (MRSA), from swab specimens or blood cultures (Patricia, 2017).

2.5. MecA gene

Penicillin binding protein (PBP) 2' is the most important mechanism of the resistance to β-lactams in MRSA, and the mecA gene is the coding gene of PBP2. Part of the structure of mecA is similar to that of the penicillinase gene. PBP-2a functions as a transpeptidase in cell wall synthesis in MRSA at high concentrations of β-lactam antibiotics that inhibit the growth of methicillin-susceptible strains with normal PBPs. This additional PBP is encoded by the structural gene mecA on the chromosome which has also

been detected in methicillin resistant strains of other *Staphylococcal* species. (Khayri *et al*., 2011).

Expression of PBP-2a is controlled by two regulator genes on mec DNA, mecI and mecR1, located upstream of mecA, which encode mecA repressor protein and signal transducer protein, respectively (Khayri *et al*., 2011).

2.6. Detection of mecA gene

There several assays that detect the *mecA* gene (which encodes methicillin resistance) in conjunction with a species-specific target gene. Several of the most commonly used MRSA test systems include the BD GeneOhm MRSA ACP and StaphSR assays (BD, Franklin Lakes, NJ), the BD MAX MRSA and Staph-SR assays, the Xpert MRSA/SA tests for nasopharyngeal swabs and blood cultures (Cepheid, Sunnyvale, CA), and the Roche Light Cycler MRSA Advanced Test (Roche Diagnostics, Indianapolis, IN) (Patricia, 2017).

2.7. Treatment

In the United States, 90% or more of *S. aureus* strains are resistant to penicillin G.Most of these strains produce β-lactamase. Such organisms can be treated with β-lactamase–resistant penicillins (e.g., nafcillin or cloxacillin), some cephalosporins,or vancomycin. Treatment with a combination of a β-lactamase–sensitive penicillin (e.g., amoxicillin) and a β-lactamase inhibitor (e.g., clavulanic acid) is also useful (Levinson, 2014).

Approximately 20% of *S. aureus* strains are methicillin-resistant or nafcillin resistant by virtue of altered penicillin-binding proteins. These resistant strains of *S. aureus* are often abbreviated MRSA or NRSA, respectively. Such organisms can produce sizable outbreaks of disease, especially in hospitals. The drug of choice for these staphylococci is vancomycin, to which gentamicin is sometimes added. Daptomycin is also useful.

Trimethoprim-sulfamethoxazole or clindamycin can be used to treat non–life-threatening infections caused by these organisms (Levinson, 2014).

Note that MRSA strains are resistant to almost all β-lactam drugs, including both penicillins and cephalosporins. Ceftaroline fosamil is the first β-lactam drug useful for the treatment of MRSA infections. Strains of *S. aureus* with intermediate resistance to vancomycin (VISA strains) and with complete resistance to vancomycin (VRSA strains) have been isolated from patients. These strains are typically methicillin-/nafcillin-resistant as well, which makes them very difficult to treat. Daptomycin (Cubicin) can be used to treat infections by these organisms. Quinupristin-dalfopristin (Synercid) is another useful choice (Levinson, 2014).

2.8. Control and prevention

The control and prevention of MRSA involves the education of all healthcare professionals and the public, fast and reliable detection in the laboratory (including perhaps the use of molecular methods), active surveillance (even universal surveillance), prompt patient isolation or cohorting when admitted to hospital (Greenwood *et al*., 2012).

Standard precautions and good professional practice by all health-care workers (including compliance with hand hygiene guidelines), effective hospital hygiene programs and antibiotic stewardship programs, e.g. avoidance of the excess use of cephalosporins and fluoroquinolones. Such measures have been very successful in Scandinavia and in the Netherlands where an aggressive ‘search and destroy’ approach involving the extensive screening of all MRSA contacts is employed (Greenwood *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

Descriptive cross sectional study.

3.2 Study area

This study was carried out in Khartoum State and samples were collected from different areas (Alkalaklah, Omdurman, Sudan University of Science and Technology, Khartoum University, Alneelain University and Alsouge Alarabi).

3.3 Study duration

Study was carried out during 2 months in the period from September to November 2018.

3.4 Ethical considerations

The study was approved by College of Medical Laboratories Science, in Sudan University of Science and Technology, and the samples were collected after agreement of retailer owners about the purpose and importance of the study.

3.5 Sample size

Four hundred samples were collected, this including 100 meat samples, 100 fish samples (50 salted and 50 raw), 100 cheese samples and 100 milk samples.

3.6 Sample collection

Fifteen grams of cheese samples were collected from different retailers by using sterile container, meat samples were collected randomly from supermarkets and restaurants using disposable blades small piece of raw meat had been splitted and transferred to the lab in sterile containers, small pieces from fish inner tissues were collected by sterile blade and placed in sterile plain containers and milk samples were collected in sterile containers and stored in refrigerator at 4°C in microbiology laboratory until examined

3.7. Samples processing

3.7.1. Cultivation

Meat, fish and cheese samples were enriched in peptone water (appendix II), incubated over night at 37°C. Then the isolates were sub cultured in mannitol salt agar (MSA) (appendix-2), and MacConkey agar (appendix-3) and incubated aerobically, at 37 °C, for overnight. Milk samples were cultivated as above in addition to blood agar.

3.7.2. Identification of organisms

3.7.2.1. Colonial morphology

S. aureus appeared on blood agar as medium to large, smooth, slightly raised, low convex, opaque, most colonies pigmented creamy yellow and some of them are beta-hemolytics.

On MacConkey agar as medium to large, smooth, slightly raised, low convex, pink and lactose fermented colonies. And large, smooth, slightly raised, low convex, golden yellow color and mannitol fermented colonies on mannitol salts agar.

3.7.2.2. Indirect Gram's stain

A drop of sterile normal saline was placed on the center of a clean glass slide; a bacterial colony was taken by a sterile wire loop and emulsified in the normal saline. Very thin film was prepared by uniform spreading of the

suspension. The smear was fixed by passing it three times over the flame and allowed to cool before staining. Staining was done by covering the smear with crystal violet (Appendix- II-1) stain for 30–60 seconds and rapidly washed off with clean water. All the water was tipped off; secondly the smear was covered with Lugol's iodine (Appendix- II-2) for 30–60 seconds then washed off with clean water. Smear decolorized rapidly for few seconds with acetone alcohol (Appendix- II-3) and washed immediately with clean water. Finally the smear covered with Safranin (Appendix- II-4) for 2 minutes and then washed off with clean water. The back of the slide whipped off, and the slide placed in a draining rack for the smear air-dry. The smear examined microscopically, firstly with the 40X objective, then by the oil immersion objective (100X) which appeared as Gram-positive cocci in cluster (Cheesbrough, 2006).

3.7.2.3 Biochemical tests

3.7.2.3.1. Catalase test

By using sterile wooden stick, well isolated colony of tested organism was removed and inserted in a tube containing 2-3 ml of 3% hydrogen peroxide appearance of immediate active air bubbling considered as positive catalase test (Cheesbrough, 2006).

3.7.2.3.2. Coagulase test

A drops of normal saline was added on each end of a slide or on two separate slides then by using wire loop colony is emulsified in each of the drops to make two thick suspensions, a drop of undiluted human plasma was added to one of the suspensions (the second suspension considered as control negative) and mixed gently. Clumping within 10 sec is considered positive coagulase test (Cheesbrough, 2006).

3.7.2.3.3. DNase test

Using sterile loop the organism with heavily spotted inoculated in DNase agar (Appendix- I-5) and incubated aerobically at 37° C for overnight, after bacterial growth the colonies were tested for DNase production by flooding the plate with a weak hydrochloric acid (Appendix- II-4). A clear zone around the colonies was considered DNase test positive (Cheesbrough, 2006).

3.7.3. Antimicrobial susceptibility test using modified Kirby-Bauer sensitivity testing method

3.7.3.1. Preparation of the bacterial suspension

Using a sterile wire loop, 1-2 well-isolated colonies of *S. aureus* were picked up and emulsified in 3-4 ml of sterile normal saline in sterile test tube. Turbidity of the suspension compared to the turbidity of 0.5% McFarland standard in presence of a good light (Cheesbrough, 2006).

3.7.3.2. Seeding of the plate and application of discs

A sterile swab was dipped into the suspension, excess fluid removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The swab was seeded evenly over the surface of the medium, using sterile forceps, the antimicrobial disc oxacillin was placed on the inoculated plate in about 15 mm from the edge of the plate each disc was lightly pressed down to ensure its contact with the agar. Within 30 minutes of discs application, the plates were inverted and incubated aerobically at 37˚C for 18-24hrs (Cheesbrough, 2006).

3.7.3.3. Reading and interpretation

After growth the zone of inhibition diameter for disc was measured and compared to zone size interpretative chart (Cheesbrough, 2006).

Antimicrobial disc used for *S. aureus* was Oxacillin (5 µg) The *S. aureus* ATCC 52923 Control strain was used (Appendix III).

3.8. Genotyping of *S. aureus* mecA gene

3.8.1. DNA Extraction

DNA was extracted by simple boiling method, in which the extracted product was done from overnight isolates on Nutrient Agar. A loop full of bacterial colony was picked from an isolate and suspended in 300µl of sterile distilled water and 10µl of proteinases K was added and incubated at 6o°C for 60 minutes. Then incubated at 100°C in a water bath for 15 minutes, and then suspension was centrifuged at high speed (10000 rpm for 10 min). The supernatant containing the genomic DNA was transferred into a fresh sterile eppendorf tube and stored at -20°C until to be used for PCR (Compain *et al*., 2014).

3.8.2. Molecular confirmation

All samples were confirmed as S. aureus by specific housekeeping gene primer (16s), negative samples were excluded and positive samples were detected for enterotoxins genes

3.8.3. Amplification of DNA

Conventional pcr technique was done amplification was done using (CLASSIC K960, UK) thermo cycler (Appendix-III). Primers were used for detection of mecA gene. DNA amplifies was done using Maxime PCR Premix kit (iNtRON, Korea), The PCR assay was carried out in a total volume of 20 µL of mixture containing 2 µL Maxime PCR Premix, 0.5 µL of each of the gene-specific primers (5 µL), 2 µL of template DNA and 13 µL of double distilled water. The amplification conditions included three steps: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 45sec, annealing at 52°C for 45sec, and extension at 72°C for 45 sec; and the final extension at 72°C for 7 min.

Table 1 Primer used in amplication

Primer name	DNA sequence (5 to 3)	Ampilicon size (bp)	Specificity
Resistance primer MecA1 – F MecA1 –R	5- AACTCTGTTATTAGGGAAGAACA-3 5- CCACCTTCCTCCGGTTTGTCACC-3	310 bp	MecA
Identification primer Staph 756-F Staph 750-R	5- AACTCTGTTATTAGGGAAGAACA-3 5- CCACCTTCCTCCGGTTTGTCACC-3	310 bp	16S rRNA

3.9. Visualization of the DNA product

Visualization of the DNA product was done by agarose gel electrophoresis as follow.

3.9.1. Preparation of 10 X and 1X TBE buffer

Amount of 108 gm. Tris base were weighed and added to 55gm of boric acid and 40ml of 0.5M EDTA then dissolved into 1 liter deionized water, pH 8.0, Ten ml of 10 X TBE buffer was added to 90 ml deionized water and heated until completely dissolved respectively (Sambrook *et al*., 1989).

3.9.2. Preparation of ethidium bromide

Ten milligrams of ethidium bromide powder were dissolved into 1 ml deionized water, and kept into brown bottle (Sambrook *et al*., 1989).

3.9.3. Preparation of agarose gel

Amount of 1 g of agarose powder dissolved by boiling in 50 ml 1X TBE buffer (2%), then was cooled to 55°C in water bath, then, 2 µl of (10mg\ml) Ethidium bromides were added, mixed well and poured on to the casting tray that has been taped up appropriately and was equipped with suitable comb to form well in place. Any bubbles were removed and the gel was allowed to set at room temperature. After solidification, the comb was gently removed and the spacer from the opened sides was removed (Sambrook *et al*., 1989).

3.9.4. Electrophoresis

The gel casting tray was put into the electrophoresis, tank flooded with 1x TBE buffer just to cover the gel surface,10 µl of PCR products from each samples was added to wells of electrophoreses, 5 µl of 100-bp DNA ladder (iNtRON, Korea),was added to the well in each run. The gel electrophoresis apparatus was connected to power supply (100 V, 500 mA, UK). The electrophoresis was carried out at 75Volts for 30 minutes and the gel tray was removed from the electrophoresis apparatus and the buffer was discarded. Then the gel was visualized for DNA bands by Ultra violet (U.V) transilluminater and photographed (Uvitec – UK).

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of four hundred samples (milk 100, cheese100, fish100, and meat 100) were collected from different areas in Khartoum state. 84 (21%) of 400 of isolates were identified as *S. aureus*, 110 (27.5%) of 400 were identified as other bacteria than *S. aureus* and (51.5%) 206 of 400 yielded no growth (Table 2).

The frequency of *S. aureus* according to the area (local) of collection as 38.1% from Khartoum, 38.1% from Omdurman, 17.4% from East Nile and 6.4% from Khartoum North (Table 3).

The antimicrobial susceptibility tests of isolated *S. aureus* were showed 31of eds84 (36.9%) were Oxacillin resistant and 53 of 84 (63.1%) were Oxacillin sensitive (Table 4).

The results for mecA gene was 15 of 84 (17%) positive and 69 (83%) negative (Table 5).

Table 2. Frequencies of samples

Sample	Number
Milk	100
Meat	100
Fish	100
Cheese	100
Total	400

Figure 3. Distribution of samples among collection areas

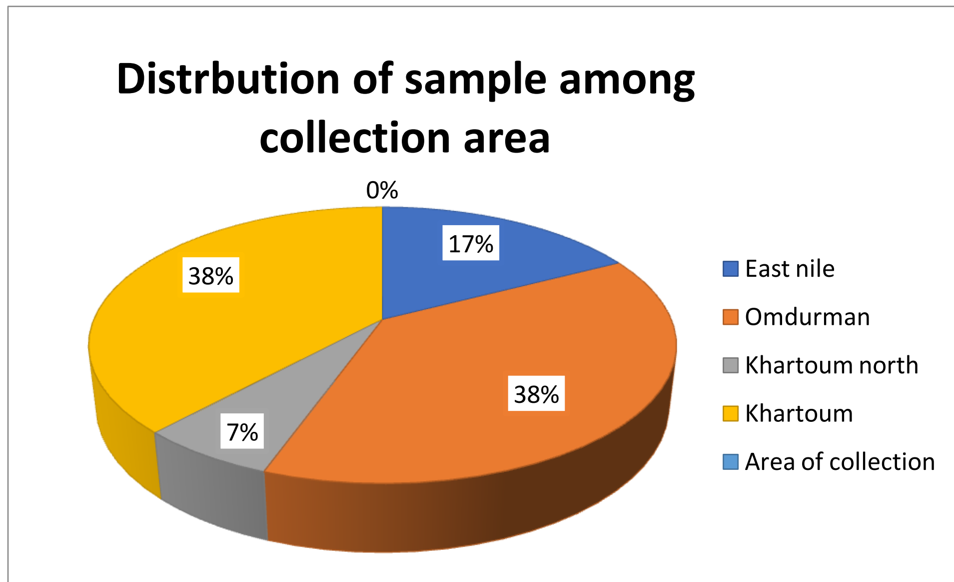

Figure 4. Frequencies of isolates

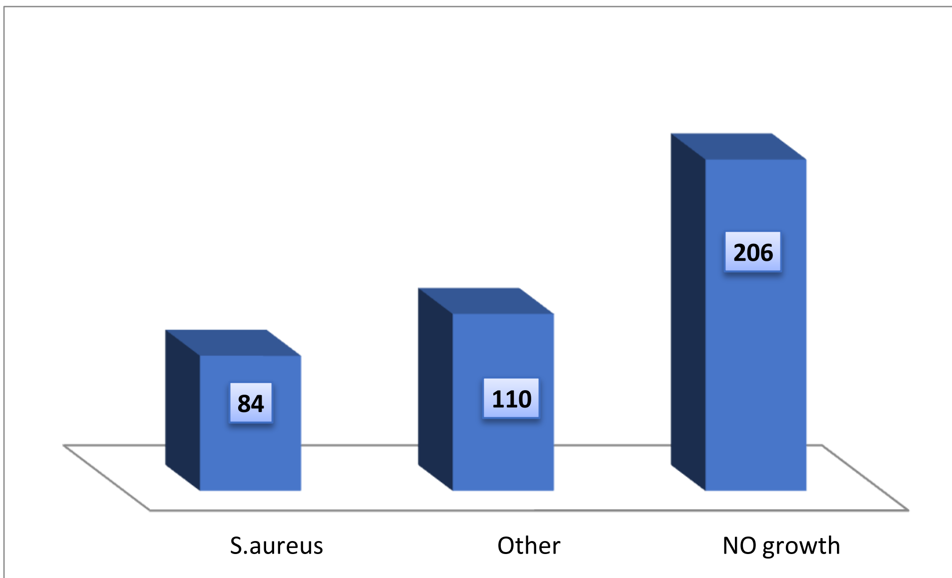

Table 3. Frequencies of mecA gene

mecA gene	Number	Percentage (%)
Positive	15	17 %
Negative	69	83 %
Total	84	

Figure 3 Gel electrophoresis of DNA product

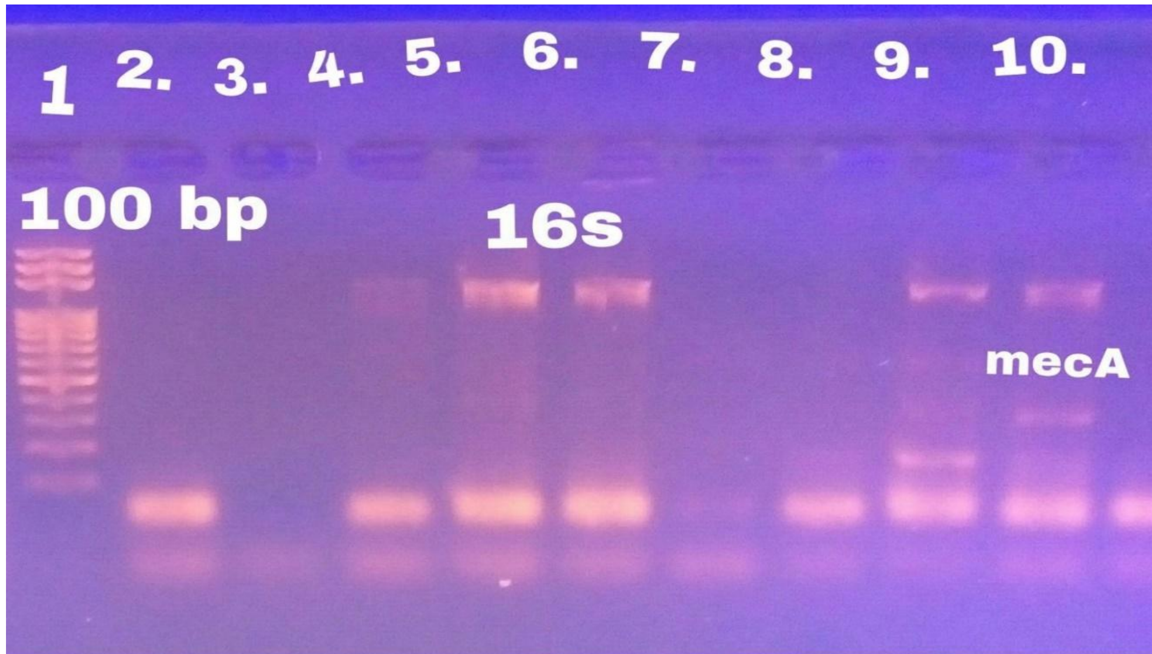

Figure …….

CHAPTER FIVE
DISCUSSION

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

In this study the prevalence of *S. aureus* and MRSA in various food samples were determined. A total of 84 (21%) *S. aureus* isolated after performing required biochemical tests.

The previous studies conducted to detect *S. aureus* in various foods revealed that the contamination levels with *S. aureus* have been observed to be lower than the results obtained in this study; as in study of Traversa and colleagues which conducted in 2015 revealed 17.1% in Italy (Traversa *et al.,* 2015), while the study of Sivakumar and colleagues revealed 12.01% in India using Enterobacterial repetitive intergenic consensus (ERIC)-PCR (Sivakumar *et al.,* 2019). Other studies showed higher prevalence of *S. aureus* in food samples as in Papadopoulos and colleagues which was 47.8% in north-central and north-eastern Greece (Paopadopouls *et al.,* 2019) and in the study of Khayri were 54.4% (Khayri, *et al,*. 2011).

Resistance gene (mecA) in *S. aureus* witch responsible of resistance to β-lactam antimicrobials was detected using PCR. The obtained results were 15 of 84 (17%) positive for mecA gene while 69 (83%) were negative.

The previous results of mecA gene showed higher prevalence than this study as in the study by Khayri was 44.4 % (Khayri, 2011) positive mecA gene, Papadopoulos and colleagues was 81.3% (Papadopoulos *et al.,* 2019) and Shahraz 89% in Iran-Tehran (Shahraz *et al.,* 2012) and Cho 20% (Cho *et al.,* 2014).

The previous results of mecA gene showed lower prevalence than this study as in Novak was 11.4% (Novak *et al.,* 2000) and 5.2% as in Kamal (Kamal

et al., 2013) and in Rizek 9% In Brazil (Rizek *et al.,* 2011) and as in song 5.6% IN Shanghai (song *et al.,* 2015) and as in Wang 7.9% (Wang *et al.,* 2017) and in Rodríguez-Lázaro 9.1% from European flight food using multiplex PCR (Rodríguez-Lázaro *et al.,* 2014) and in Basanisi 8.3% in Italy using multiplex PCR (Basanisi *et al.,* 2017).

The variation in the results may be due to variation of source of samples and different techniques including conventional PCR, multiplex PCR and intergenic consensus (ERIC Enterobacterial repetitive)-PCR.

5.2. Conclusion

The presence of high number of *staphylococcus aureus* isolates from food samples in Khartoum state also high number of MRSA become remarkably in the isolates in sudan due to presence of mecA gene.

5.3. Recommendations

1- Application of this study in larger study area, other types of food and population to better determent of MRSA.

2- Application this approaches on other bacteria since the antimicrobial resistance become worldwide concern.

3- Adaptation of proper and strict food hygiene program to all types of food as well as workers in food handling and preparation.

4- Use more advanced techniques to detect mecA gene subtypes

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Appendices

Appendices

Appendix-I: Preparation of culture media

1. Blood Agar Medium (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients

g/L HM infusion

10.0g Tryptose

10.0 g

Sodium chloride 5.0

g

Agar 15.0 g

Final pH (at 25°C) 7.3 ±

0.2 Preparation Suspend 40.0 g in 1000 ml D.W. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

2. Preparation of MacConkey agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India) Ingredients

g/l Pepton

20.00 g Lactose

10.00 g Sodium taurcholate

5.00 g Neutral red

0.04 g

Agar 20.00 g

Preparation Suspend 55.04 grams in 1000 ml distilled water. Using water bath heat the suspension to boiling to dissolve the medium completely.

Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

2. Mannitol Salt Agar Medium (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients
g/L

Proteose Peptone 10.00 g

Beef extract 1.00 g

Sodium chloride 75.00 g

D-Mannitol 10.00 g

Phenol red 0.025 g

Agar 15.00 g

Final pH (at 25°C) 7.4±0.2

Preparation Suspend 111.02 grams in 1000 ml distilled water. Using water bath heat the suspension to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

4. DNase Agar Medium (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients

g/L

Tryptone

15.00 g Soya peptone

5.00 g

Deoxyribonucleic acid (DNA) 2.00 g

Sodium chloride 5.00g

Agar 15.00 g

Final pH (at 25°C)

7.3±0.2

Preparation Suspend 42 grams in 1000 ml distilled water. Using water bath heat the suspension to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and cool to 45°C and pour in sterile Petri dish.

5. Mueller's Hinton agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India) Ingredients

g/L

Beef, infusion form 300.00 g

Casein acid hydrolysate

17.500g Starch

1.500g Agar

17.00g Final pH (at 25°C)

7.3 ± 0.1 Preparation Suspend 38.0 grams in 1000 ml distilled water. Using water bath heat the suspension to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

6. Agarose gel

Ingredients

g/ml Agarose powder

1g 1X TBE buffer

50ml Preparation Amount of 1 g of agarose powder dissolved by boiling in 50 ml 1X TBE buffer (2%), then was cooled to 55°C in water bath, then 2 µl of (10mg\ml) Ethidium bromides were added, mixed well and poured on to the casting tray that has been taped up appropriately and was equipped with suitable comb to form well in place. Any bubbles were removed and the gel was allowed to set at room temperature. After solidification, the comb was gently removed and the spacer from the opened sides was removed (Sambrook et al., 1989).

Appendix-II: Preparation of reagents

1. Crystal violet (HiMedia Laboratories Pvt. Ltd. Mumbai, India) Ingredients

g/L Crystal violet 20 g Ammonium oxalate 9 g Ethanol or methanol, absolute 95 ml Preparation Weigh the crystal violet on a piece of clean paper (pre weighed), transfer to a brown bottle pre marked to hold 1 liter, add the absolute ethanol or methanol (technical grade is suitable) and mix until the dye is completely dissolved, weight the ammonium oxalate and dissolve in about 200 ml of distilled water, add to the stain, make up to the 1 liter mark with distilled water, and mix well (Caution: Ammonium oxalate is a toxic chemical, therefore handle it with care), label the bottle, and store it at room temperature. The stain is stable for several months.

2. Lugol's iodine (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients

g/L Potassium iodide 20g Iodine 10g Preparation Weigh the potassium iodide, and transfer to a brown bottle pre marked to hold 1 liter, add about a quarter of the volume of water, and mix until the potassium iodide is completely dissolved, weight the iodine, and add to the potassium iodide solution. Mix until the iodine is dissolved (Caution: Iodine is injurious to health if inhaled or allowed to come in contact with the eyes, therefore handle it with care in well ventilated room and make up to the 1 liter mark with distilled water, and mix well. Label the bottle, and mark it Toxic. Store it in a dark place at room temperature. Renew the solution if its color fades.

3. Acetone-alcohol decolorizer (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients

ml Acetone

500ml

Ethanol or methanol, absolute 475 ml

Preparation Mix the distilled water with the absolute ethanol (ethyl alcohol) or methanol (methyl alcohol), transfer the solution to a screw-cap bottle of 1 liter capacity, technical grade is adequate, measure the acetone, and add immediately to the alcohol solution mix well (Caution: Acetone is a highly flammable chemical that vaporizes rapidly, therefore use it well away from an open flame) and label the bottle, and mark it Highly Flammable. Store in a safe place at room temperature, the reagent is stable indefinitely.

4. Safranin (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients

g/L

Safranin O 0.50 g

95% Ethyl alcohol

100.00

5. Hydrochloric acid (1mol/l) (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Tomake100 ml

Hydrochloric acid, concentrated 8.6

ml

DW to100 ml

6.10X TBE buffer

Ingredients

g/L Tris base

108g

Boric acid 55g

0.5 EDTA 40

ml

Deionized water 1

liter

PH 8.0

7. 1X buffer

10X buffer

10ml Deionized water

90ml

8. Ethidium bromide

mg/ml Ethidium bromide

10 Deionized water

1ml

APPENDIX-III colored plates

Figure 1 Growth of bacteria

Figure 2 Antimicrobial sensitivity

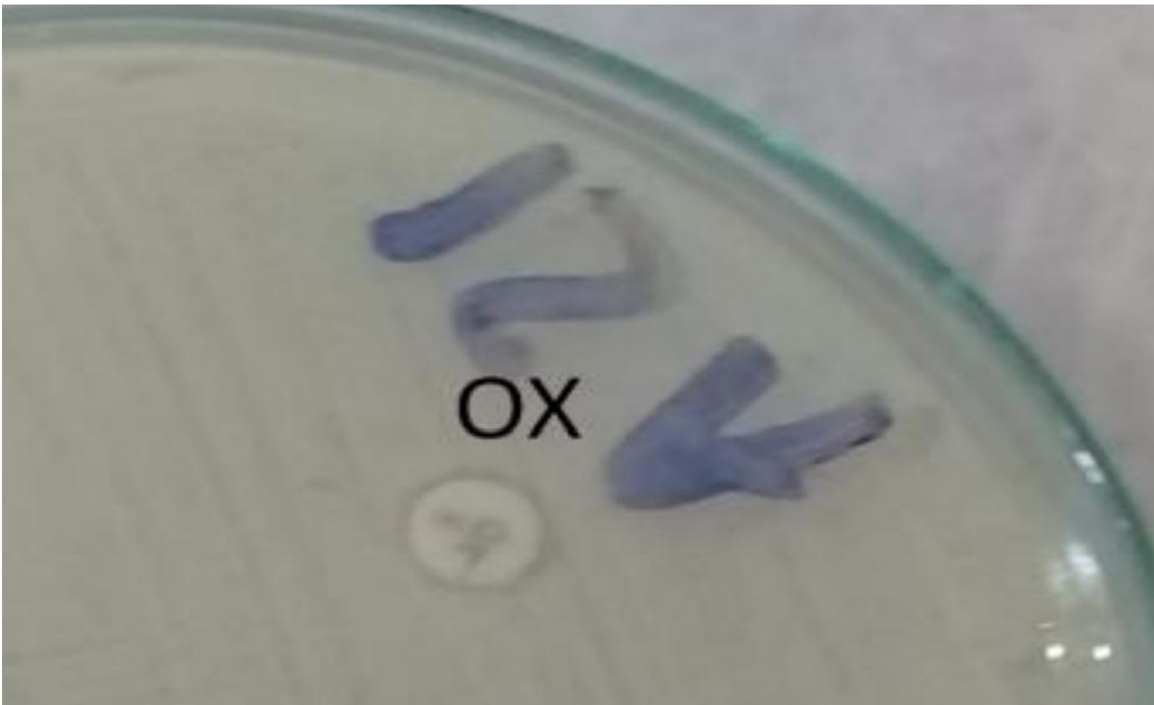

Figure 4 Trans illuminator:

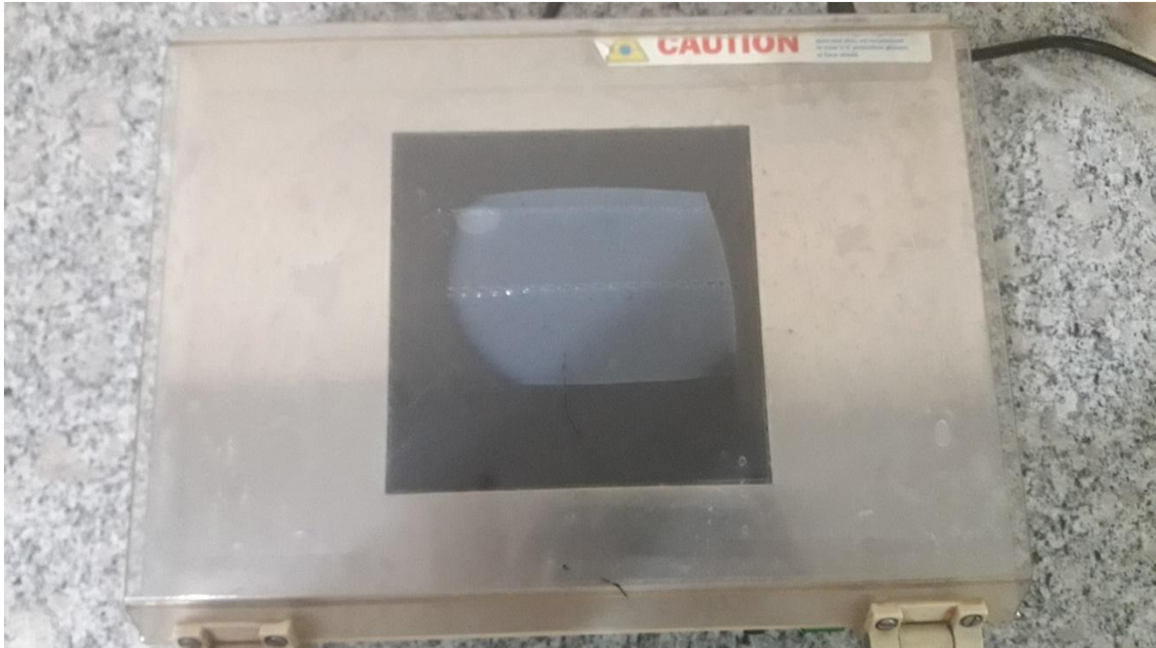

Figure 5 Thermocycler