A BACTERIOLOGIC INVESTIGATION ON

SUDANESE COINS IN KHARTOUM NORTH ROVINCE

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DEDICATION

To

Soul of my father…

My honory family…

My dear friends and colleagues…

With deep love and respect
ACKNOWLEDGEMENT

First of all my thanks are due to Allah who gave me the health and strength to conduct this study.

I am deeply grateful to my supervisor Dr. Khalid Mohammed Suleiman for his help, guidance, advice, cooperation and encouragement throughout this work.

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ABSTRACT

The objective of this study was to examine the extent of bacterial contamination of the most used coin denominations of the Sudanese currency.

A total of 72 coins of three different denominations (10, 20, and 50 SD) in circulation inside Khartoum North province were randomly studied for bacterial contamination. Sources of samples collection were groceries, cafeterias, bus drivers and elementary schools pupils during the period from February 2006 to August 2006. Samples were cultivated on appropriate media and identification of isolated bacteria was accomplished by conventional methods.

Results showed that all tested coins, except one, had bacterial growth, 84 (92%) were Gram positive bacteria while only 7 isolates (8%) were Gram negative bacteria. The predominant genus isolated was Bacillus which constituted 90.1% of the total bacteria and included B. cereus, B. mycoides, B. thuringiensis, B. subtilis, B. pumilus, B. licheniformis, and others. The other bacteria identified were Staphylococcus epidermidis and Escherichia coli (2.2%), Citrobacter freundii and Klebsiella pneumoniae (1.1%). The frequency of bacterial isolation from different sources was 20.9% for groceries, 19.8% for cafeterias, 26.4% for bus drivers and 32.9% for elementary schools. The rate of bacterial isolation was the same for all coin denominations.

For Bacillus spp subjected to antibiotic sensitivity test all isolates were sensitive to tetracycline, chloramphenicol, erythromycin and gentamycin. All Bacillus cereus group showed resistant to penicillin, ampicillin and cloxacilin. For streptomycin, all of them were sensitive except two isolates of B. licheniformis one was resistant and the other was intermediate.
هيئة هذا الدراسة إلى تحديد مدى التلوث البكتيري في فئات العملة المعدنية السودانانية الأكثر تداولًا.

تم جمع 72 عينة من ثلاث فئات مختلفة هي فئة 10، 20 و 50 دينار سوداني متداولة داخل محافظة بحري لدراسة تلوثها البكتيري. هذه العينات جمعت من مصادر مختلفة شملت البقالات، الكافتريات، ساحق الحافلات وتلاميذ مدارس الأساس في الفترة من فبراير 2006 م حتى أغسطس 2006م. زرعت العينات في الأوساط المزرعية المناسبة وعرفت البكتيريا باستخدام الاختبارات الكيميائية.

أوضح نتائج الدراسة أن جميع العملات المعدنية ملوثة بالبكتيريا عدا واحدة وحصل على 91 عزلة من بينها 84 (92%) موجبة بينما 7 فقط (8%) كانت سالبة لصبغة جرام. البكتيريا من جنس باسيلس كانت الأكثر تكراراً (90.1%) من بين العملات وضمت (باسيلس سيرس، باسيلس ميكيدز، باسيلس تيرينجنس، باسيلس سيتيلس، باسيلس بميلس، باسيلس لشينفورمز...الخ) بالإضافة إلى أنواع أخرى من البكتيريا منها استاف ابيدمردز واسشريشيا كولي (2.2%)، ستروباتكرفرداي و كليسيلا نيموني (1.1%).

أوستوح نتائج الدراسة أن معدل عزل البكتيريا من المصادر المختلفة هو 20.9% للبقالات، 19.8% للكافتريات، 26.4% لساحق الحافلات و 32.9% لتلاميذ مدارس الأساس. معدل عزل البكتيريا في جميع فئات العملات كان متوازياً.

أظهرت نتائج اختبار الحساسية للمضادات الحيوية، أن كل أنواع الباسيلس حساسة للتتراسيكلين، كلورامفينكول، ايزوبروتياميسين وجينيتاميسين. كل مجموعة الباسيلس سيرس كانت مقاومة للإيبسلين، امبيسلاين وكلوزك إسيلين. أما بالنسبة للاستريبيتاميسين، فجميع الباسيلس كانت حساسة له ما عدا اثنين في باسيلس لشينفورمز.
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CHAPTER ONE
LITERATURE REVIEW

1.1 Coins:
Along with banknotes, coins make up the cash forms of all modern money system as a medium of exchange in goods and services. They are usually used for lower valued units. Recently most of them are made of a base metal as in gold, silver, platinum coins.

The first known usage of coins comes from the kingdom of Lydia circa between 643 and 630 B.C; these first coins were made of electrum, a naturally occurring pale yellow mixture of gold and silver (Cooper, 1988).

1.2 Contamination of coins:
Until recently the studies concerning the contamination of paper notes and coins have been few and rather limited (Bonifazi, 2002; Michael, 2002) but the accumulated data obtained over the last two decades on the microbial status and survival of pathogens on coins and currency notes indicated that this could represent a potential cause of sporadic cases of food borne illness.

There are various modes of transmission of infection. Fomites are responsible for the indirect transmission of infection. Various diseases like diphtheria, trachoma, gastroenteritis, whooping cough and pathogenic agents causing diarrhea are known to be transmitted through fomites (Gilchrist MJR, 1993). Currency might also be a fomite, playing an important role in the transmission of microorganisms and also in the spread of drug resistant strains in the community (Singh DV et al., 2002).
The importance of paper notes as a means of disease transmission was realized after the outbreak of Severe Acute Respiratory Syndrome (SARS) in Asia, and the reason behind that was the belief that money played direct or indirect role in spreading disease (El-Dars and Hassan, 2005), since bacteriological studies revealed that bacteria could spread from person to person via fomites (Pope et al., 2002).

Both paper currency and coins often offer an ample surface areas to harbor bacteria (El-Dars and Hassan, 2005), and paper notes can accommodate a variety of contaminants and for longer period (Gadsby, 1998; Brown, 2003).

Survival of various microorganisms of concern on money is such that it could serve as a vehicle for transmission of disease and represents an often overlooked enteric disease reservoir (Michaels, 2002).

Jiang and Doyle (1999) demonstrated that coins could serve as potential vehicles for the transmission of pathogens. They applied E. coli 0157:H7 and Salmonella enteritidis to sterile coins and then stored at room temperature. The bacteria survived for up to 11 and 9 days respectively. Handling paper currency has long been believed to be a factor in the transmission of bacteria, and this study indicates that coins may likewise be a factor. The transmission through money handling is thought to be a particular problem for fast-food restaurants, day care centers, nursing homes and schools. The study emphasizes the importance of constant attention to hand cleanliness and prevention of cross-contamination.

1.2.1 Types of contaminants:

In the early 1970s, Abrams and others showed that 13% of US coins and 42% of paper money was found to be contaminated with what were
considered to be potential pathogens (Abrams and Waterman, 1972). Organisms identified in this study were *E. coli, P. aeruginosa, Klebsiella, Proteus* and *Staphylococcus species.*

In a more recent study, conducted in the US by Gadsby, it was found that 18% of coins and 7% of currency tested showed potential disease producing organisms including *E. coli* and *Staph aureus* (Gadsby, 1998).

Moore *et al.* (2005) investigated monetary coinage from 17 countries and demonstrated that it was contaminated by environmental Gram-positive flora, in particular *Bacillus spp* as well as *Staphylococcus spp.*

A recent Hungarian study (Havas, 2000) of the bacteriological state of 115 notes and 71 coins demonstrated that there were significantly higher numbers of bacteria on the notes than on the coins and those members of the *Enterobacteriaceae* family, *Enterococci,* and *Bacillus cereus* were found only on the notes.

Another study which investigated 100 notes and 102 coins collected from staff in New York hospital found that 3% of coins and 11% of notes were contaminated with opportunistic pathogens including *Staphylococcus spp., Bacillus spp., and Corynebacterium spp* (Pachter *et al.*, 1997).

1.3 **Bacteria contaminating coins:**

1.3.1 *Bacillus spp:*

Rods, mainly Gram-positive in young cultures, motile (some non-motile forms occur). None acid-fast and produce heat-resistant spores under aerobic conditions. Aerobic; some species facultatively anaerobic. Oxidase-variable; catalase-positive. Species differ in the manner in which they attack sugars (Barrow and Feltham, 2003).
1.3.1.1 Normal habitat:

Due to the resistance of their endospores to air-drying, to other stresses and to their long term survival under adverse conditions, most aerobic spore formers are ubiquitous and can be isolated from a wide variety of sources (Claus and Berkeley, 1872). The primary habitat of the majority of bacillus spp is the soil, from it; they can contaminate everything by dust or other means. They may play an important role in such secondary habitats in degrading polymers or other chemical compounds. As a result, Bacillus spp are especially important as food spoilage organisms (Claus and Berkeley, 1872).

1.3.1.2 Pathogenicity of Bacillus spp:

Members of the Bacillus genus are ubiquitous soil microorganisms that frequently contaminate foods (Phelps et al., 2002). With the exceptions of the Bacillus anthracis and Bacillus cereus, Bacillus species are generally perceived to be inconsequential and of little clinical significance (Drobniewski, 1993).

A number of food poisoning incidents can be attributed to B. cereus, and this bacterium is known to cause a variety of nongastrointestinal diseases as well as two different types of food poisoning (Kotiranta et al., 2000).

The relevance of other Bacillus species as food poisoning organisms and as etiological agents in nongastrointestinal infections, including local, deep- tissue, and systemic infections, is being increasingly recognized (Drobniewski, 1993).

Nongastrointestinal infections have been seen primarily in individuals who are intravenous drug abusers or immunocompromised as a consequence
of infection with human immunodeficiency virus, chemotherapy, or malignancy (Beattie et al., 1999).

Due to their endospore-forming abilities, these bacteria tolerate adverse conditions better than most bacterial enteropathogens do and may proliferate in a wide range of environments, including processed and untreated foods (Phelps et al., 2002).

1.3.1.2.1 B. cereus:

It is a cause of food poisoning which is frequently associated with the consumption of rice-based dishes. It produces an emetic or diarrhea syndrome induced by an emetic toxin and enterotoxin, respectively. Other toxins are produced during growth, including phospholipases, proteases, and hemolysins which contribute to the pathogenicity of B. cereus in non gastrointestinal disease (Drobniewski, 1993).

This organism is associated with a serious nongastrointestinal infection, particularly in drug addicts, the immunosuppressed, and neonates. Ocular infection is the commonest types of severe infection (Drobniewski, 1993). Respiratory infections involving both the lung and pleural spaces are uncommon but is potentially life-threatening (Farrar, 1963).

There have been almost 30 documented cases of Bacillus meningitis and encephalitis reported in the literature for both adults and children, with the majority attributable to B. cereus (Barrie et al., 1992).

B. cereus is a small but significant cause of endocarditis, particularly when associated with intravenous drug administration (Tuazon, Hill and Sheagren 1974). There is a reported case of pericarditis caused by B. cereus in a drug addict (Fricchione et al., 1991)
1.3.1.2.2 *B. thuringiensis:*

It produces potent protein toxins that target the mid-gut of susceptible insect species (Hofte and Whiteley, 1989).

It is not only insect pathogen but also it has been implicated in mammalian infections. Gordon (1977) reported the isolation of *B. thuringiensis* from a fatal case of bovine mastitis.

It has been associated with two cases, a wound and an ocular infection (Turnbull and Kramer and Melling, 1990).

1.3.1.2.3 *B. subtilis:*

It produces an acute-onset emetic syndrome, although a significant minority also suffer diarrhea (Kramer and Gilbert, 1989).

It was implicated in causing food poisoning similar to that due to *B. cereus* (Green-wood, Slack and Peuthere, 2002).

It is though to be involved in different mammalian infections including septicemia (Sathmary, 1958), respiratory disease (Greenberge Milne and Watt, 1970), endocarditis (Reller, 1973); pneumonia (Ihde and Armstrong, 1973).

1.3.1.2.4 *B. licheniformis:*

Cases of *B. licheniformis* food poisoning present a clinical picture similar to that of *C. perfringens* food poisoning and the diarrheal syndrome caused by *B. cereus* (Kramer et al., 1989).

It has also been associated with septicemia, peritonitis and food poisoning in humans, as well as with bovine toxemia and abortions (Turnbull and Kramer, 1995).

1.3.1.2.5 *B. sphaericus:*

It is an insect pathogen but it has also been implicated in mammalian infections. A healthy adult has been infected by *B. sphaericus* leading to...
fatal meningitis (Allen and Wilkinson, 1969). Another recorded instances of infection by this organism, which led to meningitis, bacteremia and endocarditis (Farrar, 1963).

Issacson and Jacobs (1976) reported a case of a large gelatinous pseudo tumor of the lung which found to be caused by this species.

1.3.1.2.6 *B. pumilus:*

It has been considered non pathogenic for humans, however, some strains have been detected producing toxins which experimentally had cytopathic effects in vero cells (Hoult and Tuxford, 1991).

The first report on isolation of a pumilacidin- producing *B. pumilus* strain from food implicated in food poisoning and characterization of the organism and the toxin complex of lipopeptides involved were conducted by From, Hormazabal and Granum (2007).

1.3.2 *Staphylococcus spp:*

Gram- positive cooci in clusters, non motile, non spore forming, aerobic and facultatively anaerobic. Catalase positive and usually oxidase negative. Hydrolyze arginine, produce actoin and attack sugars by fermentation (Barrow and Feltham, 2003).

1.3.2.1 *Normal habitat:*

Staphylococci are widely distributed in the environment; they form part of normal microbial flora of the skin, upper respiratory tract and intestinal tract (Quinn *et al.*, 2002).

1.3.2.2 *Pathogenicity:*

Staphylococcus is the main causative agent of postoperative wound infection (Briody, 1974). The main species of medical important is *Staph. aureus* and several other species may also cause disease including *Staph. epidermidis* and *Staph. saprophyticus* (Cheesbrough, 1987).
1.3.2.2.1 

**Staph. aureus:**

*Staph. aureus* is a major pathogen of increasing importance due to the rise in antibiotic resistance (Lowy, 1998).

It is considered to be a major pathogen that colonises and infects both hospitalised patients with decreased immunity, and healthy immunocompetent people in the community. This bacterium is found naturally on the skin and in the nasopharynx of the human body. It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening (Shulman and Nahmias, 1972). It can enter the underlying tissue if the skin is breached due to trauma or surgery creating its characteristic local abscess lesion (Elek, 1956), and if it reaches the lymphatic channels or blood can cause septicemia (Waldvogel, 1990). *Staph. aureus* can also produce a range of extracellular toxins, such as enterotoxin A-E and toxic shock syndrome toxin (Projan and Novick, 1997). Ingestion of enterotoxin produced by *Staph. aureus* in contaminated food can cause food poisoning (Howard and Kloos, 1990). *Staph. aureus* has been found to be a common cause of metal-biomaterial, bone-joint and soft-tissue infections.

1.3.2.2.2 

**Staph. epidermedis:**

The incidence of *staph. epidermedis* infection is low. It is usually linked to special circumstances, for example bacteremia following infection of cannulae, indwelling catheters, shunts, or other appliances positioned in the body. Infections are difficult to treat because of the presence in the host of foreign matter, and the antibiotic resistance of the bacteria (Cheesbrough, 1987).
1.3.3 *Escherichia coli*:

Gram-negative rods, often motile, aerobic and facultatively anaerobic. Catalase positive, Oxidase negative. Attack sugars fermentatively, with gas normally produced, usually citrate-negative (Barrow and Feltham, 2003), most E.coli strains produce indole from peptone water. Some E. coli strains are capsulated (Cheesbrough, 1987).

1.3.3.1 Normal habitat:

*E. coli* forms part of the normal microbial flora of the intestinal tract of human and animals. They can also be found in water, soil, and vegetation (Cheesbrough, 2000)

1.3.3.2 Pathogenicity:

*Escherichia coli* are a common member of the normal flora of the large intestine. Some strains that acquire bacteriophage or plasmid DNA encoding enterotoxins or invasion factors become virulent and can cause either a plain, watery diarrhea or inflammatory dysentery.

Three groups of *E. coli* are associated with diarrheal diseases. *Escherichia coli* strains that produce enterotoxins are called enterotoxigenic *E. coli* (ETEC). There are numerous types of enterotoxin. Some of these toxins are cytotoxic, damaging the mucosal cells, whereas others are merely cytotoxic, inducing only the secretion of water and electrolytes. A second group of *E. coli* strains have invasion factors and cause tissue destruction and inflammation resembling the effects of *Shigella* (EIEC). A third group of serotypes, called enteropathogenic *E. coli* (EPEC), are associated with outbreaks of diarrhea in newborn nurseries, but produce no recognizable toxins or invasion factors.
1.3.4 Klebsiella spp:

Gram negative rods which are non motile. They are aerobic and facultatively anaerobic, catalase positive, oxidase negative and attack sugars fermentatively, usually with the production of gas. VP and urease positive (Barrow and Feltham, 2003).

The main species of medical importance is Klebsiella pneumoniae. Four sub-species of klebsiella pneumoniae are recognized K. pneumoniae subsp pneumoniae, K. pneumoniae subsp areogenes, K. pneumoniae subsp ozaenae, K. pneumoniae subsp rhinoscleromatis (Cheesbrough, 2000).

1.3.4.1 Normal habitat:

Klebsiella strains can be found in the intestinal tract of humans and animals, and also in plants, soil, and water. klebsiella pneumoniae can be found as commensal in the mouth and upper respiratory tract, and also in moist environment in hospitals and else where (Carter, 1986).

1.3.4.2 Pathogenicity:

This species causes chest infections. Occasionally it causes severe pneumonia, especially in patients being treated with ampicillin. It also causes urinary tract infections, particularly those that are hospital-acquired.

It is involved in septicemia, meningitis, wound infections and peritonitis (Cheesbrough, 1987).
CHAPTER TWO
MATERIALS AND METHODS

2.1 Materials:

2.1.1 Culture media:

Most of the media were prepared according to the methods described by the Oxoid manual (1973).

2.1.1.1 Solid media:

2.1.1.1.1 Nutrient agar (Biomark lab):

The media was prepared by dissolving 28 grams of powder in 1 litre of distilled water by boiling. The medium was sterilized by autoclaving (121 °C for 15 minutes), cooled to 55 °C and then distributed into sterile Petri dishes 20 ml in each.

2.1.1.1.2 Blood agar:

Hundred ml of fresh, sterile, defibrinated blood were added aseptically to 900 ml of melted sterile nutrient agar which was cooled to 55 °C, mixed and distributed into sterile petri dishes 20 ml in each dish.

2.1.1.1.3 MacConkey agar (Hi media lab):

Fifty two grams of the media were dissolved in 1 litre of distilled water by boiling. The pH was adjusted to 7.4, after which the medium was sterilized by autoclaving at 121 °C for 15 minutes, cooled to 55 °C and distributed into sterile Petri dishes 20 ml in each.

2.1.1.1.4 Ammonium Salt Sugars (ASS) (Hi media lab):

This medium consisted of ammonium phosphate (1g), potassium chloride (0.2g), magnesium sulphate (0.2g), yeast extract (0.2g), agar (20g) and bromocresol purple, 0.2% aqueous solution (4ml). It was prepared according to Barrow and Feltham (1993) by adding the solids to 1000 ml
distilled water, dissolved completely by boiling and sterilized at 115 °C for 20 minutes. The medium was allowed to cool to about 55 °C and the appropriate sugar was added as a sterile solution to give a final concentration of 1%, the medium mixed and distributed aseptically into sterile tubes.

2.1.1.1.5 Simmons Citrate Agar (Oxoid):

Twenty three grams of powder were dissolved in 1000 ml distilled water by boiling. The pH was adjusted to 7.0, and the medium was sterilized by autoclaving at 121 °C for 15 minutes and distributed into sterile screw-capped bottles and allowed to solidify in slope position.

2.1.1.1.6 Urea Agar (Oxoid):

The medium was prepared by dissolving 2.4 grams of the powder in 95 ml distilled water by boiling. After sterilization by autoclaving at 115 °C for 20 minutes the base medium was cooled to 50 °C and aseptically 5 ml of sterile 40% urea solution was added, distributed into screw-capped bottles 10 ml each and then was allowed to set in a sloped position.

2.1.1.1.7 Kligler Iron Agar (KIA) (Hi media lab):

Fiftyfive grams of the powder were dissolved in 1000 ml distilled water by boiling. It was cooled to 50-55 °C, distributed into tubes, sterilized by autoclaving at 121°C for 15 minutes and allowed to solidify in a sloped position.

2.1.1.1.8 Starch Agar:

Ten grams of potato starch were triturated with 50 ml distilled water and then added to 100 ml molten nutrient agar. The mixture was sterilized by autoclaving at 121 °C for 15 minutes and distributed into sterilized Petri dishes.
2.1.1.9 **Diagnostic Sensitivity Test Agar (D.S.T.A) (Oxoid):**

The medium was prepared as instructed by the manufacturer. Forty grams were dissolved in 1000 ml of distilled water by boiling and autoclaved at 121°C for 15 minutes. The pH was adjusted to 7.4 and then distributed into sterile Petri dishes.

2.1.2 **Semi – Solid Media:**

2.1.2.1 **Motility medium (Oxoid):**

Thirteen grams of dehydrated nutrient broth were added to 4 grams of agar and dissolved in 1 litre of distilled water by boiling and the pH was adjusted to 7.4. The medium was distributed in 5ml amounts in test tubes containing Craigie-tubes and sterilized by autoclaving at 121°C for 15 minutes.

2.1.2.2 **Hugh and Leifson,s (O. F) medium (Oxoid):**

The medium was prepared by dissolving 10.3 grams of solids in 1 litre of distilled water by heating, and the pH was adjusted to 7.1. Filtered bromothymol blue (0.2% aqueous solution) was added and sterilized at 115°C for 20 minutes. Sterile solution of glucose was added aseptically to give a final concentration 1%, mixed and distributed aseptically into sterile tubes.

2.1.3 **Liquid Media:**

2.1.3.1 **Nutrient broth (Hi media lab):**

This medium was prepared by dissolving 13 g of the medium in 1 litre of distilled water. The pH was adjusted to 7.4 distributed into screw-capped bottles 5 ml each and sterilized at 121°C for 15 minutes.
2.1.3.2 Nitrate broth:

One gram of potassium nitrate was dissolved in 1 litre of nutrient broth, distributed into tubes and sterilized by autoclaving at 115 °C for 25 minutes.

2.1.3.3 Glucose phosphate broth (V.P Medium):

Five grams of peptone and 5 g of potassium phosphate were dissolved in 1 litre of distilled water by steaming. The pH was adjusted to 7.5 after that 5 g glucose were added and mixed. The medium was distributed into test tubes 5 ml in each and sterilized by autoclaving at 110 °C for 10 minutes.

2.1.3.4 Peptone water sugars:

Nine hundred ml of peptone water was prepared and pH was adjusted to 7.1-7.3 before 10 ml of Andrade’s indicator was added. Ten grams of the appropriate sugar were added to the mixture, distributed into tubes 5 ml in each one with inverted Durham’s tube and were sterilized by autoclaving at 110 °C for 10 minutes.

2.2 Biological Materials:

2.2.1 Sheep blood:

Sterile defibrinated sheep blood used for preparation of blood agar was obtained by vein puncture of jugular vein of donor sheep.

2.2.2 Human plasma:

This was used for the detection of coagulase production by Staphylococci.

2.3 Reagents:

All described below reagents were prepared according to Barrow and Feltham (1993).
2.3.1 Physiological saline:

This was prepared by dissolving 8.5 g of sodium chloride in 1000 ml distilled water.

2.3.2 Hydrogen peroxide:

Hydrogen peroxide was prepared as 3% aqueous solution and used for catalase test.

2.3.3 Oxidase reagent:

Tetra methyl p-phenylene diamine dihydrochloride was prepared as 1% aqueous solution and used for oxidase test.

2.3.4 Nitrate test reagent:

This reagent is composed of two types of solution:

Solution A: Sulphanilic acid 0.33% in 5N-acetic acid dissolved by gentle heat.

Solution B: Dimethyl-naphthylamine 0.6% in 5N-acetic acid.

The complete reagent was used to detect nitrate reduction.

2.3.5 Kovac’s reagent:

This reagent composed of paradimethylamino benzaldehyde, amyl alcohol and concentrated hydrochloric acid. After preparation the reagent was stored in the refrigerator at 4 °C.

2.3.6 Potassium hydroxide:

Potassium hydroxide was prepared as 40% solution and used for Voges- Proskaur (V.P) test.

2.3.7 Alpha naphthol solution:

It was prepared as 1% aqueous solution and also used for V.P test.

2.4 Indicators:

2.4.1 Andrade’s indicator:

This was prepared by dissolving 5 g of acid fuchsin in 1 litre of distilled
water, and then 150 ml of alkali solution (NaOH) was added. It was used in peptone sugar medium.

2.4.2 Bromothymol blue solution:

This was prepared as 0.2% w/v by dissolving 0.2 g of bromothymol blue powder in 100 ml distilled water. It was used for oxidation fermentation (O.F) test.

2.4.3 Bromocresol purple solution:

Bromocresol purple solution was prepared as 0.2% solution; it was used in ammonium salt sugars (ASS).

2.5 Methods:

2.5.1 Sterilization:

Screw-capped bottles, rubber caps, media solutions, normal saline…etc. was sterilized in autoclave at 121 0C for 15 minutes. 110 0C for 10 minutes was used to sterilize sugar media.

Glassware such as Petri dishes, tubes, flasks and glass rods were sterilized in hot air oven at 160 0C for one hour.

Solution of 70% alcohol and phenolic disinfectant were used for bench disinfection.

2.5.2 Collection of samples:

Seventy two coins of different denominations (10, 20, and 50 SD) were collected from the general public at four different sites: groceries, cafeterias, bus drivers and elementary schools. The coins were collected in sterile screw-capped bottles and transported to the laboratory.

2.5.3 Cultural procedures:

2.5.3.1 Primary isolation:

Coins were placed into sterile screw-capped bottles of nutrient broth,
shaked for 10 minutes, and then were removed by sterile forceps. The nutrient broth was incubated at 37°C for 24 h. Using a sterile loop, the broth culture was streaked on the blood and MacConky agars, incubated at 37°C for 24 h.

2.5.3.2 Examination of cultures:
Cultured plates were examined for detection of growth, colonial morphology, pigmentation and haemolysis.

2.5.3.3 Purification of cultures:
The primary isolates were repeatedly subcultured on nutrient agar until pure colonies were obtained for further examination.

2.5.4 Identification of isolates:
Isolates were identified using conventional biochemical methods according to the procedure described by Barrow and Feltham (1993).

2.5.4.1 Primary identification:

2.5.4.1.1 Colonial morphology:
Colony characters on solid media such as shape, size, surface, edge and chromogenesis were recorded.

2.5.4.1.2 Gram’s staining and microscopic examination:
Smears were prepared by spreading small inoculums of purified colonies of cultures in a drop of sterile normal saline on clean slides. These were dried in air, fixed by heating, and then stained with the Gram method (Barrow and Feltham, 1993). The smears were examined microscopically and the Gram reaction, shape, arrangement of cells and the presence of endospores were recorded.

2.5.4.1.3 Catalase test:
This test is used to identify bacteria which produce the enzyme catalase (Cheesbrough, 1987). One to two colonies of tested organism was
placed on a drop of 3% hydrogen peroxide on a clean slide using a wooden stick. Production of air bubbles indicated a positive result.

2.5.4.1.4. Oxidase test:

The tested organism was picked using sterile bent glass rod and rubbed on a filter paper saturated with oxidase reagent. The development of dark purple color within 10 seconds indicated a positive result.

2.5.4.1.5. Oxidation-Fermentation test (O.F):

The test was used to differentiate between oxidative and fermentative bacteria. Two tubes of Hugh and Leifsons medium were inoculated with tested organism; one of them was covered with layer of sterile paraffin oil. All tubes were incubated at 37°C and examined daily for seven days. Yellow colour in open tube only indicated oxidation of glucose, yellow colour in both tubes showed fermentation reaction and blue or green colour in open tube and green colour in sealed tube indicated production of alkali.

2.5.4.1.6 Motility test:

The Craigie tube method was used to detect the motility of isolated bacteria. The growth of the tested organism outside the Craigie tube indicated that organism was motile.

2.5.4.1.7 Sugar fermentation test:

The sugar media were inoculated with the tested organism. They were incubated at 37°C and examined daily for up to seven days. Acid production was indicated by the development of pink color in the medium and gas production was indicated by an empty space in the Durham’s tube.

2.5.4.2 Secondary identification:

2.5.4.2.1 Nitrate reduction test:

The tested organism was inoculated in nitrate broth and incubated at 37°C for two days. One ml of nitrate reagent A was added followed by 1ml
of reagent B. Development of a deep red color indicated a positive reaction. If the result was negative Zinc powder was added and the red color indicated that nitrate was present but the tested organism did not reduce it.

2.5.4.2.2 Indole test:

The tested organism was inoculated in peptone water then incubated at 37 °C for 48 h. Two to three drops of Kovac’s reagent were added to the culture and shaked well. Production of a pink color on the upper layer of the reagent was considered indole positive.

2.5.4.2.3 Citrate utilization test:

Simmons citrate medium was inoculated with the tested organism, incubated at 37 °C and examined daily for up to seven days. The development of a blue color in the medium was considered as a positive result.

2.5.4.2.4 Urease test:

The tested organisms were inoculated onto a slope of urea agar medium, and incubated at 37 °C for up to 5 days. The change of color of the medium to red or pink color indicated a positive result.

2.5.4.2.5 Hydrogen sulphide production:

The tested organism was inoculated on slope of Kligler Iron Agar (KIA) by stabbing the butt and streaking the slope, incubated at 37 °C and examined daily for up to 7 days. Blacking of the butt was considered positive for hydrogen sulphide production.

2.5.4.2.6 Voges-Proskauer test (V.P):

This test was performed to detect the production of acetyl methyl carbinol. Glucose phosphate broth was inoculated with tested organism and incubated at 37 °C for 48 h. 0.6 ml of alpha-naphthol solution and 0.2 ml of 40% potassium hydroxide solution were added to 1 ml of culture, mixed
well and examined after half an hour. A bright red color indicated a positive result.

2.5.4.2.7 Ammonium salt sugar test:

Ammonium salt sugar media was inoculated with the test bacteria and incubated at 37 °C for 7 days. The change of color of the media to yellow indicated a positive reaction.

2.5.4.2.8 Starch hydrolysis:

Starch agar plate was streaked with test culture and incubated at 37 °C for 24 h. The plate was then flooded with lugol’s iodine solution. Hydrolysis of starch was indicated by clear colorless zones around colonies. Starch which had not been hydrolyzed turned blue.

2.5.4.2.9 Tube coagulase test:

To 0.5 ml of 1/10 dilution of human plasma in saline in small coagulase tubes, 0.1 ml of an 18-24 hours broth culture of test bacteria was added. The tubes were incubated at 37 °C and examined after 1, 3 and 6 hours for coagulation. Negative tubes were left at room temperature overnight and re-examined.

2.5.4.2.10 Antibiotic sensitivity test:

Sensitivity of 34 out of 84 isolates of genus Bacillus to 8 antimicrobial agents was determined by standard disc diffusion method (Buxton and Fraser, 1977). The antimicrobial agents were commercially obtained. A fresh overnight nutrient broth culture of the test isolates was inoculated onto D.S.T medium which was completely covered using sterile swab and left for 5-15 minutes. Sterile forceps were used to apply the discs over the surface, pressed gently to ensure full contact with the surface of the culture medium and the plates were incubated at 37°C for 24 h. The diameter
of the zone of inhibition to each antibiotic against each test isolate was measured in mm.

The antimicrobial agents examined were: penicillin (1i.u.), ampicillin (10mg), cloxacillin (5mg), chloramphenicol (30mg), erythromycin (15mg), gentamycin (10mg), streptomycin (10mg), and tetracycline (30mg).
CHAPTER THREE

RESULTS

3.1 Survey:

This study investigated the extent of the bacterial contamination of coins randomly collected from groceries, cafeterias, bus drivers and elementary schools’ pupils.

Preliminary results indicated that coins were potentially contaminated with different types of bacteria; 71 out of 72 sampled coins (99%) revealed positive bacterial growth with a total number of 91 isolates. According to the Gram reaction, 84 isolates (92%) were Gram positive bacteria and only 7 isolates (8%) were Gram negative bacteria.

Isolated bacteria belonged to six genera which included *Bacillus spp* (90.1%), *Pseudomonas* (3.3%), *Escherichia*, *Staphylococcus* (2.2%), *Citrobacter* and *Klebsiela* (1.1%) (Table: 1).

The highest bacterial isolation rates were detected in coins sampled from preliminary schools pupils and bus drivers and the least was recorded in samples from cafeterias. (Table: 2 and 3).

All coin denominations under study were exhibited the same rate of bacterial isolates (Table: 4).

3.2 Biochemical properties:

Using conventional biochemical methods, 10 different bacterial species of the genus *Bacillus* (Table: 5) and one species of staphylococcus (Table: 6) were identified where as (Table: 7) showed the biochemical tests of Gram negative bacteria.
3.3 Antibiotic susceptibility:

The antibiotic sensitivity was conducted for 34 isolates of genus *Bacillus* against 8 antimicrobial agents by using the standard disc diffusion method. The sensitivity of all tested isolates to the different antimicrobial agents is shown in table 8.

Based on Arcomex interpretive standards for Gram-positive bacteria, all tested Bacillus isolates except 2 out of 5 *B. licheniformis* were completely susceptible to most of the selected antibiotics: tetracycline, streptomycin, chloramphenicol, erythromycin and gentamycin. Resistance to penicillin was observed for all isolates where as resistance to ampicillin and cloxacillin were only showed for *B. cereus* group.
Table (1): The type, number and percentage of bacterial isolates

<table>
<thead>
<tr>
<th>Types of isolates</th>
<th>Number of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. amyloliquefaciens</td>
<td>9</td>
<td>9.9%</td>
</tr>
<tr>
<td>B. cereus</td>
<td>7</td>
<td>7.7%</td>
</tr>
<tr>
<td>B. firmus</td>
<td>1</td>
<td>1.1%</td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>20</td>
<td>21.9%</td>
</tr>
<tr>
<td>B. mycoides</td>
<td>7</td>
<td>7.7%</td>
</tr>
<tr>
<td>B. pantothenicus</td>
<td>9</td>
<td>9.9%</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>8</td>
<td>8.8%</td>
</tr>
<tr>
<td>B. sphaericus</td>
<td>6</td>
<td>6.6%</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>11</td>
<td>12.1%</td>
</tr>
<tr>
<td>B. thuringiensis</td>
<td>4</td>
<td>4.4%</td>
</tr>
<tr>
<td>Staph. epidermedis</td>
<td>2</td>
<td>2.2%</td>
</tr>
<tr>
<td>Citro. freundii</td>
<td>1</td>
<td>1.1%</td>
</tr>
<tr>
<td>E. coli</td>
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<td>2.2%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
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<td>1.1%</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
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<td>3.3%</td>
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<tr>
<td>Total number of isolates</td>
<td>91</td>
<td>100%</td>
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</table>
Table (2): Number and percentage of bacterial isolates in each source

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of isolates</th>
<th>percentage</th>
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<tr>
<td>Groceries</td>
<td>19</td>
<td>20.9%</td>
</tr>
<tr>
<td>Cafeterias</td>
<td>18</td>
<td>19.8%</td>
</tr>
<tr>
<td>Bus drivers</td>
<td>24</td>
<td>26.4%</td>
</tr>
<tr>
<td>Elementary schools</td>
<td>30</td>
<td>32.9%</td>
</tr>
<tr>
<td>Total number of isolates</td>
<td>91</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (3): Types of bacterial genera isolated from different sources

<table>
<thead>
<tr>
<th>Types of isolates</th>
<th>Groceries</th>
<th>Cafeterias</th>
<th>Bus drivers</th>
<th>Elementary schools</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus spp</em></td>
<td>17</td>
<td>18</td>
<td>20</td>
<td>27</td>
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<tr>
<td><em>Pseudomonas</em></td>
<td>2</td>
<td>___</td>
<td>1</td>
<td>___</td>
</tr>
<tr>
<td><em>Staph. epidermedis</em></td>
<td>___</td>
<td>___</td>
<td>2</td>
<td>___</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>___</td>
<td>___</td>
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<td>___</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>2</td>
</tr>
<tr>
<td><em>Citro. freundii</em></td>
<td>___</td>
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<td>___</td>
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Table (4): number and percentage of bacteria isolated from different coins denomination

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<tr>
<th>Denomination</th>
<th>Number of isolates</th>
<th>Percentage</th>
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<tr>
<td>10 SD</td>
<td>28</td>
<td>30.8%</td>
</tr>
<tr>
<td>20 SD</td>
<td>33</td>
<td>36.3%</td>
</tr>
<tr>
<td>50 SD</td>
<td>30</td>
<td>32.9%</td>
</tr>
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</table>

SD: Sudanese Dinars
### Table (5): Biochemical reactions of *Bacillus* species

<table>
<thead>
<tr>
<th>Character</th>
<th><em>B. cereus</em></th>
<th><em>B. mycoides</em></th>
<th><em>B. thuringiensis</em></th>
<th><em>B. firmus</em></th>
<th><em>B. pumilus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O.F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Citrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>V.P</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
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<td>+</td>
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<td>-</td>
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<tr>
<td>Starch hydrolysis</td>
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<td>-</td>
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<tr>
<td>Acid from ASS</td>
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<td></td>
</tr>
<tr>
<td>- Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- Galactose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>- Raffinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>- Salicin</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>- Xylose</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>+</td>
</tr>
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</table>

F: Fermentative +: Positive -: Negative
<table>
<thead>
<tr>
<th>Character</th>
<th><em>B. subtilis</em></th>
<th><em>B. licheniformis</em></th>
<th><em>B. amylobiiquefaciens</em></th>
<th><em>B. pantothenticus</em></th>
<th><em>B. sphaericus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
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<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O.F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
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<tr>
<td>Indole</td>
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<tr>
<td>V.P</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Starch hydrolysis</td>
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<td></td>
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<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>Galactose</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Raffinose</td>
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<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
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<tr>
<td>Xylose</td>
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Table (6): Biochemical reactions of *Staphylococcus epidermidis*

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<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>O.F</td>
<td>F</td>
</tr>
<tr>
<td>Coagulase</td>
<td>-</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
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<tr>
<td>Urease</td>
<td>+</td>
</tr>
<tr>
<td>V.P</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate production</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
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</tr>
<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
</tr>
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<td>Trehalose</td>
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Table (7): Biochemical reactions of Gram-negative bacteria

<table>
<thead>
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<th>Character</th>
<th>E. coli</th>
<th>Cit. freundii</th>
<th>k. pneumoniae</th>
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</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
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<td>Motility</td>
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<tr>
<td>Glucose</td>
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<td>+</td>
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</tr>
<tr>
<td>O.F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Citrate</td>
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<td>+</td>
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<tr>
<td>Urease</td>
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<td>+</td>
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<tr>
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<td>- Slope</td>
<td>Y</td>
<td>R</td>
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<tr>
<td>- Butt</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>- H2 S</td>
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<td>- Gas</td>
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Table (8): Antimicrobial activity of eight antibiotics against Bacillus species isolated from coins

<table>
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<td>5</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
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<tr>
<td>B. mycoides</td>
<td>5</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
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</tr>
<tr>
<td>B. thuringiensis</td>
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<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
<td>B. pumilus</td>
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<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<td>B. subtilis</td>
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<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>B. sphaericus</td>
<td>5</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>B. licheniformis</td>
<td>5</td>
<td>R</td>
<td>1R</td>
<td>3S</td>
<td>1I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

R: Resistant  S: Sensitive  I: Intermediate
Pen: Penicillin  Str: Streptomycin  Tet: Tetracycline  Amp: Ampicillin
Chl: Chloramphenicol  Clx: cloxacillin  Ery: Erythromycin
Gen: Gentamycin
Fig.(1): *Bacillus cereus* on nutrient agar
Fig.(2): *Bacillus thuringiensis* on nutrient agar
Fig.(3): *Bacillus mycoides* on nutrient agar
Fig. (4): Antibiotic sensitivity of *Bacillus cereus*

Fig. (5): Antibiotic sensitivity of *Bacillus subtilis*
CHAPTER FOUR
DISCUSSION

There is a worldwide serious concern that money has direct or indirect role in spreading disease since paper notes and coins offer ample surface to harbor bacteria and other microorganisms.

It is well documented that bacteria can spread from person to person via fomites. The present study was designed to investigate the extent of bacterial contamination in coins rather than to study the role that coins play in transmission of diseases.

The coins collected in the current study provided a positive indications of bacterial contamination. The bacterial rates of isolation was extremely high (99%). These results revealed that the rate of contamination of Sudanese coins is higher than those reported elsewhere (Abrams and Waterman, 1972; Gadsby, 1998 and Pachter et al., 1997).

The study identified the bacteria contaminating Sudanese coins as belonging to six genera (namely: Bacillus, Staphylococcus, Pseudomonas, Klebsiella, Escherichia and Citrobacter). Similar findings were reported in previous studies except the last genus which was not reported in the available literature concerning bacteria contaminating coins. Moore et al (2005) found that the most dominant bacteria isolated from coins were Bacillus spp (40%) followed by Staph. spp (28%).

The highly resistant spore forming Bacillus have also been isolated by Pachter (1997). In this study most of the bacterial isolates were Bacillus spp and constituted 90.1% of the total isolates, and this reflected their wide
spread and survival in the environment due to their ability to form endospores.

With the exception of *Bacillus cereus* and *Bacillus anthracis*, *Bacillus* species are generally perceived to be inconsequential. However, the relevance of other *Bacillus* species as food poisoning organisms and etiological agents in nongastrointestinal infections is being increasingly recognized. A recent study (Rowan *et al*., 2003) constituted the first demonstration that *Bacillus* species was found associated with serious nongastrointestinal infections in animals may harbor and express diarrheagenic enterotoxins traditionally linked to toxigenic *B. cereus*.

In the present study 2.2% of isolated bacteria was *E. coli* which form apart of the normal microbial flora of the intestinal tract of human and animal. This organism was also isolated by Abrams and Waterman, (1972); Gadsby (1998). *E. coli* is a Gram negative enteric organism and its presence on coins is indicative of faecal contamination, cross contamination with raw products or poor personal hygiene.

3.3% and 1.1% of the bacterial isolates were *Pseudomonas* and *K. pneumoniae* respectively, both of them were also isolated by Abrams and Waterman (1972). *K. pneumoniae* is a virulent organism that is isolated from the respiratory tract of man and animal (Wilson and Miles1957). It causes both community and hospital acquired infections (Pope *et al*. 2002). Its presence on coins might be reflecting a possible contamination with nasal discharges.

*Pseudomonas* which is commonly found in water, soil, and vegetation was detected in coins in the present investigation as a source of pollution for coins. Several types of *Pseudomonas* can cause infection, especially *Pseudomonas aeruginosa*. *Pseudomonas* can cause minor skin infection or
serious, life-threatening illness. The most serious infections from Pseudomonas develop in debilitated and hospitalized people, particularly those with a weakened immune system. It can infect the blood, skin, bones, ears, eyes, urinary tract, heart valves, and lungs.

The higher rates of bacterial contamination in coins collected from elementary schools pupils and bus drivers (32.9% and 26.4% respectively) may be attributed to the fact that there are extensive exchanges of coins within these two groups leading to eventual build up of contaminants on coin surfaces.

Coins that collected from bus drivers might be potentially hazardous to the public health putting in considerations the habit of bus conductors of exchange paper notes for coins from beggars, some of them are carriers of chronic potentially pathogenic bacteria. The isolation rates of contaminating bacteria in coins in the other investigated groups were less, 20.9% for groceries and 19.8% for cafeterias. This finding is in agreement with the same group recorded by Suleiman (2006) in paper notes.

Little interest was shown in antimicrobial susceptibility of Bacillus species until recently and this was due to the low recognition of the ability of Bacillus species other than B. anthracis to cause infections.

The antibiotic susceptibility profile of Bacillus species investigated in this study to tetracycline, gentamycin, chloramphenicol and erythromycin confirmed previous reports in the literature. Coonrod et al. (1971) reported that these antimicrobials inhibited almost all Bacillus strains regardless of the species. Luna et al. (2007) reported that all B. cereus group were
susceptible to chloramphenicol, gentamycin, tetracycline and streptomycin. Reva et al. (1995) revealed that all tested strains of B. cereus, B. subtilis, B. licheniformis and B. pumilus were susceptible to chloramphenicol, gentamycin, tetracycline, erythromycin and streptomycin.

In relation to penicillin sensitivity; all Bacillus isolates were resistant, in part this is in agreement with the high proportion of resistant isolates for B. cereus group conducted by Peter et al. (2004) and in contrast with findings of Weber et al. (1988) who reported many non- B. cereus strains such as B. licheniformis, B. subtilis and B. pumilus were susceptible to penicillin. Resistant Bacillus spp. may act as a reservoir of resistant genes that might be transferred to potentially pathogenic bacteria.
CONCLUSION

- This study concluded that coins from all sources investigated were contaminated with bacteria in particular *Bacillus* spp including *B. subtilis*, *B. pumilus*, *B. cereus*, *B. licheniformis*, *B. mycoides* and others. On the other hand Gram negative bacteria were also found but as lesser contaminants, *Pseudomonas spp* and *E. coli*.
- Coins obtained from elementary schools showed the highest rate of bacterial contamination followed by bus drivers.
- All coins denominations showed the same rate of contamination.
RECOMMENDATIONS

In view of the results of the present investigation, the following is recommended:

- It is advisable to isolate the selling and money-collecting activities to prevent the potential of cross contamination.
- Regular disinfection of currency deposited in banks by ultraviolet light or formalin vapors.
- Improvement of personal hygiene by always washing hands with soap to limit infection.
- The possibility of getting infected by improper handling of currency is a potential hazard especially among children, who are known to swallow coins accidentally or just keep them in mouth while playing. Therefore, health awareness must be primarily directed towards children in the school itself through cartoons, stickers and other audio visual aids.
- As bacteria found in coins relative to other researches on paper notes were far less, it is recommended that most used denomination of paper notes as in 100 SD and 200 SD should be replaced by coins.
- Further studies would be required to provide complete picture about the extent of bacterial contamination of coins as well as its role in spreading diseases.
REFERENCES


