بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

In Vitro Assessment of AntibacterialActivity of *lactobacillus* species Against Selected Bacteria Isolated from Sudanese Patients Suffering from Urinary Infection

التقيم المعملي لنشاط البكتريا الملبنه ضد بكتريا مختارة معزولة من مرضى سودانيين يعانون من التهاب المسالك البولية

A thesis Submitted in partial Fulfillment for the Requirement of MSc in Medical laboratory Sciences (Microbiology)

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قال تعالى :

﴿ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ ﴾

صدق الله العظيم

سورة يوسف الآية 76

Dedication

To my Parents

To my husband , my baby

To my Teachers

To my Dear and Best Friend

(Maka)

To my Colleagues

Acknowledgments

Firstly I want to give my greatest thanks to Allah for his gifts which never ends.

I would like to express my gratefulness and profound gratitude to

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A special feeling of gratitude to my loving parents, and my husband ,my baby with love and appreciations whose words of encouragement and push for tenacity ring in my ears .

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LIST OF ABBREVIATIONS

DNA	Deoxyribo nucleic acid
MHA	Muller-Hinton agar
KIA	Kliger Iron Agar
DW	Distilled water
UTI	Urinary tract infection
HCl	Hydrochloric acid
MRS	deMan, Rogosa and Sharpe media
H2O2	Hydrogen peroxide
SPP	Species
MSA	Manitol salt agar
H2S2	hydrogen sulphide

Abstract

This was cross-sectional study had been carried out to isolate, identify and testing antimicrobial activity of UTI among 30 urinary tract infection patients attended, National Public Health laboratory during the period from April to May 2016 to determine antibacterial activity of *lactobacillus ssp*. against urinary tract infection causative bacteria isolate from Khartoum state. A total of 30 urine sample were collected from patient with urinary tract infection. These specimens were cultured onto Blood agar media and MacConkey's agar media incubate aerobically at 37^0C for 24 hours .The isolates were identified using conventional method, 100% sample showed significant bacterial growth. The percentage of these isolated uro-pathogen, *E. coli* was found to be the most frequently isolated pathogen 9 (30%) followed by *K. pneumoniae*8 (26.7%), *Enterococcus faecalis*6 (20%), *Staphylococcus aureus*5 (16.7%), *Psudomonasaeruginosa*1 (3.3%), *Proteus mirabilis* 1 (3.3%).When sensitivity testing was performed using stander disc diffusion method,Most of isolates was resistant to co-trimoxazole 24 (80%), norfloxacin 12 (40%), ciprofloxacin and nitrofurantoin were similar 10 (33.3%), Ninety (90%) specimens were sensitive to chloramphenicol and 10% isolated bacteria were resistant to it. For probiotics the pattern of sensitive was performed using ager well diffusion method showing following result *Lactobacillus .acidipiscis* 1 (3.3%) *Lactobacillus rhamanosus* 2 (6.9%), *Lactobacillus.lactissub.sp. hodinae* was more sensitive 4 (13.3%).

probiotic*Lactobacillus.lactissub.sp. hodinae* capable of producing antimicrobial activity against urinary tract infection bacteria and higher antibiotic produce sensitivity chloramphenicol.

ملخص الدراسة

هذه الدراسة المسحية أجريت لعزل والتعرف واختبار نشاط المضادات الحيوية للبكتيريا المسببة للاتهاب المسالك البولية لدى مرضى التهاب المسالك البولية. حيث جمعت30 عينه بول من المعمل القومي للصحة العامة في ولاية الخرطوم خلال الفترة من أبريل إلى مايو 2016 لتحديد النشاط البكتيري للبكتيريا اللاكتوباسلس ضد البكتيريا المسببة للاتهاب المسالك البولية. وأظهرت النتيجة ان ال30 عينة التي تم زراعتها في مادة الماكونكي المثخنة ومادة الدم المثخنة (في درجه حراره 37 درجه مئوية لمدة 24 ساعة) كانت نسبة البكتيريا المعزولة فيها (100%). تم العثور على الاشريكيه القولونية بنسبة 30% (9)، وتليه الكليبسيليه الرئوية 7.67% (8)، المكورة المعوية الرازيه 20%(6) مكورات العنقودية الذهبية 16.7%(5) الزائفة الزنجارية 3.3%(1)، المتقلبة الرائعة 3.3%(1)، وعندما تم إجراء واختبار الحساسية بطريقة الانتشار الطبقي القياسي، كانت معظم البكتيريا المعزولة مقاومة للكوتريموكسازول 24 (80%)، النورفلوكساسين 12 (40%)، سيبروفلوكساسين والنتروفورانتوين 10 (33.3%)، الالكلورامفينيكول 10%.

البكتيريا المتعايشة تكافليا تم اختيار فعاليتها ضد الميكروبات الاخرى بطريقة الانتشار الطبقي القياسي كانت فعاليتها كتالي : اللاكتوباسلس اسيدكيس 1(3.3%), اللاكتوباسلس رحمانوسس 2(6.9%)، للاكتوباسلس لاكتك هوردينى كانت أكثر حساسية النتيجة 4 (13.3%). البكتيريا للاكتوباسلس لاكتك هوردينى لها القدرة على انتاج مواد مضادة للبكتيريا المسببة للاتهاب المسالك البولية و الالكلورامفينيكول اكثر حساسية للبكتيريا المسببة للاتهاب المسالك البولية.

CHPTER ONE

INTRODUCTION

1.1. Background

UTI is frequently accompanied with urologic abnormalities and can cause end stage renal failure or hypertension if continued. The most common bacteria cause infection is *Escherichia coli*, and higher risk factors groups include female anatomy, sexual intercourse, diabetes, obesity, and family history. Resist of drugs cause due to using antibiotics for the purpose of controlling uro-pathogens produces even super bacteria which are multi-resistant and cause intractable infections that are difficult to treat, due to use of antimicrobial agents is not only select resistance bacteria but it can disturb the balance of body by killing friendly bacteria, when this happen bacteria and yeast can move in and flourish leading to uro-genial tract infection (In Seok*et al*., 2009; Diaz *et al*., 1993).

Multi-drug resistant bacteria are the cause of numerous clinical problems throughout the world. Increased resistance among pathogens causing nosocomial and community acquired infections is known to be related to the widespread utilization of antibiotics (Boehm*et al*., 1997).

Infectious diseases caused by resistant microorganisms are accountable for increased health costs as well as high morbidity and mortality, especially in developing countries (Jarvis and Martone, 1992; Vosahlikova*et al*., 2007).

Probiotics are microorganisms that are believed to provide health benefits when consumed .In recent years, there has been increased focus on the use of probiotic such as *Lactobacillus spp* for prophylaxis and treatment of urinary tract infection (Hill *et al.*, 2014;Uehara*et al*., 2006).

Lactobacilli are an important part of the normal flora commonly found in the mouth, gastrointestinal tract and female genitourinary tract, they also protect the female urogenital tract from pathogen colonization by competitive exclusion of pathogens from the cell surface (Dunne *et al*, 2001).

Lactobacilli produce many different bacteriocins of similar activity and are usually predominant species in the vaginas of healthy women. It play an important role in maintaining vaginal health and produce lactic acid and hydrogen peroxide, which can prevent the overgrowth of other microorganisms in the vagina including *Escherichia coli* and *Gardnerellavaginalis* (Eschenbach*et al*., 2009).

The viable *Lactobacilli* can inhibit food-borne and enteric pathogenic microorganisms by producing lactic acid and other antimicrobial substances, yogurt and milk have been considered to be healthy probiotic diets (McGroaty and Reid, 2006).

1.2. Rationale

UTI are considered to be the most common bacterial infection. According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations and estimated annual cost of community-acquired UTI is significant, at approximately $1.6 (Marelli*et al*., 2004).

In Sudan, to assess the production of antimicrobial compounds by the selected lactic acid bacteria isolated from some Sudanese fermented foods against three pathogens including Gram-positive, *Staphylococcus aureus*ATCC 43306 and Gram-negative *E. coli* ATCC 25922 and *Salmonella typhai.* Antimicrobial effects of lactic acid bacteria isolated from gariss all the 19 strains of LAB isolated from gariss samples (Camel milk) were found to show no

antimicrobial effect on both *Staphylococcus aureus*ATCC 25923 and *E. coli* ATCC25922. (Hamza *et al*., 2015; Ahmed *et al*., 2015)

Recent reports have documented the role of exogenous Lactobacilli in the prevention and treatment of some infections (Marelli*et al*., 2004).

We need to force on researchers by the longtime in developing alternative therapy to antimicrobial agents such the application of probiotics to cure urinary tract infection.

1.3 Objectives

1.3.1. General objective

To study antibacterial activity of *Lactobacillus species* against bacterial isolates of urinary tract infected patients.

1.3.2 Specific objectives

1. To isolate and identify the most common bacteria that cause urinary tract infection.

2. To identify the most commonly resistant bacteria that cause UTI and to determine their antimicrobial susceptibility testing.

3. To evaluate the antibacterial activity of *Lactobacillus species* against urinary tract infection bacteria.

CHPTER TWO

2. LITERATURE REVIEW

2.1 History of *Lactobacillus*

For centuries lactic acid bacteria have been used in the preservation of food for human consumption. In 1906, Nobel piece winner Dr. Illya Metchnikoff attributed the long life span of the Balkan people to the ingestion of large quantities of fermented foods—foods rich in lactobacilli and other lactic acid producing organisms which inhibit pathogens and detoxify one's system. In the late 1950s, Dr. KhemShahani began studying *L. acidophilus* and other lactic acid producing cultures to determine their dietary effect on humans (PBworks, 2008).

2.2 *Lactobacillus*

Lactobacillus isa genus of Gram-positive, facultative anaerobic or microerophilic rode shape non-spore forming bacteria (Makarova*et al*, 2006).

2.3Habitat

They are a major part of the lactic acid bacteria group in humans they constitute a significant component of the microbiota at a number of body sites. In women, *Lactobacillus* species are normally a part of the vaginal microbiota (Mariya*et al*., 2015).

2.4 Taxonomy of *Lactobacillus*

According to a metabolism, *Lactobacillus* species can be divided into :three groups

:Obligatory homofermentative (group I) including

L. acidophilus, L. delbrueckii, L. helveticus, L. salivarius

:Facultativelyheterofermentative (group II) including

L. casei, L. curvatus, L. plantarum, L. sakei

:Obligatory heterofermentative (group III) including

L. brevis, L. buchneri, L. fermentum, L. reuteri (Etsuzo*et al*., 1986)

2.5 Role of Lactobacillus to prevent UTI

Urinary tract infection remains a major medical problem in terms of number of human afflicted each year. About 5% of women each year suffer with the problem of painful urination and frequently UTI can be treated with broad-spectrum antibiotics. The main problem of antibiotic is the emergence of rapid increase of drug resistance microbes has made therapy of UTI difficult. This resistance problem needs a re-mewed effort to search for new antimicrobial substances from various source like a probiotics (Selvamohan*et al.*, 2010).

Lactobacilli generally have a fastidious growth requirement they prefer an acidic environment and help create one by producing lactic and other acids. In general, *Lactobacilli* have not been associated with disease and for > 100 years have been regarded as nonpathogenic In .members of the intestinal and urogenital floras (Bibel *et al*., 988) presented study, in vitro antagonistic activity of *Lactobacillus plantarum* showed the greater inhibitory effect (20 mm) against *E. coli* while the least activity (9 mm) was found against *Streptococcus ssp*. The antagonism properties of *Lactobacillus ssp*. against urinary pathogens were investigated using well diffusion method. *L. acidophilus, L.plantarum* and *Lactobacillus casei* produced a greater inhibitory effect towards all five *E. coli* isolates. *Lactobacillus plantarum* showed highest inhibitory activity against *S.typhimurium* (3.75 mm inhibitory zone) while the least activity was demonstrated against *Vibrio cholerae*(0.90 mm inhibitory zone (Selvamohan*et et al.*,2010).

2.6 Antimicrobial susceptibility of lactobacilli

The antimicrobial susceptibility of lactobacilli has received only little attention, and clinical isolates have rarely been studied. Most of the studies have included only a limited number of *Lactobacillus* strains representing different species. For example, some species, such as *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus curvatus*, and *Lactobacillus fermentum*, are inherently resistant to glycopeptides, in contrast to the susceptible *Lactobacillus acidophilus* group and *Lactobacillus delbrueckii*. In previous study, survey of 89 patients with *Lactobacillus bacteremia*, the data suggested that appropriate antimicrobial therapy would be associated with reduced mortality (Salminen*et al*., 2006).

2.7 UTI

UTI is an infection in the urinary tract causes by microbes including bacteria, virus, and fungi. Bacteria one the most common causes of UTIs. The urinary tract including two kidney and two ureters, a bladder and a urethra Bacteruria is the multiplication of bacteria in urine with in renal tract a concentration of greater than 10^5 organism/ml (Irving *et al*., 2006).

Urinary tract infection are common infections increase with age and are more common in female. The most infection causes by *Escherichia coli*, and a minority caused by *Klebsiella* species, *Proteus* species, *E. faecalis*, *S. aureus*. Risk factor including structural abnormalities of urinary tract (Irving *et al.,* 2006).

2.8 Incidence

Women have a lifetime risk of UTI of 1 in 3, and men 1 in 20.It accounts for 5% of women with cystitis will have a recurrence and most are due to re-infection. UTI is rare in men aged 20-50 years and uncommon in young boys and elderly men (National Institute for Health Care, 2007).

2.9 Risk factors

There is evidence to suggest that deregulation of candidate genes in humans may predispose patients to recurrent UTI, diabetes is also a predisposing factor Urinary catheter, urological, diabetes and immunosuppression (Irving *et al.*, 2006; Gorter*et al.*, 2010).

2.9.1 in women

Atrophic urethritis and vaginitis (postmenopausal), abnormalities of urinary tracts (indwelling catheter, neuropathic bladder, vesico-ureteric reflux,outflow obstruction, anatomical anomalies), incomplete bladder emptying (dysfunctional urination), contraception-diaphragm, history of urinary tract surgery and immune compromise (schools *et al.*, 2015).

2.9.2 in men

Abnormalities of urinary tract function, incomplete bladder emptying (prostatic enlargement, chronic indwelling catheter), previous urinary tract surgery, immunocompromised state and anal intercourse (European Association of Urology 2013).

2.10 Symptoms of a urinary tract infection (UTI) may include:

Pain or burning when you urinate. An urge to urinate frequently but usually passing only small amounts of urine.

Urine that looks cloudy, is pink or red, or smells bad. Pain in the flank, which is felt just below the rib cage and above the waist on one or both sides of the back. Fever and chills. Nausea and vomiting. Some people have bacteria in their urinary tract without having any symptoms. This is called asymptomatic bacteriuria(Thomas *et al.*, 2010**).**

CHAPTER THREE

Materials and methods

3.1Methods

3.1.1 Study approach

Qualitative approach**.**

3.1.2 Study area and Study duration

Urine specimens were collected from patients attending National Public Health laboratory, Khartoum, Sudan. At the period from April to May 2016.

3.1.3 Study design

Cross-sectional, hospital based study.

3.1.4 Study population

This study focused on urinary tract infected patients in Khartoum State of different age

3.1.5 Inclusion criteria

Symptomatic bacteria and commonly isolated bacteria in UTI patients

3.1.6 Exclusion criteria

Asymptomatic bacteria and fastidious organisms were excluded

3.1.7 Sample size

Thirty samples were collected (n=30); 7 samples were males and 23 were females suffering from UTI. Their age ranged from 20 to 80 years.

3.1.8 Sampling technique

Five ml of urine samples were collected in clean, dry universal screw cape container.

3.1.9 Method of data collection

Data were collected through in-person interview with UTI patients. The interview instrument (Questionnaire) consists of 7 questions (Appendix I).

3.1.10 Culture of the specimens

A loop-full of well mixed urine was streaked under aseptic conditions on the surface of MacConkey'sagar (HiMedia, India) (Appendix II) and Blood agar. The plates were incubated aerobically at 37°C for 24hours.

3.1.11 Isolation of causative agents

At the end of incubation period when specimens showed significant growth, the colonial morphology was studied and further identification was done.

3.1.12 Identification

3.1.12.1 Colonial morphology

Colonial morphology was studied: the color, shape, and size, of bacterial colonies were identified.

3.1.12.2 Gram's stain

The fixed smear covered with crystal violet stain for 30–60 seconds. Rapidly washed off the stain with clean water. Then the smear was covered with Lugol's Iodine for 30–60 seconds and washed off with clean water, then decolorized rapidly (few seconds) with acetone–alcohol and washed immediately with clean water then covered with neutral red stain for 2 minutes and the stain was washed off with clean water. The back of the slide was wiped and placed in a draining rack to air-dry the smear. The smear was examined microscopically, first with the 40X objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells (Cheesbrough, 2006).

3.1.12.3 Biochemical Tests

3.1.12.3.1Kligler Iron Agar (KIA)

3.1.12.3.1.1Principle

KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide. A yellow butt (acid production) and red-pink slope indicates the fermentation of glucose only. Cracks or bubbles in the medium indicate gas production from glucose fermentation. A yellow slope and yellow butt indicate fermentation of lactose and possibly glucose. A red-pink slope and butt indicate no fermentation of glucose and lactose. Blackening along the stab line or throughout the medium indicates hydrogen sulphide (H_2S) production (Ahmed and Yasmeen, 2009).

3.2.12.3.1.2Procedure

By using of sterile straight loop the organism under test was inoculated onto butt of KIA tube and zigzag on the slope was made.

The tubes was incubated aerobically at 37°C overnight (Cheesbrough, 2000).

3.2.12.3.2Indole test

3.2.12.3.2.1Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria (Cheesbrough, 2006).

3.2.12.3.2.2Procedure

The tested organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water, and incubated at 35–37 _C for up to 48 h. Then was tested for indole by adding 0.5 ml of Kovac's reagent and shacked gently. Then was examined for a red color in the surface layer within 10 minutes (Cheesbrough, 2006).

3.2.12.3.3 Urease test

3.2.12.3.3.1 Principle

The tested organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink-red (Cheesbrough, 2006).

3.2.12.3.3.2Procedure

The organism cultivated using straight wire on agar medium containing urea and phenol, when bacteria is urease producer it will break down the urea and change the color into pink (Cheesbrough, 2000)

3.2.12.3.4Citrate utilization test

3.2.12.3.4.1Principle

The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen in the presence of bromthymol blue as indicator. Positive results appeared in the change of the indicator color from green to blue (Ahmed and Yasmeen, 2009).

3.2.12.3.4.2Procedure

The test based on the ability of organism to utilize citrate as the sole sore if carbon and ammonium salt as sole source of nitrogen. Simmons citrate agar inoculated using straight wire, incubated aerobically at 37°C overnight. Presence of bright blue color indicate positive result and no change indicate negative result (Cheesbrough, 2000).

3.2.12.5 Catalase test

3.2.12.5.1Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old (Cheesbrough, 2006).

3.2.12.5.2Procedure

Two 2–3 ml of the hydrogen peroxide solution was poured into a test tube. Sterile wooden stick or a glass rod was usedand removed several colonies of the tested organism and immersed in the hydrogenperoxide solution. Then looked for immediate bubbling (Cheesbrough, 2006).

3.2.12.3.6 Manitol salt agar (MSA)

3.2.12.3.6.1Principle

The test is based on fermentation of manitol when culturing the organism in MSA medium. Yellow color indicates fermentation of manitol while pink color indicates no fermentation (Ahmed and Yasmeen, 2009).

3.2.12.3.6.2 Procedure

The organism was inoculated using a wire loop on mannitol salt agar medium, and incubated aerobically at 37°C overnight to show the presence of yellow color (mannitol fermenter) or pink color (non mannitol fermenter) (Cheesbrough, 2000).

3.2.12.3.7Deoxyribonuclease (DNase) test

The test is used to help in identification of*Staphylococcusaureus*which produces DNA-ase enzyme from other *staphylococci* Spp.

3.2.12.3.7.1Principle

The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNA-ase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates non hydrolyzed DNA. DNA-ase producing colonies are therefore surrounded by clear areas due to DNA hydrolysis (Cheesbrough, 2006).

3.2.12.3.8 Procedure

DNA-ase plate was divided into the required number of strips by marking the underside of the plate. Sterile loop or swab was used to inoculate the test and control organisms. The plate was incubated at 35–37°C overnight. The surface of the plate was covered with 1 mol/l hydrochloric acid solution. The result appearance clearing around colonies was examined within 5 minutes of adding the acid (Cheesbrough2006).

3.2.13 Antimicrobial sensitivity testing

3.2.13.1 Kariby - Baure disk diffusion method

Sterile wire loop was used to touch 3–5 well-isolated colonies of similar appearance to the tested organism and emulsified in 3–4 ml of sterile physiological saline or nutrient broth. In a good light the turbidity of the suspension was matched to the turbidity standard .When comparing turbidities it is easier to view against a printed card or sheet of paper. Plate of Mueller Hinton agar was (MHA) inoculated by using a sterile swab and removed excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension the swab was streaked evenly over the surface of the medium in three directions and rotating the plate approximately 60̊ to ensure even distribution. With the Petri dish lid in place, was allowed 3–5 minutes for the surface of the agar to dry. Sterile forceps, needle mounted in a holder, or a multidisc dispenser was used to place the appropriate to ensure the discs are correctly placed. Within 30 minutes of applying the discs the plate was, inverted and incubated it aerobically at 35˚C for 16–18 hours .After overnight incubation, the control and tested plates was examined to ensure the growth was confluent or near confluent. The ruler was put on the underside of the plate to measure the diameter of each zone of inhibition in mm. The endpoint of inhibition was where growth starts (Cheesbrough, 2006).

3.2.14. Isolation and identification of lactobacillus *spp*

Gram stain and catalase test were used for purified isolates. When strains are: gram +ve and catalase –ve they were picked off into suitable broth medium MRS. The obtained culture were reexamined by gram-stain and re-streaked on the solid media MRS (Man, *et al*., 1960) and were incubated anaerobically in anaerobic jar at 37˚C for 2-3 days. Biochemical tests were used for identification LAB.

Phenotypic identification, Anaerobic jar was used for an incubated culture using gas generating kits (H_2+ CO_2) (Oxioid) and incubated at 37˚C for 2 to 3 days (Algadi*et al*., 2016).

3.2.15. Antagonistic Activity of *Lactobacillus* spp.

3.2.15.1 Agar-well diffusion method

3.2.15.1.1Principles

The antibacterial present in the *lactobacillus spp.* are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular around well. Will be a confluent growth. The diameter of zone of inhibition can be measured in millimeters.

3.2.15.2 REAGENTS

3.2.15.2 .1Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm Petriplates (25-30ml/plate) while still molten. (Selvamohan*et al*, 2010).

3.2.15.2. 2. Nutrient broth (1L)

One liter of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to be dissolve completely. The medium was dispensed and sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm Petriplates (25-30ml/plate) while still molten, used for sub subculture test organism.

3.2.15.2.3deMan, Rogosa and Sharpe (MRS) medium

This medium supports luxuriant growth of lactobacilli from oral, fecal, dairy, and other sources. (deMan *et al*, 1960).

3.2.15.2.3.1Principles

Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract are the carbon, nitrogen, and vitamin sources used to satisfy general growth requirements in Lactobacilli MRS medium. Dextrose is the

fermentable carbohydrate. Sodium Acetate is an inhibitory agent. Sodium Acetate and Ammonium Citrate act as selective agents as well as energy sources. Potassium Phosphate is the buffering agent. Magnesium Sulfate and Manganese Sulfate provide cations used in metabolism. Polysorbate 80 is a surfactant, facilitating uptake of nutrients by lactobacilli (deMan et al, 1960).

3.2.16Invitro Antagonistic Activity of Lactobacillus spp.

Petridishes containing 20ml Muller Hinton medium were seeded with 24hours culture of bacterial strains. wells were punched over the agar plates using sterile gel puncher (4mm diameter) then put inside the edge of each well 24hr culture of lactobacillus *spp.* suspended in 5 ml distal water and match with turbidity standard (Selvamohan*et al*., 2010).

The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993).

CHAPTER FOUR

4. Result

The study included 50 patients with UTI, were surveyed in this study, then 30 samples were chosen for the study 23(%) of patients were females and 7 (%) were males. They were within the age range of 20-80 years.

4.1 The percentage

From 30 isolates organisms demonstrated that among these isolated uro-pathogen, *K.pneumoniae*8 (26.7*%), E. faecalis*6 (20%), *S.aureus*5 (1

4.2 Antimicrobial susceptibility testing of isolateagainst various

The results in table 4:1 illustrate the sensitivity and resistance patterns of isolated bacteria to the antibiotics (Ciprofloxacin, Co-trimoxazole, Chloramphenicol, Nitrofurantoin, Norfloxacin) and probiotics *Lactobacillusacidipiscis, Lactobacilluslactissub.sp. hodinaeLactobacillus rhamanosus*). Most of isolatewere resistant to Co-trimoxazole 24 (80%), Norfloxacin 12 (40%), Ciprofloxacin and Nitrofurantoin were similar 10 (33.3%), Ninety (90%) isolated bacteria were sensitive to Chloramphenicol and 10% isolated bacteria were resistant to it. For probiotics the pattern of resistance was as following *Lactobacillusacidipiscis* 29 (96.7%) *Lactobacillus rhamanosus* 28 (93.3%),*Lactobacillus.lactissub.sp. hodinae*26 (86.7%) but the isolated bacteria was more sensitive it 4 (13.3%) ***** p-value = 0.036 (p > 0.05).

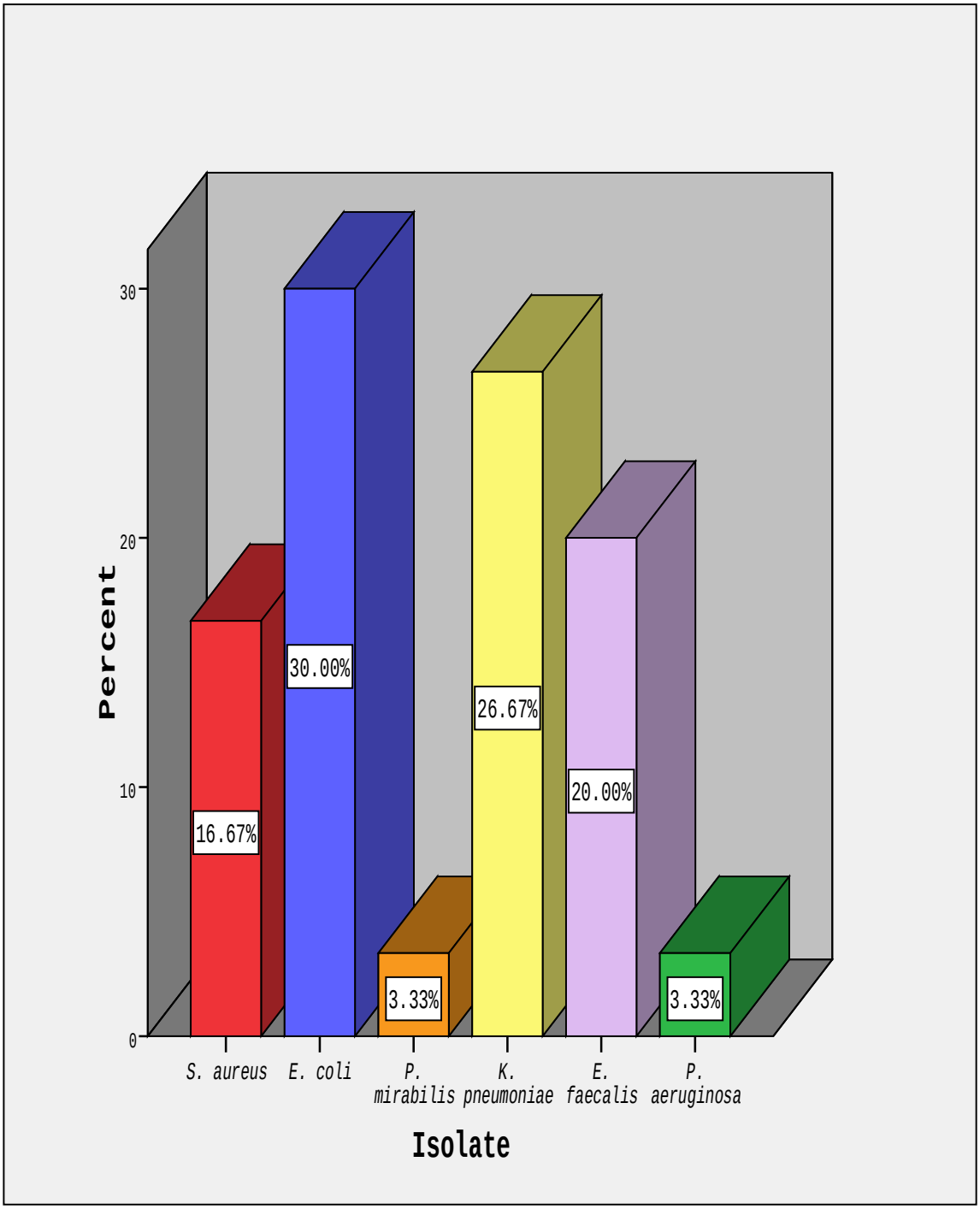

Fig.1 The percentage of frequent of isolated bacteria in Khartoum state.

Table: 1 correlation between antibiotic and probiotic against urinary tract infection causative bacteria isolate.

Isolate	Norfloxacin		*Lactobacillus rhamnosus*		*Lactobacillus acidiscis*		*Lactobacillus cocciuslacticspp*	
	S	R	S	R	S	R	S	R
S.aureus	3	2	2	3	4	1	3	2
E. coli	6	3	0	0	9	0	1	8
P. miribilis	0	1	0	1	1	0	0	1
K.pneumoniae	5	3	0	8	8	0	0	8
E.faecalis	3	3	0	6	6	0	0	6
P.aeruginosa	1	0	0	1	1	0	0	1
Total	18	12	2	28	29	1	4	26

R: resistance

S: sensitive

Isolate	Ciprofloxacin		Chloramphenicol		Cotrimoxazole		Nitrofurantoin	
	S	R	S	R	S	R	S	R
S.aureus	4	1	3	2	1	4	2	3

Table: 2 correlation between antibiotic and probiotic against urinary tract infection causative bacteria isolate.

E. coli	6	3	8	1	2	7	8	1
P. miribilis	0	1	1	0	0	1	1	0
K.pneumoniae	4	4	8	0	1	7	7	1
E.faecalis	5	1	6	0	1	5	1	5
P.aeruginosa	1	0	1	0	1	0	1	0
Total	20	10	27	3	6	24	20	10

R: resistance

S: sensitive

CHAPTER FIVE

5. Discussion

In the present study we found that the predominant uro-pathogen is *E. coli* 9 (30%), followed by *K. pneumoniae*8 (26.7*%), E. faecalis*6 (20%), *S.aureus*5 (16.7%), *P. aeruginosa*1 (3.3%) and *P. mirabilis* 1 (3.3%). This result in agree with a study conducted by Naderi*et al*, (2014) (Iran) in UTI patients who found *E. coli* represents 32.33%, followed by *K. pneumoniae*(30.9%). Also we agree with a study done by Hamdan*et al.*, (2011) in Khartoum, found that *E. coli* (42.4%) was the most predominant organism in UTI patients, and other isolates were *S.aureus* (39.3%), *K.pneumoniae* (9%), and *P.aeruginosa* (3%).

Most of isolated organisms were resistant to co-trimoxazole 24 (80%), norfloxacin 12 (40%), ciprofloxacin and nitrofurantoin were 10 (33.3%), this result was in agree with other study conducted by Mandal*et al*, (2010) in India, who found 73.04% of *E. coli* was resistant to Ciprofloxacin, 26.9% resistant to Nitrofurantoin, and also found 54.8%of *K. pneumonia* resistant to Ciprofloxacin and 58.6% to Nitrofurantoin. Ninety percent of (90%) isolated bacteria were sensitive to Chloramphenicol and 10% were resistant to it. This finding is agrees with a study in Nepal, where Chloramphenicol was sensitive to 50.0% (Rai*et al*., 2007). The antimicrobial activity of lactic acid bacteria was as follows; Lactobacillus *acidipisci*s was active against one isolate (3.3%) and *lactobacillus rhamanosus* was active against two organisms (6.6%). This may be due to the presence of diacetyl that has been noted as a metabolite produced by LAB which contributes to their antimicrobial activity against pathogenic organisms (Olaoye, 2016; Adejumo, 2014; Emereini*et al*., 2014). While Lactobacillus.lactissubsp. *hordinae*was more active (13.3%), this may be due to Lactobacillus.lactis*subsp.* hordinae produce more concentration of diacetylthan other species(Olaoye, 2016). The *Lactobacillus.lactissubsp. hordinae*was more active against *S. aureus*

than *E. coli*, this agree with Olaoye, 2016, Adejumo (2014) and Khandare and Patil (2015).

Conclusion

The presence of *E. coli* among urinary tract infection patients (1 was 30%.

Chloramphenicol showed highly sensitivity against UTI (2 isolated organisms.

Norfloxacin, Ciprofloxacin and Nitrofuranto in were showed (3 low effective against UTI isolated organisms.

*Lactobacillus.lactissub.sp. hodinae*was more active 4 (13.3%) (4 against selected isolates.

Recommendations

It is import to increase efforts to monitor and control the spread (1 of antimicrobial resistant strains in hospitals and community.

Needs to force on researchers to use probiotic bacteria to (2 resolve problem of drugs resistance.

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Appendix I

Questionnaire model

Sudan University of Science and Technology

antibacterial activity of *lactobacillus ssp* against urinary tract infection.

Appendix (1): Questionnaire

Patient name

……
…

Age

…….

Isolated bacteria

……
……

M Gender

Female

Antimicrobial sensitivity test

Antibiotics Sensitive to

……
……
……
……

Antibiotics Resistance to

……
……
……
……..

Appendix (2): Culture media

MacConkey's agar

Ingredients:	gms/litter
Peptic digest of animal tissue	17.00
Protease peptone	3.00
Lactose	10.00
Bile salts	1.50

Sodium chloride	5.00
Neutral red	0.03
Agar	15.00

:Preparation

Suspend 51.53 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs .pressure (121°C) for 15 minutes. Mix well before pouring

Blood agar

Content:	grams/litter
Nutrient agar	95ml
Sterile defibrinated blood	5ml

.Weight the nutrient agar about 2.8gm in 95ml distilled water

Preparation: Prepared 95ml from nutrient agar as above, cooled to 50^{o} C, added aseptically 5ml from the sterile blood and mixed gently .poured in sterile Petri dishes

Gram's stain

:Crystal violet .0.1

Crystal violate	20 gram
Ammonium oxalate	99 gram
Ethanol	95 ml
D.W	IL

:Lugols iodine .0.4

Potassium iodine	20 gram
Iodine	10 gram

Distilled water	1L

:0.3Acetone - alcohol decolorize

Acetone	500 ml
Ethanol or methanol absolute	465 ml
Distilled water	25 ml

:0.4Saffranin

Saffranin	0.54 g
Distilled water	100 ml

Peptone water

Component:	Gems/liter
Peptic digest of animal tissue	1.00
Sodium chloride	5.00

PH 7.2 at 25c

15.0grams in 1000 ml distilled water, mix well and sterile by .autoclaving at 12-15 Ibs pressure (118-121˚C) for 15 minute

Simmon's citrate

Components:	Grams/L
Magnesium sulphate	0.20
Ammonium hydrogen phosphate	1.00
Di potassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08

Agar	15.00

Final PH 6.8 at 25°C

:Preparation

Suspended 24.28 grams in 1000 ml distilled water. Heat to boiling to .dissolve the medium completely

.Sterilize by autoclaving at 15 Ibspressure(121°C) for 15 mins

Urea agar base

Component:	Grams/liter
Peptic digest of animal tissue	1.00
Dextrose	1.00
Sodium chloride	5.00
Di sodium phosphate	1.20
Mono potassium phosphate	0.80
Phenol red	0.012
Agar	15.00

PH 6.8 at 25°C

:Preparation

Weight 24.0 grams of medium in 95 ml distilled water, mix well and sterile by autoclaving at 12-15 Ibs pressure (118-121°C) for 15 .minutes, cool to 50°C and aseptically add 50 ml of sterile 40% urea

Kligler iron agar

Component:	Grams/liter
Peptic digest of animal tissue	15.00
Sodium chloride	5.00

Beef extract	3.00
Yeast extract	3.00
Protease peptone	5.00
Lactose	10.00
Dextrose	1.00
Ferrous sulphate	0.20
Sodium thiosulphate	0.30
Phenol	0.024
Agar	15.00

PH 7.4 at 25c

:Preparation

Suspend 42.524 grams in 1000 ml distilled water. Sterilize by autoclaving at 15 Ibs pressure (121°C) for 15 minutes. Cool to about 50̊ C. Mix well and pour into sterile tubes. Allow the medium to .solidify in a slope position to give a butt and slope

Manitol salt agar

Component:	Grams/liter
Meat extract	1.00
Casein peptone	5.00
Sodium chloride	75.00
Meat peptone	5.00
Manitol	10.0
Phenol red	0.025
Agar	15.00

Suspended 111 grams in 1000 ml distilled water mix well and sterile .by autoclaving at 12-15 Ibs pressure (118-121˚C) for 15 minutes

DNA-ase test agar base

Component	Grams/liter
Casein enzyme hydrolyte	15.00
Papic digest of soya bean meal	5.00
Deoxyribonucleic acid (DNA)	2.00
Sodium chloride	5.00

.PH 7.3 at 25˚C

Suspended 42.0 grams in liter distilled water mix well and sterile by .autoclaving at 12-15 Ibs pressure (118º-121ºC) for 15 minutes

deMan, Rogosa and Sharpe (MRS) media .

Formula/Liter

Enzymatic Digest of Animal Tissue..... 10 g

Beef Extract ..... 10 g

Yeast Extract ..... 5 g

Dextrose ..... 20 g

Sodium Acetate ..... 5 g

Polysorbate80 ..... 1 g

Potassium Phosphate ..... 2 g

Ammonium Citrate ..... 2 g

Magnesium Sulfate ..... 0.1 g

Manganese Sulfate ..... 0.05 g

Final pH: 6.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

:**Appendix (3): Reagents**

:1-Hydrochloric acid 1mol/L

Concentrated hydrochloric acid 8.6 ml and 100 ml distilled water

Hydrogen peroxide 3ml in 100 ml)Hydrogen peroxide (3%H_2O_2 -2
.distilled water

:3-Kovacs reagent

.P-di methyl aminobenzaldehide 2 grams

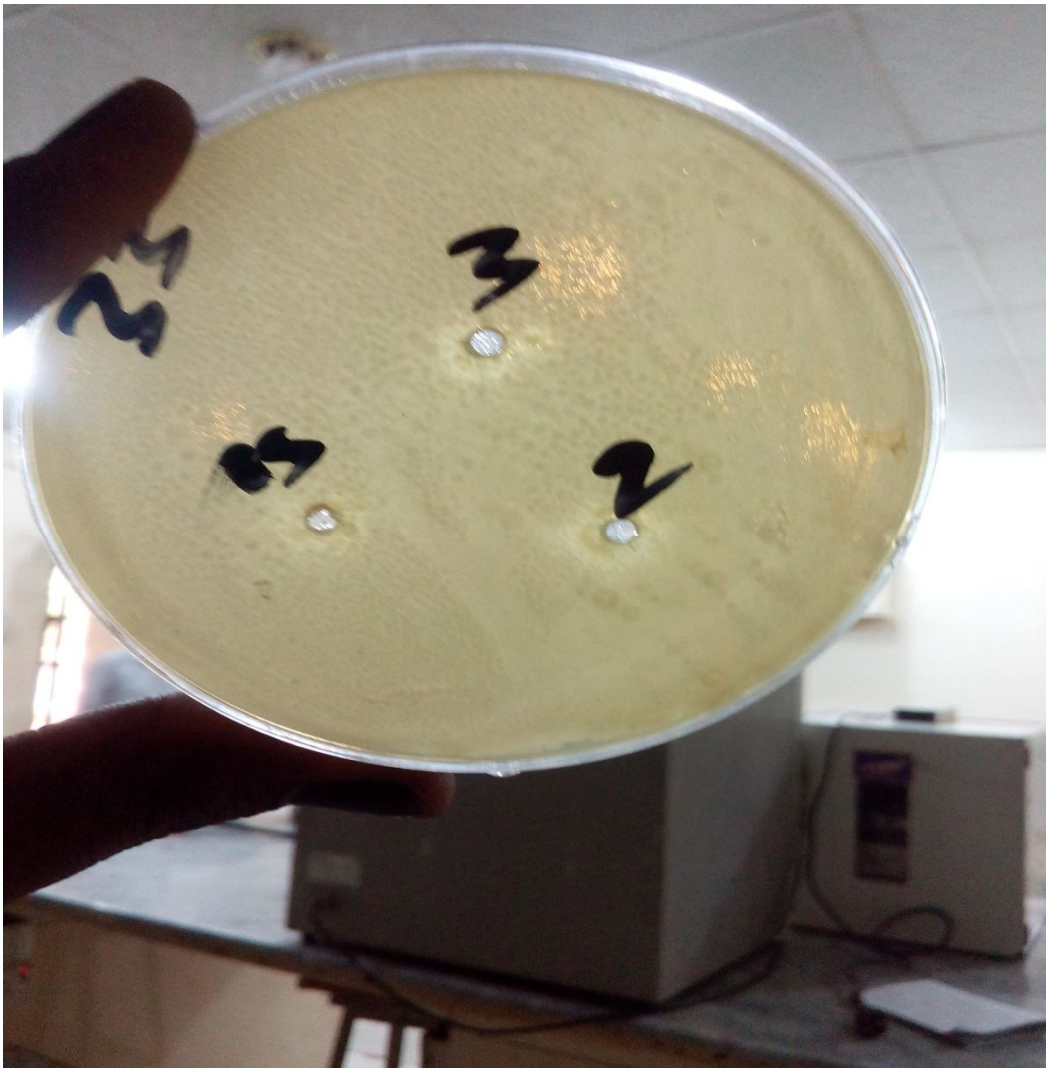

Plate (1): Show *Escherichia coli* produce inhibition zone with three lactobacillus*species.*

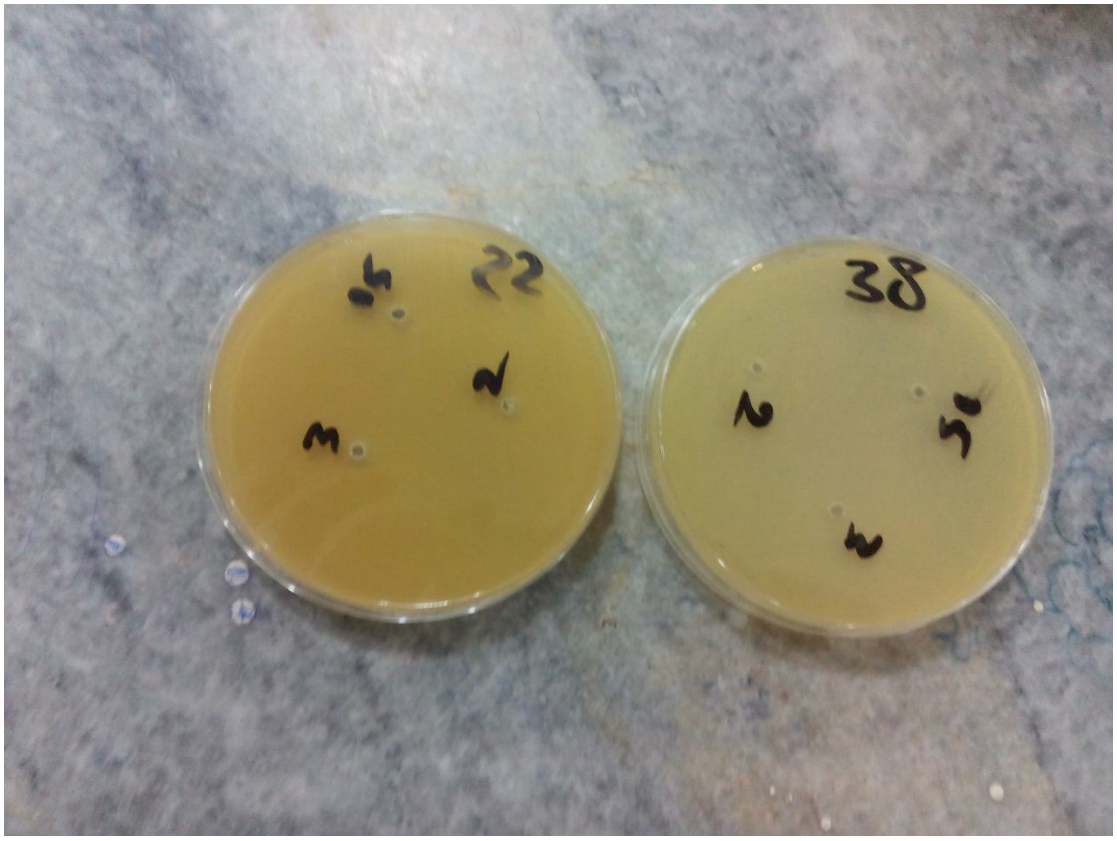

Plate (2): Show isolated bacteria no inhibition zone with three-

Lactobacillus species.