Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.6, 2013

Isolation, Identification and Characterization of Urinary Tract Infectious Bacteria and the Effect of Different Antibiotics

Geoffrey Arasa Ouno[1*] Scolastica Chepngetich Korir[2] Joan C.Cheruiyot[3] Dr.Ongechi Donald Ratemo[4]
Benard Maronga Mabeya[5] Godfrey Omare Mauti[6] Eliakim Mbaka Mauti[7] , Sabella J. Kiprono[8]

1. School of Medicine, Department of Medical Microbiology- Maseno University.
2. School of Medicine, Department of Medical Microbiology- Maseno University.
3. School of Medicine, Department of Nursing-Maseno University.
4. Kisii Level 5 Hospital.
5. School of Health Sciences- Kampala International University, Dar es Salaam.
6. School of Health Sciences- Kampala International University, Dar es Salaam.
7. School of Health Sciences- Kampala International University, Dar es Salaam.
8. Masinde Muliro University of Science and Technology, Department of Medical Laboratory Science

* E-mail of the corresponding author: arasa_ouno@yahoo.com

Abstract

Introduction: Urinary Tract Infection (UTI) defines a condition in which the urinary tract is infected with a pathogen causing inflammation which is a common, distressing and occasionally life threatening condition. UTI affects people of all ages and both gender. In all patients with UTI are reported with asymptomatic bacteriuria. Female are more susceptible to UTIs compared to male. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic is susceptibility **is mandatory.**

Methods: This study focused on the frequency of uropathogens and their antibiotic susceptibility in different gender in Madurai District. Cultural and biochemical characterization of uropathogens revealed the prevalence of both gram-positive and gram-negative organisms

Results: *E. coli* was the predominant isolate isolated from the urine specimen followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis* and *Enterococcus faecalis***.** Among the antibiotics tested, chloraphenicol and ciprofloxacin (100%) were found to be effective for empirical treatment of UTI and has covered the majority of urinary pathogens followed by tetracycline, gentamycin and kanamycin (83%), Ampicillin (67). Streptomycin, Rifampicin and amoxicillin were less effective (50%).

Conclusion: Some of the isolates were resistant to penicillin-G, Streptomycin, rifampicin and amoxicillin which are more frequently prescribed and indicates that increased consumption of a particular antibiotic leads to acquisition of resistance by the uropathogens. Resistance rates among common uropathogens continue to evolve and appear to be increasing too many commonly used antimicrobial agents and a continued surveillance of resistance rates among uropathogens is needed to ensure appropriate recommendations for the treatment of the urinary tract infection**s.**

Keywords: Urinary tract infection, Drug resistence, Uropathogens, Biochemical tests.

INTRODUCTION

Urinary Tract Infection (UTI) is caused by pathogenic invasion of the urinary tract which leads to an inflammatory response of the urothelium. Proliferation of bacteria in the urinary tract is the cause of **urinary tract infection**. The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organism(s), the severity of the infection and the patient's ability to mount an **immune response** to it (Foxman and Brown, 2003). Signs and symptoms may include fever, chills, dysuria, urinary urgency, frequency and cloudy or malodorous urine. Infections are almost always ascending in origin and caused by bacteria in the periurethral flora and the distal urethra. These bacteria inhabit the distal gastrointestinal tract and colonize the perineal area. *E. coli* usually causes a child's first infection (Brkic *et al*., 2010) but other gram-negative bacilli and *Enterococci* may also cause infection. *Staphylococcal* infections, especially those due to *Staphylococcus saprophyticus* (Assel *et al*., 2009) are common causes of **urinary tract infection** among female adolescents.

Under normal circumstances the kidney, uterus and the urinary bladder of mammals are sterile. Urine within the urinary bladder is also sterile. However, in both male and female, a few bacteria are usually present in the distal portion of the urethra. The factors for sterility are: Urine kills some bacteria

due to its low pH and the presence of urea and other metabolic end products and much enzymes. The kidney medulla is so hypertonic that few organisms can survive. The lower urinary tract is flushed with urine and some mucus 4-5 times each day, eliminating potential pathogens. In males the anatomical length of the urethra (20cm) provides a distance barrier that excludes microorganisms from the urinary bladder. Conversely, the short urethra (5cm) in females is more readily transverse by microorganisms; this explains why general urinary tract infections are 14 times more common in females than in males. Under the influence of oestrogen, the vaginal epithelium produces increased amount of glycogen that acid-tolerant ***Lactobacillus acidophilus*** bacteria called Doderlein's bacilli degrade to form lactic acid. Normal vaginal secretions contain up to 10^8 Doderlein's bacilli per ml. thus an acidic environment (pH 3.5) unfavorable to most organisms established. Cervical mucus also has some antibacterial activity. Most UTIs are thought to be caused by organisms originating from the patient's own bowel.

Urinary tract infection is one of the most common frequently occurring nosocomial infections. Normally UTIs are caused by a variety of Gram-negative and Gram-positive bacteria. The Gram-positive bacteria includes ***Staphylococcus sp, Streptococcus sp*** *and* ***Enterococcus sp.*** Gram-negative includes a large number of aerobic bacilli such as ***Escherichia sp, Klebsiella sp, Enterobacter sp, Citrobacter sp, Proteus sp, Serratia sp, Salmonella sp and Pseudomonas sp.*** Among this 80-90% of UTI is caused by ***E. coli*** (H.G.I Rushton 1997) and in ambulatory patients and of nosocomial infections, ***Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Enterococcus faecalis*** are the most frequently isolated.

The pathogenesis of UTIs involves complex interaction between an organism, the environment and the potential host. The symptoms of a person with urinary tract infections depend on the age and the location. Chronic and acute infection of urinary tract leads to high blood pressure, kidney damage and results in death. Chronic manifestations of the UTIs are acute and chronic pyelonephritis (a disease process resulting from the effect of infection of parenchyma and pelvis of the kidney), cystitis, renal carbuncle, urethritis and prostatitis.

Treatment of UTIs depends upon sensitivity of bacteria towards a variety of antibiotics such as Trimethoprim-Sulfamethoxazole (TMP-SMX). However prolonged use of antibiotic causes side effects in the patients and the pathogens develop resistance through plasmid and or mutational changes due to course.

The sequence of steps in performing a complete study of microorganisms in urine sample includes: Aseptic collection of specimens, Quantitative analysis, Isolation of pathogens, Identification of pathogens and Antimicrobial sensitivity testing

Treatment of UTIs depends on the bacteria responsible for infection because all the bacteria possess their own susceptibility towards a variety of antibiotics.

MATERIALS AND METHODS

Media--The media used were purchased from Hi media Laboratories, pvt Mumbai. The following media were used: Blood agar, Casein agar, Simmons Citrate agar, Nutrient Gelatin agar, Kovac's reagent, Mueller-Hinton agar, MR-VP broth, MacConkey agar, Nutrient broth/agar, Peptone broth, Starch agar, Triple Sugar Iron agar, Thioglycollate fluid, Hi-Crome UTI Agar, Baird Parker agar, Cetrimide agar.

Antibiotics used--The antibiotics generally used for the treatment of UTIs are selected for the disc sensitivity method (Hi-media discs). The antibiotics are as follows: Ampicillin, Gentamycin, Septrin, Amoxicillin, Ciproflaxin, Cefatoxin, Nalidixic acid, Streptomycin, Tetracycline, Kanamycin, Penicillin-G, Rifampicin, Chloramphenicol and Norfloxacin

Aseptic collection of urine specimens: In this procedure, the patients were asked to clean their external genitalia with liquid soap containing chlorhexidine. Midstream urine was collected in sterile bottles and closed tightly.

Transportation of urine specimens: The collected urine specimens were transported with the temperature of 4-8^{0}C with coolant pack to the laboratory. All urine specimens brought to the microbiology laboratory were examined at once. The remaining specimens were placed in a refrigerator at 4-8^{0}C until they were examined.

Physical examination of urine--The physical parameters of collected urine specimens were analyzed such as the volume, pH, color, odor and appearance.

Quantitative analysis-This was done by two methods simultaneously; Plate count method (spread plate technique) where by Sterile Nutrient agar plates were prepared. The urine samples were mixed well by shaking in shake bottles. The samples were serially diluted by transferring 1.0ml of each sample into the shake bottle containing 99ml sterile distilled water and mixed well by shaking. Using a sterile pipette 0.1ml from each dilution was transferred onto the surface of nutrient agar plates separately. Using a sterile L-rod spreader, the samples were spread evenly on the surface of each nutrient agar. The plates were incubated at 37^0C for 24 hours. After incubation, the number of colonies in each plate was counted.

Direct microscopic study of the fresh urine sample was done (Simple staining and Gram staining). Separate smears from each urine specimen were prepared on glass slides and heat fixed. The slides were held over basin/staining tray. The smears were flooded with crystal violet dye for one minute. The stain was rinsed off with distilled water into basin by using wash bottle. The slides were blot dried using a blotting paper and examined under microscope using oil immersion.

Separate smears from each urine specimen were prepared on glass slides and heat fixed. The smears were stained/ flooded with crystal violet for 30 sec to 1 minute. The stain was washed off with distilled water using wash bottle. Each smear was flooded with gram's iodine for 1 minute. Excess gram's iodine was washed off with distilled water. The slides were tilted and decolorized with gram's decolorizer, draining into basin for 10 seconds. The decolorizer was washed off with distilled water. The smears were flooded with safranine for 20-30 seconds. The slides were rinsed with distilled water, blot dried and examined under oil immersion.

Isolation of pathogens: Pathogens were isolated from the urine sediment. The urine sample was shaken well to resuspend the organisms and 10ml was decanted into a centrifuge tube. The tube was kept closed to avoid contamination. The sample was centrifuged at 2000 RPM for 10 minutes. The entire sample was decanted but 0.5ml sediment from the tube was resuspended with a sterile wire loop. A loopful of the sediment was inoculated in a tube of thioglycollate medium.

Identification of pathogens

Cultural observation---Color, size, and colony morphology are observed from the incubated plates.

Microscopical Examination of urine specimen---Slides were prepared from each different colonies observed on the plates and gram staining was performed. The results such as the gram positive or gram negative, shape of the bacteria are observed from the examinations.

Microbiological analysis of urine specimen---The uropathogens were identified by swabbing/streaking the urine specimens on various selective and differential media such as Hi-Crome UTI agar, Baird Parker Agar, Blood Agar, Cetrimide agar, MacConkey agar based on their color morphology after the incubation period.

Biochemical Examination

The selected colonies based on the cultural, microscopic and microbiological examinations, were subjected to biochemical examination (starch hydrolysis, lipid hydrolysis, casein hydrolysis, triple sugar iron agar test,) oxidase test,) catalase test, nitrate reduction test, indole production test, methyl red test, voges-proskauer test, citrate utilization test, urease test**)** for confirmation of the pathogens.

Antimicrobial sensitivity testng (kirby-bauer method)

The susceptibility of the entire isolated organisms to selected antibiotics which were normally used to treat uropathogens was tested by Kirby-Bauer Method.

Sterile Mueller-Hinton agar plates were prepared and Various antibiotic discs were selected. Identified pathogens were inoculated in peptone water tubes separately and incubated at 37^0C for 1 hour. Using sterile cotton swabs for each test organism, incubated test organisms were inoculated on the surface of Mueller-Hinton agar plates three times, rotating the plate 60^0 after each streaking. Finally the swab was run around the edge of the agar. The cultures were allowed to dry on the plate for 5-10 minutes at room temperature. Various antibiotic discs were placed on the surface of the agar medium by gently pressing using a sterile forceps on the top of the discs (for better contact and effective diffusion of the antibiotics into the medium). Make sure that contact is made between the antibiotic disc and the culture. The plates were incubated in an inverted position for 16-18 hours at 37^0C.

RESULTS

The management of UTI is very important because the prevalence of the pathogenesis and development of drug resistance caused by the uropathogens are increasing in a higher magnitude. As per

the reports documented by different countries, *E.coli* was found to be the most predominant uropathogen isolated from the patients with UTI and the development of multi drug resistance among uropathogens that causes a complicated UTI. The urine specimens showed greater variation in color, odor, appearance and pH when they were subjected into physical examination (Table 1). The urine specimens were subjected to different selective and differential media. The uropathogens were identified based on color morphology (Table 3). Biochemical tests were carried out to confirm the presence of the species of uropathogens from the selected groups. The results show that ***E.coli*** were predominant isolates isolated from the urine specimen followed by ***P. aeruginosa, K. pneumoniae, S. aureus, P. mirabilis*** and ***E. faecalis*** (Table 4). The biodiversity of the uropathogens from the study group shows that populations were suffering from acute urinary tract infection. Urine samples of patients of age group 30-35 years showed presence of the highest number of uropathogens. Age group 20 years and below and 50 years and above showed the lowest number of uropathogens present (Table 2)

The plates were observed for zone formation around the discs and the diameter of growth inhibition zone was measured and recorded in mm as shown in Table 6.

The isolated uropathogens were sensitive to the various antibiotics. Among the isolated pathogens all of them shows 100% sensitivity towards Chloramphenical and Ciprofloxacin. Followed by Tetracycline, Kanamycin and Gentamycin which show 83% sensitivity, Ampicillin 67%, Streptomycin, Rifampicin and Amoxicillin showed 50% the lowest sensitivity. ***Staphylococcus aureus*** was sensitive to all the antibiotics.

E.coli showed resistance to Tetracycline, Kanamycin and Rifampicin. ***S. aureus*** showed no resistance to any of the antibiotics. ***K. pneumoniae*** was resistant to Ampicillin, Streptomycin, Rifampicin and Amoxicillin. ***P. mirabilis*** showed resistance to Ampicillin and Amoxicillin. ***P. aeruginosa*** showed resistance to Streptomycin and Amoxicillin. ***Enterococcus faecalis*** resisted Streptomycin, Rifampicin and Gentamycin (Table 5).

DISCUSSION

Urinary tract infections are serious health problems affecting millions of people every year. UTI can be categorized as complicated or uncomplicated. Urinary tract infections occur in patients with structurally or functionally abnormal tracts (complicated UTI) and in patients with anatomically normal urinary tracts (uncomplicated UTI). The pathogens of urinary tract infection involve complex interaction of organisms, the environment and the potential host. Gram negative aerobic organisms are the organisms involved in urinary tract infections. These organisms usually originating from the normal microbial flora and 80-90% of first infection, *E.coli* is usually isolated. The outer genital and periurethral bacterial flora usually reflects the gut flora. With subsequent infections, ***E. coli*** is seen in around 70% of cases although more unusual organisms occur after antibiotic therapy, surgery or the presence of obstruction or stones. ***K. pneumoniae, P. aeruginosa, P. mirabilis, S. aureus*** and ***Enterococcus faecalis*** are the next predominant uropathogens isolated. Proteus species are often associated with renal calculi. Results from the studies shows that about the entire positive analyzed, 5 common bacterial pathogens were isolated. Among them ***E.coli*** is predominant followed ***P. aeruginosa***, ***K. pneumoniae, S. aureus, P. mirabilis*** and ***E. faecalis.*** The report of physical parameters of urine specimen shows many of the urine samples was pale yellow, few samples were white and red. This reveals that many of the patients have renal diseases and haemoglobinuria. In the present analysis of urine specimen reveals more pus cells indicating that patients were suffering from pyuria.

Recently there is a wide spread distribution of UTI all over the world due to multi-drug resistant uropathogens caused by Resistance (R) plasmid. The effects of urinary tract infectious pathogens on urinary tract organs are: erethritis, cystitis, ureteritis and pyelonephritis.

Conclusion: Drink plenty of water daily, wipe from front to back to prevent bacterial around the anus from entering the vagina or urethra, avoid smoking, clean genital area before sexual intercourse, avoid using feminine hygiene sprays and scented douches which may irritate urethra

Antibiotics for 1-2 days, longer treatment needed by patients infected with Mycoplasma which is treated with tetracycline, kidney infections requires several weeks of antibiotic treatment, and heating pads may help in some cases.

TABLES

TABLE 1
PHYSICAL PARAMETERS OF URINE SPECIMENS COLLECTED FROM THE PATIENTS

Patient Code	Age	Volume in ml	Color	Odor	Appearance	pH Acidic/Alkaline
01	35	10-20	Red	Fruity	Turbid	Acidic
02	27	10-20	Pale yellow	Ammonical	Clear	Acidic
03	30	10-20	Pale yellow	Aromatic	Clear	Acidic
04	54	10-20	Pale yellow	Aromatic	Turbid	Acidic
05	20	10-20	White	Aromatic	Clear	Acidic

TABLE 2
PRESENCE OF UTI PATHOGENS IN THE PATIENT'S URINE SAMPLE

PATIENT'S CODE	AGE	UROPATHOGENS ISOLATED	NUMBER OF UROPATHOGENS PRESENT
Patient 01	35	*Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Escherichia coli* *Staphylococcus aureus*	4
Patient 02	27	*Escherichia coli Klebsiella pneumonia Pseudomonas aeruginosa*	3
Patient 03	30	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Enterococcus faecalis* *Escherichia coli*	4
Patient 04	54	*Klebsiella pneumonia* *Pseudomonas aeruginosa* *Escherichia coli*	3
Patient 05	20	*Proteus mirabilis* *Escherichia coli*	2

TABLE 3

THE COLOR OF COLONIES FORMED ON SELECTIVE & DIFFERENTIAL MEDIA

MEDIA	UROPATHOGEN	COLOR
1 Baird parker agar	*Staphylococcus aureus*	-Black, shiny and convex colonies with a clear halo (clear zone)
	E.coli	-No growth
	Klebsiella pneumoniae	-No growth
	Enterococcus faecalis Proteus mirabilis	-Poor black colonies, no halo
	Pseudomonas aeroginosa	-Black colonies without halo
2 MacConkey agar	*Staphylococcus aureus*	No growth Red/pink
	E. coli	colonies Red/pink colonies
	Klebsiella pneumoniae	No growth
	Enterococcus faecalis Proteus mirabilis Pseudomonas aeroginosa	White/colorless colonies Colorless colonies
3 Blood agar	*Staphylococcus aureus*	Clear zone surrounding the colony (beta hemolysis)
	E. coli	Clear zone surrounding the colony (beta hemolysis)
	Klebsiella pneumoniae	No hemolysis giving a brown color (gamma)
	Enterococcus faecalis Proteus mirabilis	No hemolysis giving a brown color (gamma) Swarming growth on blood agar
	Pseudomonas aeroginosa	A wide clear zone surrounding the colony (beta hemolysis)
4 Hi-Crome UTI agar	*E. coli*	Violet
	Klebsiella pneumoniae Proteus mirabilis Staphylococcus aureus	Green White
	Pseudomonas aeruginosa	Cream yellow Cream
	Enterococcus faecalis	white Greenish blue
5 Cetrimide agar	*Staphylococcus aureus*	No growth
	E. coli	No growth
	Klebsiella pneumoniae	No growth No growth No growth
	Enterococcus faecalis Proteus mirabilis Pseudomonas aeruginosa	Blue, yellow-green to blue- green pigments

TABLE 4
IDENTIFICATION OF ISOLATED MICROBES USING BIOCHEMICAL TEST

BIOCHEMICAL TESTS	***E.coli***	***Klebsiella pneumoniae***	***Staphylococcus aureus***	***Proteus mirabilis***	***Pseudomonas aeruginosa***
Simple staining	Rods	Rods	Coccus	Rods	Rods
Gram staining	-	-	+	-	-
Lactose utilization	+	+	+	-	-
TSI	**A**	**A**	**AG**	**A**	**NC**
Starch Hydrolysis	-	-	-	-	+
Lipid Hydrolysis	-	-	-	-	-
Casein Hydrolysis	+	-	-	-	-
Oxidase Test	-	-	-	-	+
Catalase Test	+	+	-	-	+
Nitrate Reduction Test	+	+	-	+	+
Indole Production Test	+	-	-	+	-
MR Test	+	+	+	+	+
VP Test	-	+	-	-	-
Citrate utilization	-	+	-	-	+
Urease Test	-	+	-	+	-

A--Acid slant NC---No change
G--Gas production

TABLE 5
ANTIMICROBIAL SUSCEPTILITY TEST AGAINST ISOLATED PATHOGENS

Antibiotic	***E.coli***	***S.aureus***	***K. pneumoniae***	***P.mirabilis***	***P.aeruginosa***	***E.faecalis***	**% of sensitivit**
Ampicillin	-	-	+	+	-	-	6
Streptomycin	-	-	+	-	+	+	5
Tetracycline	+	-	-	-	-	-	8
Kanamycin	+	-	-	-	-	-	8
Rifampicin	+	-	+	-	-	+	50
Gentamycin	-	-	-	-	-	+	83
Chloromphenic	-	-	-	-	-	-	100
Amoxicillin	-	-	+	+	+	-	50
Ciprofloxacin	-	-	-	-	-	-	100

- = **Sensitive**

\+ = **Resistant**

Table 6: interpretation of zones of inhibition for Kirby-Baurer antibiotic susceptibility test (principles of microbiology by Ronald M.Atlas)

ANTIBIOTIC	DISC CONCENTRATION	DIAMETER OF GROWTH INHIBITION ZONE (mm)		
		SUSCEPTIBLE (S)	INTERMEDIATE (I)	RESISTANT (R)
Mikacin	0.01mg	14 or more	12-13	11 or less
Ampicillin	0.01mg	14 or more	12-13	11 or less
Bacitracin	10 units	13 or more	9-11	8 or less
Cephalothin	0.03mg	18 or more	15-17	14 or less
Chloramphenicol	0.03mg	18 or more	13-17	12 or less
Erythromycin	0.015mg	18 or more	14-17	13 or less
Gentamycin	0.01mg	13 or more	-	-
Kanamycin	0.03mg	18 or more	14-17	13 or less
Vancomycin =	0.002mg	15 or more	10-14	9 or less
Methicillin	0.005mg	14 or more	10-13	9 or less
Nalidixic acid	0.03mg	19 or more	14-18	13or less
Neomycin	0.03mg	17 or more	13-16	12 or less
Nitrofurantoin	0.3mg	17 or more	15-16	14 or less
Penicillin G (Staphylococci)	10 units	29 or more	21-28	20 or less
Penicillin (other organisms)	10 units	22 or more	12-21	11 or less
Polymyxin	300 units	12 or more	9-11	8 or less
Streptomycin	0.01mg	15 or more	12-14	11 or less
Sulfanomides	0.3mg	17 or more	13-16	12 or less
Tetracycline	0.3mg	19 0r more	15-18	14 or less
Vancomycin	0.03mg	12 or more	10-11	9 or less

BIBLIOGRAPHY/REFERENCES

Paolo. F. Albuquerque,et al.,UI: Review of 16,154 Consecutive patients, Journal of (USA),Vol.103,Feb.1970.

Pryles.C.V-Discussion before joint sessions of sections of Pediatrics, Medicine, Obstetries and Gynerology and Orology, American Medical Association San Fransisco, California,1964.Ronalds M. Atlas- Principles of Microbiology, second edition,

Wm.c.Brown Publishers-1997. Stamey T.E,et al., The localization and treatment of urinary tract infections; the role of bactericidal urine levels as opposed to serum levels. Medicine,44:1,1965.Winberg.J, Andersen.H.J, Bergstrom.T.et al., Epidemiology of symptomatic urinary tract infection in childhood. Acta paediatr Scand 252(suppl):1,1974. Naber.K.G, Therapy of complicated urinary tract infections, Wien-med Wochenschr.1991;141(23-24):552-5.

P.Gunasekeran-Laboratory Manual in microbiology 1995. Holt. J.G- Bergy 1984-1989-Bergey's Manual of Systematic Bacteriology-Waverly press Inc.Cappuccino and Shreeman-

Laboratory Manual in Microbiology- Third edition- Benjamin cummings publishing company,1992. Cox C.E and Hinman.-F.,Jr,; Experimental with induced bacteriuria,vesical emptying and bacterial growth on the mechanism of bladder defense against infection.J.Urol.,86;739,1968.

Flanagan.P.G; Rooney. P.J; Davis.E.A;Stout.Rw; A comparison of single dose versus conventional-dos antibiotic treatment of bacteriuria in elderly women; Age-Aging.1991May;20(3);206-11. Harley-Prescott: Laboratory Exercises in Microbiology, Fifth Edition. Benson: Microbiological Applications Laboratory Manual in General Microbiology, Eight Edition

World Health Organization Geneva (2003) -Basic Laboratory Procedures in Clinical Bacteriology, Second Edition.