WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 9, 810-825 Research Article ISSN 2278 – 4357

ISOLATION AND MOLECULAR CHARACTERIZATION OF BACTERIA FROM URINARY TRACT INFECTIONS

Wadhah Kadhim Hamzah*[1] and Mohamed Yahya Khan[2]

[1]Ministry of Housing, Construction and Public Municipalities, Babylon, Iraq.

[2]Kalams Institute of Sciences, Tarnaka, Hyderabad, India.

Article Received on
25 June 2017,

Revised on 16 July 2017,
Accepted on 07 Aug. 2017,

DOI: 10.20959/wjpps20179-9639

***Corresponding Author**
Wadhah Kadhim Hamzah
Ministry of Housing, Construction and Public Municipalities, Babylon, Iraq.

ABSTRACT

Urinary tract infection (UTI) is one of the common infections diagnosed in community and hospitals. Due to increasing antibiotic resistance amid uropathogens, it is significant to have community and hospital based knowledge of the microorganisms causing UTI and their antibiotic sensitivity pattern to select right course of therapy. The objective of present study is to isolate bacteria from patient samples suffering with UTI and specially to focus on isolation and characterization of UTI pathogens total of 25 urine samples collected from patients reported with UTI from University health Center, OU and RTC Hospital Tarnaka, Hyderabad. A total of 25 bacterial isolates were obtained by pure culture techniques based on morphological variation. All the 25 bacterial isolates were cultured on UTI specific medium (CLED agar). They were further characterized and identified by biochemical methods. It was found that 8 isolates were *Eschericha coli*, 7isolates were *Klebsiella* species, 3 were *Proteus* species, 3 were *Pseudomonas* species and 4 were *Staphylococcus* spp. All the 25 bacterial isolates were characterized for antibiogram pattern using Gentamycin, streptomycin, norfloxcin, oflaxacin and amoxicillin. Percentages of isolates indicate *E.coli* as the dominant pathogen of UTI compare to others and the isolate which showed maximum resistant to antibiotic was subjected to molecular identification and it was identified to be *E. coli*.

KEYWORDS: Urinary Tract Infection, *Klebsiella, Pseudomonas, Eschericha Coli.*

INTRODUCTION

Urinary tract infection (UTI) is a common bacterial infection known to affect the different parts of the urinary tract and the occurrence is found in both males and females. Nevertheless,

both the genders are susceptible to the infection; women are mostly vulnerable due to their anatomy and reproductive physiology.[1] The infection is usually caused as a consequence of bacterial invasion of the urinary tract including the lower and the upper urinary tract. Among the bacterial species *Escherichia coli* account to 80% to 85% of the infection followed by other bacteria.[2] These organisms are mainly from the external genitalia, vagina, the genital tract, rectum, and gastro-intestinal tract. Treatment of UTI varies with the type but is usually empirical because of the common spectrum of uropathogens.[3]

A variety of parameters are related to UTI which include age, parity, gravidity, pregnancy and association of diseases augment the condition of the infection.[4] The diagnosis of UTI is primarily based on symptoms. Individually, symptoms are rarely diagnostic, but in combination their accuracy is greater. Signs and examination findings are generally unhelpful in uncomplicated cystitis, but have more value where upper UTI is possible.[5] Laboratory culture has historically been the benchmark for diagnosis of UTI, but studies have shown that women who respond to treatment may have bacterial counts below the traditional threshold for diagnosis.[6]

The evaluation of children after a UTI was once thought to be quite straightforward and focused primarily on detecting and treating vesicoureteral reflux in order to prevent end-stage renal disease from reflux nephropathy. Hutch in 1958 and Hodson in 1960 were among the first to describe a relationship between reflux and renal scarring. Subsequently, a relationship was established between reflux and chronic pyelonephritis.[7-10] Until recently, further evaluation of UTI has centered on the search for reflux with anatomic studies, including ultrasound and voiding cysto-urethrogram (VCUG). As newer radiological tests have become available, this routine work-up has been challenged, and in their recent review, the American Academy of Pediatrics subcommittee recognized the evolving nature of this area, although continuing to recommend a VCUG and ultrasound for all children under the age of 2 presenting with a febrile urinary tract infection.[11]

Urinary tract infection is the growth and multiplication of microorganisms within the urinary tract that includes organs which collect, store, and void urine from the body i.e. the kidney, ureter, bladder, and the urethra. Urinary tract infection affects millions of people world-wide children and adults, male and female.

However it is more prevalent in females because of the shorter and wider female urethra and its proximity to the anus. Bacteria from the rectum can easily cross over to the urethra and cause infection. Urinary tract infection in women is more prevalent during pregnancy, with a rate of 12- 35%. This is due to the various anatomical, physiological, and biochemical changes of pregnancy along-side the structural alterations caused by the gravid uterus in the pelvis. The increased progesterone levels lead to reduced ureteral, bladder, and urethral tone with dilatation and urine stasis.[12] The increased glomerular filtration rate leads to increased urine volume, glycosuria and proteinuria that form good culture media for bacteria; the gravid uterus compresses the ureter causing stasis and dilatation leading to infection in the kidneys. Urinary tract infection can occur as asymptomatic bacteriuria with a prevalence of 2-13%. This is when up to 100 000 colony forming units (cfu) of pathogenic bacteria are cultured from the urine without any urinary symptoms. When left untreated, 20-30% develops into pyelonephritis.[13-15] Urinary tract infection also occurs in the symptomatic form as pyelonephritis involving the kidneys, as cystitis involving the bladder with clinical symptoms of dysuria, frequency supra-pubic and loin pains along with fever, nausea and vomiting. Predisposing factors to urinary tract infection include the female sex, pregnancy, poor general and perineal hygiene, young age, multiparty, *Diabetes mellitus*, sickle cell disease, and previous treatment for UTI, low socio-economic status, asymptomatic bacteriuria and sexual intercourse.[16,17]

MATERIALS AND METHODS

Collection of samples and isolation of bacteria

Urine samples were collected from RTC (Road Transport Corporation) hospital, Tarnaka, Hyderabad, Telangana, India. The patients visiting the General Practitioner were employees of RTC and were of different age group. Predominant samples were from adult (male and female) patients followed by children. Urine samples were collected with the sample number given by the hospital according to enrolment of patients visiting the same. For our convenience and clarity we maintained the same numbers for this study and considered the sample no. as the strain no. of bacteria.[18]

The samples were collected in sterilized vials before processing. The patients were asked to provide the mid-stream urine (MSU) to avoid contamination with normal flora and others.

The samples obtained for this study were collected for about 6 month period. However, the samples were immediately processed for further studies as mentioned below.

A total of 25 urine samples were collected from patients with UTI as recommended by the general practitioner. The samples were streaked onto CLED agar medium with BTB indicator to see the bacterial population. If the growth was profused, dilution was performed. Wherever, the growth was scanty; the sample was processed as such using streak plate technique to isolate different bacteria associated with patient samples of UTI. Based on the colony morphology on CLED media different bacteria selected for further work.

Identification of bacteria isolates

The different bacteria colonies were identified on the basis of their cell morphology (Shape, Motility and Gram staining). Further identification was done using standard biochemical tests (IMVIC) including Urease, Catalase, Oxidase, H_2S and sugar fermentations.[19]

GRAM STAINING

The Gram stain, a differential stain was developed by Dr. Hans Christian Gram, a Danish physician, in 1884 that is why Gram staining. It is a very useful stain for Identifying and classifying bacteria into two major groups: the gram-positive and gram-negative. In this process the fixed bacterial smear is subjected to four different reagents in the order listed: crystal violet (primary stain), iodine solution (mordant), alcohol (decolorizing agent) and safranin (counter stain). The bacteria which retains the primary stain (appear dark blue or violet) (i.e. not decolorized when stained with Gram's Method) are called gram-positive, whereas those that lose the crystal violet and counter stained by safranin (appears red) are referred to as gram-negative.

Molecular identification

The isolate which showed maximum resistant to antibiotic was farther identified by using 16s r RNA sequencing.

DNA was isolated from the culture –**WHBU7**. Quality was evaluated on 0.8% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of *16S rDNA*was amplified by PCR using **8F**and **1492R** from the above isolated DNA. A single discrete PCR amplicon band of **1500bp** was observed (**Figure 1**). The PCR amplicon was purified and further process for the sequencing. Forward and Reverse DNA sequencing reaction of PCR amplicon was carried out with **704F**and **907R** primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of **1033bp***16S rDNA* was generated from forward and reverse sequence data using aligner software. The *16S rDNA* sequence was

used to carry out BLAST alignment search tool of NCBI genbank database. Based on maximum identity score first Fifteen sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA5.

RESULTS AND DISCUSSION

Isolation of pathogen from Urinary tract infection (adult and children) samples

A total of 25 urine samples were collected adult and children patients suffering from urinary tract infections from Tarnaka Hospital, Hyderabad. The urine samples were inoculated onon CLED medium with BTB and the colonies that showed yellow color and yellow zone on plate were picked up for further study. This medium is recommended for use in urinary bacteriology, promoting the growth of all urinary pathogens on the basis of lactose fermentation pH of the media play major role in this method. Shigella type species may not grow in this media. All the samples were spread onto CLED agar medium with BTB indicator and growth was monitored. Later on, where ever there is growth of bacteria, it was recorded as Motility (M), Morphology and Gram staining.

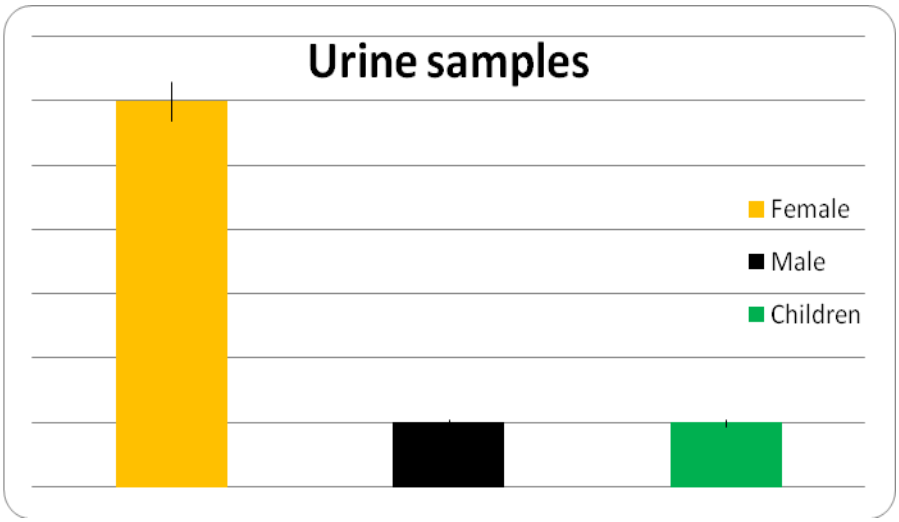


Fig. 1: Isolation of pathogen from Urinary tract infection (adult and children) samples.

Among 25 samples 15 of females, 5 of Males and 5 of children patients were screened. After checked the bacterial growth of all samples 15 of Females, 5 of Male and 5 of Children were positive of urinary tract.

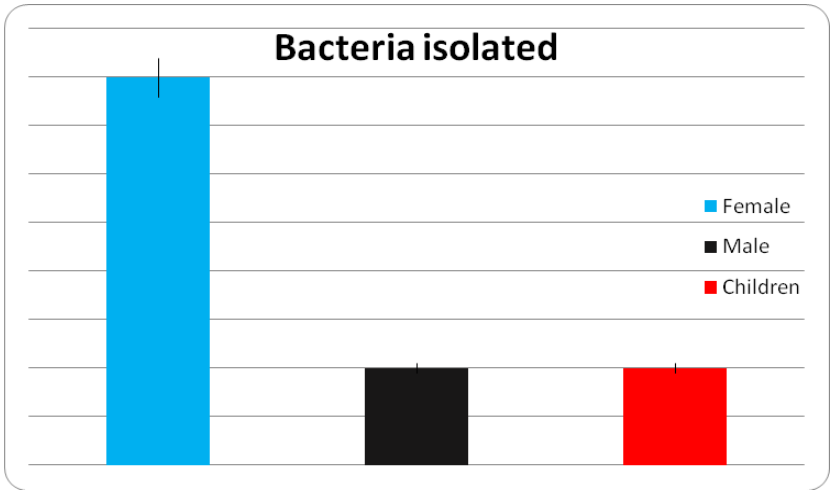


Fig. 2: Bacteria isolated from adults and children's.

Growth of bacteria from UTI samples (adults) on CLED agarmedium

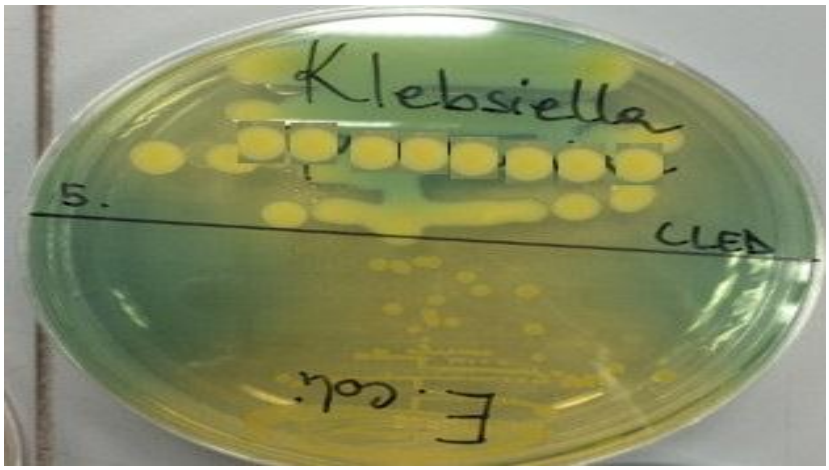


Fig. 3: *E. coli*and K*lebsiella*on CLED agar medium.

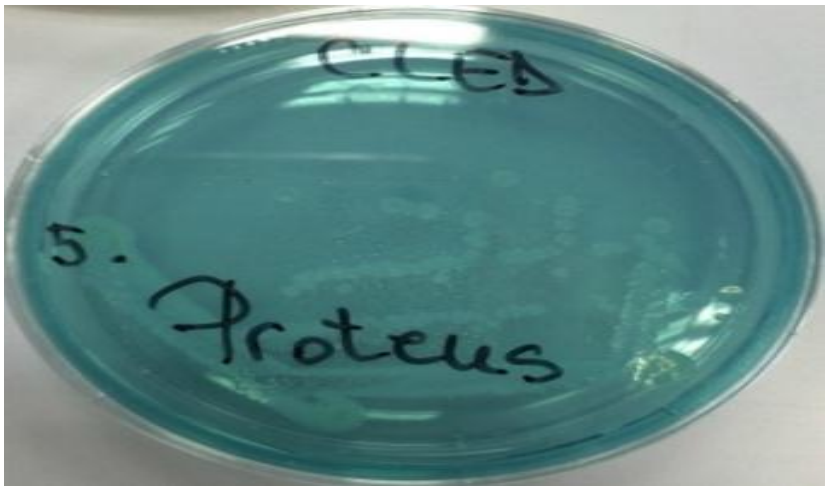


Fig. 4: *Proteus*on CLED agar medium.

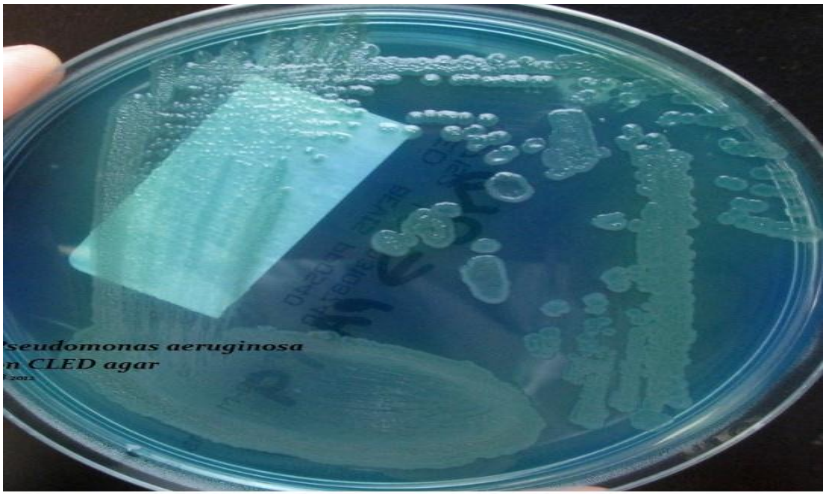


Fig. 5: *Pseudomonas*on CLED agar medium.

Table. 7: Morphological characteristics of bacterial isolates from UTI samples.

S. No	Isolate	Shape	Gram staining	Motility
1.	WHBU 1	Rods	Negative	Motile
2.	WHBU 2	Rods	Negative	Motile
3.	WHBU 3	Rods	Negative	Non-motile
4.	WHBU 4	Rods	Negative	Non motile
5.	WHBU 5	Rods	Negative	Motile
6.	WHBU 6	Rods	Negative	Motile
7.	WHBU 7	Rods	Negative	Motile
8.	WHBU 8	Cocci	Positive	Non Motile
9.	WHBU 9	Rods	Negative	Motile
10.	WHBU 10	Rods	Negative	Motile
11.	WHBU 11	Rods	Negative	Non motile
12.	WHBU 12	Rods	Negative	Motile
13.	WHBU 13	Rods	Negative	Non Motile
14.	WHBU 14	Cocci	Positive	Non Motile
15.	WHBU 15	Rods	Negative	Motile
16.	WHBU 16	Rods	Negative	Motile
17.	WHBU 17	Rods	Negative	Non Motile
18.	WHBU 18	Cocci	Positive	Non Motile
19.	WHBU 19	Cocci	Positive	Non Motile
20.	WHBU 20	Rods	Negative	Motile
21.	WHBU 21	Rods	Negative	Non Motile
22.	WHBU 22	Rods	Negative	Motile
23.	WHBU 23	Rods	Negative	Motile
24.	WHBU 24	Rods	Negative	Non Motile
25.	WHBU 25	Rods	Negative	Non Motile

Further these isolates were identified by different biochemical tests such as IMViC, Sugar fermentation, catalase, oxidase, urease, gelatinase which is presented in the tables below.

Table. 8: Biochemical tests of the bacterial isolates from UTI samples.

IMViC TESTS				
Isolate Number	**Indole Production**	**Methyl Red**	**Vogues Proskeur**	**Citrate Utilization Test**
WHBU 1	Negative	Negative	Positive	Positive
WHBU 2	Negative	Negative	Negative	Positive
WHBU 3	Negative	Negative	Positive	Positive
WHBU 4	Negative	Negative	Positive	Positive
WHBU 5	Positive	Positive	Negative	Negative
WHBU 6	Positive	Positive	Negative	Negative
WHBU 7	Positive	Positive	Negative	Negative
WHBU 8	Negative	Positive	Negative	Negative
WHBU 9	Positive	Positive	Negative	Negative
WHBU 10	Positive	Positive	Negative	Negative
WHBU 11	Negative	Negative	Positive	Positive
WHBU 12	Negative	Negative	Negative	Positive
WHBU 13	Negative	Negative	Negative	Positive
WHBU 14	Negative	Positive	Negative	Negative
WHBU 15	Positive	Positive	Negative	Negative
WHBU 16	Positive	Positive	Negative	Negative
WHBU 17	Negative	Negative	Positive	Positive
WHBU 18	Negative	Positive	Positive	Negative
WHBU 19	Negative	Positive	Negative	Negative
WHBU 20	Positive	Positive	Negative	Negative
WHBU 21	Negative	Negative	Negative	Positive
WHBU 22	Positive	Positive	Negative	Negative
WHBU 23	Positive	Positive	Negative	Negative
WHBU 24	Negative	Negative	Positive	Positive
WHBU 25	Negative	Negative	Positive	Positive

Table. 9: Sugar Fermentation test of the bacteria isolates from UTIsamples.

Carbohydrate fermentation test				
Isolate	**Glucose**	**Lactose**	**Manitol**	**Sucrose**
WHBU 1	Acid and gas	Acid and gas	-	Acid
WHBU 2	-	-	-	-
WHBU 3	Acid and gas	Acid and gas	-	Acid and gas
WHBU 4	Acid and gas	Acid and gas	-	Acid and gas
WHBU 5	Acid and gas	Acid and gas	-	-
WHBU 6	Acid and gas	Acid and gas	-	Acid
WHBU 7	Acid and gas	Acid and gas	-	Acid
WHBU 8	Acid	Acid	Acid	Acid
WHBU 9	Acid and gas	-	-	Acid and gas
WHBU 10	Acid and gas	Acid and gas	-	-
WHBU 11	Acid and gas	Acid and gas	-	Acid and gas
WHBU 12	-	-	-	-
WHBU 13	Acid and gas	Acid and gas	-	Acid and gas
WHBU 14	Acid	Acid	Acid	Acid
WHBU 15	Acid and gas	Acid and gas	-	Acid

WHBU 16	Acid and gas	Acid and gas	-	Acid
WHBU 17	Acid and gas	Acid and gas	-	Acid and gas
WHBU 18	Acid	Acid	Acid	Acid
WHBU 19	Acid	Acid	Acid	Acid
WHBU 20	Acid and gas	-	-	Acid and gas
WHBU 21	Acid and gas	Acid and gas	-	Acid and gas
WHBU 22	Acid and gas	Acid and gas	-	-
WHBU 23	Acid and gas	Acid and gas	-	Acid
WHBU 24	Acid and gas	Acid and gas	-	Acid and gas
WHBU 25	Acid and gas	Acid and gas	-	Acid and gas

Table. 10: Biochemicals tests (Catalase, Urease, Oxidase, H_2S and Gelatin test of the bacterial isolates from UTI samples).

Isolates	Urease	Catalase	Oxidase	H2S	Gelatin
WHBU 1	Negative	Positive	Negative	Negative	Negative
WHBU 2	Negative	Positive	Positive	Negative	Positive
WHBU 3	Positive	Positive	Negative	Negative	Negative
WHBU 4	Positive	Positive	Negative	Negative	Negative
WHBU 5	Negative	Positive	Negative	Negative	Negative
WHBU 6	Negative	Positive	Negative	Negative	Negative
WHBU 7	Negative	Positive	Negative	Negative	Negative
WHBU 8	Negative	Positive	Negative	Negative	Positive
WHBU 9	Positive	Positive	Negative	Positive	Positive
WHBU 10	Negative	Positive	Negative	Negative	Negative
WHBU 11	Negative	Positive	Negative	Negative	Negative
WHBU 12	Negative	Positive	Positive	Negative	Positive
WHBU 13	Positive	Positive	Negative	Negative	Negative
WHBU 14	Negative	Positive	Negative	Negative	Positive
WHBU 15	Negative	Positive	Negative	Negative	Negative
WHBU 16	Negative	Positive	Negative	Negative	Negative
WHBU 17	Negative	Positive	Negative	Negative	Negative
WHBU 18	Negative	Positive	Negative	Negative	Positive
WHBU 19	Negative	Positive	Negative	Negative	Positive
WHBU 20	Positive	Positive	Negative	Positive	Positive
WHBU 21	Positive	Positive	Negative	Negative	Negative
WHBU 22	Negative	Positive	Negative	Negative	Negative
WHBU 23	Negative	Positive	Negative	Negative	Negative
WHBU 24	Positive	Positive	Negative	Negative	Negative
WHBU 25	Nagative	Positive	Negative	Negative	Negative

Based on the above morphological and biochemical tests performed, it was noticed that out of the twenty five isolates eight were *Escherichia coli*, seven were *Klebsiella*speciesthree are *Pseudomonas* species, four were*Staphylococcus* species and three were*Protease* species. Further antibiotic sensitivity test was carried out and the *Escherichia coli* spp which showed resistance was selected molecular identification.

Table. 11: Influence of Temperature, pH, and Salt concentration of growth of bacterial isolates.

S.no	Bacterial Isolate	Effect of temperature			Effect of pH			Effect of NaCl		
		30° C	40° C	45° C	5	8	9	5%	8%	9%
1.	WHBU 1	+	+	-	+	+	-	-	-	-
2.	WHBU 2	+	+	-	+	+	-	-	-	-
3.	WHBU 3	+	+	-	+	+	-	-	-	-
4.	WHBU 4	+	+	-	+	+	-	-	-	-
5.	WHBU 5	+	+	-	+	+	-	-	-	-
6.	WHBU 6	+	+	-	+	+	-	-	-	-
7.	WHBU 7	+	+	-	+	+	-	-	-	-
8.	WHBU 8	+	+	-	+	+	-	+	+	+
9.	WHBU 9	+	+	-	+	+	-	-	-	-
10.	WHBU 10	+	+	-	+	+	-	-	-	-
11.	WHBU 11	+	+	-	+	+	-	-	-	-
12.	WHBU 12	+	+	-	+	+	-	-	-	-
13.	WHBU 13	+	+	-	+	+	-	-	-	-
14.	WHBU 14	+	+	-	+	+	-	+	+	+
15.	WHBU 15	+	+	-	+	+	-	-	-	-
16.	WHBU 16	+	+	-	+	+	-	-	-	-
17.	WHBU 17	+	+	-	+	+	-	-	-	-
18.	WHBU 18	+	+	-	+	+	-	+	+	+
19.	WHBU 19	+	+	-	+	+	-	+	+	+
20.	WHBU 20	+	+	-	+	+	-	-	-	-
21.	WHBU 21	+	+	-	+	+	-	-	-	-
22.	WHBU 22	+	+	-	+	+	-	-	-	-
23.	WHBU 23	+	+	-	+	+	-	-	-	-
24.	WHBU 24	+	+	-	+	+	-	-	-	-
25.	WHBU 25	+	+	-	+	+	-	-	-	-

Table. 12: Colony characteristic on C.L.E.D agar medium.

Bacteria	Colony Characteristics
E.coli	Opaque yellow colonies with a slightly deeper yellow center
Proteus mirabilis	Translucent blue colonies
Klebsiella spp.	Yellow to whitish-blue colonies, extremely mucoid
Pseudomonas aeruginosa	Green colonies with typical matted surface and rough periphery
Staphylococcus aureus	Pale yellow to deep yellow colonies
Enterobacter	Grows wet, grey colored colonies in non-selective medium

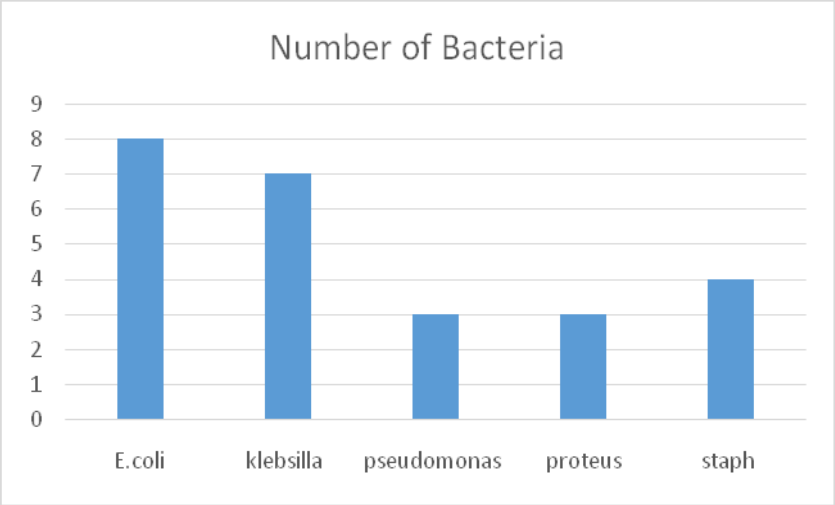


Figure. 6: No. of Bacterial isolates from patients with UTI.

Table. 13: Biochemical identification of bacterial isolates.

S. No	Isolate	Identification of the isolate
1	WHBU 1	*Enterobacter*
2	WHBU 2	*Pseudomonas*
3	WHBU 3	*Klebsiella*
4	WHBU 4	*Klebsiella*
5	WHBU 5	*E. coli*
6	WHBU 6	*E. coli*
7	WHBU 7	*E. coli*
8	WHBU 8	*Staphylococcus*
9	WHBU 9	*Proteus*
10	WHBU 10	*E. coli*
11	WHBU 11	*Enterobacter*
12	WHBU 12	*Pseudomonas*
13	WHBU 13	*Klebsiella*
14	WHBU 14	*Staphylococcus*
15	WHBU 15	*E. coli*
16	WHBU 16	*E. coli*
17	WHBU 17	*Enterobacter*
18	WHBU 18	*Staphylococcus*
19	WHBU 19	*Staphylococcus*
20	WHBU 20	*Proteus*
21	WHBU 21	*Klebsiella*
22	WHBU 22	*E. coli*
23	WHBU 23	*E. coli*
24	WHBU 24	*Klebsiella*
25	WHBU 25	*Enterobacter*

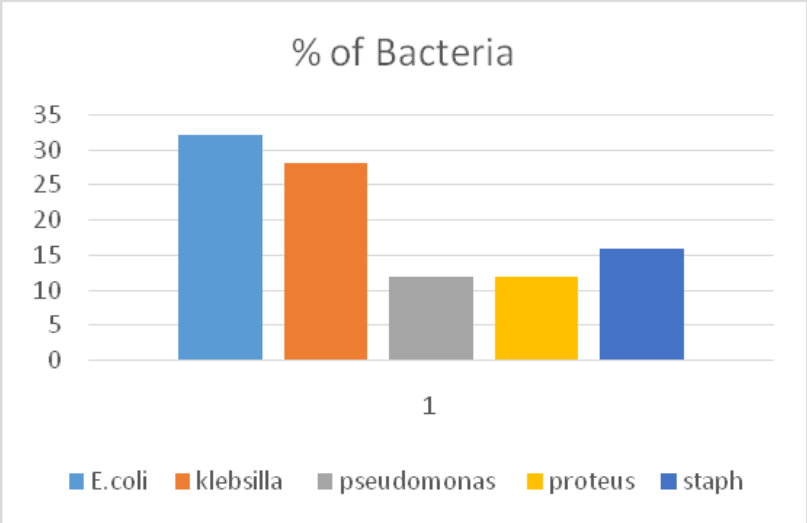
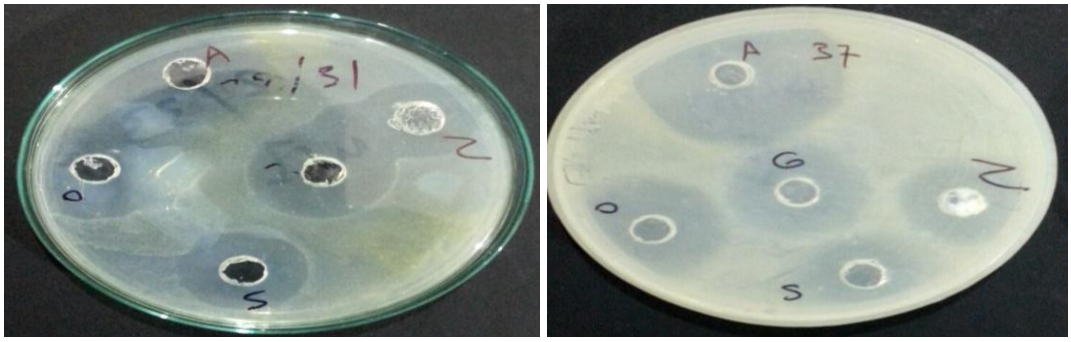


Figure. 7: Percentage of bacterial isolates showing UTI.

Table. 14: Anti-biogram pattern of Bacterial isolates.

S. NO	Isolate	GENT	STREPT	NORFLOX	OFLOXA	AMOXY
1.	WHBU 1	1	0.8	0.1	0.3	-
2.	WHBU 2	0.7	0.9	0.5	1	-
3.	WHBU 3	0.6	0.3	0.3	0.2	-
4.	WHBU 4	0.5	0.3	0.4	0.3	-
5.	WHBU 5	0.9	0.7	0.2	0.3	-
6.	WHBU 6	1	0.8	0.1	0.3	-
7.	WHBU 7	1	0.8	0.3	0.2	-
8.	WHBU 8	1.1	1	0.9	1	0.3
9.	WHBU 9	0.7	0.6	0.4	0.8	-
10.	WHBU 10	0.9	0.8	0.4	0.7	-
11.	WHBU 11	1	0.8	0.5	0.3	-
12.	WHBU 12	0.8	0.7	0.4	0.6	-
13.	WHBU 13	0.5	0.4	0.4	0.3	-
14.	WHBU 14	0.9	0.8	0.9	1	0.4
15.	WHBU 15	1	0.8	0. 1	0.3	-
16.	WHBU 16	0.9	0.9	0.2	0.4	-
17.	WHBU 17	0.9	0.9	0.5	0.3	-
18.	WHBU 18	0.8	0.9	1	0.9	0.2
19.	WHBU 19	1	0.7	0.9	0.8	0.4
20.	WHBU 20	0.7	0.8	0.3	0.3	-
21.	WHBU 21	0.6	0.3	0.3	0.2	-
22.	WHBU 22	1	0.8	0.1	0.3	-
23.	WHBU 23	0.9	0.9	0.2	0.3	-
24.	WHBU 24	0.5	0.4	0.4	0.2	-
25.	WHBU 25	1	0.8	0.5	0.3	-

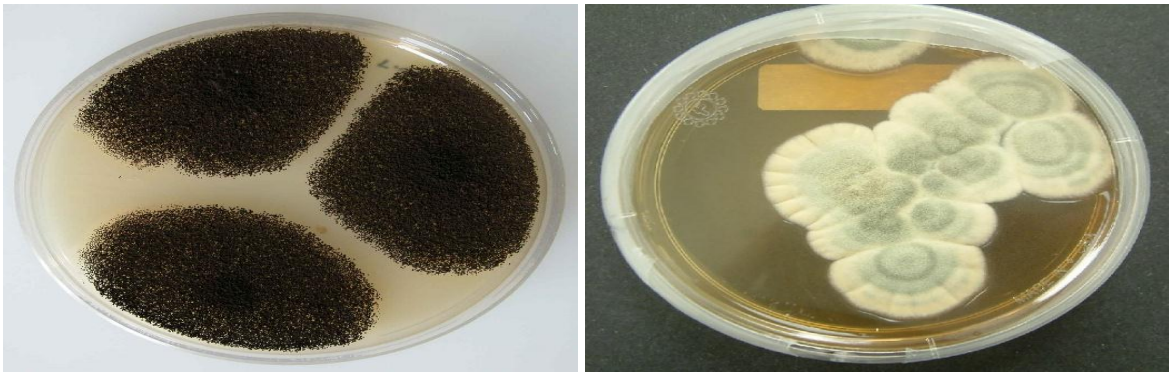
GENT=Gentamycin; Strepto=Streptomycin, Norflox=Norfloxacin, Oflax=Oflaxocin, Amox=Amoxycilli.



Antibiogram pattern of bacterial isolates from UTI.

Fungi isolated and identifiedfrom ice creamsamples.

S.No	Totalnumber ofsamples	Number of positive samples	Name of the Organisms	Number of the organism
1	10	4	*Aspergillusniger* *Penicilliumspp*	2 2



Aspirgillus niger ***pencillium***

Molecular identification of *Escherichia coli:* The culture, which was labeled as **WHBU7**showed similarity with***Escherichia coli*, strain UT2 16S** (**Accession Number: KP276715.**) based on nucleotide homology an Phylogenetic analysis. Information about other close homolog's for the microbe can be found from the Alignment View table.

A. Quality Check on 0.8% Agarose gel.

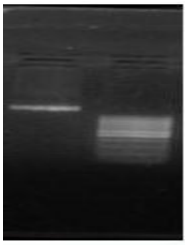


Figure 13: 0.8% Agarose gel showing single 1.5 kb of *16S rDNA* amplicon. Lane 1: WHB7 2: 100 kb Ladder.

DISCUSSION

The overall prevalence of urinary tract infection among male, female and children of sample collected from University Health centre, Tarnaka, OU revealed that percent screened were 5 of male patients, 15 of females and 5 children samples complaining with abdominal pain and burning in urine were collected and tested. The samples of children with age group 3-13 was screened, however, this study did not show any positive UTI. This corroborates with previous studies where, mostly adults have the UTI infection due to their life style, hygienic condition and sexual intercourse. This is comparable to studies done elsewhere in the world. The prevalence of UTI reported in Addis Ababa, Ethiopia was 11.6 %[17], and in a study in Northern Tanzania was 16.4 %[18], Mwanza, Northwestern Tanzania (14.6 %)[19], and Khartoum North Hospital, Sudan (14.0 %).[20] this variation may be explained by the differences in the environment, social habits of the community, the standard of personal hygiene and education. Also the fact that this study was conducted among women who had lower abdominal pains, which is a symptom of UTI, it explains the slightly higher prevalence of 66.7% in our study. In this study, Gram-negative bacteria isolate were more prevalent compared to Gram positive bacteria. However, previous study shows the presence of Gram positive *Staphylococcus aureus* in few UTI.[25]

E. coli is the most common microorganism in the vaginal and rectal area. Anatomical and functional changes and difficulty of maintaining personal hygiene may increase the risk of acquiring UTI from E.coli. In this study, susceptibility pattern of Gram-negative bacteria showed that most of the isolates were sensitive to gentamycin, oflaxacin and streptomycin. Urinary tract infection is a common contagion among both genders with higher prevalence among women due to their physiology and pregnancy enhances the occurrence of the infection due to a variety of physiological changes during the course of pregnancy. In addition, age is an important factor where elderly people with urinary devices like catheters are prone to the infection. Patients undergoing long term treatment are also vulnerable to the infection due to moist hospitalized conditions. In addition, diabetes enhances the incidence due to elevated blood sugar levels and other factors like parity, gravidity, hormonal imbalance, immunosuppressant and geographical location also has a significant role in the incidence of the infection. Though antibiotic usage has proven to be beneficial in counteracting the infection, plant source like cranberry juice and probiotics is equally effective in fighting the infection and can be used as an alternative to counteract the pathogen causing UTI.

CONCLUSION

Bacteria associated with UTI were isolated from patient samples. A total of 25 bacterial isolates were obtained by pure culture techniques based on morphological variation. All the 25 bacterial isolates were cultured on UTI specific medium (CLED agar). They were further characterized morphologically and identified by biochemical methods. It was found that 8 isolates were *Eschericha coli*, 7isolates were *Klebsiella* species, 3 were*Proteus* species 4 were *staphylococcus* and 3 were *Pseudomonas* species. All the 25 bacterial isolates were characterized for antibiogram pattern using Gentamycin, streptomycin, norfloxcin, oflaxacin and amoxicillin. The isolate which showed maximum antibacterial activity is identified by 16s r RNA sequencing.

REFERENCES

1. Abramoff, M. D., P. J. Magelhaes, et al. "Image processing with ImageJ "Biophotonics International, 2004; 7: 36–42.
2. Agace, W. W., S. R. Hedges, et al. "Interleukin-8 and the neutrophil response to mucosal gram-negative infection." The Journal of clinical investigation, 1993; 92(2): 780-785.
3. Alteri, C. J., S. N. Smith, et al. "Fitness of Escherichia coli during urinary tract infection requires gluconeogenesis and the TCA cycle." PLoS pathogens, 2009; 5(5): e1000448.
4. Andersen-Nissen, E., T. R. Hawn, et al. "Cutting edge: Tlr5-/- mice are more susceptible to Escherichia coli urinary tract infection." The Journal of immunology, 2007; 178(8): 4717-4720.
5. Anderson, G. G., J. J. Palermo, et al. "Intracellular bacterial biofilm-like pods in urinary tract infections." Science, 2003; 301(5629): 105-107.
6. Apodaca, G. "The uroepithelium: not just a passive barrier." Traffic, 2004; 5(3): 117- 128.
7. Arinzon, Z., S. Shabat, et al. (2011). "Clinical presentation of urinary tract infection (UTI) differs with aging in women." [Published online ahead of print, Sept 29] Archives of gerontology and geriatrics.
8. Becker W, L. N., Pedersen AN, Aro A, Fogelholm M, Tórsdottír I, Alexander J, Anderssen SA, Meltzer HM, Pedersen JI (2005). Nordic Nutrition Recommendations 2004 Copenhagen, Nordic Council of Ministers.
9. Belderbos, M. E., M. L. Houben, et al. "Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis." Pediatrics, 2011; 127(6): e1513-1520.

10. Bent, S., B. K. Nallamothu, et al. "Does this woman have an acute uncomplicated urinary tract infection?" JAMA: the journal of the American Medical Association, 2002; 287(20): 2701-2710.
11. Bergsten, G., M. Samuelsson, et al. "PapG-dependent adherence breaks mucosal inertia and triggers the innate host response." The Journal of infectious diseases, 2004; 189(9): 1734-1742.
12. Bergsten, G., B. Wullt, et al. "Do type 1 fimbriae promote inflammation in the human urinary tract?" Cellular microbiology, 2007; 9(7): 1766-1781.
13. Blanco, J., M. P. Alonso, et al. "Virulence factors of bacteraemic Escherichia coli with particular reference to production of cytotoxic necrotising factor (CNF) by P-fimbriate strains." Journal of medical microbiology, 1990; 31(3): 175-183.
14. Bode, W., C. Fernandez-Catalan, et al. "Insights into MMP-TIMP interactions." Annals of the New York Academy of Sciences, 1999; 878: 73-91.
15. Brauner, A., S. H. Jacobson, et al. "Urinary Escherichia coli causing recurrent infections--a prospective follow-up of biochemical phenotypes." Clinical nephrology, 1992; 38(6): 318-323.
16. Sreenath Konanki, Jayasimha Rayalu Daddam, Anitha S And Muralidhararao Dowlathabad Analytical Method Optimization For Biologics Mitteilungen Klosterneuburg, 2014; 64(7): 135-151.
17. Sreenath Konanki , Jayasimha Rayalu Daddam, Anitha S And Muralidhararao Dowlathabad Modelling And Ligand Interaction Studies Of Endo-1,4-Beta-Xylanase From Bacillus Subtilis IJPAES, 2014; 4(1): 19-24.
18. Brauner, A., M. Katouli, et al. "Production of cytotoxic necrotizing factor, verocytotoxin and haemolysin by pyelonephritogenic Escherichia coli." European Journal of clinical microbiology and infectious diseases, 1990; 9(10): 762- 767.
19. Bressan, S., B. Andreola, et al. "Procalcitonin as a predictor of renal scarring in infants and young children." Pediatric nephrology, 2009; 24(6): 1199-1204.