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To Isolate and Identify the Etiological Agents and Establish the Co-Relation between Pyuria and Significant Bacteriuria among Patients Suspected of Urinary Tract Infection

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Abstract:- Urinary tract infection (UTI) one of the most common infectious diseases has been seen as a global burden. Escherichia coli (E. coli) is responsible for causing majority of the UTIs. This study was conducted at Kantipur Hospital Pvt. Ltd. Kathmandu from July 2016 to December 2017.The research was conducted to study about the relation between pyuria and bacteriuria among patients suspected of urinary tract infection. A total of 464 mid-stream urine samples were collected. Out of total processed sample 97/464 was positive while 263 were culturally negative. E. coli accounted for 76.28 %(74) of the growth while K. pneumoniae accounted for 9.27% (9) of the total bacterial growth. Other organism which were found were P. vulgaris 8.24 (8) S. aureus 3.09 %(3), Coagulase negative staphylococcus (CoNS) 1.03% (1), E. facealis1.03% (1) and P. aeruginosa 1.03% (1). Amikacin was most susceptible drugs for E. coli with 90.54% (67) success followed by gentamicin 81.08 %(60). Both amikacin and gentamicin proved susceptible for other gram negative isolates too. Among the total isolates 73 of them were found to Multi Drug Resistant (MDR) were E. coli 92.85% (66) has most number of MDR cases followed by 3.96 %(4).It was found that female patient were more affected (79) than male patient (18).While on age group basis people aging between 21-30 years mostly had infection (31). Among the 464 samples, 78.67% (365) of samples showed insignificant pyuria. However, 4.12% of samples gave positive culture results. The highest Culture positivity, 28 (75.67%) samples out of 37 was detected in urine samples having pus cells of 6-10/hpf, whereas 5(33.33%) urine samples with more han 50 pus cells/hpf was culture positive. Female are more susceptible for UTI than Male and presence of pyuria can be good indicator of urine infection, though Culture should always be considered as gold standard.

Keywords:- *Urinary Tract Infection; Pyuria; Bacteriuria; Urine Culture.*

I. INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious diseases diagnosed in outpatient as well as hospitalized patients and utilizes large proportion of antimicrobial drug consumption for treatment[1]. Around 150 million people are diagnosed worldwide with UTI each year, which cost global economy in excess of 6 billion US dollars [2]. UTI is defined as the presence of multiplying microorganisms in the tract and should be accompanied by laboratory findings (bacteriuria, leucocyturia, and positive urine culture) [3, 4]. The urinary tract consists of the kidneys, ureters, bladder and urethra and infection can occur in any part, however entire urinary tract may be prone for bacteria invasion in case any one of urinary tract part gets infected [5, 6].

The Enterobacteriaceae are the most frequently detected pathogen responsible for causing around 84.3% of UTI [7]. *Escherichia coli* (*E. coli)* a common member of this family is responsible for causing around 75.0-90.0% of all UTIs. Other organisms like Streptococci, *Pseudomonas* spp., Staphylococci, *Candida albicans*, and *Enterococcus* spp. can also be responsible for causing UTI [8, 9]. *E. coli* that resides on GastroIntestinal (GI) tract as a commensal provide the pool for initiation of UTI and certain serotypes of *E. coli* like Uropathogenic *E. coli* is responsible for uropathogenicity [10]. *E. coli* is predominant facultative aerobes of the human colonic microflora. Most *E. coli* strains are harmless to humans, but pathogenic strains can cause various ailments like; gastroenteritis's, Urinary Tract Infection (UTI), Hemolytic Uremic Syndrome (HUS), Mastitis, Septicemia, Peritonitis, Gram negative pneumonia and in rare cases can also lead to neonatal meningitis [11].

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The most common agents for causing UTI are the Gram negative bacilli. Eighty percent of acute infections in patients is caused by *Escherichia coli* even without catheters, urologic abnormalities or calculi. A very small portion of uncomplicated infection can be caused by other Gram negative rods, especially *Proteus* and *Klebsiella*, and occasionally *Enterobacter*. About 10% to 15% of acute symptomatic UTI in young females are caused by *Staphylococcus saprophyticus* whereas, in case of hospitalized patient, *E. coli, Proteus, Pseudomonas, Klebsiella, Serratia, Staphylococci, Enterococci* and *Candida* accounts 10% to 15% catheter related UTI [12].

The gender and sexual anatomy are among the major determinants of UTI. Women have higher chances of getting UTI in comparison to men this can be as the matter of fact that women urethra is much shorter and very close to the anus, which is a constant source of fecal bacteria. Whereas, in case of male UTI is rare unless microorganisms are introduced artificially with catheters. UTI among the preschool children is approximately 2% and its incidence is 10 times more common in girls. Likewise, in case of school aged girls the percentage increases to 5%. The largest group of patients with UTI is adult women. Rates of infection are also high in post-menopausal women further incidence increases with age and sexual activity. 1 in every 3 women in their mid 20's will experience more than one episode of UTI, and approximately sixty percent women's report having had UTI during their lifetime and in case of males younger than 50 years UTI is generally unusual[13, 14].

The genitourinary tract, which runs from the kidney's renal cortex to the urethral meatus, is the site of microbial invasion that causes a variety of diseases known as urinary tract infections. Pyuria and bacteriuria are the two most significant markers of urinary tract infections. Bacteriuria is defined as the presence of more than 105 colonies of a single pathogen per milliliter of urine, whereas Pyuria is defined as the presence of white blood cells (WBCs) in the urine. A more current definition is the presence of as few as 103 CFU/ml in symptomatic patients or when a specimen is obtained by sterile catheterization [15, 16, 17, 18].

A study conducted in Bangladesh demonstrated the rate of UTI to be 48.61% [19]. The overall prevalence of UTI in Turkey was found out to be 1.7% in hospitalized patient and 65.4% of UTI were associated with urinary catheters [20]. A study from Germany reported that the most common nasocomial infection was UTI (28%) [21].

Nepal, being a developing country is lagging in the concept of hygiene and so is always vulnerable to infections. Further the healthcare system in Nepal is also in developing phase; the facilities like urine culture and antimicrobial susceptibility testing are also not available in many parts of Nepal, thus leading to incorrect diagnosis, mismanagement of UTI and antibiotics are usually given empirically before the laboratory results of urine culture are available. Further, in Nepal people are not serious regarding routine health check-up and generally pursue medical attention when the disease symptoms begin to become noticeable or aggravate diseases which can ultimately leads to serious complication [22, 23].

The fact being bacteria resistance to three or more antibiotics among six commonly prescribed drugs have lead to Multiple drug resistant (MDR) which have become an emerging problem throughout the world and has been seen as emergence of treatment problem [24].

Antimicrobial resistance, which has been developing and has been connected to a higher likelihood of clinical failure, is one of the primary issues surrounding UTIs. Resistance to beta-lactam, fluoroquinolone, and trimethoprim-sulphamethoxazole (TMP/SMX) is increasingly concerning. Additionally, reports show that cotrimoxazole resistance is more common than 15.0% and may reach 25.0% [25, 26].

The objective of this study is to isolate and identify the etiological agents of Urinary tract infection and establish the co-relation between pyuria and significant bacteriuria. The result of this study can be helpful to health care professionals to facilitate the treatment of patients and manage the symptoms that are associated with UTIs. The result will also help to show pyuria can be alternative for bacterial cultural when cultural in not available in certain setups.

II. METHODOLOGY

A. Study Site

This study was conducted at Microbiology laboratory of Kantipur Hospital Pvt. Ltd. Kathmandu Nepal from July 2016 to December 2017. A total of 464 urine samples from patients suspected of UTI were collected and processed accordingly.

B. Data Collection

Patients visiting Kantipur Hospital Pvt. Ltd for urine microscopy and culture were taken for study.

C. Inclusion and Exclusion Criteria

Patients from out-patient and in-patient of all age and sex were included in the study whereas patients under antibiotic therapy and those specimens who do not meet standard acceptance criteria as described by Cheesebrough were excluded from study [27].

D. List of Equipment and Materials

- Equipment: Weighing machine, Water bath, Glass ware, Inoculating wire and loop, Autoclave, Incubator, Hot air oven, Microscope, Refrigerator,
- Microbiological Media: Sulfer Indole Motility Media, Meullar Hinton Agar, Urea Agar Base, Simmon's Citrate Agar, Blood Agar, Chocolate Agar, Mac Conkey Agar, Triple sugar iron Agar, Muellar Hinton Broth, Hugh and Leifson's Media.
- Chemical and Reagent: Barritt's reagent (40% KOH, 5% alpa-napthol in a ratio of 1:3), Barium chloride, Conc.Sulfuric acid, Gram's reagent, Catalase reagent (3% H2O2), Oxidase Reagent (1% Tetramethyl p-phenylene diamine dihydrochloride), Kovac's reagent.

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- Antibiotic discs: Co-trimoxazole (25 µg), Gentamycin (10 µg), Imipenam (10 µg), Nalidixic acid (30 µg), Nitrofurantoin (30 µg), Piperacillin/Tazobactam, Amikacin (30 µg), Amoxicillin (10 µg), Ampicillin (30 µg), Cefotaxime (30 µg),

E. Specimen Collection

Every patient received a sterile, dry, clean, and leak-proof container along with instructions on how to properly collect their samples. The patient was asked to provide a clean, midstream urine sample of 20 milliliters. Patients were asked to offer their first urine pass (i.e., the first urine of the day) for evaluation whenever possible, as this specimen is better suited for analysis. The patient's midstream pee was collected, and it was processed right away. Samples were taken from young children (under the age of five) following appropriate parental instruction [28].

F. Specimen Evaluation

For Specimen evaluation single urine specimen was collected from each patient and bacteriological culture was performed. Further, routine microscopic observation was done after inoculation.

Specimen was properly labeled that include; full name, age, sex, serial number, date and time of collection. Likewise, for visible signs of contamination include; turbidity, particles and blood cells [28].

G. Sample Processing

➢ *Routine Macroscopic Examination*

Urine sample was collected and Macroscopic examination was done by observing its color, turbidity and appearance and reported accordingly [28, 29].

➢ *Routine Microscopic Examination*

A clean, sterile centrifuge tube was filled with approximately 5 ml (roughly half of the sample), and the sample was centrifuged for 10 minutes at 3000 rpm. The supernatant was discarded. After that, the sediment was investigated via wet mount preparation, which allowed for the detection of RBC and WBC (pus cells). White blood cell and red blood cell counts were calculated using the High Power Field Urine (HPF), which measures the number of items visible through a 40X microscope objective [28, 29].

H. Culture of Specimen

In order to identify significant bacteriuria using standard methods, a semi-quantitative culture technique was employed. This involved using an inoculating loop with a standard dimension of 2 mm to inoculate a known volume (0.001 ml) of mixed, uncentrifuged urine onto the surfaces of Blood Agar (BA) and Mac Conkey Agar (MA) at a fixed (±10% error was accepted) rate. The inoculated MA and BA plates were then incubated aerobically for the entire night at 37º C. The bacterial count was then recorded in accordance with Vandepitte et al.'s protocol. Considering the fact that BA may quickly identify infections and facilitate the separation of gram-positive organisms such as hemolytic streptococci, it is favored over MA in these situations [27, 28, 29].

I. Biochemical Tests

To identify bacterial isolates, various biochemical assays were performed. Tests for Oxidase, Coagulase, and Catalase were used to identify isolates that were Gram-positive. Similarly, tests for Gram-negative bacteria isolates included those for catalase, oxidase, indole, methyl red, Voges Proskauer, citrate utilization, oxidation/fermentation, triple sugar iron (TSI), motility, gas production, hydrogen sulfide production, and urease. Additionally, test findings were documented when pure colonies of bacteria were injected on media plates using various biochemical medium. [28].

J. Antibiotic Susceptibility Testing [30]

Mueller Hinton Agar was made and then sterilized for the purpose of assessing antibiotic susceptibility. The medium's pH was adjusted to between 7.2 and 7.4. The medium depth in the petridish was kept at 4 mm, or roughly 25 ml per plate. A sterile wire loop was used to inoculate a single isolated colony into a Mueller Hinton broth tube for the sensitivity pattern, and the tube was then incubated for two to four hours at 37ºC.

Next, the suspension's turbidity was assessed against the Mc Farland tube number 0.5 standard. After utilizing the carpet culture method to inoculate a plate of MHA with the bacterial suspension, the plate was allowed to dry on the agar surface for approximately five minutes. After that, the suitable antimicrobial discs (6 mm diameter) were equally dispersed on the inoculation plates using sterile forceps, and no more than 6 discs were placed on a Petri dish with a 90 mm diameter. The diameter of each zone of inhibition was measured in millimeters and compared with a standardized zone interpretive chart after the plates were checked for growth.

K. Purity Plate

The same inoculums were sub-cultured in respective medium and incubated when doing biochemical tests. Appearance of pure growth of organisms was checked in the media in order to ensure whether the inoculums used for performing biochemical tests were done in an aseptic condition or not.

L. Quality Control

The quality of each agar plate prepared was maintained by incubating one plate of each batch in the incubator. For identification test, control strains from American Type Culture Collection (ATCC 25922) were used and for the standardization of Kirby-Bauer test and also for correct interpretation of inhibition zones of diameter. The thickness of MHA was adjusted at 4mm and the pH at 7.2-7.4 to maintain quality of sensitivity test. Similarly antibiotics discs having correct amount as indicated was used. All the procedure was carried out under strict aseptic condition. Temperature of the incubator, refrigerator and freezer was checked properly and major focus was given to quality control in order to obtain reliable microbiological results.

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M. Data Analysis

Win Pepi (version 11.43) was used to statistically analyze all of the collected data. The Chi-square ($\chi 2$) test was used to identify any significant associations between the various factors that could be responsible for a UTI.

A p-value of less than 0.05 was considered to be statistically significant ($p<0.05$), while p-value more than 0.05 was considered to be statistically non-significant (NS) ($p>0.05$).

III. RESULTS

A. Distribution of Significant Growth among Cases Examined

Among the total 464 UTI suspected urine samples, 367 urine samples were from outdoor patients while 64 urine samples were taken from for indoor patients. Culture positive cases for outdoor and indoor patients were 81 and 16 respectively, which is shown in Table 1. There was no association between culture positivity and types of cases.

Table 1: Distribution of Significant Growth among Cases Examined

Patients	Total samples	Positive Samples	P-value
Indoor	64	16	
Outdoor	400	81	P=0.548
Total	464	97	

B. Age and Sex Wise Distribution of Bacterial Isolates in Positive Cases

Out of 97 positive growth cases, 18 (18.55%) were from male patients while 79 (81.44%) were from female patients. In male, maximum number of isolates (n=7) was observed in age group 21-30 years, and in female, maximum number of isolates (n=31) was observed in age group 21-30 years. (p=0.548).

Table 2: Age and Sex-Wise Clinical Distribution of Bacterial Isolates in Cultural Positive Cases

Age of patients (in years)	Number of Bacterial Isolates			
	Male	Female	Total No. (%)	
<10	1	3	4(4.13%)	
11-20	2	7	9(9.28%)	
				(p=0.548)
21-30	7	31	38(39.17%)	
31-40	5	20	25(25.78%)	
41-50	0	11	11(11.34%)	
51-60	1	05	6(6.18%)	
61-70	2	02	4(4.12)	
Total	18 (17.46%)	79(81.44%)	97	

C. Pattern of Bacterial Isolates in Processed Urine Samples

A total of 7 different genus of bacteria were isolated from significant bacteriuria urine samples, which are shown in Table 3. Among the isolates, *E. coli* 74(76.29%) was found to be the most predominant organism followed by *Klebsiella pneumoniae* 9 (9.27%), *Proteus vulgaris 8* (8.24%), *Staphylococcus aureus* 3 (3.09%),*Pseudomonas aeruginosa* 1 (1.03%), Coagulase negative staphylococcus (CoNS) 1 (0.36%) and *Enterobacter fecalis1* (1.03%), as seen in Table no 3.

Table 3: Pattern of Bacterial Isolatesin Processed Urine Sample

Bacterial isolates	Number of isolates	Percentage of isolates
E. coli	74	76.29%
K. pneumoniae	9	9.27%
P. vulgaris	8	8.24%
S.aureus	3	3.09%
CoNS	1	1.03%
E. facealis	1	1.03%
P. aeruginosa	1	1.03%
Total	97	100%

D. Comparison of Pyuria and Significant Bacterial Growth

Among the 464 processed samples, 78.67% (365) of samples showed insignificant pyuria. However, 4.12% (4/365) of insignificant pyuria samples gave positive culture results. Similarly, 99 (21.33%) of total processed samples showed significant pyuria. Among those showing significant pyuria, 79.06% (93/97) samples gave positive culture result and 6 samples showed negative growth results.

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Table 4: Comparison of Pyuria and Significant Bacterial Growth

Pyuria (%)	Culture positive (%)	Culture negative (%)	Total
Significant (≥ 5 WBC/HPF)	93 (95.87)	6 (1.37)	99 (21.33
Insignificant (<5 WBC/HPF)	4(4.12)	361 (98.63	365 (78.67)
Total	97 (20.90%)	367 (78.10)	464 (100.0)

E. Antibiotic Susceptibility Pattern of E. Coli against Different Antibiotic

The antibiotic susceptibility pattern of isolated *E. coli* against different antibiotics were tested and analyzed accordingly. *E. coli* showed the highest i.e. 90.54% susceptibility against Amikacin followed by 81.08% Gentamicin and 78.37 % Piperacillin/Tazobactam whereas *E. coli* was fully resistant against ampicillin.

Table 5: Antibiotic Susceptibility Pattern of *E. coli* against Different Antibiotic

Antibiotics	Sensitive Frequency	%	Resistant Frequency	%
Ampicillin	0	0	74	100
Amikacin	67	90.54	7	9.46
Cotrimoxazole	45	60.81	29	39.19
Ciprofloxacin	17	22.97	29	39.19
Cefotaxime	28	37.83	46	61.17
Nitrofurantoin	59	79.72	15	20.18
Gentamicin	60	81.08	14	18.91
Norfloxacin	28	37.83	46	62.17
Piperacillin/Tazobactam	58	78.37	16	21.63

F. Antibiotic Susceptibility Pattern of other Gram Negative Isolates

Gram negative bacterial isolates were most susceptible to Amikacin 16 (88.88%) followed by Gentamicin 15 (83.33%) while Ampicillin was least effective with 17 isolates being resistant to it as shown in Table 6.

Table 6: Antibiotic Susceptibility Pattern of Gram Negative Bacterial Isolates

Antibiotic	Susceptible		Resistant	
	No	Percent	No	Percent
Ampicillin	1	5.56%	17	94.44%
Amikacin	16	88.88%	2	21.12%
Ciprofloxacin	12	66.67%	6	33.33%
Cotrimoxazole	12	69.67%	6	30.33%
Ceftriaxone	10	55.55%	8	44.45%
Gentamicin	15	83.33%	3	83.33%
Nitrofurantoin	9	50%	9	50%
Nalidixic Acid	5	27.78%	12	72.22%
Norfloxacin	11	61.11%	7	38.89%

G. Multi Drug Resistance Profile Among Gram Negative Bacterial Genera

Among the total positive cases, 73 were found to be multi drug resistant bacteria. Among the 73, MDR cases high number of the cases were found to be from *E. Coli* 66 followed by *K. pneumonia 4*, which is shown in Table 7.

Table 7: Multidrug Resistance Profile of Bacterial Strains from Different Samples

S. No.	Organisms	Total Isolates	No. of MDR Strains
1	*E. coli*	74(85.02%)	66(92.85%)
2	*K. pneumoniae*	9(7.69%)	4(3.96%)
3	*Ps. aeruginosa*	8(2.02%)	3(0%)
4	*P. vulgaris*	1(0.40%)	0(0%)
5	*Enterobacter*spp	1(0.80%)	0(0%)
TOTAL		**93**	73

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IV. DISCUSSION

Among 464 urine samples, only 97 (20.90%) urine samples were found positive and 367 (79.10%) samples did not show any growth. Similarly, previous studies have also reported the low growth rate of 21.8% [31]. This might be due to the now clinical presumption given by the patient to the physician.

Among the 97 UTI positive cases, the percentage of positive uropathogens were found higher in out-patients (81) than in in-patients (16), which were found statistically insignificant.(P=0.548)

In this study, 81.44% (79/97) female and 18.56% (18/97) male were affected by UTI which was similar to a study done by Gupta et al [32]. Further, this study shows the age group 20-30 years has got high prevalence of UTI. A total of 31 female and 7 male positive cases of UTI were found in this age group. A study done at Kathmandu also showed highest numbers of positive samples were from this age group with 32.57% [33, 34]. This result suggests that sexually active men and women and women of childbearing age are more susceptible to UTI. A study revealed that married women have high prevalence of getting UTI in-comparison with nuns and unmarried women [35]. The study also suggests that sexual intercourse is an important factor regarding pathogenesis of UTI and the female group has a more uniform distribution as well as elevated incidence in twenties and forties can be due to obstetric and gynecological causes respectively [1]. However, based on study, the risk of infection may increase as the age of the men increases since the age group of 31-40 (5) has shown the second highest positive cases in male patients while in female the age group of 31-40 (20) showed second highest positive cases.

Among the isolates, *E. coli* 74(76.29%) was found to be the most predominant organism which were in agreement with various studies [32, 38]. Similarly a study reported that *E. coli* represented about 70% to 90% of the causative agents of UTI [36]. A study revealed that proposition of *E coli* has been rising as in the year 1991 there were 69% of positive cultures which had increased to 75% in 1994 and likewise, 81% in 1997 [37].

Klebsiella pneumoniae was next common causes of UTI in our study i.e. 9 (9.27%). A study from Bangladesh found that *Klebsiella pneumoniae* isolation rate was nearer to 6.7%, which supported our findings [38]. Similarly, various studies found isolation rate of 32.20% and 17.6% for *Klebsiella* spp [39, 40] which were not in agreement with the present study.

This study also found the incidence of Gram positive cocci of about 4.67% where 3.54% were for *Staphylococcus aureus*. A study conducted on India showed the lower incidence (1.5%) for *Staph. Aureus* [41] and study conducted on Turkey showed the isolation rate to be (4.8%) which was contrary to our study [42]. This finding agreed with other study done in Nepal [31, 43, 44, 45]. The result is in agreement with various studies [41, 46, 47, 48, 49, 50, 51] which shows gram negative bacteria mostly *E. coli* was the commonest bacteria isolated in patients with UTI. However, differ from the study which reported *Pseudomonas aeruginosa* and *Klebsiella* spp respectively as the predominant bacteria [52, 53]. Whereas, various studies have still reported higher incidence of *E. coli* 71.3%, 73.0% and 76.8% respectively in urine sample [54, 55, 56].

In this investigation, K. pneumoniae was the second isolate other than E. coli that has the ability to manufacture the urease enzyme, which is responsible for catalyzing the hydrolysis of urea and releasing ammonia [57].

In this study amikacin was found to be most effective drugs 90.5% against *E. coli.* Other antibiotics such as Gentamicin, Nitrofurontoin showed 81.08% and 79.72% susceptibility against the bacterium respectively. Ampicillin was found to be 100% resistant to the organism whereas, combination of Trimethoprim and Sulphamethoxazole was not found effective for UTI treatment and all the uropathogens from inpatients and outpatients showed high degree of resistance to Cotrimoxazole [32]. A study also showed 54.8% of *E. coli* isolates were resistant to Ampicillin, 28% to Cotrimoxazole and 9% to Ciprofloxacin [58]. A study revealed that *E. coli* isolates were found least susceptible to Nalidixic acid and the most potent antibiotics were found to be Nitrofuratoin and Norfloxacin [59]. Various studies found resistant to Ampicillin and also observed that Ampicillin resistance was present in more than 93.0% isolates of *E. coli.* up to an extent of 93.0% [60, 61, 62].

In a study done in Turkey, *E. coli* showed two types of Multi drug resistance (MDR) against different antibiotics one type against three antibiotics viz., Ampicillin, Nalidixic acid and Norfloxacin and other type against four antibiotics viz., Ampicillin, Nalidixic acid, Ciprofloxacin and Nofloxacin [63]. In this study MDR isolates were accounted 53.27 % of the total samples which differs from the study done in Nepal, where MDR isolates accounted for 35.2% cases [64].

Since this study is carried out in Kantipur Hospital Pvt Ltd, this study cannot be generalized for whole country. A similar study can be done using more than one hospital for generalization with in Nepal. *E.coli* is the predominant pathogen of urinary tract infection so further analysis of *E. coli* on its extended spectrum Beta lactamase (ESBL) can be done.

V. CONCLUSION

The study showed that the female were more susceptible to UTI than the male patients. Microscopy of urine can be useful tool for early diagnosis of urinary tract infection when culture facilities are not available however presence of pyuria can be considered as diagnostic criteria for urinary tract infection. The drug of choice to treat urinary tract infection caused by *E. coli* can be Amikacin and Gentamycin and Nitrofurantoin can be used as alternative drug. Multi drug resistant have been an emerging problem therefore rampant use of antibiotic should come to an end and appropriate guideline should be followed regarding prescribing pattern of antibiotic for UTI.

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- Conflict of Interest**:** None

Fig 1: Isolated Colonies of Staphylococcus Aureus in Blood Agar (Isolate no 79)

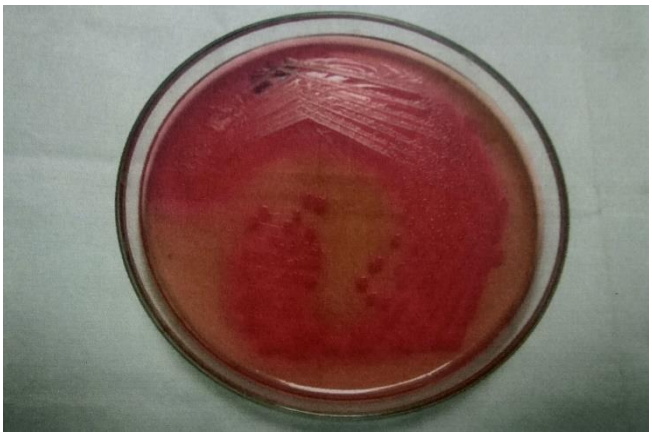


Fig 2: Isolated Colonies of E. Coli in Macconkey Agar (Isolate no 118)

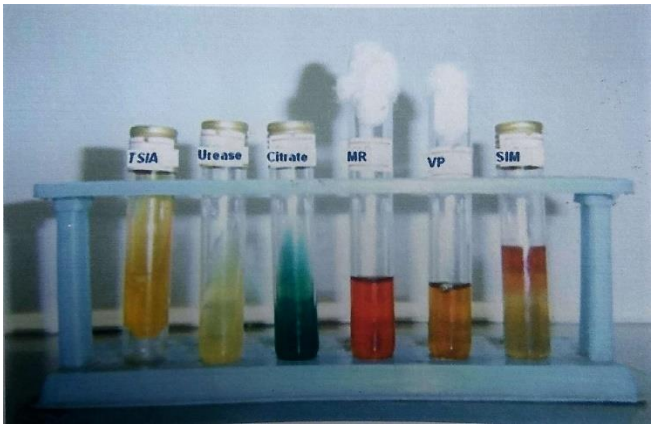
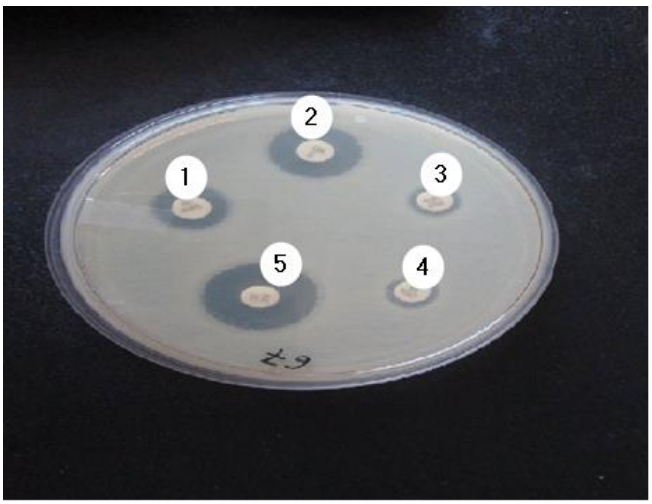


Fig 3: Biochemical Tests of *E. coli* (From Left to Right TSIA:Acid/Acid, Gas Production and H_2S Negative; Urease Negative, Citrate Negative, Methylene Red Positive,VP Negative, Motile and Indole Positive.)



1 Nitrofurantoin (300µg) 2 Gentamicin (10µg) 3 Norfloxacin (10µg) 4 Ceftriaxone (30µg) 5 Amikacin (30µg)

Fig 5: Antibiotic Susceptibility Pattern of E. Coli

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