Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Microbiology

Prevalence of Bacterial Isolates Specimens from Suspected Patients of Urinary Tract Infection in Both Outpatient Department and in Patient Department in MMCH, Mymensingh, Bangladesh

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DOI: 10.36347/sjams.2021.v09i10.013 | **Received:** 09.09.2021 | **Accepted:** 12.10.2021 | **Published:** 15.10.2021

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Abstract **Original Research Article**

Background: Urinary tract infection (UTI) is among the most common bacterial infections and posses significant healthcare burden. Escherichia coli is the most common cause of UTI accounting for about 70%% and a variable contribution from Proteus mirabilis, Pseudomonas aeruginosa and Klebsiella pneumoniae Patients are often treated as soon as bacteria are shown to be present by microbiological culture. ***Objective:*** To identify the prevalence of bacterial isolates specimens from suspected patients of urinary tract infection in both outpatient department and in patient department. ***Methods:*** This study was carried out in the department of Microbiology, Mymensingh Medical College during the period from July 2016 to June 2017. Urine specimens were collected and isolation and identification of major uropathogens (Escherichiacoli Klehsiella pneumonias, Proteusmirabilis, and Pseudomonas aeruginosa) were done by standard microbiological procedure a biochemical tests. The antibiotic sensitivity pattern of the isolate according to age and sex. ***Results:*** Out of 250 urine specimens, 200 specimens were isolated and identified by culture and different biochemical methods which were supported by microscopical examination and at the same time PCR could detect species specific genes in 201 specimens directly from urine of suspected UTI patient Escherichia coli was responsible as a leading causative pathogen in both outpatient department and in patient department with a higher prevalence of 71.8% for outpatient department. On the other hand prevalence of Proteus mirabilis was lowest and it was 1.8 % in outpatient population. Culture positivity of urine specimens was higher in female in both out patient population and inpatient population. Culture positivity of in patient population among the male (45.5%) was slighty higher than that of outpatient population (34.5%). The predominant age group suffered from UTI in case of outpatient population was >15-30 but for the in patient population, the age group was 60 years and above. ***Conclusion:*** The prevalence of the UTI in female patients was predominant in both out-patient and in-patient department in the present study. The prevalence of high UTI in the in-patient department has several associated risk factors indicating the catheterization is the most predominant followed by the physiological and pathogenic conditions like pregnancy, menopause, diabetes, and immobility. It also observed that almost all patients of in-patient department who developed UTI had used urinary catheters, other factors associated with the development of these infections is due to prolonged use of urinary catheter, female patient and prolong stay in intensive care unit of hospitals.

Keywords: Prevalence of Bacterial isolates, UTI, Risk Factors.

I INTRODUCTION

UTI is the second most common infectious presentation in community medical practice after the respiratory tract infections [1].Worldwide about 150 millions peoples are diagnosed with UTI each year and are classified as uncomplicated or complicated [2]. As the most common healthcare-associated infection, UTI accounts for more than 30% of infections reported by acute-care hospitals [3]. A study was done in South India and it showed higher prevalence of UTI in women (47.9%) than men (34.1%) [4]. UTI is more prevalent in

female (65.7%) than male (34.4%) in Bangladesh. Twenty five percent to 35% of all female suffer from UTI at some stages in their lives. A higher incidence (16.8%) of UTI was noted among adult women aged above 19 years. Predominant age group is 20-30 years. Prevalence of UTI in infant and young (0-15 age group) is very few [5, 6]. Prevalence of urinary tract infections is dependent on factors like gender, age, disease, race and nutritional status. Females are generally more predisposed to renal infections, compared to males, except at the extremes of early childhood and geriatric stage [7]. This is basically due to the shorter length of the urethra in females and its closeness to the excreta passage, which is a source of pathogens that can colonize the urethra. Again, sexual intercourse, pregnancy and childbirth contribute to the increase in UTI in females [8, 9]. It is estimated that about 50% of all women are likely to experience an UTI in their lifetime [10]. At the first three months after birth, boys have a higher UTI prevalence of 2.77% as compared to0.7% in girls [11]. However, just before puberty, girls are at higher risk with3-5% UTI prevalence while it remains unchanged at 2.T96 among males of the same age [12, 13]. During the pubertal stage and adulthood, females maintain a higher prevalence than males [14]. Other studies indicate a10-12 fold increase in risk of UTI in uncircumcised male infants and this is thought to be likely due to the colonization of the mucosal surface of the foreskin with bacteria [15-17]. However, recent works disprove this assertion [18, 19]. White children have been estimated to have a relatively higher incidence, compared to black children [20]. Identified the prevalence of UTI in white children to be higher in both sexes with white girls having rates as high as 16-17 %. This racial difference in UTI prevalence among girls is attributed to genetic differences in secretion of carbohydrates like mannose that inhibit the adherence of bacteria to the urinary tract. White girls have been found to lack the gene responsible for the secretion of these carbohydrates [21, 22]. Individuals, especially children who are malnourished are more susceptible to UTI. Various studies in developing countries have presented data that indicate the prevalence of UTI to be 8-35 % higher in malnourished children [23-25]. The severity of malnourishment also has a strong correlation with the level of bacteriuria and this is thought to be due to a weekend immune system which is overwhelmed by infections [25]. Although the detection of UTI by microbiological culture method is well established, major drawback of it is the increased time consumption (48 to 72 hours). In addition culture methods sometimes cannot reveal two or more organisms in the same culture medium if there is an overgrowth by predominant species [26].The difficulty in rapid detection by conventional culture based biochemical methods has stimulated research into molecular diagnostic approaches.

II MATERIALS AND METHODS

A Cross sectional observational study was carried out Department of Microbiology, Mymensingh Medical College, Mymensingh from July 2016 June 2017. A total of 250 patients irrespective of age and sex admitted in Mymensingh Medical College Hospital and outpatient department (OPD) was included in this study on the basis of following criteria: burning sensation during micturition, lower abdominal pain, urgency, frequency, dysuria. All relevant history, clinical findings and laboratory records of every subject was systemically recorded in a by a pre-designed data sheet. A pre-tested datasheet was filled up by interviewing the patients with written consent of the patient or his/her guardian. A significant positive culture was taken that was 250 samples which belong both OPD and IPD.

The clean catch mid-stream technique was employed to collect urine samples. Following the verbal consent of the patient /attendants, urine sample was collected in a sterile container. a) For female patients-After proper positioning of thigh, patient was instructed to spread the labia with one hand and cleanse the area with soaped swabs with the other hand, then pass a small amount of urine into toilet and finally urinate into the wide mouthed container. b) For male patient-After washing his hands, clean catch mid-stream urine was collected with foreskin separated. c) For catheterized patient-urine was collected through the draining portal of the urinary catheter using aseptic precaution [26]. Approximately 20 ml of urine was collected aseptically in a sterile wide mouthed container. Each sample in the container was properly labeled with patients name, ID number etc. The specimens were then transferred to the laboratory as quickly as possible, usually within 1 hour after collection [27]. Wet film preparation for centrifuged urine: Five ml of urine samples were poured into a clean and dry 15 ml centrifuge tubes by sterile pipette and centrifuged at 3000 RPM for 5 minutes. The supernatant fluid was discarded and one drop of sediment was transferred to a clean labeled glass slide, covered with a clean cover slip and then examined under a light microscope using 10X and40X magnifications. On the basis of findings of pus cells/ HPF, urine samples were categorized into 3 groups. Group A included all those urine samples having a pus cell count equal or less than 5/ HPF. Group B included pus cell counts ranging between 6 to 10 /HPF and group were us cell counts above 10/ HPF [28]. The strip was just immersed completely in urine simple. Then it was extracted from container and left to stand for the time necessary for the reaction to occur, usually I to 2 minutes. Finally the colors that appear were compared against the chromatio scale provided by the manufactureren.m.wikipedia.org/wiki/urine test strip).

After 24 hours incubation, all the bacterial isolates were identified by colony morphology in MacConkey agar media and CLED agar media.Urine samples were shaken well in their sterile containers for even distribution of organisms. A calibrated wire loop with internal diameter 3.26 mm that hold 0.004 ml of urine were inoculated into the above media. The inoculums were spread with the wire loop on the media plate. They were incubated aerobically at 37°C for 24 hours [29]. All isolates were subjected to gram staining for initial identification of organism according to their gram reaction, colony morphology and finally by biochemical test. Gram negative bacteria were identified by motility test, in dole production, citrate utilization test, urease production and reaction in TSI media [29, 30]. In MacConkey agar Escherichia coli was isolated and identified by smooth pink colonies and in CLED agar by smooth, circular, 1.5 mm diameter, yellow opaque colonies.

In TSI agar it was identified by yellow slant & butt with gas production. It was indole positive, motile and no producer of urease in MIU agar medium, Citrate negative and Oxidase-Negative. In MacConkey agar Klebsiella pneumonia was isolated and identified by mucoid pink colonies and in CLED agar by mucoid yellow colonies. In TSI agar it was identified by yellow slant &butt with gas production. It was in dole negative, non-motile and slow producer of urease in MIU agar medium. It was Citrate positive and Oxidase-Negative. In MacConkey agar and CLED agar Proteus mirabilis was isolated and identified by non-lactose producing pale coloured colonies. In TSI agar it was identified by red slant and yellow butt with gas and H.S production. It was in dole negative, motile and urease producer in MIU agar medium. Citrate Positive and Oxidase-Negative. In MacConkey agar Pseudomonas aeruginosa was isolated and identified by non-lactose fermenting pale colony and in CLED agar non-lactose fermenting green colony. In TSI agar it was identified by red slant and butt without gas and H S production. It was motile, citrate and oxidase positive. The PCR products was analysed by 1.5% agarose gel (Alpha Imager, Germany electrophes and photographed using a gel documentation system (Alpha Imager, Germany). The PCR products was analyzed by 1.5% agarose gel electrophoresis to detect specific band.

III RESULTS

This study shows- results of culture of 250 clinical specimens. Of which 150 from outpatient department and 100 from in patient department. Out of the total 250 specimens 200 (80%) became positive by culture. Out of 150 specimens from outpatient department 110 (66.6%) and out of 100 specimen from in patient department 90 (90%) became culture positive (Table-1).

Table 1: Results of culture of clinical specimens (n=250)

Type of patient	Urine cultured	Culture positive cases
Outpatient department	150	110 (66.6%)
In patient department	100	90 (90%)
Total	250	200 (80%)

Table-2: Distribution of bacterial isolates both in outpatient and in-patient department population.

Isolated organism	Out patient population (n=110)	Inpatient population (n=90)
Escherichia coli	79(71.8%)	61(67.8%)
Klebsiella pneumonia	15(13.6%)	5(5.5%)
Pseudomonas aeruginosa	14(12.7%)	19(21.1%)
Proteus mirabilis	2(1.8%)	5(5.6%)

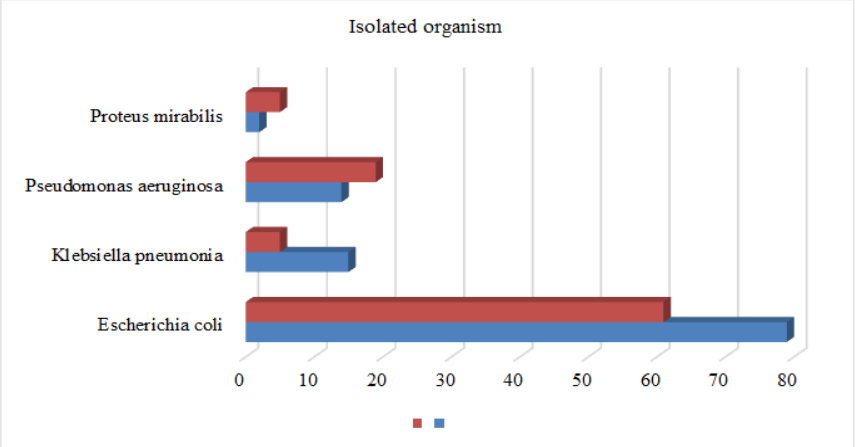

Fig-1: Distribution of bacterial isolates both in outpatient and in-patient department population.

(Table-2) shows among the isolated pathogens, E.coli was responsible as a leading causative pathogene in both outpatient and inpatient population with a highest prevalence of 71.8 % for the out patient population. On the other hand prevalence of Proteus mirabilis was lowest and it was 1.8 % in outpatient population.

Table 3: Age and sex distribution of the culture positive urine samples in case of outpatient population (n=110) and in patient population (n=90)

Age	Outpatient population			In patient population		
	Male	Female	Total	Male	Female	Total
0-15	5	15	20 (18.2%)	4	2	6(6.7%)
>15-30	10	30	40 (36.4%)	7	11	18(20%)
>30-45	11	15	26 (23.6%)	6	17	23(25.5%)
>45-60	6	10	16 (14.5%)	8	10	18(20%)
60+	6	2	8 (7.3%)	16	9	25(27.8%)
Total	38(34.5%)	72(65.5%)	110 (100%)	41(45.6%)	49(54.4%)	90(100%)

(Table-3) shows the culture positivity of urine specimens was higher in female in both out patient population and inpatient population. Culture positivity of in patient population among the male (45.5%) was slighty higher than that of outpatient population (34.5%). The predominant age group suffered from UTI in case of outpatient population was >15-30 but for the in patient population, the age group was 60 years and above.

IV DISCUSSION

Urinary tract infections (UTIS) are considered to be the most common bacterial infection. Women are significantly more likely to experience UTI than men [30]. UTIs area severe public health problem and are caused by a range of pathogens, but most commonly by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus [31]. In the present study the specimens were collected from outpatient and inpatient department of Mymensingh Medical College Hospital (MMCH). About 250 specimens were subjected for culture and 200 were culture positive. In a study Bijan Moshaver *et al*., [32] reported 79 (37.8%) culture positive specimens out of 209 total specimens tested, suggesting dissimilarity with the present study. This might be due to the adoption of the better selection criteria in the present study. The prevalence of the UTI in female patients was predominant in both outpatient and in-patient department in the present study. It revealed in the study that 65.5% female and 34.5% male suffered from UTI in the outpatient department using culture method which was almost similar to the findings of Gupta *et al*., (2002) where female and male were reported to be82.7% and 18.9% respectively. In the present study 54.5% female and 45.5% male suffered from UTI in in-patient population suggesting vulnerability of women patients with uropathogens. The male suffered more from UTI in in- patient department in comparison to outpatient department. In female the predominant age group suffered from UTI was young and middle aged whereas, elder aged (more than 60 male group) suffered more from UTI. Guptaet *et al*., [32] also reported almost similar prevalence of the UTI, 51.23% for female and 48.76%for male in in-patient department. The higher incidence of UTI in elder aged groupmalepatients may be due to urinary tract pathologies like prostate disorders and in female patients the higher incidence was in young and middle aged group due to the obstetric and gynecological causes respectively [33]. The prevalence of high UTI in the in-patient department has several associated risk factors indicating the catheterization is the most predominant followed by the physiological and pathogenic conditions like pregnancy, menopause, diabetes, and immobility. It also observed that almost all patients of inpatient department who developed UTI had used urinary catheters, other factors associated with the development of these infections is due to prolonged use of urinary catheter, female patient and prolong stay in intensive care unit of hospitals [34]. The urinary catheterization was the most important and the leading causes of UTI due to instrumentation. In this study 60% cases were associated with the urinary catheters. On the other hand the present study revealed that E. coli was responsible for 61(67.8%) in inpatient population followed by several other pathogen namely Pseudomonas spp 19 (21.1%), Proteus spp 5(5.6%) and klebsiella spp 5 (5.5%). The findings of E. coli were in agreement with the study reported by Sharmin *et al*., [35]. Another study done by Hasan *et al*., [36], showed 50.7% incidence in case of in patient population caused by E. coli, which was nearer to the present study. Leblebicioglu and Esen *et al*., [37], reported that E. coli (32.4%) was the mostly responsible for UTI and N. Anbumani and Mallika *et al*., [38], reported that it was around 33% Gastmeier *et al*., [39], revealed E. coli as the causative agent of UTI in case of in- patient department by 56.7% suggesting E. coli has been the predominant organism ever isolated and no significant change has occurred in this picture over the last couple of decades. From the above data it could be concluded that the frequency of UTI caused by E. coli in outpatient populationis higher than that of in- patient population. The reason of highest rate of isolation of E. coli causing UTI is due to the fact that most of the pathogens causing UTI originate from the faecalflora and among these facultative anaerobes, E coli

constitutes the major portion superimposed by various virulence factors that facilitate the ascent of bacteria from faecal flora, introitus or periurethral area, and beyond the urethra into the bladder and less frequently allow the organisms to reach the kidneys to induce symptomatic inflammation [40]. Although E. coli was the most common cause of UTI in both outpatient and inpatient department, Klebsiella spp and Pseudomonas spp posses the second position as the causative agents in outpatient population and in patient population respectively, Klebsiella spp was the 3rdcommon causes in case of in patient population in the present study. N. Anbumani and Mallika *et al*., [38], found isolation rate of 17.6% for Klebsiella spp which supported our findings.

V CONCLUSION

Prevalence of urinary tract infections is dependent on factors like gender, age, disease, race and nutritional status. Females are generally more predisposed to renal infections, compared tomales, except at the extremes of early childhood and geriatric stage. Thisis basically due to the shorter length of the urethra in females and its closeness to the excreta passage, which is a source of pathogens that can colonize the urethra. Again, sexual intercourse, pregnancy and childbirth contribute to the increase in UTI in females. It is estimated that about 50% of all women are likely to experience an UTI in their lifetime. The prevalence of the UTI in female patients was predominant in both out-patient and in-patient department in the present study. It revealed in the study that 65.5% female and34.5% male suffered from UTI in the out-patient department using culture method. It also observed that almost all patients of in-patient department who developed UTI had used urinary catheters, other factors associated with the development of these infections is due to prolonged use of urinary catheter, female patient and prolong stay in intensive care unit of hospitals.

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