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Different Laboratory Diagnostic Procedures Including PCR Directly from Urine Specimens from Suspected Patients of UTI

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Abstract: Background: Urinary tract infection (UTI) is among the most common bacterial infections and possess significant healthcare burden. Hence a study was necessary to apply PCR technology directly to clinical specimens to allow early and accurate identification of pathogens of UTI. Objective: Diagnosis of major uropathogens by different laboratory diagnostic procedures including polymerase chain reaction (PCR) directly from urine specimens from suspected patients of urinary tract infection. 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 anbiochemical tests. PCR was performed by using standard protocol with species specific primer for detection offim H gene for Escherichia coli, fimK gene for Klehsiella paeunomiae, UreC for Proteus mirabilis and ETA for Pseudomonas aeruginasa. 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. 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. 90 culture positive in patient population, 43 (95.5%) were from the gynae unit, 23 (92%) were from surgery unit, 8 (72.7%) were from the medicine unit, 12 (85.7%) were from orthopaedic unit and 4 (80%) were from paediatric unit. On the other hand Pseudomonas aeruginosa Profeus mirabilis and Klebsiella pneumonia were more prevalent in in-patient department and it was 21.1%, 5.6% and 5.5% respectively, Among the 50 culture negative urine specimens, 14 (28%) showed PCR positive for Escherichia coli, Klehsiella pneumonia and Pseudomonas aeruginosa. Conclusion: This study revealed that, the prevalence of UTI is high n MMCH Single pathogen base uniplex PCR was found superior than standard culture and less time consuming. Because uniplex PCR could detect many (28%) culture negative cases.

Keywords: Major Uropathogens, Different Laboratory Diagnostic, Polymerase Chain Reaction (PCR)

1. Introduction

Urinary tract infections (UTls), of which Escherichia coli are the major causative agent, are among the most common human infections. At least 10 to 20% of women experience an acute symptomatic UTI at some point during their lifetimes [1]. Global records on the disease show that among children, the infection is more common in young girls, except in the neonatal age group where boys predominate [2]. It is also estimated that about 20% of women develop an UTI during their lifetime; the incidence increases at puberty and remains high throughout the adult life [3]. Uropathogenic organisms are more likely to colonize anatomically and functionally in normal urinary tracts, however individuals with obstructed and abnormal urinary tract structures have a higher risk of UTI [4]. Many human diseases are as a result of infections caused by bacteria pathogens, either externalor internal of the human host. One of such bacterial infection is the Urinary Tract Infection (UTI), involving the presence of bacteria in the urinary tract (UT), which is naturally sterile [4]. The pathogens responsible for infection comprise 70-90%of uropathogens Escherichia coli and the reminder a variable contribution from Proteus, Klebsiella, Pseudomonas aeruginosa, Staptylococcus Sapropyicus and Enterococcus [5]. UTI is the second most common infectious presentation in community medical practice after the respiratory tract infections [6]. Worldwide about 150 million peoples are diagnosed with UTI each year and are classified as uncomplicated or complicated [7]. As the most common healthcare-associated infection, UTI accounts for more than 30% of infections reported by acute-care hospitals [8]. A study was done in South India and it showed higher prevalence of UTI in women (47.9%) than men (34.1%) [9]. 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 [10]. Infections of the lower urinary tract, is a cause of morbidity, with increasing incidence of urinary tract infections predisposing the host to more severe renal cell Damage [11]. The more severe renal damage frequently leads to renal scarring and hypertension with high mortality rate [12]. Detection of UTI organism always remains an essential element in clinical diagnosis. The development of rapid screening tests and automated systems continues, but at present, microscopy and culture remain the most important techniques for laboratory diagnosis. 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 [13]. To date, molecular biology techniques such as PCR are used to complement conventional culture methods, especially with regard to shortening the time to result [14, 15]. PCR is the best known and most successfully built nucleic acid detection technology to date. Amplification of individual species specific gene in different reactions by uniplex PCR is powerful and widely used tool for rapid and specific identification of pathogenic bacteria. This study used species-specific primer for specific detection of Excherichia coli, Pseudomonas aeruginosa, Klebsiellapn eumoniae and Proteus mirabilis targeting gene sequence of fimH, ETA, fimK, ureC respectively. Single pathogen base uniplex PCR reaction assay was performed leading to individual detection of four UTI pathogens in clinical isolates. This study established the PCR directly from urine specimens collected from suspected UTI patients.

2. Objectives

2.1. General Objective

Diagnosis of major uropathogens by different laboratory diagnostic procedure including polymerase chain reaction (PCR) directly from urine specimens from suspected patients of urinary tract infection.

2.2. Specific Objectives

To determine the prevalence of UTI in suspected individuals.

To isolate and identified UTI by microscopic examination and dipstick method.

To isolate and identify major uropahogens by culture and other biochemical test.

To detect commonly encountered uropathogens by amplitying species specific genes using PCR directly from urine specimens.

To compare positivity among culture and PCR.

3. Materials and Methods

Type of study: Cross sectional observational study.

Place of study: Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh.

Period of study: The study was carried out from July 2016 June 2017.

Subjects/ cases: 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.

Sampling technique: Non probability purposive type of sampling.

Sample size: Two hundred fifty (250).

Specimen: Urine of patient was collected as specimen.

Data collection and recording: 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. The confidentiality of the patient was fully maintained. In this study, there was no such potential risk of the patient regarding physical, psychological or legal aspect. The subject were informed about the nature, purpose and procedure of the study and the privacy of the patient was fully maintained.

Collection of urine specimen: 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 [12]. The final stage was an extension cycle at 72°C for 2 min. 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.

4. Results

A total of 250 patients of all age groups clinically diagnosed as UTI were studied to isolate and identify bacteria from urine. This study was carried out in the department of Microbiology, Mymensingh Medical College during the period from July 2016 to June 2017. The specimens were collected from in and out patient departments of Mymensingh Medical College Hospital (MMCH), Mymensingh, Bangladesh.

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%)

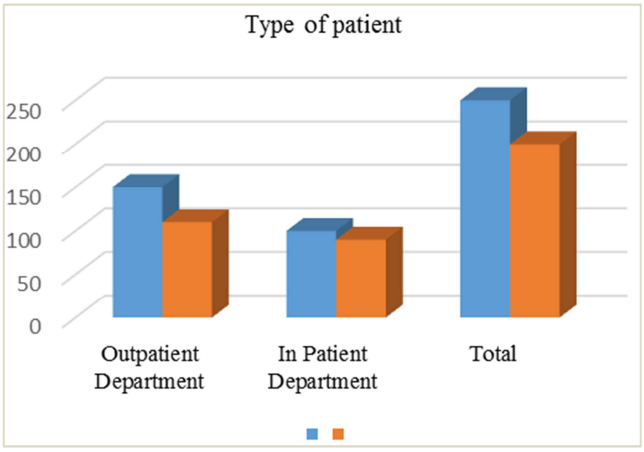

Figure 1. *Results of culture of clinical specimens.*

Table 1 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 2. *Correlation between pyuria and urine culture.*

Groups	Total Number of cases	Culture Positive cases	Culture Negative cases
Group A (Pus cell count ≤5/ HPF)	60	25 (42%)	35 (58%)
Group B (Pus cell count 6-10/HPF)	90	75 (84%)	15 (16%)
Group C (Pus cell count > 10/HPF)	100	100 (100%)	0 (0%)

Table 2 shows- patients were classified into three groups (Group A, Group B and Group C) depending on the presence of puscell / HPF in centrifuged urine deposite and the relationship between pyuria and culture positivity. Culture positivity was directly proportional to pus cell.

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.

Table 4. *Distribution of culture positivity in different in-patient departments.*

Department	Total Number of cases	Number of Positive culture	Percentage of positivity among different in-patient department
Gynae	45	43	95.5
Surgery	25	23	92
Medicine	11	8	72.7
Orthopedics	14	12	85.7
Paediatrics	5	4	80
Total	100	90	

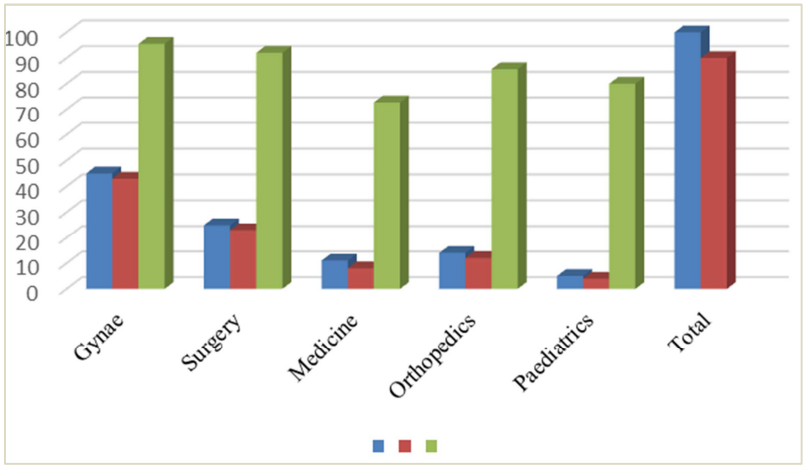

Figure 2. *Distribution of culture positivity in different in-patient departments.*

Table 4 shows among 90 culture positive in patient population, 43 (95.5%) were from the gynae unit, 23 (92%) were from surgery unit, 8 (72.7%) were from the medicine unit, 12 (85.7%) were from orthopaedic unit and 4 (80%) were from paediatric unit.

Table 5. *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%)

Table 5 shows among the isolated pathogens, E.coli was responsible as a leading causative pathogene in both outpatient and in-patient 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 6. *Results of PCR among culture positive and culture negative specimens (n=250).*

Culture type	PCR positive	PCR negative	Total
Culture positive (n=200)	187 (93.5%)	13 (6.5%)	200
culture negative (n=50)	14 (28%)	36 (72%)	50
Total (n=250)	201 (79.6%)	49 (20.4%)	250

Table 6 shows-among 200 cultures positive specimens 187 were PCR positive and 13 were negative sample 14 were PCR positive and 36 were negative.

5. Discussion

Urinary tract infections (UTIS) are considered to be the most common bacterial infection. Women are significantly more likely to experience UTI than men [2]. 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 [16]. In the present study the specimens were collected from outpatient and in-patient department of Mymensingh Medical College Hospital (MMCH), Bangladesh. About 250 specimens were subjected for culture and 200 were culture positive. In a study Bijan Moshaver et al. [17] 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. In the present study the rate of culture

positivity has significantly increased with the presence of increased number of pus cell/HPF from sediment of urine after centrifugation. It was observed the Pus cell >10/ HPF were found in all the 100 (100%) of the cases and all were culture positive, whereas only 25 (42%) patients with insignificant pyuria of 55/ HPF had culture positive (Table 2) which may be due to pus cell had not yet produced sufficiently. About fifteen (16%) patients with significant pyuria of 6-10/HPF had negative culture despite being symptomatic which indicate the recent use of any antibiotics. Chaudhury [18], in Bangladesh reported that centrifuged urinary specimen containing pus cell >10/ HPF, 6-10/ HPF and ≤5/HPF became culture positive by 100%, 36.3% and 0% respectively. Similarly Raco and Barez et al. [19] reported significant number of cases (64.6%) were culture positive containing pus cell>10/ HPF whereas only (35.4)% cases were culture positive containing pus cell <10/ HPF. 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 and34.5% male suffered from UTI in the outpatient department using culture method which was almost similar to the findings of Gupta et al. [20] where female and male were reported to by 82.7% 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. In the present study the organisms mostly isolated from out patient population was E. coli. 79 (71.8%) followed by Klebsiella species 15 (13.6%), Pseudomonas aeruginosa 14 (12.7%) and Proteus species 2 (1.8%). Gupta et al. [20] in USA found 75% E. coli, which is in agreement with this study. Another study reported by Gupta et al. [20], E. coli represented about 70% to 90% of the causative agents of UTI in outpatient department. Dyer et al. [21] reported that the proposition of E. coli in the current decade has increased significantly and accounted for 69% of positive cultures in 1991, which increased to 75% in 1994 and 81% in1997. 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 klebsiellaspp 5 (5.5%). Although E. coli was the most common cause of UTI in both outpatient and inpatient department, Klebsiellaspp and Pseudomonas supposes the second position as the causative agents in outpatient population and in patient population respectively, Klebsiellasp was the 3rd common causes in case of in patient population in the present study. N. Anbumani and Mallika et. al. [22] found isolation rate of 17.6% for Klebsiellaspp which supported our findings. In the present study among the culture positive specimens, 13 specimens were found PCR negative. Among these 13 specimens, 3 were Klebsiella species and remaining 10 were E.coli. Similarly Padmavaty et al. [5] found 2 Klebsiella pneumoniae negative by PCR out of 41 specimens, which were culture positive. This may be due to inherent difficulty in rupturing the Klebsiella cell wall. In case of E.coli it may be due to presence of inhibitory factors in urine specimens. Urea inhibits PCR in concentrations of 50 mM, and the normal concentration of ureain adult is about 330 mM [5]. The novelity of the research is the use of molecular method namely Polymerase Chain Reaction (PCR). In this study out of the 50 culture negative urine specimens of suspected UTI patients 14 (28%) showed PCR positive for Escherichia coli, Klebsiella pneumoniae and Proteusmirabilis, suggesting the sensitivity of PCR both in culture positive and negative specimens. Culture negativity may be due to use of antibiotics or due to absence of sufficient bacteria in specimens. Van der zee et al. [23] reported that out of 211 specimens, 62 were positive in PCR and 44 of those had a positive culture, 18 were PCR positive but no significant culture result could be obtain. In this study among 50 culture negative specimens, Escherichia coli was PCR positive in 85%cases, Klebsiella pneumoniae was positive in 7.1% cases and Proteus mirabilis was positive in7.1% cases, suggesting the higher prevalence of E.coli in UTI and other organisms were less prevalent in our settings.

6. Conclusion

This study tried multiplex PCR for several times but could not be succeeded may be due to different primer interaction. So further study may be performed by more sensitive Real Time Multiplex PCR using different set of primer, modified thermal and cycle condition Analyzing the different findings, the present study revealed that the prevalence of UTI was high in MMCH. Multiplex PCR for diagnosis of uropathogens was not successful. Pathogen base uniplex PCR method was found superior diagnostic tool for detecting UTI and less time-consuming compared to the standard culture. Because uniplex PCR could detect many (28%) culture negative cases. Multiplex real time PCR could be better option.

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