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Original Research Article

PCR Results among Culture Positive and Culture Negative Specimens of Suspected UTI Patients in Mymensingh Medical College Hospital, Mymensingh, Bangladesh

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Abstract

Background: Urinary tract infection (UTI) is among the most common bacterial infections and possess 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 assess THE PCR results among culture positive and culture negative specimens of suspected UTI patients. ***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 isolationand identification of major uropathogens (Escherichia coli Klehsiella pneumonias, Proteusmirabilis, and Pseudomonas aeruginosa) were done by standard microbiological procedure a biochemical tests. PCR was performed by using standard protocol with species specific primer for detection of fimH gene for Escherichia coli, fimK gene for Klehsiella paeunomiae, UreCfor Proteus mirabilis, 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 timePCR could detect species specific genes in 201 specimens directly from urine of suspected UTIpatient 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 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.

Keyword: PCR Results, Culture Positive, Culture Negative, Suspected UTI Patients.

I INTRODUCTION

UTI often presents as a clinical conundrum. It is rarely fatal, yet highly morbid and affects all patient demographics. To date, molecular biology techniques such as PCR are used to complement conventional culture methods, especially with regard to shortening the time to result [1]. Although effective treatments are available, the associated urinary symptoms are nonspecific and overlap with numerous other non-infectious entities. Furthermore, the presence of bacteria in urine does not always necessitate treatment, yet differentiation of asymptomatic bacteriuria from UTI is subjective, particularly in patient with urinary catheter sand other neurological or anatomical impairments of the bladder. These challenges, coupled with the inherent delay of urine culture, contribute to widespread misuse and overuse of antibiotics, which has accelerated the selection of resistant pathogens and decreased the lifespan of antibiotics [2]. 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 [3,4]. The difficulty in rapid detection by conventional culture based biochemical methods has stimulated research into molecular diagnostic approaches. The advancement of molecular biology has led to the development of highly sensitive techniques, including Polymerase Chain Reaction (PCR), which is widely applied in the field of diagnostic microbiology. PCR mimics the in vivo process of DNA replication. The technique thus enables amplification of DNA sequences from any organism. Separation of the PCR products by electrophoresis allows determination of polymorphism and cloning of amplified genes. Increasing trend in genome sequencing and analysis has facilitated the increased usage of PCR in molecular diagnostics. In addition, substantial work has been done to ascertain host-pathogen information at molecular level [4-6]. Comparatively, PCR requires much smaller quantities of specimen for analysis. 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, Klebsiella pneumoniae 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.

II MATERIALS AND 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 isolationand identification of major uropathogens (Escherichiacoli Klehsiella pneumonias, Proteusmirabilis, and Pseudomonas aeruginosa) was done by standard microbiological procedure abiochemical tests. PCR was performed by using standard protocol with species specific primer for detection of fimH gene for Escherichia coli, fimK gene for Klehsiella paeunomiae, UreC for Proteus mirabilis, ETA for Pseudomonas aeruginasa.

PCR was performed by using standard protocol with species specific primers for detection of Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas aeruginosa species. Four pairs of multiplex PCR oligonucleotide primers were used. The sequence 5 to 3 ends of these oligonucleotide primers were as follows:

Gene	Primers (5'-3')	Product size (bp)
fimK (K pneumoniae)	TGCTCTATCAGGTGAGTCAT AAAATCGATAGTITCAGCAT	746
fimH (E.coli)	TCGAGAACGGATAAGCCGTGGGCAGTCACCTGCCCTCCGGTA	508
UreC (proteus mirabilis)	GITATTCGTGATGGTATGGG ATAAAGGTGGTTACGCCAGA	317
ETA (P. aeruginosa)	GCCTTCGAACATCAAGGTGT CCATGACCACGCTGACC	207

III RESULTS

A total of 250 patients of all age groups clinically diagnosed as UTI were studied to isolate and identify bacteria from urine. The specimens were collected from in and out patient departments of Mymensingh Medical College Hospital (MMCH), Mymensingh, Bangladesh. (Table-1) shows-among 200 cultures positive specimens 187 were PCR positive and 13 were negative sample 14 were PCR positive and 36 were negative. 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 sufficientbacteria in 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 (Table-2), suggesting the higher prevalence of E.coli in UTI and other organisms were less prevalent in our settings.

Table-1: 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

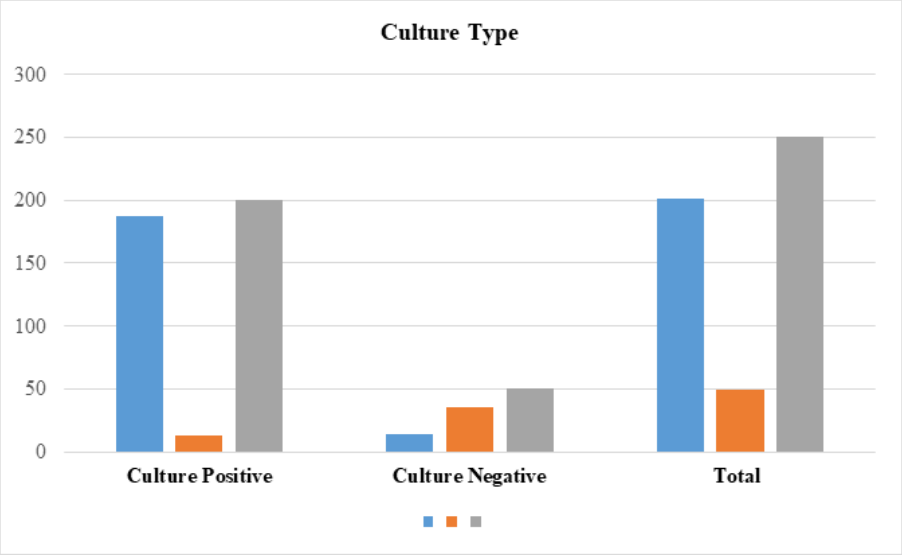

Fig-1: Results of PCR among culture positive and culture negative specimens

Table-2: Results of PCR of culture negative specimens (n=14)

Organisms	PCR positive	Percentage (%)
Escherichia coli	12	85.8
Klebsiellapneumoniae	1	7.1
Proteus mirabilis	1	7.1
Total	14	100

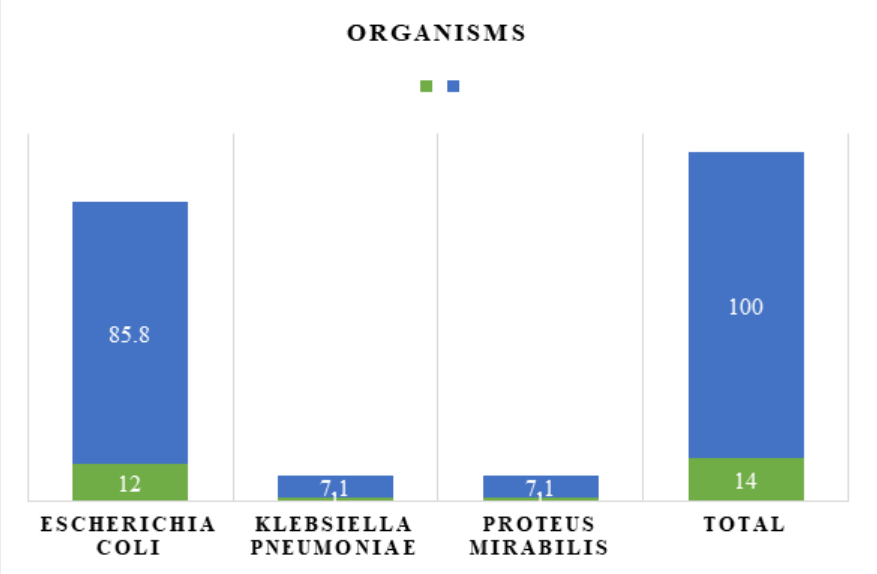

Table-2: Results of PCR of culture negative specimens

IV DISCUSSION

In the present study the specimens were collected from outpatient and in-patient 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*., [7] 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 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*., [3] found 2 Klebsiella pneumoniae negative by PCR out of 41specimens, 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 urea in adult is about 330 mm [3]. 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 Proteusmira bilis, 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*., [8] 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 in 7.1% cases, suggesting the higher prevalence of E.coli in UTI and other organisms are less prevalent in our settings.

V 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. Because uniplex PCR could detect many (28%) culture negative cases. Multiplex real time PCR could be better option.

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