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DETERMINATION AND ASSESSMENT OF BACTERIAL INFECTION AND ITS ANTIMICROBIAL RESISTANCE IN PREGNANT WOMEN IN QUETTA, BALOCHISTAN

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Abstract

The aim of this study was to assess the pathogenicity of UTI-causing bacteria and to identify drugs with significant biomedical importance for treating these infections. A sample of 100 pregnant females from Quetta city, Balochistan, was examined using two methods: the conventional method and molecular method, to identify the microbial pathogens responsible for UTIs.

The presence of S. aureus was identified in 65% of cases using the conventional/biochemical method and in 70% of cases using the PCR method. E. coli was observed in 59% of cases using the conventional/biochemical method and in 63% of cases using the molecular method. The presence of E. fecalis was recognized in 51% of cases using the conventional/biochemical method and in 55% of cases using PCR, while K. pneumoniae was detected in 25% of cases using the conventional/biochemical method and in 36% of cases using PCR.

The antimicrobial results revealed that all isolated bacterial pathogens (E. coli, S. aureus, K. pneumoniae, and E. fecalis) were resistant to Amoxicillin. S. aureus was sensitive to Erythromycin (a Macrolide antibiotic). Ciprofloxacin, a Fluoroquinolone antibiotic, showed sensitivity to all four strains of bacterial pathogens: K. pneumoniae, E. fecalis, S. aureus, and E. coli. Another Fluoroquinolone antibiotic, Norfloxacin, showed sensitivity to K. pneumoniae and E. fecalis. Gentamycin, an Aminoglycoside antibiotic, was sensitive to K. pneumoniae and S. aureus, while Aminoglycoside antibiotics were sensitive to E. fecalis.

The study suggests that PCR testing is the most specific and sensitive method for identifying bacteria causing UTIs in urine samples. In conclusion, maintaining personal hygiene and using appropriate and recommended drugs are necessary to prevent UTI infections.

Keywords: *Antimicrobial activity, Bacterial infection, Pregnant women, Quetta, Urinary tract infection*

INTRODUCTION

Bacterial infection is most common among pregnant women triggering infection of urinary tract and bladder (1). UTI has been categorized into two types of infection i.e., lower UTI and upper UTI. Lower urinary tract infection occurs in bladder and urethra while upper urinary tract infection occurs in the kidney, pelvis and ureter. Upper urinary tract infection is the commonest among the two types (2, 3). Low immunity in pregnancy leads to the growth of microorganisms (4). Increase in the volume of plasma and decrease in the concentration of urine during pregnancy lead to glucosurea this factor also increase growth of bacteria in urine in 70% pregnant females (5). Pregnant women suffering from urinary tract infection may lead to death or death of her fetus (6). Gram -ve and Gram +ve bacteria are major pathogens causing urinary tract infection. These bacteria include facultative anaerobes of stool, skin flora, and vaginal flora (7). Uropathogen *Escherichia coli* (UPEC) is majorly found in the patients with urinary tract infections

accounting for up to 70 – 80% of infections in communities and up to 40 – 60% of infections regarding healthcare (8).

It is necessary to collect urine samples through clean approach because contamination from urethra and vagina makes the diagnosis very complicated (9). In a study conducted (10), some urine specimens were taken directly in a container and some from catheter and compared thoroughly in premenopausal ladies. They determined that E. coli easily passed through catheter and some findings gave result about K. Pneumonia and Staphylococcus. While some results showed that Enterococcus and streptococci can make the contamination easily. Traditional approach to the diagnosis of UTI was to find 150 colonies forming pathogens / CFUs from urine which now has been changed (11); (12). Recent studies showed that ladies with less number of CFUs also have been diagnosed as UTI patients (13). Incidence of bacteria may be reduced with recurrent draining of urine in the sign of dysuria where infection still perseveres in the UTI patients (9). The research study was aimed to detect and isolate pathogenic bacteria associated with urinary tract infection and to determine their antibiotic susceptibility pattern from pregnant women.

MATERIALS AND METHODS

SAMPLE COLLECTION

This descriptive study was conducted at largest Public Hospital of the Province (Bolan Medical Complex Hospital), Quetta for a period from September to December 2019. A total 100 were samples collected in Falcon tubes from suspected UTI positive patients. The samples were investigated at CASVAB, university of Balochistan Quetta.

SAMPLE PROCESSING

The urine samples were processes for analysis of pH, specific gravity, bilirubin, glucose, albumen, blood and leukocytes. The pallete of urine was separated from supernatant through centrifugation (operated for 3-4 minute), and then 1ml nutrient broth was added in the pallete. After the completion of above mentioned process, the urine samples were incubated at 37 °C for 24 hours.

URINE EXAMINATION

Midstream urine was tested using a dipstick, and the results were examined for PH, bilirubin, blood, albumen, and leukocytes. Samples were analyzed, then placed in a sterile centrifuge and centrifuged for three to four minutes at 3000 rpm. One milliliter of broth was added to the pallet after the urine pellets were separated from the supernatant using centrifugation. Urine samples were then incubated for 24 hours at 37°C. The urine sample was incubated for 24 hours at 37°C before being grown in various media plates and observed for growth. Staining was used to monitor and identify the development of bacteria in medium plates, and several biochemical tests were then conducted. microorganisms confirmed by microscopic analysis (catalase, oxidase, indole, voges-proskauer, methyl red, nitrate reduction test, sugar fermentation test).

DNA EXTRACTION

Using boiling method DNA was extracted. Colonies were collected from media plates, mixed with 30 ul TE buffer and then vortexed thoroughly. Then mixture was boiled inside water bath for 10 minutes at 95°c. After boiling samples were centrifuge at 6000rpm for 3 to 4 minutes. DNA supernatant was transferred in new eppindrof tube and kept at -20°v.

PCR AND GEL ELECTROPHORESIS

Primers, Master Mix, extracted DNA, and Molecular Grade water were combined in PCR tubes, and the mixture was vortexed for a brief period of time to execute PCR. The negative control was achieved using distilled water. The touch PCR down technique was applied. After PCR, the product was run using gel electrophoresis. For the gel electrophoresis, a 2% agrose gel was prepared. For this, 50ml of 1X TAE buffer was used to dissolve 1g of agrose powder. After adding 7 ul of ethidium bromide and letting the solution cool, the mixture was cooked in a microwave oven and then poured into a casting tray to solidify. Following solidification, the PCR product was put into the walls, and the gel was then placed within an electrophoresis

chamber with a 35-minute current supply of 120 volts at 400 mA. Following the. After the completion of electrophoresis. Under UV light illuminator in gel documentation system the gel was visualized.

ANTIBIOTICS SUSCEPTIBILITY TEST

Kirby Bauer disc method was used to determine the susceptibility or resistance of bacteria to different antibiotics. The media used were Muller-Hinton Agar. The antibiotic contents of multidisc were Clarithromycin (CLR), Tetracycline (TE), Amoxicillin (AML), Vancomycin(VA), Erythromycin (E), Ampicillin (AMP), Norflacin , Kanamycin, Gentamycin (CN), Ciprofloxacin, Cefix\)ime, Chloromphenicol.

RESULTS

In current study 100 pregnant females were observed for UTI caused by bacterial pathogens at BMCH Quetta. Samples were collected from those patients who visited the BMCH for diagnosis & treatment. The data was analyzed by two methods, conventional method & molecular method to detect the presence of bacterial pathogens that were responsible for UTIs.

Table I. Detection rate of Isolation bacteria from Urine samples

Total n=100	***S. aureus***	***E. coli***	***E. Fecalis***	***K. pneumoniae***
Conventional	P=65	P=59	P=51	P=25
method	n=35	n=41	n=49	n=75
	65%	59%	51%	25%
PCR	P=70	P=63	P=55	P=36
	n=30	n=41	n=45	n=65
	*5	*4	*4	*11
Percentage	70%	63%	55%	36%

Four bacterial pathogens *S. aureus E. coli, E. fecalis* & *K. pneumoniae* responsible for UTIs were observed in current study. Out of 100, samples presence of S.aureus was detected 65% by conventional /biochemical method and 70% by PCR method. *E. coli* was observed as 59% by conventional/biochemical method and 63% through molecular method. The presence of *E. fecalis* was identified as 51% through conventional/biochemical method and 55% by PCR method while *K. pneumoniae* were detected as 25% by conventional/biochemical method and 36% through PCR as shown in (Table I)

ANTIBIOTIC SUSCEPTIBILITY TESTING

Results shown that all the isolated bacterial pathogens (*E. coli, S. aureus, K. pneumoniae* and *E. fecalis*) were found to be resistant for Amoxicillin an antibiotic which belongs to the class penicillin. For Erythromycin (Class Macrolides) *S. aureus* was identified as sensitive while the other three isolates (*E. coli, K. pneumoniae* and *E. fecalis*) were detected as resistant. Ciprofloxacin an antibiotic of class Fluoroqinlone was proved to be sensitive for all four strains of bacterial pathogens like *K. pneumoniae, E. fecalis, S. aureus* and *E. coli*. Another antibiotic of class Fluoroqinlone, Norfloxacin showed sensitivity for pathogens *K. pneumoniae, E. fecalis, E. coli* while proved to be resistant for S.aureus. Aminoglycoside antibiotics like Gentamycin were identified as sensitive for *K. pneumoniae* and *S. aureus* but revealed resistance against *E. coli* and *E. fecalis*. Chloramphenicol antibiotics of class Amphenicol, Vancomycin (class Glycopeptides), Clarithromycin of Macrolides and antibiotics of Tetracycline were found to be resistant for all isolates (*K. pneumoniae, E. fecalis, E. coli* and *S. aureus*. Antibiotics of class Aminoglycoside were detected as sensitive for *E. fecalis* while resistant for *S. aureus, E. coli* & *K. pneumoniae* as shown in (Table II).

DISCUSSION

In the current study the urine samples of 100 pregnant females were observed for the UTI which is certainly caused due to bacterial pathogens at Quetta. However, the molecular and conventional methods were used to detect and determine the prevalence of the bacterial pathogens which are accountable for UTIs. Therefore, the four bacterial pathogens such as *E. coli E. fecalis, S. aureus, and K. pneumoniae* were selected for observation. The results of the study display that among 100 samples, the conventional method detected *E.*

coli by 59%, while 63% was detected by PCR method. *S. aureus* bacteria was detected 65% through conventional method and 70% was detected through molecular method. About 51% of *E. fecalis* species were identified through conventional method and 55% were identified through molecular method. About 25% *K. pneumoniae* was detected by conventional method and 36% was identified through PCR. The results of (14) display that *E. coli* was found to be the most dominant gram-negative bacteria. The finding is highly consistent with the current study where the sample size was about 100 patients experiencing UTIs that were generally caused by 4 pathogens of bacteria such as *S. aureus* 58%, *K. pneumoniae* 11%, *E. fecalis* 25% and *E. coli* 54%.

Table II. Results of antibiotic susceptibility of isolated bacteria

S. no.	Antibiotics	Class	*S. aureus*	*E. coli*	*E. fecalis*	*K. pneumoniae*
1	Ampicillin	Penicillin	-	0mm	0mm	-
2	Amoxicillin	Penicillin	0mm	0mm	0mm	0mm
3	Erythromycin	Macrolides	24mm	0mm	0mm	10mm
4	Ciprofloxacin	Fluoroqinlone	23mm	28mm	22mm	27mm
5	Norfloxacin	Fluoroqinlone	-	24mm	20mm	26mm
6	Gentamycin	Aminoglycoside	22mm	12mm	-	22mm
7	Chloramphenicol	Amphenicol	14mm	-	-	-
8	Vancomycin	Glycopeptides	-	-	0mm	-
9	Clarithromycin	Macrolides	16mm	-	-	-
10	Tetracycline	Tetracycline	16mm	10mm	12mm	10mm
11	Kanamycin	Aminoglycoside	18mm	-	20mm	-

The prevalence of urinary tract infection worldwide varies. In Egypt it was reported 35%, 31.3% in Zigzag University Hospital, Egypt, 30%, 28% in Pakistan, 11.6% in Ethiopia (15-17). The study of Humayun and Iqbal 2012, reveals that these UTI are particularly challenging and complex for women as about 1/3 of the women likely to experience these UTI to some extent in their life (14). However, proper treatment of infection requires concrete classification regarding the position of infection and the infection impairment. The UTI imposes severe health complexities for the patient with a high social cost. Thus, the UTI is considered as the most frequent infection which exists in healthcare. Therefore, the current study was aimed to isolate and identify the bacterial pathogens associated with the UTI among pregnant women in Quetta.

Furthermore, the study's findings highlight the importance of utilizing both molecular and conventional diagnostic methods to accurately identify bacterial pathogens responsible for UTIs. The higher detection rates observed with molecular methods like PCR underscore the need for advanced diagnostic techniques in clinical settings to ensure accurate diagnosis and appropriate treatment. This is particularly crucial in regions like Quetta, where healthcare resources may be limited, and accurate diagnosis is essential for effective management of infections. Additionally, understanding the prevalence and antimicrobial resistance patterns of these pathogens can inform public health strategies and antibiotic stewardship programs, ultimately reducing the burden of UTIs among pregnant women in the region.

The molecular detection method, particularly PCR, offers higher sensitivity and specificity compared to conventional methods. This advantage is critical in accurately identifying bacterial strains that may otherwise be missed, leading to better-targeted treatments. This study's use of PCR revealed higher detection rates for all four bacterial pathogens, suggesting that molecular techniques should be integrated into routine diagnostic protocols to improve the accuracy of UTI diagnosis. As a result, pregnant women can receive more timely and appropriate treatments, potentially reducing complications associated with UTIs during pregnancy.

In addition, the study underscores the necessity of ongoing surveillance and research to monitor the prevalence and resistance patterns of UTI-causing pathogens. With the rise of antibiotic-resistant bacteria, continuous monitoring is essential to adapt treatment guidelines and ensure effective management of UTIs. This research contributes to the growing body of evidence on the significance of using molecular diagnostics

in detecting UTIs and highlights the need for healthcare systems to invest in such technologies to enhance patient care and outcomes.

CONCLUSION

Based on the present study, it is concluded that *S. aureus*, *E. coli*, *E. faecalis*, and *K. pneumoniae* are frequently found in UTIs among pregnant women. The PCR method was identified as a rapid and sensitive technique, showing more positive results compared to conventional methods used in this study. *S. aureus* emerged as the most dominant species responsible for UTIs, with an estimated prevalence of 70%. *E. coli* was found in 63% of cases, *E. faecalis* in 55%, and *K. pneumoniae* in 36%. The study also revealed that the isolated bacteria were sensitive to antibiotics such as Amoxicillin, Ciprofloxacin, Fluoroquinolone Norfloxacin, and Aminoglycosides. The results suggest that maintaining personal hygiene during pregnancy is crucial to prevent UTIs. Additionally, if an infection occurs, proper examination and culture sensitivity testing are necessary to ensure that only those antibiotics to which the isolated pathogens are sensitive are used.

Conflict of Interest:

Authors have no conflict of interest.

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