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Isolation and Identification of Microbial Agents Causing Urinary Tract Infection (UTI) in Women

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Abstract

Urinary tract infection (UTI) is linked to a high rate of morbidity and long-term consequences in children, including renal scarring, hypertension, and chronic renal failure. UTI manifests with vague symptoms in paediatric patients, making the diagnosis more difficult. As a result, the high prevalence of untreated and inadequately managed UTI in children is a source of clinical and societal concern.

Aims of that project are Isolation & Identification of bacterial flora in Urine samples from pregnant & non pregnant women both in study & Control group. Its drugs sensitivity testing and compare our data with other studies. This study was conducted in Helix Biogenesis Pvt. Ltd. Noida & samples were collected from Different Private Hospital & Nursing Home, Noida. In this study 15 cases were selected (10 cases Pregnant women in study group & 05 cases non-pregnant women in control group) of Urine sample from different age groups.

Keywords: UTI; Women; Isolation; Identification; Microbes

Introduction

Urinary tract infections (UTIs) are one of the most common issues that family physicians face. UTIs during pregnancy are one of the most frequent health issues globally, particularly in underdeveloped nations (Abrutyn E et al., 1998) [1]. Urinary tract infections (UTI), which are caused by the presence and proliferation of germs in the urinary system, are one of the most frequent bacterial illnesses in humans and in pregnancy; they can affect the lower urinary tract or the bladder. UTI is reported in 20% of pregnant women and is the most prevalent reason for admission to obstetrical wards [2].

In an asymptomatic patient, UTI is defined as the presence of at least 100,000 organisms per millilitre of urine, or more than 100 organisms/ml. of urine with associated pyuria (>5 WBCs/HPF) in a symptomatic patient. A positive culture for auro pathogen should support a diagnosis of UTI, especially in healthy individuals [3]. Many physiological, anatomical, and interpersonal variables all play a role in this issue throughout maternity [4]. Urinary tract infection during pregnancy is a major cause of maternal and perinatal morbidity. Urinary tract infection during pregnancy is linked to abortion, small birth size, maternal anaemia, hypertension, premature labour, phlebitis, thrombosis, and persistent pyelonephritis [5].

Gram-negative enteric bacilli are the most common pathogens that cause UTI in children. The most prevalent etiological agents are *Escherichia coli* and *Klebsiella*. However, in recent years, Enterococci, yeasts, and *Staphylococcus aureus* have emerged as significant agents, and many of them are resistant to various antibiotics 3, 4. Because urine includes urea, prolonged infection with urea splitting organisms such as Proteus and *Klebsiella* species may develop to urinary calculi in patients1. As a result, early detection of UTI is required to prevent illness and death, as well as the treatment load on patients. This study is being conducted in response to the aforementioned issue.

Material and Method

Hanging drop preparation: Firstly take a clean groove glass slide and a clean cover slip after that with the help of sterile inoculating loop take a full of liquid culture and put it in the centre of the cover slip. Then carefully lift the slide along with drop hanging from the under surface of the cover slip. Examine the preparation under the microscope. The first focus in 10X then turn to high power objective and observe the motility of the bacteria at the edge.

Inoculation of the urine samples: Inoculation of the Urine sample on different medium including MacConkey's Agar, Nutrient Agar Medium, Savoured Dextrose Agar Medium. After inoculation culture plate kept in incubator at 37°C for 24-72 hrs. After incubation time were completed and observe the colonies morphology (Figures 1-4), respectively. Gram's staining, Motility and performed the biochemical test for final identification. After that perform the Sensitivity test.

Biochemical identifications:

Indole production In 5 ml of peptone water 1 ml of the inoculum was inoculated and incubated at 37°C for 24 hours. After incubation 5 drops of Kovac's reagent was added to the surface.

Result - reddening of medium (Figure 5)

Methyl Red test 1 ml of inoculums were inoculated into 5 ml of Glucose Phosphate broth and incubated at 37°C for 48 hours. 5 drops of Methyl red indicator were applied to the surface after incubation. To disseminate the methyl red, the tube was rolled between the palms of the hands.

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Figure 1: Growth of Bacteria on MacConkey agar.

Figure 2: Growth of Bacteria on Nutrient agar.

Figure 3: Growths of *Klebsiella sps.* on MacConkey agar.

Figure 4: Growths of *Candida sps.* on Sabouraud Dextrose agar medium.

Figure 5: Result of Indole production.

Result – bright red medium (Figure 6)

Voges-Proskauer test In 5ml of Glucose Phosphate broth, 1 ml of inoculums was inoculated and incubated at 37°C for 48 hours. After incubation 0.6 ml of alpha-naphthol was added and shacked. Then 0.2 ml of 40% potassium hydroxide was added, mixed gently and left 10-15 minutes for colour development.

Result – pink colour slowly develops in the medium (Figure 7).

Oxidase test A piece of filter paper was placed on a cleaned microscopic slide. Then added 2-3 drops of oxidase reagent was placed on the filter paper. The isolated colony was smeared with a loop on the filter paper. The presence of a dark purple colour was observed. The reaction positive, if the smear turns purple within 10-30 second.

Result – deep purple colour on the strips or medium (Figure 8).

Catalase test A drop of 3% hydrogen peroxide was placed on to a clean microscopic slide. The isolated colony was smeared with an inoculated loop in to hydrogen-peroxide. Then observe the bubbles.

Result – release of oxygen bubbles (Figure 9)

Urease test The inoculums was inoculated in the Christensen's Media with help of loop and incubated at 37 degree centigrade for 48 hours. If the red colour comes test is positive.

Result – red pink medium (Figure 10)

Figure 6: Result of Methyl red test.

Figure 7: Voges Proskauer test.

Figure 8: Oxidase test.

After final identification of the bacteria drugs sensitivity pattern using the following drugs (Table 3).

G-Gentamycin, CF-Ciprofloxacin, TE-Tetracycline, TB-Tobramycin, Nx-Norfloxacin, AK-Amikacin, NZ-Ofloxacin, AX-Amoxiclin, NT-Netilmycin, AP-Ampicillin

Drug sensitivity test

Kirby- Bauer disc diffusion method: Nutrient agar plate is taken. The inoculums are prepared by touching 3-5 colonies and inoculated into sterile peptone water which is incubated at 37°C for 4hrs. The nutrient agar plate is inoculated with the help of sterile cotton swabs. Allow the plate to dry before antibiotics discs are applied. The plates are then incubated at 37°C for 24hrs and then the zone of inhibition is observed (Figure 11).

This methodology is adopted from Bauer A et al. [6].

After that we also performs AST to check their sensitivity result are shown in table 3.

Figure 9: Catalase test.

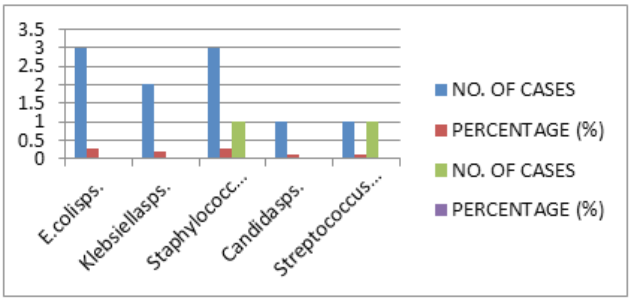
Figure 10: Urease test.

Observations

The present study is based on the isolation and identification of Microorganisms from Pregnant & Non-Pregnant women in Study group (10 cases) & Control group (05 cases) (Table 1) in different hospital in Noida region and its drugs sensitivity pattern (Table 3) from isolated bacteria. The observation as follows:-

Table-1 Master table

Table-2 Isolation rate of bacterial flora in study & control group



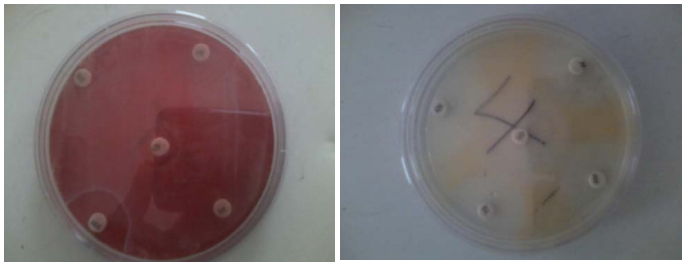


Figure 11: Disc diffusion method result.

Table 1: Shows 10 cases of pregnant and 05 cases were normal women.

S.No	Name of patient	Age	Complains	Date of collection	Bacteria/Fungus	Media		
						Macconkey agar	Nutrient agar	Sabouraud agar
Pregnant Women (Study Group)								
1	Reema	22	Blood in urine	20-10-2019	*Staphylococcus sps.*	+	+	-
2	Meena	39	Mucus in urine	22-10-2019	*E.coli sps.*	+	+	-
3	Ekta	27	Pain in urination	28-10-2019	*Candida sps.*	-	-	+
4	Devika	32	Pain in lower abdomen	13-11-2019	*E.coli sps.*	+	-	-
5	Reshma	36	Pain in urination	24-11-2019	*Stapylococcus sps.*	+	+	-
6	Sheetal	27	Pain in lower abdomen	29-11-2019	*Klebsiella sps.*	+	+	-
7	Reena	22	Burning in urination	03-12-2019	*Streptococcus sps.*	+	+	-
8	Deepa	28	Burning in urination	10-12-2019	*Klebsiella sps.*	+	+	-
9	Chahya	33	Blood in urine	15-12-2019	*E.coli sps.*	+	-	-
10	Kusum	37	Burning in urination	19-12-2019	*Stapylococcus sps.*	+	+	-
Non-Pregnant Women (Control Group)								
11	Rita	32	Non pregnant woman	19-04-2017	*Sterile*	-	-	-
12	Prachi	25	Non pregnant woman	20-04-2017	*Sterile*	-	-	-
13	Suman	44	Non pregnant woman	22-04-2017	*Staphylococcus sps.*	+	-	-
14	Sunita	24	Non pregnant woman	22-04-2017	*Sterile*	-	-	-
15	Radha	40	Non pregnant woman	25-04-2017	*Streptococcus sps.*	+	-	-

Table- 3 Antibiotic senstivity pattern

Result

In our study Table 1 shows the distribution of study and control group. A total of 10 bacteria were isolated (Table 2) with *E. coli* & *Staphylococcus sps*. (30.0%) respectively being the major organism. Next organism of importance is the *Klebsiella sp.* (20.0%). In control group only (5.0%) were isolate of *Staphylococcus albus* & *Streptococcus* respectively. Our finding According to Tamalli M et al. [7].

Conclusion

The physiological characteristics that sensitize women to UTI during pregnancy were explored in conjunction with other characteristics such as age, sex acts, past record of UTI, & socio - economic status. As a consequence of the findings of this research, the prenatal care doctor should emphasise personal hygiene instruction to every pregnant females, particularly those from poor socioeconomic backgrounds.

Urine culture must be performed at the initial prenatal appointment, and cultures should be acquired at various trimesters because treated individuals' urine may not remain sterile throughout the pregnancy.

To minimise maternal-fetal problems, pregnant women should be treated with appropriate antibiotic medication based on sensitivity testing when bacteriuria is detected.

The most prevalent urine symptoms in pregnancy, according to this study, were irregular nullifying trend and irritative manifestation. The majority of urinary symptoms were caused by changes in the urinary system caused by pregnancy. UTI risk factors included a history of UTI, sexual activity, being from a lower socioeconomic group, and having several children. UTI in pregnancy is definitely linked to the chance of developing symptomatic pyelonephritis later in pregnancy, and it may also be linked to other maternal and foetal problems. A prenatal care inquiry should include a urine check.

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Conflict of Interest

No author has any conflict of interest.

Ethics of approval statement

No need to take approval of ethics

Patient consent statement

All patient gives their consent.

Permission to reproduce material from other sources

No data taken from other sources

Clinical trial registration and ethical approval information

None

Author contributions

Sumit Sheoran (SS) designed the experimental work Swati Arora (SA), Nishi Aggarwal (NA), SamsonRaj R (SR) and Lopamudra Ruth (LR) do the lab works and write the final manuscript along with SS.

Table 2: Reveals that presence of UTI Pathogen in control group are sterile i.e. 0% while the percentage of bacteria in study group are different that are *E.coli* (30%), *Klebsiella* (20%), *Staphylococcus sp.* (30%), *Candida sp.* (10%) *Streptococcus sp.* (10%).

S.No	Name of Bacteria	Study Group		Control Group	
		No. of Cases	Percentage (%)	No. of Cases	Percentage (%)
1	*E.coli sps.*	3	30%	0	0%
2	*Klebsiella sps.*	2	20%	0	0%
3	*Staphylococcus sps.*	3	30%	1	5%
4	*Candida sps.*	1	10%	0	0%
5	*Streptococcus sps.*	1	10%	1	5%

Table 3: Showed percentages of bacterial strains that were resistant to G-Gentamycin (60%), CF-Ciprofloxacin (90%), TE-Tetracycline (40%), TB-Tobramycin (60%), Nx-Norfloxacin (100%), AK-Amikacin (90%), NZ-Ofloxacin (70%), AX-Amoxicllin (50%), NT-Netilmycin (90%), and AP-Ampicillin (40%).

S. No	Name of Organism	Name of the Drugs									
		G	CF	TE	TB	NX	AK	NZ	AX	NT	AP
1	*Staphylococcus sps.*	20mm	26mm	R	R	24mm	28mm	18mm	20mm	26mm	R
2	*E.coli sps.*	18mm	20mm	R	R	20mm	-	-	-	-	-
3	*Candida sps.*	-	-	-	-	-	-	-	-	-	-
4	*E.coli sps.*	R	20mm	16mm	22mm	24mm	18mm	22mm	R	22mm	18mm
5	*Staphylococcus sps.*	20mm	24mm	20mm	22mm	26mm	28mm	22mm	18mm	24mm	16mm
6	*Klebsiella sps.*	R	18mm	R	20mm	26mm	18mm	R	R	20mm	R
7	*Streptococcus sps.*	20mm	26mm	R	18mm	22mm	28mm	20mm	R	24mm	18mm
8	*Klebsiella sps.*	R	R	R	20mm	18mm	22mm	R	R	22mm	R
9	*E.coli sps.*										
10	*Stapylococcus sps.*	16mm	22mm	R	R	20mm	26mm	20mm	18mm	26mm	R
11	Sterile	-	-	-	-	-	-	-	-	-	-
12	Sterile	-	-	-	-	-	-	-	-	-	-
13	*Staphylococcus sps.*	24mm	18mm	22mm	26mm	28mm	20mm	24mm	20mm	20mm	R
14	Sterile	-	-	-	-	-	-	-	-	-	-
15	*Streptococcus sps.*	R	22mm	26mm	R	22mm	26mm	28mm	24mm	20mm	18mm

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