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Original Research Article

Evaluation of antibacterial susceptibility profile in pregnant women with asymptomatic bacteriuria in Kadapa

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ABSTRACT

Background: The bacterial urinary tract infection in pregnancy may be symptomatic (cystitis, pyelonephritis) or asymptomatic (bacteriuria without symptoms). Empirical antimicrobial therapy may be affected by resistance of uropathogens due to the irrational use of antibiotics. This prevalence of resistance is rising and varies across the globe due to different treatment recommendations which may be determined by taking into account frequently occurring infections, susceptibility patterns, evidence, physician acceptance, antimicrobial stewardship norms, availability of formularies, and antimicrobial prices. So the current research was carried out.

Materials and Methods: A cross-sectional study was conducted among pregnant women with UTI attending antenatal care (ANC) by excluding pregnant women who had taken antibiotics within the two weeks prior to their ANC follow-up and symptomatic UTI pregnant women. The antibiotic sensitivity of the uropathogens and their identification were evaluated by conventional culture, microbiological techniques and sensitivity tests. The results were analysed by SPSS software for descriptive statistics.

Results: 377 pregnant women with UTI participated in the present study. The results demonstrated that most of the participants who lived in urban areas (64%) were affected. The results also suggest that 42% of participants who had secondary education as the highest level of education, 45% of participants belonged to an upper middle-class family and, 40% of working professionals in the study were affected. Out of 103 isolates, 79 isolates were gram negative & 24 were Gram positive. Out of seventy-nine gram negative isolates, the major contribution was by *Escherichia coli* (36), *Klebsiella spp*. (26). Among 24 gram positive isolates, the major contribution was by Coagulase Negative Staphylococci (CONS) (12). The uropathogens were shown diverse antibiotic sensitivity for the study test drugs.

Conclusion: This study concludes that the antibacterial susceptibility and resistance of uropathogens may vary in different geographical areas based on antibiotic usage and circumstances. This study also demonstrated the imperative prerequisite of periodic assessments.

1. Introduction

Urinary tract infection (UTI) is the foremost prevailing bacterial infection during pregnancy. The urinary tract infection in pregnancy may be symptomatic (cystitis, pyelonephritis) or asymptomatic (bacteriuria without symptoms). Based on the research evidence available, the prevalence of symptomatic and asymptomatic urinary tract infections in pregnant women has been 17.9% and 13% respectively.[1,2] The urethra's small length, lack of

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prostatic secretions, pregnancy, and the probability of urinary tract contamination by faecal bacteria make women more prone to UTIs than men.[3] Asymptomatic bacteriuria can lead to maternal and neonatal complication like preterm labour, pre-eclamptia, IUGR, Preterm babies.[4,5] Enterobacteriaceae, which are frequently present in the gastrointestinal system, are the source of most infections; 80–90% of these instances are caused by *Escherichia coli*, or *E. coli*. Other bacteria, such as coagulase-negative *Staphylococcus*, *Klebsiella pneumoniae*, Group-B *Streptococcus saprophyticus*, Proteus mirabilis and, Staphylococcus aureus, are present in smaller amounts.[6]

The empirical antimicrobial therapy commenced prior to the laboratory antibiotic sensitivity tests outcomes of urine culture, may leads to increase in resistant among uropathogens.[7] The prevalence of resistance among UTI patients is rising and varies according on the patient's geographic & regional area.[8] Treatment recommendations are often determined by taking into account frequently occurring infections, susceptibility patterns, evidence, physician acceptance, antimicrobial stewardship norms, availability of formularies, and antimicrobial prices.

Choosing the appropriate antibiotic for each patient while considering the safety profile of both the mother and the newborn is crucial, and treatment recommendations for UTIs have been produced globally to help clinicians in this regard. The regular screening of antibacterial susceptibility of available therapeutic agents may help to prevent antibacterial resistance and, to furnish revised antibacterial guidelines for the rationale approach to UTI in pregnancy. So the study was conducted.

2. Materials and Methods

Cross-sectional study was conducted at antenatal care (ANC) in Fathima Institute of Medical Sciences Kadapa, from December 2022 to December 2023. Being pregnant and having an ANC follow up were involved in the study. The study excluded pregnant women who had taken antibiotics within the two weeks prior to their ANC follow-up and symptomatic UTI pregnant women. Competent nurses collected clinical and sociodemographic data using a structured questionnaire. A sterile container with a wide opening was used to collect 10 ml of clean catch midstream urine samples from every study participant. The urine sample that was obtained was labeled and transported to the hospital laboratory less than one to one and half hour.[9] By calibrated wire loops (0.001 ml), urine samples were plated on blood agar, MacConkey, and Cystine Lactose Electrolyte-Deficient (CLED) media. After that, all plates were aerobically incubated at 37°C for 24 hours. The bacterial growth in all plates was examined under microscope and, if the count of colonies is more than 10^5 CFU, and is deemed to be a significant growth.

The species level identification was done by employing the conventional microbiological procedures, colony features, Gram-staining response, and pattern of biochemical profiles to all significant positive cultures. The KIA agar method, H2S generation, carbohydrate fermentation, urease test, indole production, motility test, citrate consumption and, oxidase test were used to identify the enterobacteriaceae. Catalase and coagulase tests were employed to identify the Gram positive bacteria. A tube with 4-5 mL of nutrient broth was filled with three to five pure colonies, gently mixed, and cultured for two to six hours at 35–37 °C. To standardize the size of the inoculum, the suspension's turbidity was evaluated using McFarland 0.5 tubes.[7] Inoculate the whole Mueller-Hinton agar (MHA) (Oxide Ltd, Hampshire, UK) surface equally by dipping a sterile cotton swab into the solution. The inoculated plates were left at room temperature to dry for 3–15 min.[6,10] Following the organism inoculation and disc implantation, plates were incubated for twenty-four hours. The inhibitory zones were evaluated 24 hours later.

The antibiotics that were assessed in this study were: Nitrofurantoin (300 μg), Azithromycin (15 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Norfloxacin (10 μg), Trimethoprim-Sulphamethoxazole (co-trimoxazole) (1.25-23.75μg), Ceftriaxone (30 μg) and Amoxycillin (10 μg), Amoxycillin-Clavulinic Acid, Tetracycline (30 μg), Meropenem (10 μg). In accordance with the Clinical Laboratory Standards Institute (CLSI) the data were evaluated.[11] Before every patient's samples were collected, informed consent was sought both verbally and in writing. The research was initiated with the necessary institutional ethics committee permission. The American Type Culture Collection (ATCC) 25922, *Staphylococcus* aureus (ATCC 25923), Enterococcus fecalis (ATCC 29212), and, Pseudomonas aeruginosa (ATCC 27853) were utilized as standard strains for quality control. The antibiotic discs, quality control stains and, culture media were attained from HiMedia Laboratories, Mumbai, India.

2.1. Statistical analysis

The survey data reviewed carefully, coded and imported into excel. The data was exported to SPSS version 25 for descriptive statistics.

3. Results

According to the sampling technique described in the methodology, 377 pregnant women participated in the present study. Among them 243 urban participants and 134 rural participants from Kadapa district. Out of the participants, 86, 151 and, 140 participants were <25 years, 25-30 and, >30 years old respectively. Among study participants, 73 were illiterates, 99 had primary education, 158 had secondary education, and 47 had higher education.

According to family income, the participants were poor (76), middle class (110), upper middle class (168), and rich.[12] Out all study participants, 96 were housewives, 125 were businesswomen, 151 were working professionals and 5 participants were students (Table 1).

According to Table 2, second trimester (176), multigravida (291) participants were in majority number. Few participants recruited with history of Catheterization, diabetes mellitus, abortion, obstetric and gynecologic surgery, premature labor, previous UTI.

The uropathogens isolated from the urine samples of the participants were tabulated and represented in the Table 3. In the current research 77 percentage (79) of microorganism isolates were gram negative and 23 (24) percentage was Gram positive among 103 microorganism isolates. Out of seventy-nine isolates 36, 26, 7, 6, 1 and, 3 were *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Pseudomonas* spp., *Proteus* spp., and *Enterobactor* spp respectively. Out of 24 gram positive isolates 6, 12, and 6 isolates were enterococcus, Coagulase Negative Staphylococci (CONS), and *Staphylococcus aures.*

The antibacterial sensitive of 6 gram negative and 3 gram positive organisms were depicted in relation with Nitrofurantoin (300 μg), Azithromycin (15 μg) Gentamicin (10 μg) Ciprofloxacin (5 μg) Norfloxacin (10 μg) (co-trim) (1.25 23.75μg) Ceftriaxone (30 μg) Amoxycillin (10 μg) Amoxycillin-Clavulinic Acid Tetracycline (30 μg) Meropenem (10 μg) and the same was tabulated in Table 4.

The major uropathogenic bacteria in the present study were *Escherichia coli* (36), *Klebsiella spp* (26) and Coagulase Negitive Staphylococci (CONS) (12) belongs to gram negative and positive bacteria respectively.

3.1. Escherichia coli

The evaluated antibacterial sensitivity of *Escherichia coli* was grater with, Meropenem (10 μg), Nitrofurantoin (300 μg), Ceftriaxone (30 μg), co-trimoxazole (1.25 - 23.75μg), Norfloxacin (10 μg), Azithromycin (15 μg) Gentamicin (10 μg), Ciprofloxacin (5 μg). *E coli* showed resistance to Amoxycillin (10 μg), Tetracycline (30 μg) and the antibacterial sensitivity was moderate to Amoxycillin-Clavulinic Acid.

3.2. Klebsiella species

The strains in the *Klebsiella* species of this study were sensitive to co-trimoxazole (1.25 23.75μg), Azithromycin (15 μg), Norfloxacin (10 μg), Meropenem (10 μg), Ceftriaxone (30 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Nitrofurantoin (300 μg). *Klebsiella* spices demonstrated intermediate antibacterial sensitivity to Amoxycillin-Clavulinic Acid, and were highly resistant to Tetracycline (30 μg), Amoxycillin (10 μg).

3.3. Coagulase Negative Staphylococci (CONS)

Coagulase Negative Staphylococci (CONS) is a major gram positive bacterium observed in the present study was sensitive to Nitrofurantoin (300 μg), Azithromycin (15 μg), co-trimoxazole, Ceftriaxone (30 μg), Meropenem (10 μg), Gentamicin (10 μg), Norfloxacin (10 μg), Ciprofloxacin (5 μg) and resistant to Amoxycillin (10 μg), Tetracycline (30 μg), Amoxycillin-Clavulinic Acid.

4. Discussion

The proper diagnosis and treatment are preferred to impede the risk of morbidity and fatal conditions due to uropathogens in pregnancy. There are better antibiotics available for treatment of UTIs in pregnancy, majority of them included in category B of the US-FDA guidelines sense that they have negligible evidences of untoward effects, some in category C have to be cautious in their usage.[13] The availability of better antibiotics for UTI is always in the front line to discuss due to the resistance developed by the uropathogens. The prevalence of UTI and susceptibility of its causative agents vary with geographical distribution.[14] In this context, resistance is an issue that is steadily becoming worse around the globe and has intriguing patterns.[15] Irrational and prior usages of antibiotics are great risk factors. Resistance, irrational, miss use were driving forces to conduct the study in Kadapa region of Andhra Pradesh, India.

The current study results demonstrated that participants lived in urban area (64%) was affected. The results also suggest that 42% of participants who had secondary educations as highest level of education, 45% of participants belong to upper middle-class family and, 40% of working professionals in the study were affected. Due to limited participants, the association of obstetrics-clinical features such as diabetes mellitus, history of catheterization, obstetric and gynecologic surgery, abortion, premature labor, previous UTI was observed in fewer participants.

This study was supported by Matalka et al a retrospective study stated that *E. coli* and CoNS were the utmost acknowledged bacteria. The significant increase in antibacterial resistance in Enterobacter species was established.[16] This pattern of isolates was supported by de Souza et al systematic review by includig 67 studies. Their review results suggested that major isolate bacterial species were *Escherichia coli*, *Klebsiella* spp. *Staphylococcus* sp., excluding *Staphylococcus* aureus, Proteus mirabilis and *Enterobacter* spp.[17,18]

The Gram-staining response, colony features, and pattern of biochemical profiles, together with conventional microbiological procedures, were used to identify all significant positive cultures of bacteriuria (>10^5 CFU colonies) at the species level. In this study out of 103 isolates 79 isolates were gram negative & 24 were Gram

Table 1: Sociodemographic characteristics of pregnant women

Variable		Frequency	Percentage
Age	< 25	86	23
	26-30	151	40
	>31	140	37
	Total	377	100
Residence	urban	243	64
	Rural	134	36
	Total	377	100
Educational Status	Illiterate	73	19
	Primary Education	99	26
	Secondary Education	158	42
	Higher Education	47	12
	Total	377	100
Family Income	Poor	76	20
	Middle Class	110	29
	Upper middle class	168	45
	Rich	23	6
	Total	377	100
Occupational Status	Housewife	96	25
	Businesswomen	125	33
	Employee	151	40
	Student	5	1
	Total	377	100

The frequency and percentage analysis was done by SPSS software

Table 2: Clinical characteristics of study participant

Variable		Frequency	Percentage
Gestational period	1^{st} trimester	92	24
	2^{nd} trimester	176	47
	3^{rd} trimester	109	29
	Total	377	100
Gravida	Primigravida	86	23
	Multigravida	291	77
		377	100
History of Catheterization	No	362	96
	Yes	15	4
		377	100
History of Diabetes Mellitus (DM)	No	367	97
	Yes	10	3
		377	100
History of Abortion	No	354	94
	Yes	23	6
		377	100
History of Obst. & Gynec surgery	No	360	95
	Yes	17	5
		377	100
History of premature labor	No	372	99
	YES	5	1
		377	100
History of previous UTI	No	320	85
	Yes	57	15
		377	100

The frequency and percentage analysis was done by SPSS software

Table 3: Prevalence of uropathogen in pregnant women

Micro organism	Frequency	Percent
Gram Negative Organism	79	77
Escherichia coli	36	34.95
Klebsiella spp.	26	25.24
Citrobacter spp.	7	6.80
Pseudomonas spp.	6	5.83
Proteus spp.	1	0.97
Enterobactor spp.	3	2.91
Gram Positive Organism.	24	23
Enterococcus spp.	6	7.77
Coagulase Negative Staphylococci (CONS)	12	11.65
Staphylococcus Aures	6	3.88
	103	100.00
The frequency and percentage analysis was done by SPSS software		

positive. Out of seventy-nine gram negative isolates the major contribution was by *Escherichia coli* (36), *Klebsiella* spp. (26). Among 24 gram positive isolates, the major contribution was by Coagulase Negative Staphylococci (CONS) (12).

This study results were supported by Ali, A.H et al study demonstrated that "most of the gram negative were sensitive to meropenem (95.9%), ceftriaxone (79.6%), Norfloxacin (77.5%), gentamicin (75.5%), nitrofurantoin (75.5%) and ciprofoxacin (71.4%), and were resistance to tetracycline (71.4%), trimethoprim–sulfamethoxazole (57.1%), amoxicillin clavulanic acid (55.1%) and nalidixic acid (51%).[19] Analogous research conducted in diverse geographical area like in Addis Ababa, Ethiopia that showed highly sensitive to meropenem (75.2%), nitrofurantoin (93.1%), gentamicin (85.2%), ceftriaxone (82.2%), cefuroxime (79.3%), and ciprofloxacin (75.2%).[20]

The evaluated sensitivity of *Escherichia coli* was grater with, Meropenem (10 μg), Nitrofurantoin (300 μg), Ceftriaxone (30 μg), co-trimoxazole (1.25 - 23.75μg), Norfloxacin (10 μg), Azithromycin (15 μg) Gentamicin (10 μg), Ciprofloxacin (5 μg). *E coli* showed resistance to Amoxycillin (10 μg), Tetracycline (30 μg) and the sensitivity was moderate to Amoxycillin-Clavulinic Acid. In contrast Belete MA et al study showed increased *E. coli* resistant to gentamicin, sulfamethoxazole, Trimethoprim, Nitrofurantoin, Norfloxacin, Ciprofloxacin, ampicillin, Ceftriaxone, amoxicillin, and nalidixicacid (20). This may be the usage of antibacterial at that particular geographical area.

The strains in the strains in the *Klebsiella* species of this study were sensitive to co-trimoxazole (1.25 23.75μg), Azithromycin (15 μg), Norfloxacin (10 μg), Meropenem (10 μg), Ceftriaxone (30 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Nitrofurantoin (300 μg). *Klebsiella* spices demonstrated intermediate sensitivity to Amoxycillin-Clavulinic Acid, and were highly resistant to Tetracycline (30 μg), Amoxycillin (10 μg). This was supported with little variation by Johnson B et al. and, Moyo SJ et al. studies.[21,22] The findings of Derese et al. study stated that *Klebsiella* species were entirely (100%) resistant to amoxicillin, ampicillin, and nitrofurantoin, nevertheless 66.7% of bacteria were resistant to tetracycline, and chloramphenicol.[12]

According to current study among the Gram Positive bacteria Coagulase Negative Staphylococci (CONS) is a foremost bacterium perceived in the current research study was sensitive to Nitrofurantoin (300 μg), Azithromycin (15 μg), co-trimoxazole, Ceftriaxone (30 μg), Meropenem (10 μg), Gentamicin (10 μg), Norfloxacin (10 μg), Ciprofloxacin (5 μg) and resistant to Amoxycillin (10 μg), Tetracycline (30 μg), Amoxycillin-Clavulinic Acid. This study was supported by Aseffa, A et al by stating that CoNS was the foremost gram positive bacterium (55%) was found 63% to 81% sensitive to erythromycin, cefriaxone, cefoxitin, nitrofurantoin, ciprofloxacin, and gentamicin. However the study conducted in Ethiopia was shown Cons resistant to nitrofurantoin (26.7%).[8,23] According to Shaheen et al study, CoNS is exceedingly unaffected with ampicillin (81.8%), tetracycline (54.5%), and amoxicillin clavulanic acid (45.4%) but affected by cefoxitin (81.8%), erythromycin (81.8%), nitrofurantoin (72.7%), ceftriaxone (72.7%) and gentamicin (72.7%), ciprofloxacin (63.6%) and co-trimoxazole (54.5%).[19]

5. Conclusion

This study demonstrated and supported that the antibacterial susceptibility and resistant of uropathogens may be vary in different geographical areas based on the antibiotic usage, and also demonstrated the imperative prerequisite of periodic assessments to determine the susceptibility configurations of bacteria that are responsible for bacteriuria in pregnant women. Operative health policies have to be designed to reduce irrational use of antibiotics and to

Table 4: Antibacterial susceptibility configuration of isolated uropathogens in the study

Microorganism	Pattern	Nitrofurantoin (300 μg)	Azithromycin (15 μg)	Gentamicin (10 μg)	Ciprofloxacin (5 μg)	Norfloxacin (10 μg)	(Co-trim) (1.25 23.75μg)	Ceftriaxone (30 μg)	Amoxycillin (10 μg)	Amoxycillin-Clavulinic Acid	Tetracycline (30 μg)	Meropenem (10 μg)
Gram Negative Organism												
Escherichia coli (36)	S	31	27	26	24	27	28	29	3	15	7	33
	I	1	3	4	3	2	3	2	1	3	1	2
	R	4	6	6	9	7	5	5	32	18	28	1
Total		36	36	36	36	36	36	36	36	36	36	36
Klebsiella spp (26)	S	16	21	19	17	20	22	19	2	10	8	20
	I	1	1	0	1	2	2	2	3	4	14	2
	R	9	4	7	8	4	2	5	21	12	4	4
Total		26	26	26	26	26	26	26	26	26	26	26
Citrobacter spp. (7)	S	7	5	5	4	5	5	5	0	1	3	6
	I	1	1	0	1	0	1	1	1	2	0	0
	R	0	1	2	2	2	1	1	6	4	4	1
Total		8	7	7	7	7	7	7	7	7	7	7
Pseudomonas spp. (6)	S	4	3	4	4	4	5	5	0	2	0	5
	I	1	1	1	1	0	0	1	0	1	1	1
	R	1	2	1	1	2	1	0	6	3	5	0
		6	6	6	6	6	6	6	6	6	6	6
Proteus Spp. (1)	S	0	1	1	0	1	1	1	0	0	0	1
	I	1	0	0	1	0	0	0	0	1	0	0
	R	0	0	0	0	0	0	0	1	0	1	0
Total		1	1	1	1	1	1	1	1	1	1	1
Enterobactor Spp. (3)	S	2	2	1	1	2	2	1	0	1	0	1
	I	0	1	1	1	0	1	1	1	0	1	0
	R	1	0	1	1	1	0	1	2	0	0	0
Total		3	3	3	3	3	3	3	3	1	1	1
Gram Positive Organism												
Enterococcus Spp (6)	S	4	4	4	3	4	5	5	0	2	1	5
	I	0	0	1	1	0	1	0	1	1	2	0
	R	2	2	1	2	2	0	1	5	3	3	1
Total		6	6	6	6	6	6	6	6	6	6	6
Coagulase Negitive Staphylococci (CONS) (12)	S	10	9	8	7	8	9	9	1	5	1	9
	I	1	2	1	2	2	2	1	1	1	2	2
	R	1	1	3	3	2	1	2	10	6	9	1
Total		12	12	12	12	12	12	12	12	12	12	12
Staphylococcus Aures (6)	S	4	3	4	3	4	5	4	0	2	0	5
	I	1	1	1	1	2	1	1	1	1	2	0
	R	1	2	1	2	0	0	1	5	3	4	1
Total		6	6	6	6	6	6	6	6	6	6	6

The number of strains that were sensitive was displayed in the table. S- Sensitive strains, I – Intermediate Strains, R- Resistance strains

provide revised strategies on empiric management of UTIs in pregnant women. There should be practices to educate antibiotic usage in pregnant women and to prevent prior usage of antibiotics to its susceptibility test.

6. Source of Funding

None.

7. Conflict of Interest

No.

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