BACTERIAL PROFILE OF ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE ISOLATES OBTAINED

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ABSTRACT

BACKGROUND

Urinary tract infection in pregnancy is associated with significant morbidity for both the mother and the baby. The aim of this study was to determine the incidence of asymptomatic bacteriuria, the bacterial profile and antibiotic sensitivity pattern of the urinary pathogens isolated from pregnant women attending the OPD of a teaching hospital in a semi-rural area in the outskirts of Visakhapatnam city.

MATERIALS AND METHODS

The study group included 500 asymptomatic antenatal women in their first or second trimester of pregnancy. They were screened for bacteriuria by the catalase method. Their mid-stream clean-catch samples of urine were cultured by the standard loop semi-quantitative method. Antibiotic sensitivity was tested by the disc-diffusion method. Culture positive cases were advised to strictly follow treatment to avoid future complications.

RESULTS

48 (9.6%) of the 500 samples were culture positive. 66.66% (32 cases) of the positive cases were primigravida. The incidence was also high in the less than 20 years age group. The frequency of isolating coagulase negative Staphylococcus has increased in the present study. Many of the isolates proved to be ESBLs.

CONCLUSION

The findings of the study re-confirm the results of the earlier studies conducted in Visakhapatnam and elsewhere, and call for an even more vigilant approach to the problem. Prevalence of CONS was not encountered in the earlier study conducted in a similar demographic area, nor was that of the ESBLs. It has thus been proven that early screening of all pregnant women for urinary tract infection is mandatory for those visiting the obstetrician for antenatal checkups.

KEYWORDS

Asymptomatic Bacteriuria, Screening, ESBLs, Empirical Treatment.

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BACKGROUND

Urinary tract infection (UTI) is an infection caused by the presence and growth of micro-organisms anywhere in the urinary tract. The infections are usually caused by the bacteria from the digestive tract. These bacteria ascend up the urethral opening, and start multiplying in any part of the urinary system, to cause infection.[1,2] Urinary tract is second only to the respiratory tract in acquiring infection, especially in the females. It is more common in pregnant than in non-pregnant women.[3,4] Various studies conducted in different parts of the world have indicated that UTI, during pregnancy, leads to low birth-weight in the new-born, increased perinatal mortality and premature birth, along with acute and chronic sequelae in the mother.[5] This study was conducted to verify the present incidence of UTIs in asymptomatic antenatal women, check the bacterial profile of the UTIs, and their patterns of antibiotic sensitivities.

The mechanical, hormonal and physiological changes during pregnancy contribute to such changes in the urinary tract that lead to bacteriuria during pregnancy. Asymptomatic bacteriuria (ABU) is characterized by the isolation of the same uropathogen in two consecutive mid-stream, clean voided urine samples, in significant numbers ($\geq 10^5$ cfu/ml), in a patient without the classical symptoms of UTI (burning micturition, frequency of micturition and bouts of pyrexia).

Escherichia coli is the most common agent causing UTI, followed by Klebsiella, Staphylococcus aureus, Proteus, Pseudomonas, coagulase negative Staphylococci, Enterococci, Group B Streptococci.[6,7]

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Management of UTIs, without performing culture and sensitivity testing, usually leads to usage of antibiotics to which the causative organism is already resistant. Antimicrobial resistance among the pathogens that cause UTIs is increasing and is a major health problem in the treatment of UTI.[8,9] Culture and sensitivity testing is a must to ensure a favourable outcome of the treatment regime.

There is growing concern worldwide regarding resistance to antimicrobial agents, particularly of Escherichia coli, which is the dominant causative agent of UTI in pregnant women.[10] Moreover, it is the responsibility of the microbiologist to identify the extended spectrum beta lactamase (ESBL) producers as and when they occur. The availability of the ESBL Identification Kit, where the combination disc method is followed.[11] underlines the prevalence and significance of these organisms. However, they are few in numbers among the community strains.

Aims and Objectives

Urinary tract infections in pregnancy may lead to unfavourable pregnancy outcomes and complications like preterm delivery, low birth weight, pre-eclamptic toxaemia and anaemia. They should therefore always be screened for and treated in time.[12] In developing countries like India, screening for UTIs in pregnant women is not considered as an essential part of antenatal care. The present study is carried out to determine the bacterial profile and antibiotic susceptibility pattern of uropathogens among pregnant women, in a semi-rural area in the outskirts of Visakhapatnam city, in north coastal Andhra Pradesh, to provide empirical guidance for treating these cases. Antenatal women attending the obstetric out-patient department in Gitam Institute of Medical Sciences and Research (GIMSR), were selected for the study. Five hundred antenatal women were requested to submit their urine sample for testing, even though they were not suffering from any symptoms suggestive of UTI. The samples were collected over a period of one year, one month. The women submitting the samples belong to lower socio-economic status, and belong to the villages of Endada and Rushikonda, to which the hospital is close by.

Unlike the other situations, it is not possible to prepare and present antibiograms, as the patients are always outpatients, and there is no issue of a hospital acquired infection of already resistant strains. Also, it is to be remembered that the antibiotics to be avoided during pregnancy include chloramphenicol, sulphonamides and trimethoprim.

MATERIALS AND METHODS

Five hundred asymptomatic antenatal women were chosen to take part in the study as subjects. Their data was collected in a suitably designed and printed pro forma. Other than the identifying and personal details, important details like age and literacy, were enquired about. Other relevant details included obstetric history, gestational week, gynaecological history, past history of UTI, relevant and significant family history. These have an impact on the treatment outcome of the condition.

Women in either their first or second trimesters of pregnancy only were included in the study.

The patients were instructed to clean their genital area with a mild soapy solution, and then thoroughly with water, before collecting a mid-stream sample of urine. The patients were given sterile, screw-capped, plastic, wide-mouthed containers to collect the mid-stream samples. These samples were immediately transferred, by a specially designated attendant, to the laboratory in the floor below the OPD, and the sample processing was always initiated within half an hour after collection of the sample.

All the samples received were subjected to a preliminary screening test, the catalase test, which suggests the presence of bacteriuria, even before the culture results. A small volume, around 2 ml, of urine sample, was taken in a test-tube, and a few drops of hydrogen peroxide (10 vol) was added with a Pasteur pipette. The presence of prompt frothing indicates the presence of the enzyme catalase, and of the infecting uropathogens that are producing the catalase.

The semi-quantitative method of counting viable bacteria was used to decide whether the bacteriuria was significant or not. Samples were plated using the standard loop method. Calibrated platinum wire loop of 4.0 mm diameter was used to transfer a fixed volume (0.01 ml) of the well stirred urine sample, onto the 10 cm diameter culture plate. Nutrient agar, blood agar and MacConkey agar were inoculated by streak culture and incubated overnight (18 hrs), at 37°C. Plates then were examined for growth. Plates showing a hundred colonies denote the presence of one lakh cfu (colony forming units) per ml of urine. In the case of Staphylococcus aureus, a count as low as 20,000 cfu/ml of urine, is said to be significant. The isolated growths were identified by colony characteristics, and by some biochemical tests.

Escherichia coli-colony characteristics of the growth on MacConkey agar, and the IMViC reactions (indole, methyl red, Voges-Proskauer and citrate).

Klebsiella-same as Escherichia coli.

Pseudomonas-Colony characteristics and pigment production by the growth on nutrient agar, and the oxidase test.

Proteus-colony characteristics of the growth on MacConkey agar and blood agar, and indole test for species identification (Proteus mirabilis is indole negative).

Staphylococcus aureus-colony characteristics of the growth on nutrient agar, and the coagulase test.

Coagulase negative Staphylococci-colony characteristics of the growth on nutrient agar, the coagulase test and novobiocin sensitivity test.[13]

Enterocooci- Growth characteristics on MacConkey agar and blood agar, Gram stained smear examination of the growth and the bile aesculin hydrolysis test.

In the past, coagulase-negative Staphylococci were generally considered to be contaminants, having little clinical significance. Over the past four decades, however, these

organisms have become recognized as important agents of human disease. Staphylococcus saprophyticus is a well-documented pathogen. It primarily causes acute urinary tract infection in sexually active women. Staphylococcus saprophyticus is also the most frequently identified coagulase negative Staphylococcus in asymptomatic bacteriuria in antenatal women. It is identified by the colony characteristics on nutrient agar and blood agar, the coagulase test which is negative, and the novobiocin resistance on blood agar.

Antibiotic sensitivity testing was then carried out, which takes another 24 hrs. for completion. Mueller-Hinton agar plates were used to carry out the sensitivity testing by the Kirby-Bauer disc diffusion technique as recommended by the WHO technical report. As the strains causing asymptomatic bacteriuria in antenatal women are community acquired, it is expected to come across little resistance to routinely used antibiotics. Thus, lists with limited number of antibiotics only were tested for sensitivity of the urinary isolates in the present study. The antibiotic discs used included penicillin-10 units/disc, ampicillin-10 mcgs, amoxicillin-clavulanic acid combination 20/10 mcgs, cefotaxime-30 mcgs, cefotaxime-clavulanic acid combination-30/10 mcgs.[11]

Colistin-10 mcgs, nitrofurantoin 300 mcgs, gentamicin-10 mcgs, amikacin-30 mcgs, imipenem-10 mcgs, cefazolin-30 mcgs, ciprofloxacin-5 mcgs, vancomycin-30 mcgs, oxacillin-1 mcg, tetracycline-30 mcgs and novobiocin-5 mcgs.[13] Five microgram novobiocin disc was used to test the sensitivity of coagulase negative Staphylococci on blood agar plates.

Combination Disc Method- Several manufacturers have developed ESBL detection tests based on the combination disc method. The principal of this method is to measure the inhibition zone around a disc of cephalosporin and around a disc of same cephalosporin plus clavulanate. Depending on the disc type, a difference of > or =5 mm between the two diameters is indicating ESBL production. The test is easy to perform and its interpretation is straightforward. Sensitivity and specificity of this method is 96% and 100% respectively.[11]

Depending on the bacterial growth isolated, six most appropriate antibiotic discs were used while testing for antibiotic sensitivity. They included...

For Escherichia coli-Imipenem, amikacin, cephazolin, cefotaxime alone, cefotaxime-clavulanic acid combination and nitrofurantoin.

For Coagulase negative Staphylococci- Penicillin G, oxacillin, Ciprofloxacin, gentamycin, vancomycin, tetracycline, and novobiocin.

For coagulase positive Staphylococcus aureus: Penicillin G, cefazolin, cefotaxime, cefotaxime-clavulanic acid, vancomycin and oxacillin.

For Klebsiella pneumoniae- Colistin, imipenem, amikacin, cefotaxime alone, cefotaxime-clavulanic acid combination and nitrofurantoin.

For Pseudomonas aeruginosa- Colistin, imipenem, ciprofloxacin, cefotaxime alone and cefotaxime-clavulanic acid combination.

For Enterococci: Ampicillin, cefotaxime alone and cefotaxime-clavulanic acid combination, nitrofurantoin, amikacin, vancomycin and oxacillin.

For Proteus: Ampicillin, cefotaxime alone and cefotaxime-clavulanic acid combination, amikacin, ciprofloxacin and nitrofurantoin.

Each of the culture positive cases was advised to submit a second sample; if the second sample also yielded a positive growth, the patient was treated for the infection. The patients who were advised treatment were also educated regarding the necessity to comply with the treatment and about the serious side effects that would follow if the condition is not treated; for which, charts with diagrams were used.

RESULTS

Out of the 500 samples tested, 48 were culture positive, i. e, 9.6%. Of the above 48 culture positive cases, 32 were primi-gravida and 16 were second gravida.

Sl. No.	Gravida	Cases	Percentage
1	Primi	32	66.6
2	Second	16	33.4

Table 1. Gravida

Sl. No.	Organism	Culture Positives	Percentage
1	Escherichia coli	24	50%
2	CONS	8	16.6%
3	Staphylococcus aureus	4	8.3%
4	Klebsiella pneumoniae	4	8.3%
5	Pseudomonas aeroginosa	3	6.25%
6	Enterococcus	3	6.25%
7	Proteus mirabilis	2	4.16%
	Total	**48**	**100%**

Table 2. The Organisms Isolated During the Study Were

The interpretation of the results of the antimicrobial susceptibility tests were based on the national committee for Clinical and Laboratory Standards Institute (CLSI, 2005) criteria. The standard reference strains used are Staphylococcus aureus (ATCC25923), Escherichia coli (ATCC25922) and Pseudomonas aeruginosa (ATCC27853).[14]

The following tables show the sensitivity and resistance patterns of the different organisms isolated in the study-

Name of the Antibiotic	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Cephazolin	0	0%	24	100%
Cefotaxime	6	25%	18	75%
Cefotaxime-Clavulanic acid	24	100%	0	0%
Imipenem	15	62.5%	9	37.5%
Amikacin	12	50%	12	50%
Nitrofurantoin	18	75%	6	25%

Table 3. Sensitivity and Resistance Pattern of Escherichia Coli Isolates (24)

Name of the Antibiotic	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Penicillin G	0	0%	8	100%
Oxacillin	4	50%	4	50%
Ciprofloxacin	4	50%	4	50%
Gentamicin	6	75%	2	25%
Vancomycin	8	100%	0	0%
Tetracyclin	2	25%	6	75%

Table 4. Sensitivity and Resistance Pattern of Coagulase Negative Staphylococcus Isolates (8)

All were resistant to novobiocin, when tested on blood agar plates; thus all of them were Staphylococcus saprophyticus.

Name of the Antibiotic	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Penicillin G	0	0%	4	100%
Cephalexin	0	0%	4	100%
Cefotaxime	1	25%	3	75%
Cefotaxime-Clavulanic acid	2	50%	2	50%
Vancomycin	4	100%	0	0%
Oxacillin	4	100%	0	0%

Table 5. Sensitivity and Resistance pattern of Staphylococcus Aureus (4)

Thus none of the Staphylococcus aureus isolates were resistant to vancomycin (they may not be counted as VRSA).

Name of the Antibiotic	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Cefotaxime	2	50%	2	50%
Cefotaxime-Clavulanic acid	4	100%	0	0%
Colistin	4	100%	0	0%
Imipenem	4	100%	0	0%
Amikacin	3	75%	1	25%
Nitrofurantoin	4	100%	0	0%

Table 6. Sensitivity and Resistance Pattern of Klebsiella Pneumoniae (4)

Note: Klebsiella pneumoniae carbapenemases (KPCs) are absent according to the findings in the above table.

Name of the Antibiotic	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Cefotaxime	0	0%	3	100%
Cefotaxime-Clavulanic acid	3	100%	0	0%
Colistin	3	100%	0	0%
Imipenem	2	66%	1	33%
Tetracycline	0	0%	3	100%
Nitrofurantoin	2	66%	1	33%

Table 7. Sensitivity and Resistance Pattern of Pseudomonas aeruginosa (3)

Name of the Antibiotic	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Ampicillin	2	66%	1	33%
Cefotaxime	0	0%	3	100%
Cefotaxime-Clavulanic acid	0	0%	3	100%
Amikacin	3	100%	0	0%
Vancomycin	3	100%	0	0%
Nitrofurantoin	3	100%	0	0%

Table 8. Sensitivity and Resistance Pattern of Enterococcus (3)

During the study, vancomycin resistance however, was never come across in the Enterococcus isolates.

Name of the Antibiotic	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Ampicillin	1	50%	1	50%
Cefotaxime	2	100%	0	0%
Cefotaxime-Clavulanic acid	2	100%	0	0%
Amikacin	2	100%	0	0%
Ciprofloxacin	2	100%	0	0%
Nitrofurantoin	1	50%	1	50%

Table 9. Sensitivity and Resistance Pattern of Proteus Mirabilis Isolates (2)

DISCUSSION

Pregnant women are at an increased risk of developing urinary tract infection mainly because of a shift in the position of the urinary tract. The hormonal changes during pregnancy make it easier for the bacteria to travel up the urethra to the urinary bladder, ureters and the kidneys, and lead to the development of bacteriuria, which may be symptomatic or asymptomatic.[10,2] Unless intervention is made on time, UTI will show serious side effects, both in the health of the mother as well as the fetus. Therefore, early screening and antimicrobial treatment are the best preferred interventions.[12]

The incidence of asymptomatic UTIs in the present study is 9.6%. It is slightly higher than the incidence obtained in the earlier study of 8.4%.[7] The most important reason for this increase could be the more meticulous procedures followed in the present study, by well-trained technical staff. This resulted in a better isolation of the uropathogens.

However, it is much lower than the incidence obtained in a study conducted elsewhere, where it was 18.9%.[15]

The most prevalent organism isolated was Escherichia coli, which is similar with previous works.[15] followed by CONS, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococci and Proteus mirabilis. The major reason for frequently isolating E. coli is due to urine stasis in pregnancy, which favours colonization of the organism. Another reason could be due to poor genital hygienic practices by the pregnant women who find it difficult to maintain the genital hygiene because of the pregnancy.

When we compare the rate of isolation of Gram negative and Gram positive bacteria, Gram negative bacteria were the dominant causative agent of UTI which is in confirmation with the findings of other similar studies.[13,15]

Higher rate of antibiotic resistance was identified for Gram negative bacterial isolates, of which Escherichia coli was most frequently resistant to most antibiotics, confirming its resistance pattern to that of ESBL producers. Extended spectrum beta lactamase producers are resistant to all penicillins, and 1st, 2nd and 3rd generation cephalosporins and monobactam.[16]

Selective inhibition of cell wall synthesis is the best understood action of beta lactam drugs, which are active against growing bacteria. Resistance to these drugs is determined by the ability of the organism to produce penicillin destroying enzymes, (beta lactamases), which open the beta-lactam ring of penicillins and cephalosporins and abolish their anti-microbial activity. These enzymes have been described for many gram positive and gram negative bacteria. Some beta lactamases are plasmid mediated, eg penicillinase of Staphylococcus aureus. Some beta lactamases are chromosomally mediated, eg many species of gram negative bacteria. Plasmid mediated beta-lactamase of enterococci has a propensity to move from and to the given species of Enterococci. Chromosomally mediated betalactamases may be constitutively produced, as in Bacteroides, or may be inducible as in Pseudomonas. Extended spectrum of beta lactamases is a group of betalactamases that may be found in Klebsiella pneumoniae and Escherichia coli. They are so named because they confer upon the bacteria the additional ability to hydrolyse the beta lactam rings of cefotaxime, ceftazidime or aztreonam. Cloxacillin is a penicillin that has a high affinity for beta lactamases. Of the most concern is the emergence of Klebsiella pneumoniae carbapenemases (KPC) which are the ESBL-type enzymes that confer resistance to third and fourth generation cephalosporins and carbapenems. Staphylococci and certain Streptococci show tolerance to beta lactam drugs (that is, they are inhibited but not killed). Agents that act by inhibition of cell-wall synthesis are penicillins, cephalosporins, vancomycin and cycloserine. Bacitracin, vancomycin and novobiocin inhibit the early stages in the biosynthesis of the peptidoglycan. These drugs must penetrate the cell membrane to be effective, because the early stages of cell-wall synthesis occur in the cell membrane.[17]

Among the Gram positive bacteria, CONS showed higher rate of resistance to ciprofloxacin, norfloxacin, amoxicillin-clavulanic acid which is similar to the pattern in other studies.[13] The low rate of resistance to nitrofurantoin could be due to the less frequent use of this antibiotic in the study area.

Resistance to antibiotics was unknown in one of the earlier studies.[7] However, the more recent studies.[13,15] quote resistance rates comparable with the present study.

CONCLUSION

In conclusion, the prevalence of UTI in pregnant women in a semi-rural area in the outskirts of Visakhapatnam, city located in the north-coastal region of the state of Andhra Pradesh, India, is significant, and calls for the regular screening tests. Resistance to commonly used antibiotics is also prevalent, and an antibiotic sensitivity testing should always be conducted, before starting the antibiotic course, to prevent an increase in multidrug resistance rate. Therefore, early screening, identification of the infecting agent and antibiotic sensitivity testing are important interventions to prevent complications that may endanger the life of both the pregnant woman and the fetus.

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