



ANTIBIOGRAM OF ISOLATES OBTAINED FROM PREGNANT WOMEN WITH ASYMPTOMATIC BACTERIURIA AT A TERTIARY CARE HOSPITAL

Microbiology

Ms. Shagufta N. Kaskar *	Research Student, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital. Mumbai Central, Mumbai-40008 *Corresponding Author
Ms. Meghana R. Tendolkar	Research Student, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital. Mumbai Central, Mumbai-40008
Dr. Shashikant P. Vaidya.	Assistant Director, Clinical Pathology Department, Haffkine Institute, Acharya Donde Marg, Parel (East), Mumbai-4000 12
Dr. Subhash A. Angadi	Professor, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital. Mumbai Central, Mumbai-40008
Dr Geeta V. Koppikar	Dean , T. N. Medical College and B. Y. L. Nair Charitable Hospital. Mumbai Central, Mumbai-40008

ABSTRACT

Study was conducted to understand contemporary levels of resistance to commonly used antimicrobial agents for aerobic bacteria obtained from urine samples of pregnant (3000) and non- pregnant women (300) in treatment of Asymptomatic bacteriuria, in Nair Hospital, Mumbai. Microscopic examination of urine and identification of isolates & its susceptibility to antibiotics were carried out by standard methods. In both groups, *E. coli* strains were predominant, followed by *Klebsiella* spp. *E. coli* (100%), *Pseudomonas aeruginosa* (83.3%), *Proteus mirabilis* strains (100%) were sensitive to Piperacillin + Tazobactam. *Klebsiella pneumoniae* (70.2%), *Staphylococcus epidermidis* (93.8%) and *Enterococcus faecalis* (93.4%) strains showed sensitivity to Nitrofurantoin, while *Klebsiella oxytoca* (80%) strains to Amikacin, *Acinetobacter baumannii* (81.9%) strains to Ceftriaxone and Cefotaxime and *Staphylococcus aureus* (100%) strains to Vancomycin. Antibiotic susceptibility tests are intended to be guide for clinician, not a guarantee that an antimicrobial agent will be effective in therapy

KEYWORDS

Asymptomatic Bacteriuria, Antimicrobial Agents, Antibiotic Susceptibility Tests, *E. coli*

1. INTRODUCTION

Asymptomatic bacteriuria (ASB) is often a dynamic process, it may wax and wane, in particular women and is defined as 10^5 bacteria per ml of one or more on two clean-catch cultures taken on separate days (Faro et al, 1998) During pregnancy ASB can be associated with a variety of adverse obstetrics outcomes and medical conditions. In early pregnancy it has a 20–30-fold increased risk of developing pyelonephritis, compared with women without Bacteriuria. These women also are more likely to experience premature delivery and have infants of low birth weight (Nicolle et al, 2000). Prospective, comparative clinical trials have consistently reported that antimicrobial treatment of ASB during pregnancy decreases the risk of subsequent pyelonephritis from 20% - 35% to 1% - 4% (Small et al, 2001).

Studies from different parts of India have indicated that UTI during pregnancy leads to preterm labor, low birth weight, preeclampsia, perinatal mortality and premature births along with acute and chronic sequelae in mothers (Nath et al, 1996). The prevalence of bacteriuria not only increases with age but also with sexual activity and parity. The epidemiology, risk factors and etiologic agents of bacteriuria in pregnancy is similar to that seen in non-pregnant women. Most cases of UTIs are caused by *Enterobacteriaceae* especially *Escherichia coli*, *Klebsiella species* and *Enterobacter species*. These organisms account for approximately 90% of all UTIs encountered in pregnancy. (Lavanya et al, 2002)

Because of the dangers that drugs may pose to the fetus or the newborn, in consequence, few antibiotics and those well attested, are used in obstetric practice (Lambert et al, 1992). Selection of an antimicrobial agent to treat bacteriuria must be made with special considerations given to maternal and fetal toxicity. Only the penicillins and cephalosporins, given orally or parenterally, are thought to be safe and effective during any phase of pregnancy. When a prophylactic or suppressive agent is selected, contraindications imposed by pregnancy should still be considered. Therefore, it is generally accepted that all pregnant women should be screened with quantitative urine cultures to prevent complications associated with bacteriuria in pregnancy. (Schaeffer AJ, 2002).

The choice of an initial antibiotic treatment often is empirical, being supported by knowledge of epidemiological and clinical data. But empirical treatment of ASB without proper microbiological confirmation of diagnosis may be hazardous to the patient. (Lambert et al, 1992). Hence, objective of this study was to determine susceptibility pattern of isolates obtained in patients suffering with ASB in order to provide prophylactic treatment and understanding the resistance pattern for epidemiological purpose.

2. Material and methods :

2.1 Place of work :

Study was carried out for two years, from January 2003 to December 2004 after taking permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai, in Department of Microbiology in association with Department of Obstetrics and Gynecology.

2.2 Participants :

Patients from Gynecology department, recruited for bacteriologic evidence of ASB, 3000 married pregnant (study group) and 300 married non-pregnant women (control group) were included in study. Subjects showing symptoms of UTI, suffering from diabetes, under antibiotic or steroids treatment were excluded from study. Counseling of subjects for enrollment and their detailed data were recorded in a specially formulated structured proforma.

2.3 Collection and microbiological analysis :

Collection, transportation and culturing of urine samples were carried out by standard methods. (Koneman EW et al, 1997) Identification of obtained isolates from urine was done on basis of morphological, cultural characteristics and biochemical tests. (Isenberg HD Et al, 1992).

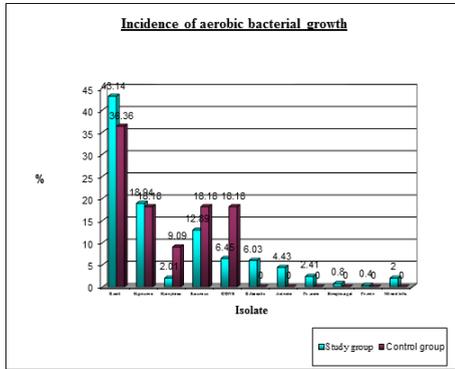
2.4 Antibiotic sensitivity testing (AST):

AST of all the isolates were done in Muller Hinton Agar by modified Kirby – Bauer disc diffusion method as per CLSI guidelines using antibiotic discs. (NCCLS guidelines, 2001). Antibiotics used for all, 200 Gram positive and 52 Gram negative bacteria, were Ampicillin (A)(10) Cephalexin (CF)(30), Norfloxacin (NRF)(10), Nitrofurantoin (

NF)(300), Cotrimoxazole (CTM)(1.25+ 23.75), Augmentin (AU)(30), Ceftriaxone (CFTR)(30), Cefotaxime(CFT)(30), Piperacillin + Tazobactam (P+T)(100 + 10) , Amikacin (AM) (30), Cefuroxime (CFX)(30). While Nalidixic acid(NA)(30) for Gram positive and Ciprofloxacin (CP)(5), Erythromycin (E)(15), Vancomycin(V) (30), Penicillin G (Pg)(10 units) were added extra for Gram negative bacteria. Value in bracket indicates antibiotic content in disc in mcg/ml.

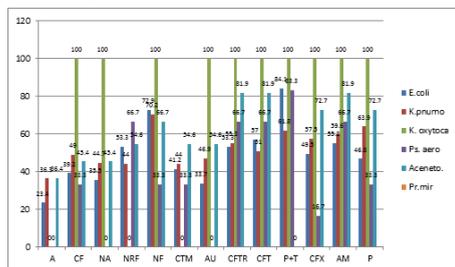
3. RESULTS

GRAPH 1: Isolates obtained in study and control group with ASB Study Group =248 Control Group= 11



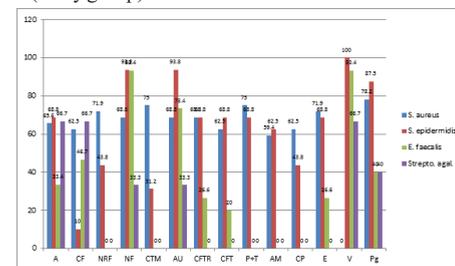
In study group, *E.coli* was predominant isolate (43.14%) followed by *Klebsiella pneumoniae* (*K.pneumo*) (18.94%), *Staphylococcus aureus* (*S.aureus*) (12.89%) and least was *Proteus mirabilis* (*Pr.mir*) (0.4%). Mixed infection of two pathogens was detected (2%) here. While in control group, *E.coli* was predominant isolate (36.36%) followed by *K.pneumo* (18.18%) & CONS (18.18%) & least was *Klebsiella oxytoca* (*K.oxytoca*) (0.4%). In this group *Enterococcus faecalis* (*E.faecalis*), *Acinetobacter baumannii* (*Acinet*), *Pseudomonas aeruginosa* (*Ps.aero*), *Streptococcus agalactiae* (*Str.agal*), *Pr.mir* and mixed infection was not detected. Etiology of bacterial agents was similar in both pregnant as well as non-pregnant women.

GRAPH2:Antibiotic susceptibility pattern of gram-negative organisms (study group)



E.coli strains was highly sensitive to P + T (84%) followed by CFT (57%), AM (55.2%) and NRF (53.3%).*K. pneumoniae* was highly sensitive to NF (70.2%) followed by P + T (61.8%), P (63.9%), AM (59.6%), CFX (57.5%) and CFTR(55.3%) while *K. oxytoca* strains were highly sensitive to AM (80%) followed by CF (60%) and NF (60%). *Ps. aero* strains were highly sensitive to P + T (83.3%) followed by NRF (66.7%), CFT (66.7%) and AM (66.7%). *Acinet* strains were highly sensitive to CFTR (81.9%), CFT (81.9%) followed by P (72.7%) and CFX (72.7%). *Pr.mir* strains were found sensitive only to P + T while it showed resistance to all other antibiotics.

GRAPH3 : Antibiotic susceptibility pattern of gram-positive organisms (study group):



S. aureus strains were highly sensitive to V (100%) followed by P (78.2%), CTM (75%), P + T (75%), NRF (71.9%), E (71.9%), NF (68.8%), AU (68.8%), CFTR (68.8%), AM (65.6%), CFT (62.5%) and CP (62.5%) While *S. epidermidis* strains were found to be sensitive to NF (93.8%) followed by AU (93.8%), P (87.5%). About 69% strains were sensitive to A, CFTR, CFT, P + T and E. *E. faecalis* strains showed sensitivity to NF (93.4%) followed by AU (73.4%) while *Strept.agal* strains were highly sensitive to A (66.7%) and CF (66.7%).

4. DISCUSSION :

In general, there is a need to start treatment before final microbiological reports are available, which may lead to frequent misuse of antibiotics. For better decision making physician must have current knowledge about uropathogens and should advice a bacteriological examination of urine sample to know trend of antibiogram of uropathogens in their region to update appropriate treatment and to prevent development of multi drug resistant strains. The primary objective of using antimicrobials is to eradicate offending microbe and thus ensure total clinical recovery of patient without giving a chance for microbe to develop resistance. The antimicrobial sensitivity pattern of bacteria can be useful as an epidemiological marker for tracing strains. (Sarasu I VP and , S. Ramalatha SR, 2017). In study group most of patients were cured. Besides, common antimicrobial agents like A, AM and Pg; CF and NF was also introduced in antibiotic regime, which was active against uropathogens. A decrease in colony count was observed after administration of antibiotics. All treated patients were followed up to end of third trimester. Antibiotic sensitivity of uropathogens obtained among study group is mentioned in graphs. Antibiotic sensitivity of uropathogens obtained among control group was as follows. *E.coli* strains were highly sensitive to P + T (100%) followed by CF (100%), NF (75%), CTM (75%), CFTR (75%) and AM (75%). *K. pneumo* strains showed 100% sensitivity to CF followed by NA, NRF, NF, AU, CFTR, P + T, CFX, AM and P. While *K. oxytoca* strains showed 100% sensitivity to CF followed by NA, NF, P + T, CFX, AM and P. *S. aureus* strains were found to be 100% sensitive to CF followed by NRF, NF, CTM, AU, CFTR, CFT, P + T, AM, V and P. While *S. epidermidis* strains were 100% sensitive to A followed by CF, NF, AU, CFTR, CFT, P + T, AM, E, V and P. According to Gruneber et RN, 1984, Nalidixic acid resistance is becoming more important proportion of gram positive urinary pathogens. While study in Libya, (Khaled et al, 2017), the most effective antibiotics were gentamycin (87.5%), azithromycin (75%) and CP (68.75%) and the less effective antibiotics were CF (6.25%) and A (12.5%). However, Akram et. al, in 2007 from India have reported 100% activity of imipenem against *E. Coli* and similar findings were also reported by Ullal et. al. in 2009 from Pakistan. Resistance to A and co-trimoxazole (C-T) was high and showed only 11% and 13% sensitivity. Studies from USA, Europe and most other countries have shown better susceptibility against C-T. (Sahm DF et al, 2001) But, in this region of the world C-T has shown poor activity (Tankhivale SS et al, 2004) as C-T has been extensively used in this region in the past. Hence, C-T cannot be recommended as an empiric therapy for the treatment of UTI in India. Study by Sarasu I VP and Ramalatha SR, in 2017 observed, most effective antibiotics against all isolates were imipenem (100%) followed by amikacin (84%), levofloxacin (83%), cefepime (81%), cefoxitin (76%), nitrofurantoin (61%) and ciprofloxacin (48%). Changing pattern of antibiotic resistance among common uropathogens is being noticed worldwide. With wider use of antibiotics more and more resistant strains are causing disease. This problem is due to inappropriate use of drug and partly to circumvent by advent of newer antibiotics, which are coming up at a rapid pace. Nevertheless, making a decision regarding choice of antibiotic in a particular patient may not be very easy, especially when results of culture sensitivity are not available. A rational approach with proper understanding of pharmacology, dosage, untoward side effects and proper indications is warranted (Anandkumar et al, 2003).

5. CONCLUSION

Microbiologists are of great assistance to clinicians as they evaluate *in vitro* interactions between an isolated microbe and antimicrobial agents that would be appropriate for treatment of infection *in vivo*. AST are intended to be a guide for clinician, not guarantee that antimicrobials will be effective in therapy. A contemporary level of resistance was studied to commonly use antimicrobial agents in treatment of UTI for obtained aerobic bacteria. In order to prevent development of resistance, antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate

regimen to treat UTI and to avoid complications. Also institutional antibiotic policy can be developed to achieve superior therapeutic outcome. (Sarasu VP and , S. Ramalatha SR, 2017).

6. ACKNOWLEDGEMENT :

Authors are grateful to Nair Golden Jubilee Research Foundation for providing financial help to carryout this study. Also to Dr. Asha Dalal, Ex-Professor and Head, Department of Obstetrics & Gynecology and Dr. Saraswati karr Ex- Professor and Head, Dept of Microbiology of T. N. Medical College and B. Y. L. Nair Charitable Hospital for their moral support

REFERENCES

1. Faro S., and Fenner DE. Urinary Tract Infections. *Clinical Obstetrics and Gynecology*,1998;41(3):744-754.
2. Nicolle LE. Epidemiology of urinary tract infections. *Clin Microbiol News*. 2002; 24:135-140.
3. Small F. Antibiotics for asymptomatic bacteriuria in pregnancy. *Cochrane Database Syst Rev* 1,2001, 2:CD000490
4. Nath G., Chaudhary M., Pandey JPL, and Singh T B. Urinary tract infection during pregnancy and fetal outcome. *Indian Journal of Medical Microbiology*. 1996; 14(3): 158-160.
5. Lavanya SV.,and Joglakshmi D. Asymptomatic bacteriuria in Antenatal women. *Indian Journal of Medical Microbiology*.2002; 20 (2), 105-106.
6. Lambert HP and O'Grady FW. Antibiotic in obstetrics. In: *Antibiotic and Chemotherapy*. Churchill Livingstone, Edn 6,1992; 416-487.
7. Schaeffer AJ. Infections of the urinary tract. In: *Campbell's Urology*. Edn 8 , Edited by Retik AB, Vaughan ED, Walsh PC, Wein AJ. W.B. Saunders , 2002; 1(17): 515-588
8. Koneman EW, Allen SD., Janda WM. Urinary tract infections. In: *Color atlas and textbook of diagnostic microbiology*. J. B. Lippincott Company; Edn 14,1997: 136-141.
9. Isenberg HD. Urine Culture Procedure. In: *Clinical Microbiology Procedures*, American Society for Microbiology Press, 1992;1: (1.4.10- 1.4.11).
10. National Committee for Clinical Laboratory Standards . Performance standards for antimicrobial susceptibility testing. Wayne; 11th Informational Supplement.2001; 21(1): 40-54.
11. Sarasu VP, Ramalatha SR. Bacteriological profile and antibiogram of urinary tract infections at a tertiary care hospital. *International Journal of Medical Microbiology and Tropical Diseases*, 2017;3(3):106-112
12. Gruneber RN. Antibiotic sensitivity of urinary pathogens. *Journal of Antimicrobial Chemotherapy*, 1984;14: 17.
13. Khaled AA., Ramadan HA., Faisal FI. Bacteriuria in Pregnant and Non Pregnant Women in Benghazi Acomparative Study. *Journal of Pharmacy and Biological Sciences*. 2017;12(1)(Ver. I), 133-137
14. Akram M., Shahid M. and Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in N N M C Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobiols*. 2007; 6(4):1-7.
15. Ullah F, Malik SA. and Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North- West Pakistan. *African Journal of Microbiology Research*. 2009; 3(11): pp. 676–680.
16. Sahn DF., Thornsberry C, Mayfield DC, Jones ME and Karlowsky JA. Multi- drug resistant urinary tract isolates of E. coli. Prevalence and patient demographics in the united states in 2000. *Antimicrob. Agents Chemother*. 2001; 45(5):1402-06.
17. Tankhiwale SS., Jalgaonkar SV., Ahamad S. and Hussani U. Evaluation of extended spectrum B -lactamase in urinary isolates. *Indian J. Med. Res*, 2004; 120(6):553–556.
18. Anandkumar H., Dayanand A., Vinodkumar C., Kapur I. In vitro activity of norfloxacin against uropathogens and drug efficacy in simulated bladder model under diabetic conditions. *Indian Journal of Medical Microbiology*.2003; 21 (1), 37-42.