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ASYMPTOMATIC SIGNIFICANT BACTERIURIA AMONG PREGNANT WOMEN IN ADO-EKITI, EKITI STATE, NIGERIA

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Microbiological culture examination of urine samples from 502 pregnant women attending antenatal clinic resulted in the isolation of seven genera of bacterial species. They were *Staphylococcus aureus* 16 (21.3%), *Escherichia coli* 12 (16.0%), *Staphylococcus spp* 11(14.7%), *Klebsiella spp* 8 (10.7%), *Pseudomonas aeruginosa* 7 (9.8%), *Streptococcus faecalis* 6 (8.0%), and *Citrobacter spp* 1 (1.3%). Asymptomatic significant bacteriuria occurred in 16 (12.22%), pyuria in 10 (2.0%) while significant bacteriuria with pyuria occurred in 1(0.2%). The antibiogram indicated that ofloxacin, ciprofloxacin and nalidixic acid were in that order the most effective of the antibiotics tested. All isolates showed multiple resistance to most of the antibiotics tested. Plasmid DNA was detected in *Ps. aeruginosa* and *E. coli* with an estimated molecular weight of between 4.5 and 6.5 kb. The result indicated a significant rise in the frequency of S. *aureus* in asymptomatic bacteriuria.

Keywords: Asymptomatic bacteriuria, urinary tract infections, pregnant women

INTRODUCTION

Asymptomatic bacteriuria in women is a frequent occurrence that results when urinary tract pathogens such as *Escherichia coli*, amongst others enter the bladder without causing symptoms (1). The pathogens, which are usually eliminated by host defense factor, may persist for a short, but rarely a long time resulting in symptomatic urinary tract infection (UTI). Studies on asymptomatic bacteriuria in healthy women and school girls showed no adverse outcomes, whereas adverse outcomes have been reported in well defined groups such as pregnant women, catheterized or elderly patients (2.)

Asymptomatic bacteriuria during pregnancy has been associ-

ated with an increased risk of developing pyelonephritis (3,4,5), maternal and infant morbidity, preterm labour and low birth weight (6,7,8). Adverse outcomes have been prevented with antimicrobial drug therapy. However, the efficacy of antimicrobial drug treatment is limited by increased antimicrobial resistance in community-acquired strains of *E. coli* particularly to ampicillin, sulphonamides, and trimethoprim-sulfamethoxazole (9).

The present study examined the incidence of asymptomatic bacteriuria in pregnant women in Ado-Ekiti, Ekiti State, Nigeria. The antibiotic resistance pattern of the isolates and the presence of plasmid DNA were also investigated.

MATERIALS AND METHODS

Collection of samples

Sample of clean-catch early morning voided mid-stream urine were collected from 502 pregnant women attending antenatal clinic at the state specialist hospital, and Okesa maternity centre, Ado-Ekiti, Ekiti state, Nigeria. The patients were apparently healthy and symptom-free at the time of sample collection. All samples were collected aseptically, stored at 4°C and examined within two hours of collection. Information about patients such as age, gestation age, occupation, blood group and parity were collected from the hospital case note. Informed consent of individual involved in this study was obtained.

Microscopic examination of urine

Three loopful of well-mixed uncentrifuged urine were placed on a clean grease-free slide and covered with a coverslip. The preparation was examined under the microscope for bacteria and pus cells.

Isolation of Bacteria

Samples were examined using standard methods (10). Briefly a calibrated wire loop capable of delivering 0.001 ml of urine was used for culturing on Cysteine-Lactose Electrolyte Deficient (CLED) and MacConkey agar. The culture plates were incubated aerobically at 37°C for 24 hours Culture plates without visible growth were further incubated for additional 24 hours before being discarded. The number and types of colonies growing on the medium (CLED) were recorded as being insignificant, doubtful (contaminated) or significant when 1 or less colony, < 10 colonies or 10 or more colonies

were counted respectively.

Identification of isolates

Identification of bacterial isolates was based on the combination of cultural, morphological and biochemical characteristics (11).

Antimicrobial Assay

Antimicrobial susceptibility testing of the isolates was done by the Kirby-Bauer disc diffusion technique (12), with the following antibiotics; Ampicillin, Cotrimoxazole, Gentamycin, Chloramphenicol, Nalidixic acid, Vibramycin, ofloxacin and ciprofloxacin.

Plasmid Analysis

Isolation of plasmid from the isolates that showed multiple resistance to antibiotics was done as described by Birnboim and Doly (13). This plasmid DNA was resolved on agarose gel electrophoresis using lambda DNA cleaved with *Hind* III restriction enzyme as the molecular weight marker.

RESULTS

Of the 502 urine samples examined, 16 (12.2%) yielded significant growth, 228 (45.4%) had insignificant growth, 15 (2.9%) had mixed growth while 193 (38.45) had no growth (Table 1). There was pyuria in 10 (2.0%) urine samples while significant bacteriuria with pyuria occurred in 1 (0.2%) (Table 2). Table 3 shows the various bacterial isolates with their percentage distribution. *S. aureus* was the most common organism with an isolation rate of 21.3% followed by *E. coli* with 16.0% isolation rate while *Salmonella spp* had the least prevalence rate. Table 4 shows the susceptibility patterns of the bacteria pathogens to the eight antimicrobial agents employed. Ofloxacin was the most effective drug on all the tested organisms followed by ciprofloxacin and vibramycin. Most of the isolates exhibited multiple antibiotic resistance (MAR) patterns. *Providencia* and *Enterobacter spp* showed the single R-type with 100% resistance to both cotrimoxazole and ampicillin. All the other organisms showed multiple resistant pattern ranging from 2 to 8 antibiotics. *Ps. aeruginosa, Citrobacter* and *Salmonella spp.* were 100% multiple R-type while *E. coli* and *Proteus spp.* demonstrated 91.7% and 83.3% resistance respectively and *S. aureus* showed 81.3% resistance (Table 5).

Ps. aeruginosa, which was resistant to four antibiotics, harboured three plasmid bands. *E. coli* resistant to two antibiotics had one plasmid DNA band. All other isolates had no detectable plasmid (Table 6).

Table 1: Occurrence of significant bacteriuria in pregnant women in Ado-

CASES	TOTAL NO OF PATIENTS	%
Significant growth	61	12.2
Insignificant growth	228	45.4
Mixed growth	15	2.9
No growth	193	38.4
Growth of *Candida albicans*	5	0.9
TOTAL	502	100

Table 2: Relations between pyuria and significant bacteriuria

CASES	TOTAL NO OF PATIENTS	%
Significant bacteriuria	61	12.2
Pyuria	10	2.0
Significant bacteriuria with pyuria	1	0.2
Significant bacteriuria without pyuria	60	11.9
Pyuria without significant bacteriuria	9	1.8
TOTAL	502	100

Table 3: Profile of bacteria isolated from cases of significant bacteriuria in pregnant women.

BACTERIAL ISOLATES	TOTAL NO OF ORGANISM	%
Staphylococcus aureus	16	21.3
Escherichia coli	12	16.0
Staphylococcus spp.	11	14.7
Pseudomonas aeruginosa	7	9.3
Streptococcus faecalis	6	8.0
Klebsiella spp.	8	10.7
Proteus spp.	6	8.0
Citrobacter spp.	3	4.0
Enterobacter spp.	2	2.7
Providencia spp.	3	4.0
Salmonella spp.	1	1.3
TOTAL	75	100

Table 4: Pattern of resistance of bacterial isolates to antimicrobial agents

Bacterial isolate	No of isolate	AMP	CHL	COT	GEN	NAL	CIP	OFX	VIB
S. aureus	16	10(62.5)	7(43.7)	7(43.7)	5(31.3)	3(18.8)	1(6.3)	0	1(6.3)
E.coli	12	6(50.0)	2(16.7)	3(25.0)	4(33.3)	1(8.3)	1(8.3)	0	7(58.3)
Staphylococcus spp.	11	6(54.5)	1(9.1)	9(81.8)	2(18.2)	0	1(9.1)	1(9.1)	3(27.3)
Klebsiella spp.	8	5(62.5)	1(12.5)	7(100.0)	2(25.0)	2(25.0)	1(12.5)	1(12.5)	0
P. aeruginosa	7	7(100.0)	7(100.0)	7(87.5)	4(57.1)	7(100.0)	0	0	4(57.1)
S. faecalis	6	5(83.3)	4(66.7)	2(33.3)	2(33.3)	2(33.3)	0	0	1(16.7)
Proteus spp.	6	3(50.0)	2(33.3)	6(100.0)	2(33.3)	1(16.7)	0	0	1(16.7)
Citrobacter spp.	3	1(33.3)	2(66.7)	2(66.7)	2(66.7)	0	0	0	2(66.7)
Providencia spp.	3	0	0	2(66.7)	0	0	0	0	0
Enterobacter spp.	2	1(50.0)	1(50.0)	0	1(50.0)	0	0	0	0
Salmonella spp.	1	1(100)	1(100)	1(100)	1(100)	0	0	0	1(100)
TOTAL	75	45	28	46	25	16	4	2	20

AMP = Ampicillin COT = Cotrimoxazole GEN = Gentamycin CHL = Chloramphenicol
NAL = Nalidixic acid OFX = Ofloxacin CIP = Ciprofloxacin VIB = Vibramycin
( ) = % resistance

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Table 5: Prevalence of multiple antibiotic resistance (MAR) strains among bacterial isolates from pregnant women

Bacterial isolate	Total No isolate	Single R-type (%)	Antibiotics	Multiple R-type (%)	Antibiotic range	Antibiotics to which multiple resistance was demonstrated
S. aureus	16	1(18.8)	Chl, Amp	13(81.3)	2-5	Chl, Gen, Nal, Cip, Cot
E.coli	12	1(8.3)	Amp	11(91.7)	2-5	Amp, Chl, Gen, Nal, Cot, Cip
Klebsiella spp.	8	2(33.3)	Amp, Cot	6(66.6)	2-4	Amp, Nal, Gen, Cot, Cip, Ofx
P. aeruginosa	7	0(0)		7(100)	4-6	Amp, Nal, Vib, Chl, Cot, Gen, Ofx
S. faecalis	6	1(16.7)	Cot	5(83.3)	2-4	Amp, Cot, Nal, Gen, Chl
Proteus spp.	6	1(16.7)	Cot	5(83.3)	2-4	Gen, Cot, Vib, Amp, Nal, Chl
Staphylococcus spp.	11	3(27.3)	Cot	8(72.7)	2-4	Gen, Cot, Amp, Vib, Chl, Ofx, Cip
Citrobacter spp.	2	-	-	2(100)	4-5	Vib, Gen, Chl, Cot, Amp, Vib
Providencia spp.	2	2(100)	Cot	0	-	-
Enterobacter spp.	2	1(50)	Amp	0	-	Gen, Chl.
Salmonella spp.	1	0(0)	-	1(100)	5	Vib, Gen, Chl, Cot, Amp.

AMP = Ampicillin COT = Cotrimoxazole GEN = Gentamycin CHL = Chloramphenicol
NAL = Nalidixic acid OFX = Ofloxacin CIP = Ciprofloxacin VIB = Vibramycin
( ) = % resistance

Table 6: **Plasmid analysis of some of the bacterial species from asymptomatic significant bacteriuria of pregnant women**

Bacterial isolate	Antibiotic resisted	No of bands/plasmid	Estimated molecular weight of plasmid kilobase pair less than 6.5
E.coli	Vib, Cot	1	Less than 6.5
E.coli	Amp	-	
Proteus spp.	Vib, Gen; Cot, Amp	-	
Proteus spp.	Cot	-	
Klebsiella spp.	Cot, Amp	-	
Klebsiella spp.	Cot, Chl	-	
Citrobacter spp.	Vib, Gen, Chl, Cot, Amp	-	
P. aeruginosa	Chl, Amp, Cot, Vib	3	Between 4.5 - 6.5

DISCUSSION

Urinary tract infection (UTI) is one of the most common bacterial infections in both pregnant and non-pregnant women. It is estimated that 10-20% of all women suffers from UTI at some point in life (14). The prevalence of bacteriuria in female varies from less than 1% in infants to 10% and even more in older women. The prevalence of asymptomatic bacteriuria in pregnancy ranges from 4% to 7% depending on the population studied (15). The overall isolation rate of bacteria in the population studied was12.2%. Famurewa (16) earlier reported significant bacteriuria in 12 (2.5%) non-pregnant women, 108 (22.2%) pregnant women and 120 (24.7%) asymptomatic women. Olusanya *et al* (17) also reported significant bacteriuria in 122 (23.9%) pregnant women and 37 (12.2%) non-pregnant women in Sagamu, Ogun State, Nigeria. The result of this study further affirms that variation in frequency may be ascribed to population characteristics such as age, parity, socio-economic status, sexual activity (multiple sexual partners) and health care during pregnancy (17, 18, 19,20).

S. aureus was the most prevalent bacterium (21.3%), which agrees with the report of earlier workers (16, 17, 21,22). It is important to note that *E. coli* which is implicated in 75% of out-patients with urinary tract infections is the second most prevalent in this study. Anderson *et al* (23) observed that although *E. coli* was not among the organisms found in the periurethral area, yet it was one of the common organisms causing significant bacteriuria because of its ability to outgrow other organisms that could cause similar problems. Akinyemi *et al* (24) reported the occurrence of coagulase negative staphylococci (CNS) and their importance as pathogens rather than contaminants in the urinary tract. This has been confirmed in this study and in reports from USA (25), Sweden (26), UK (27) and Nigeria (16). *S. saprophyticus* has been considered the most frequent agent of urinary tract infection in young sexually active women after *E. coli* (28).

The *in vitro* susceptibility pattern of the isolates revealed that the quinolone class of antimicrobials is highly effective against all isolates demonstrating less than

10% resistance. The newer quinolones (ciprofloxacin, ofloxacin) are excellent agents for treatment of UTI (20) although the quinolones are not generally safe in pregnancy and should probably be avoided (29). The high prevalence of resistance to β-lactam (ampicillin) has long been appreciated. This has been confirmed by this study and as such the continued use of β-lactams should be discouraged as much as possible in the empirical treatment of uropathogens. Of considerably greater concern is the increasing prevalence of resistance to trimethoprim-sulfamethoxazole (co-trimoxazole) as observed in this study. This data suggests that this drug also may not be acceptable for empirical therapy. The resistance pattern seen in this study further affirms the need to discourage empirical treatment of UTI. The prevalence of resistance among uropathogens has been shown to be on the increase. The prevalence of resistance to trimethoprim-sulfamethoxazole rose from more than 9% in 1992 to more than 18% in 1996 for *E. coli*. It rose from 8% to 16% among all isolates combined while for ciprofloxacin, it did not change significantly during the 5 years period (9). The high resistance to these commonly used and relatively cheap antibiotics (ampicillin, co-trimoxazole etc) in Nigeria could be a reflection of widespread and indiscriminate usage of these drugs. Of obvious concern is the likelihood that increased use of fluoroquinolones for the treatment of UTI in women may likely promote the emergence of resistance to these drugs. A rapid development of resistance to nalidixic acid in Ontario, Canada after widespread use has been reported (30), but resistance to this drug is relatively low from this study. Inappropriate use of antimicrobial agent has been identified as a major risk factors for the development of resistance (31), hence the correlation between antimicrobial resistance and antimicrobial use.

An important finding in the current study is the lack of plasmid-mediated resistance to quinolones. Despite an increased widespread usage, resistance has not emerged (32). Although other studies have reported increasing fluoroquinolone resistance amongst *E. coli* (33, 34), low resistance rate was observed amongst the *E. coli* in this study. The presence of plasmid

DNA in some of the isolates shows that this antimicrobial resistance could be plasmid mediated. Co-transfer of plasmid borne resistance amongst *E. coli* from UTI has been reported (30). Plasmid bands were detected in both *Ps. aeruginosa* and *E. coli*. The plasmid DNA in both organisms had identical size and both isolates were resistant to vibramycin, suggesting that this is likely to be plasmid-mediated resistance. The increase in antibiotic resistance among community-acquired isolates is worrisome and new approaches to possibly prevent or control the emergence of resistance should be developed. Hence, the World Health Organization (35) recognized the importance of studying the emergence and the need for strategies for its control.

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REFERENCES

1. Hooton TM, Scholen D, Stapleton AE, *et al.* A prospective study of asymptomatic bacteriuria in sexually active young women. *N. Engl. J. Med.* 2000; 343: 992-997.

2. Nicolle LE. Asymptomatic bacteriuria-important or not? *N. Engl. J..Med.* 2000; 343: 1037-1039.

3. Zhanel GG, Harding GKM, Guay DRP. Asymptomatic bacteriuria: which patients should be treated? *Arch. Intern. Med.* 1990; 150: 1389-1396.

4. Mitterndorf R, Williams MA, Kass EH. Prevention of preterm delivery and low birth weight associated with asymptomatic bacteriuria. *Clin. Infect. Dis.* 1992; 14: 927-932.

5. Gratacos E, Jorres PJ, Vila J, Alonso PL, Cararach V Screening and treatment of asymptomatic bacteriuria in pregnancy prevent pyelonephritis. *J. Infect. Dis.* 1994; 169: 1390-1392.

6. Ronald AR. Optional duration of treatment for kidney infections. *Ann. Intern. Med.* 1987; 106: 467-468.

7. McGregor JA, French JI, Richter R, Franco Buff A, Hiller S. Antenatal microbiology and maternal risk factors associated with prematurity *Ann. J. Obstetr. Gynaecol.* 1990; 163: 1465-1473.

8. Schults R, Read AW, Straton JAY, Stanley FJ, Morich P. Genitourinary tract infection in pregnancy and low birth weight: case control study in Australian aboriginal women. *Brit. Med. J.* 1991; 73: 576-582.

9. Gupta K, Scholes D, Stamm WE. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *J. Am. Med. Assoc.* 1999; 250: 3007-3011.

10. Cheesbrough M. Medical laboratory manual for tropical countries. London, Butterworth & Co. Ltd., 1985.

11. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria. 3rd edition. Cambridge University Press, London, 1993.

12. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 1966; 45: 493-496.

13. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 1979; 7: 1513-1523.

14. Weisseenbacher ER, Reisenberger K. Uncomplicated urinary tract infection in pregnant and non-pregnant women. *Curr. Opin. Obstetr. Gyn.* 1993; 5: 513-516.

15. Bint AJ, Hill D. Bacteriuria of pregnancy; an update, significance, diagnosis and management. *Antimicrob. Agents Chemother.* 1994; 33 (Suppl. A): 93-97.

16. Famurewa O. Prevalence of urinary tract infection in women in Ado-Ekiti, Ondo State, Nigeria. *L'Igiene Moderna.* 1992; 97: 580-591.

17. Olusanya O, Ogunledun A, Fakayo TA. Asymptomatic bacteriuria in women in Sagamu Nigeria. *West Afr. J. Med.* 1993; 12: 27-33.

18. Wong ES, Stamm WE. Sexual acquisition of urinary tract infection in a man. *J. Am. Med. Assoc.* 1983; 250: 3007-3011.

19. Strom BL, Collin M, West SL, Kreisberg J, Weller S. Sexual activity, contraceptive use and other factors for asymptomatic bacteria. *Am. J. Intern. Med.* 1987; 107: 816-823.

20. Andriole VT, Patternson TF. Epidemiology, natural history and management of urinary tract infections in pregnancy. *Med. Clin. N. Am.* 1991; 75: 359-373

21. Baurnfeind A, Nabor K, Saverwein D. Spectrum of bacteria pathogens in uncomplicated and complicated urinary tract infections. *Eur. Urol.* 1987; 13 (Suppl.1): 9-14.

22. Gruneberg RN. Changes in urinary pathogens and their antibiotic sensitivities 1971-1992. *J. Antimicrob. Chemother.* 1994; 33 (Suppl.A1): 51-52.

23. Anderson JD, Eftekhar F, Aird MY, Hammon J. Role of bacterial growth rate in the epidemiology and pathogenesis of urinary tract infection in women. *J. Clin. Microbiol.* 1979; 10: 766-771.

24. Akinyemi KO, Alabi SA, Taiwo MA, Omonigbehin EA. Antimicrobial susceptibility pattern and plasmid profile of pathogenic bacterial isolate from subject with urinary tract infection in Lagos, Nigeria. *Nig. Qt. J. Hosp. Med.* 1997; 7: 7-11.

25. Latham RHK, Running G, Stamm WE. Urinary tract infections in young adult women caused by *Staphylococcus saprophyticus*. *J. Am. Med. Assoc.* 1983; 250: 3063-3066.

26. Hovelius B, Mardh PA. *Staphylococcus saprophyticus* as a common cause of urinary tract infection. *Rev. Infect. Dis.*1984; 6: 328-337.

27. Paed L, Maskell R, Marris J. *Staphylococcus saprophyticus* as a urinary pathogen; a six year prospective survey. *Brit. Med. J.* 1985; 291: 1159-1169.

28. Melocristino JAG, Torres-Pereira A, Andrade LG. Diversity of plasmids in *Staphylococcus saprophyticus* isolated from urinary tract infection in women. *Epidemiol. Infect. Dis.* 1989; 102: 413-419.

29. Chow AW, Jewesson PO. Pharmacokinetics and safety of antimicrobial agents during pregnancy. *Rev. Infect. Dis.* 1985; 7: 287-313.

30. Harnett N. Transferable high-level trimethoprim resistance among isolates of *Escherichia coli* from urinary tract infection in Ontario, Canada. *Epidemiol. Infect.* 1992; 109: 473-481.

31. Bronzwaer SLAM, Cars O, Buchholz U, *et al.* A European Study on the relationship between antimicrobial use and antimicrobial resistance. *Emerg. Infect. Dis.* 2002; 8: 278-282.

32. Abbas AMA, Chandrau-Dongaonkar PP, Goel PK, *et al.* Ciprofloxacin versus amoxycillin-clavulanic acid in the treatment of urinary tract infection in general practice. *J. Antimicrob. Chemother.* 1989; 24: 235-239.

33. Thomson KS, Sanders WE, Sanders CC. USA resistance patterns among UTI pathogens. *J. Antimicrob. Chemother.* 1994; 33 (Suppl.): S9-S15

34. Winstanley TG, Limb DT, Eggington R, Hancock FA. Ten-year survey of antimicrobial susceptibility of urinary tract isolates in the UK. The Microbe Base Project. *J. Antimicrob. Chemother.* 1997; 40: 591-594.

35. World Health Organization. Reporton infectious diseases: Overcoming antimicrobial resistance 2000; http://www.who.int/infectious-disease-report/index.html. Accessed Sept. 2001.