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EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING UROPATHOGENS IN ASYMPTOMATIC PREGNANT WOMEN ATTENDING ANTENATAL CARE IN AN URBAN COMMUNITY SECONDARY HEALTH FACILITY

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Abstract

Background: The prevalence of Extended Spectrum Beta-Lactamases (ESBLs) production by uropathogenic bacteria have increased overtime raising a global concern in the therapeutic management of infections caused by these organisms. ESBLs contribute to multi drug resistance among the organisms and the detection of ESBLs is necessary in analyzing the antibiogram pattern of ESBLs producing isolates to enable better treatment. The aim of this study was to determine the prevalence of ESBL producers in pregnant women attending antenatal clinic at Saint Luke's Hospital, Anua, Uyo, Nigeria. This is a well attended Urban Community Secondary Health facility providing antenatal care for pregnant women.

Materials and Method: Three hundred and sixty five clean catch mid-stream urine specimens (n=365) were collected from pregnant women attending antenatal clinics at St Lukes Hospital, Anua, following an ethical approval by the relevant authorities. Identification of significant bacteriuria isolates was done using Microbact 24E (Oxoid, UK) system. The isolated bacteria were tested for their antibiotic susceptibility using Clinical laboratory standard institute (CLSI) recommended disc diffusion method. A double disk synergy test (DDST) was performed to determine ESBL production.

Results: The predominant bacterial pathogens were *Escherichia. Coli* (40%) followed by *Klebsiella pneumonia* (20%), *Klebsiella oxytoca* (11%), *Citrobacter spp.* (5%), *Proteus mirabilis* (1%), *Enterobacter spp.*(14%) and *Acinetobacter baumanii* (9%). Sixteen (20%) out of the 80 uropathogenic isolates were found to be ESBL producers. *Klebsiella pneumonia* 8(50%) was the most prevalent ESBL producer. Other producers include *Escherichia coli* 6(38%), *Klebsiella oxytoca* 1(6%) and *Enterobacter cloacae* 1(6%).These ESBL producing isolates showed resistance to Trimethoprim-sulphamethoxazole (100%), Ceftazidime (100%) and Cefotaxime (100%). They were however sensitive to Imipenem (100%), Azetronam (100%) and Ofloxacin (56%). Some these antimicrobials have restricted use during pregnancy.

Conclusion: The prevalence of Community acquired Extended Spectrum beta lactamases (CA-ESBLs) causing asymptomatic bacteriuria during pregnancy is high in our locality and are probably the cause of multidrug resistance and treatment failures.

Key Words: ESBLs, asymptomatic bacteriuria and multidrug resistance

SPECTRE ELARGI BETA - LACTAMASES PROVOQUANT UROPATHOGENES DANS. DES FEMMES ENCEINTES ASYMPTOMATIQUES EN CONSULTATION PRENATALES AU CENTRE DE SANTE SECONDAIRE DE LA COMMUNAUTE URBAINE.

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RESUME

Contexte: La prévalence de spectre élargi Beta- Lactamases(BLSE) produisant par bactéries uropathogenes a augmenté au fil du temps soulevant une préoccupation mondiale dans la gestion thérapeutique des infections causées par ces organismes. BLSE contribuent a la multirésistance parmi les organismes et la détection de BLSE est nécessaire dans l'analyse du motif d'antibiogramme de BLSE qui produisent les isolats. Le but de cette recherche était déterminer la prévalence des producteurs de BLSE dans les femmes enceintes en consultation clinique prénatales a l'hôpital Saint Luke, Anua, Uyo, Nigeria -un centre de santé secondaire de la communauté urbaine bien assisté qui fournir des soins prénatales aux femmes enceintes.

Matériels et méthode: Trois cent soixante cinq échantillons d'urine du milieu capture propres(n=365) ont été collectés des femmes enceintes en consultation cliniques prénatales a l'hôpital Saint Luke, Anua, suite a une approbation éthique par les autorités compétentes. L'identification des isolats de bactériurie significative ont été faits en utilisant le système Microbact 24E. Les bactéries isolées ont été testées pour leur sensibilité aux antibiotiques utilisant la méthode de diffusion de disque recommandée par Clinique institut standard de laboratoire (CLSI). Un test de synergie double disque(DDST) a été effectué de déterminer la production de BLSE.

Résultats: Les pathogènes bactériens étaient *Escherichia coli* (40%) suivi par *Klebsiella pneumonia*(20%), *Klebsiella oxytoca*(11%), *Citrobacter spp.*(5%), *Proteus mirabilis* (1%), *Enterobacter spp.*(14%) et *Acinetobacter baumanii*(9%). Seize (20%) sur les 80 isolats uropathogenes ont été trouvés d'etre producteurs de BLSE. Klebsiella pneumonia 8(50%) était le producteur de BLSE le plus prévalent. Les autres producteurs comprennent *Escherichia coli* 6 (38%), *Klebsiella oxytoca* 1 (6%) et *Enterobacter cloacae* 1(6%). Ces isolats de la production de BLSE ont montré une résistance aux Trimethroprime – sulphamethoxazole (100%), Ceftazidime (100%) and Cefotaxime (100%). Néanmoins, ils étaient sensibles aux Imipenem (100%), Azetronam (100%) Ofloxacin (56%).Certains de ces antimicrobiens ont l'usage restreint pendant la grossesse.

Conclusion: La prévalence de Communautaire acquise spectre élargi des beta lactamases (CA - BLSE) provoquant bactériurie asymptomatique pendant la grossesse est élevée dans notre localité et sont probablement la cause de la multirésistance et les échecs de traitement.

Mots - clés: Les BLSE, bactériurie asymptomatique et multirésistance.

INTRODUCTION

Urinary tract infections (UTIs) are caused by the presence and growth of micro-organisms in the urinary tract and are the single common bacterial infection of mankind (1). In pregnancy, UTIs may involve the lower urinary tract or bladder (2). The three clinical manifestations of UTIs in pregnancy are asymptomatic bacteriuria, acute cystitis and pyelonephritis (3). Antibiotic resistance of urinary tract pathogens have been known to be on the increase worldwide especially to commonly used antimicrobials (4). Resistance to extended spectrum beta-lactams has been found among the strains of *Klebsiella pneumonia* and *Escherichia coli* isolates that produce Beta-lactamases which are resistant to penicillins, cephalosporins and monobactams(azetronam)(5).

Beta-lactamases has been divided into four groups on the basis of substrate type and physical characteristics such as molecular weight and isoelectric point (5). Extended Spectrum Beta Lactamases is one of these groups and its appearance is due to consumption of third generation cephalosporins. Extended Spectrum Beta Lactamases was first reported in in early 1980s in Europe and now they are being reported all over the world (5, 6). Extended Spectrum Beta Lactamases has variants of primary enzymes namely;TEM-1, TEM-2 and SHV-1.This variation is based on changes in one or more amino acids (5).

In Nigeria, reports in literature have described the different epidemiological distributions of ESBL producing organisms. One study reported prevalence of 44.6% in Enugu State, another study reports a prevalence of 35% and an incidence of 9% in Nsukka (7, 8). Some other studies have also reported a prevalence of 72.5% in Lagos state and 15.4% in Kano State (9, 10). The paucity of such reports from the South – South region of Nigeria especially especially Akwa Ibom State necessitated this study.

MATERIALS AND METHODS

The study was carried out in a well attended secondary Health facility in Uyo, the capital of Akwa Ibom State located in the South-south region of Nigeria. All consenting pregnant women attending antenatal clinic at St Luke's Hospital Anua, Uyo Akwa Ibom State and who are not on any antibiotics therapy during the 6 months period of were included after obtaining ethical approval from the Research Ethics Committees of the Health care facilities. Three hundred and sixty five clean catch mid-stream urine specimens were collected and processed following standard procedure. Identification of bacterial isolates was carried out using Microbact 24E (MB24E). Disc diffusion antibiotic susceptibility test was performed using the Kirby-Bauer method according to CLSI guideline using commercially available disc (Oxoid Ltd.) such as Ceftazidime (30ug), Cefotazime (30ug), Azetronam (30µg), Trimethoprim sulfamethoxazole (25µg), Imipenem (10 µg) and Ofloxacin (5 µg).

All isolates that showed reduced susceptibility to 3rd generation cephalosporins were screened for ESBL production using Double Disc Synergy Test Method (DDST).The test was carried out on Muller Hinton agar. A Muller Hinton agar was inoculated with a 0.5 McFarland turbidity of

presumptive bacteria. A Ceftazidime 30ug disc was placed on the plate 20mm (center to center) from Augumentin 30ug disk (amoxicillin and clavulanate 20ug/10ug). After incubation for 18- 24 hours at 37^{o} C a clear extension of the edge of ceftazidime disc inhibition zone towards the disk containing clavulanate is described as synergy indicating the presence of an ESBL.

RESULTS

The 356 processed mid stream clean catch urine samples yielded 80 clinical isolates including *Escherichia coli* 32(40%) followed by *Klebsiella pneumoniae*16(20%), *Citrobacter spp.*4(5%), *Proteus mirabilis* 1(1%), *Enterobacter spp.*11(14%) and *Acinetobacter baumanii*7(9%) were obtained (Table 1). Out of the 80 clinical isolates that were screened for ESBL production, 20% was positive for ESBL production. These positive ESBL isolates were *Klebsiella pneumonia* 8(50%), *Escherichia coli* 6(38%), *Klebsiella oxytoca* 1(6%) and *Enterobacter cloacae* 1(6%)(Table 2).

The ESBL producing isolates showed resistance to Trimethoprim-sulphamethoxazole (100%), Ceftazidime (100%) and Cefotaxime (100%). They were however sensitive to Imipenem (100%), Azetronam (100%) and Ofloxacin (56%) (Table 3).

TABLE 1: FREQUENCY OF GRAM-NEGATIVE ISOLATES

Bacterial Isolates	Total No. (%)
E. coli	32 (40)
K. pneumoniae	16 (20)
K. oxytoca	9 (11)
C. sakazakii	2 (3)
C. freundii	2 (2)
P. mirabilis	1 (1)
Enterobacter cloacae	11 (14)
A. baumanii	7 (9)
Total	**80 (100)**

TABLE 2: ESBL PRODUCERS AMONG BACTERIAL ISOLATES

Bacterial Isolates	ESBL Producers Total No. (%)
E. coli	6 (38)
K. pneumoniae	8 (50)
K. oxytoca	1 (6)
C. sakazakii	0 (0)
C. freundii	0 (0)
P. mirabilis	0 (0)
Enterobacter cloacae	1 (6)
A. baumanii	0 (0)
TOTAL	**16 (100)**

TABLE 3: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESBL PRODUCING ISOLATES

Antimicrobials (µg)	Antibiotic Susceptibility Pattern by the Isolates					
	E. coli (n=6)		*Klebsiella spp.*(n=9)		*Enterobacter cloacae* (n=1)	
	S%	R %	S%	R %	S %	R %
IPM (10)	6(100)	0	8(89)	1(11)	1(100)	0
ATM (30)	6(100)	0	5(56)	4 (44)	0	1(100)
SXT (25)	0	6 (100)	1(11)	8(89)	0	1(100)
CTX (30)	0	6(100)	6 (67)	3(33)	0	1(100)
OFX (5)	3(50)	3 (50)	5(56)	4(44)	0	1(100)
CAZ (30)	0	6(100)	8(89)	1(11)	0	1(100)

Key: IPM-Imipenem, ATM- Azetronam, STX-Trimethoprim-sulphamethoxazole, CTX- Cefotaxime, OFX- Ofloxacin, CAZ-Ceftazidime

DISCUSSION

Although community acquired Extended Spectrum Beta Lactamase (CA-ESBL) - producing *E. coli* and *K. pneumonia*e has been reported both from Nigeria and other lands, the revelation from this study can be said to be of interest. Of note is the fact that while ESBL producing *E. coli and Klebsiella* species are mostly implicated in hospital acquired UTI, (11,12), they were found to be the major cause of ESBL UTI in the community, a trend that should be taken as dangerous. The overall prevalence of CA-ESBL UTI in this study was 20% while similar prevalence of 22% and 27.7% respectively has been reported by Agarawal (13) and Esimone (14) as is also with other studies (15,16). Although these are higher than what this study revealed, increasing the sample size in furtherance to this study may reveal higher prevalence. This study also revealed that ESBL producing isolates are completely resistant to sulfonamide (SXT)-Trimethoprim-sulphamethoxazole (100%), Cefotaxime (100%), and Ceftaxidime (100%), such resistance for ESBLs producing bacteria is often encoded on plasmids and can easily be transferred to other non-beta- lactams including fluroquinolones, aminoglycosides and cotrimoxazole (17). Obviously some of these drugs especially cephalosporins are safe in pregnancy but will not be effective if indicated. Carbapenems in this study and from other study are the drug of choice for infections caused by ESBLs producing gram negative bacteria (18,19). However, apart from being very expensive and not easily available in our environment, these drugs are not too safe during pregnancy.

In conclusion: Although Uropathogens producing ESBLs are not routinely tested for in our locality, the prevalence of Community acquired Extended Spectrum beta lactamases (CA-ESBLs) causing asymptomatic bacteriuria during pregnancy is high and are probably the cause of multidrug resistance and treatment failures.

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