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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.

REFERENCES

1. Theodore, M. (2007). Prevalence and Bntibiogram of Urinary Tract Infections among Prison Inmates in Nigeria. *The Internet Journal of Microbiology*,3(2): 12-13.
2. Brook, G. S., Butel, J. S. and Moges, S. A. (2001). *JawetzMelmick and Adelberg's Medical Microbiology*. New York: Mc Graw Hill. pp. 637-638.
3. Loh, K. Y. andSilvalingam, N. (2007). Urinary Tract Infections in Pregnancy. *Malaysian Family Physician*, 2(2): 54-57.

4. Kahlmeter, G. (2003). An International Survey of the Antimicrobial Susceptibility of Pathogens from Uncomplicated Urinary Tract Infections, the ECO, SENS Projects. *Journal of Antimicrobial Chemotherapy,* 18(51):69-71.
5. Paterson, D. L. and Bonomo, R. A.(2005). Extended-Spectrum beta-lactamase: A Clinical Update, Clin. Microbiol Rev. 18: 657-686.
6. Harris, A. D., Kotetishvili, M., Shurland, S., Johnson, J. A. Morris, J. G. Nemoy, L. L. et al., (2007). Patient-to-patient transmission is important in Extended-Spectrum Beta-lactamase-producing KlebsiellapneumonieaAcquision. Clin Infect. Dis. 45:1347-1350.
7. Iroha, I. R., Oji, A. E., Afiukwa, T. N., Nwuzo, A. C. and Ejikeugwo, P. C. (2009). Extended Spectrum Beta-Lactamase (ESBL) Mediated Resistance to Antibiotics among *Klebsiellapneumoniae* in Enugu Metropolis. *Macedonian Journal of Medical Sciences,* 9(3):198-199.
8. Afunwa, R. A., Odimegwu, D. C.,Iroha, R. I. and Esimone, C. O. (2011). Antimicrobial Resistance Status and Prevalence Rate of Extended Spectrum Beta-Lactamase (ESBL) Producers Isolated From a Mixed Human Population. *Basic Medical Science,*11(2): 91-96.
9. Egbebi, A. O. and Famurewa, O. (2011). Prevalence of Extended Spectrum Beta-Lactamase (ESBL) production among *Klebsiella* Isolated in Some Parts of South West Nigeria. *Journal of Micrbiology Biotechnology and Research,*16(2):64-68.
10. Tijjani, J., Arzai, A. H. and Sadiq, W. M. (2012). Antimicrobial Susceptibility pattern of Extended Spectrum Betalactamase Producers in Gram-negative Urogenital Isolates in Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences,*5(1): 20-25.
11. Babypadmini, S. and Appalaraju, B. (2004). Extended Spectrum Beta-lactamases in Urinary Isolates of *E. coli and Klebsiella pneumonia*-Prevalence and Susceptibility Pattern in A Tertiary Care Hospital. *Indian Journal of Medical Microbiology*, 22(3): 172-174.
12. Irah, J. T., Sohbhan, W. V., Morovat, S. T., Nkohoda, V. S., Hamid, T. S., Mohammad, H. S. and Neda, W. T. (2011).Treatment of Urinary Tract Infections. *Journal of Urology,*17(2): 372-382.
13. Agarawal, P., Ghosh, A. N., Kumar, S., Basl, B. and Rapila, K. (2009). Prevalence of Extended-Spectrum Beta-Lactamases among *E. coli* and *Klebsiella pneumonia* Isolates in Tertiary Care Hospital. *Indian Journal of Pathology and Microbiology,* 13(51): 139-142.
14. Ejikuegwu, P. C., Ikegbunam, N. M., Ugwu, C. M., Iroha, I. R., and Esimone, C. O. (2012). Extended-Spectrum Beta-lactamase producing *E. coli* Isolatesfrom Suspected Community Acquired Urinary Tract Infections, *European Journal of Scientific Research*, 84(4): 565-571.
15. Ramazanz, R. (2010). Etiologic agents and Extended- Spectrum Bêta-lactamase Production in Urinary Tract Infections in Sanandaj, Iran. *Eastern Journal of Medicine,* (15): 57-62.
16. Shamim, M., Mumtaz, A., Irum, A., Naeem, A., Masood, U. L. H. and Abdul, H. (2010). Extended Beta-Lactamases in Enteric Gram-negative Bacilli: Related to Age and Gender. *Medicine College Abbottaad,* 19(4): 107-111.
17. Al-jasser, A. M. (2006). Extended Spectrum-Beta-lactamases (ESBLs). A Global Problem. *Kuwait Medical Journal,* 38(3): 171-185.
18. Nicoula, D. P. (2008).Use of Doripenem against ESBLs, *Antimicrobial Agent and Chemotherapy,* 54(5): 2119-2124.
19. Shah, P. (2008). Antimicrobial Susceptibility Pattern and ESBL Prevalence.*SultanQaboos Universal Medical Journal,*8(2): 171-177.

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PLASMID PROFILE AND ANTIMICROBIAL RESISTANCE RATINGS OF *ENTEROCOCCI* ISOLATES FROM PIGS AND POULTRY BIRDS IN ABIA STATE, NIGERIA

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ABSTRACT
Our aim was to isolate and investigate the resistance ratings of enterococci poultry and pig isolates to various antimicrobial agents as well as to determine their plasmid profiles. Antimicrobial resistance ratings and the plasmid profiles of Enterococci isolated from poultry birds and pigs were analyzed. Three hundred and thirty enterococci isolates from poultry birds and pigs were obtained from the three zones in Abia State. Antimicrobial resistance ratings, transformation, curing and plasmid extraction for enterococci were done. The result showed that in both animal species multi-resistance to antimicrobials occurred in more than 40% of enterococci isolates. The enterococci isolates were resistant to floxapen (90%), ceprofloxacin (70%) and norfloxacin (80%). It also showed that the organisms were sensitive to lincocin (100%), chloramphenicol (85%) and gentamicin (75%). There were significant differences (P<0.05) in some reactions of some *Enterococcus* isolates to certain antimicrobial agents especially to chloramphenicol, rifampicin and gentamicin. Some isolates that was sensitive to gentamicin, rifampicin and chloramphenicol during pre-curing were resistant after curing though not significant (P>0.05). There was significant difference (P<0.05) among the isolates during pre-transformation and post-transformation process. Plasmid profile analysis of *Enterococcus* spp. revealed plasmid DNA bands ranging in size from 800 to 2000bp which appeared as bright bands. Large plasmid were lost during cell storage, some were plasmid less. No correlation could be made between plasmid patterns and antimicrobial resistance. The studies show good prospects for further research in the same area to explore and assign definite cause for antimicrobial resistance and multi-drug resistance.

Keywords: *Enterococcus* spp., plasmid, poultry birds, pigs, antimicrobial resistance.

PROFIL PLASMIDIQUE ET LES EVALUATIONS DE LA RESISTANCE AUX ANTIMICROBIENS ISOLATS ENTEROCOCCI DES PORCS ET VOLAILLES A L'ETAT D'ABIA, NIGERIA.

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RESUME:
Notre but était d'isoler et d'examiner les évaluations des isolats entérocoque des volailles et des porcs aux plusieurs agents antimicrobiens aussi bien que déterminer leurs profils plasmidique. Les évaluations de la résistance aux microbiens et les profils plasmidiques d'Entérocoque isolé des volailles et des porcs ont été analysés. Trois cent trente entérocoque isolats des volailles et des porcs ont été obtenus de trois zones différentes a l'État d'Abia. Les évaluations de la résistance aux antimicrobiens, la transformation durcissement et plasmide extraction pour entérocoques ont été faits. Le résultat a montré que dans les deux espèces animales, multirésistance aux antimicrobiens se produit dans plus de 40% des isolats d'entérocoques. Les isolats d'entérocoques étaient résistants a floxapen(90%), également Ceprofloxacin (70%), et norfloxacin(80%). Il a aussi montre que les organismes étaient sensibles a Lincocin(100%), Chloramphenicol(85%) et la gentamicine(75%). Il y avait des différences significatives (P<0,05) dans certaines réactions des isolats d'enterolocoques aux certains agents antimicrobiens en particulier a chloramphénicol, a rifampicine et gentamicine. Certains isolats qui étaient sensibles a la gentamicine, a rifampicine et chloramphénicol pendant pré- durcissement étaient résistants après le durcissement bien qu'insignifiant (P>0,05). Il y avait une différence significative (P<0,05) parmi les isolats lors de la pré- transformation et post-transformation. L'analyse du profil plasmidique d'*Enterococcus spp.* a révélé les bandes d'ADN plasmidique allant de la taille de 800 a 2. 000 bp qui semblait bandes brillantes. Les grands ont été perdus lors de stockage de cellules,, certains étaient moins plasmide. Pas de corrélation entre schémas de plasmides et la résistance aux antimicrobiens. Les recherches montrent de bonnes perspectives pour des

recherches plus approfondies dans la même domaine d'explorer et d'attribuer la cause précise pour la résistance. Aux antimicrobiens et la multirésistance.

Mots- clés: *Enterococcus spp.*, Plasmide, Volailles, Porcs, la résistance aux antimicrobiens.

INTRODUCTION

Enterococcus species are ubiquitous, commensal inhabitants of the gastrointestinal tract of humans and animals. Their intrinsic ruggedness allows them to persist and spread in the environment. Once viewed as a genus of minimal clinical impact, enterococci, have surfaced as organisms of importance due to the emergence of multi-drug-resistant strains that are associated with significant morbidity and mortality of human and animals (6). Currently, it is responsible for approximately 12% of all nosocomial infections in the United States (7, 8). Furthermore, their ability to acquire antimicrobial resistance through transfer of plasmids and transposons, chromosomal exchange, or mutation presents a significant challenge for therapeutic measures (9). Multiple antimicrobial resistances in bacteria are most commonly associated with the presence of plasmids which contain one or more resistance genes.

These bacteria are of particular concern in human and animal medicine because some strains have constitutive antimicrobial resistance tracts (10). Another concern is that these organisms can transfer resistance genes to other bacterial species including pathogens (11). Transmission of resistance genes from normally more virulent pathogenic species to non-pathogenic organisms is very common with the animal and human intestinal tract micro-flora (12).

Although opinions differ in defining the source of resistant antimicrobial *Enterococcus* spp. increasing development of antimicrobial resistance and transferable resistance genes are points of concern (13). Furthermore, the use of antimicrobials perpetuated antimicrobial resistant plasmids in countries like Nigeria, where there is an unrestricted use of antimicrobials.

Understanding the molecular epidemiology of resistance plasmids has been a major issue since investigators/scientists became aware of its (plasmids) role in the spread of antimicrobial drug resistance. Molecular characterization of plasmids and other genetic elements are also epidemiologically useful. The plasmid replication system that dictates the plasmid's behavior (host range, cope number) is the major plasmid classification and identification (Novick, 1987). However, their number (plasmid copies) also plays a critical role in imparting various characteristics to the pathogen, such as resistance towards different antimicrobials. Therefore, the present study considered the use of *Enterococcus* species to evaluate antimicrobial resistant patterns in pigs and poultry. It was also designed to genetically characterize the isolates by using molecular techniques, such as plasmid profile analysis.

MATERIALS ANDMETHODS

Sample collection and bacterial isolation: samples were collected once each week from the three senatorial zones in Abia State. Pigs and poultry birds were randomly selected without bias in each farm visited. The faecal material was collected with a swab (EVE PON) from the rectum (pig), cloaca (poultry birds), and transported to the laboratory and were inoculated into enterococcosal broth and incubated at 35^{o}C for 24 hours. Presumptive identification of the *Enterococcus* species were performed by using the following characteristics; Gram stained reaction, colony morphology, growth and blackening of bile-aesculin agar, growth in the presence of 6.5% Nacl and growth at 10^{o}C and 45^{o}C, the presence or absence of catalase and acidification of glucose with the production of gas (1).

Antimicrobial susceptibility testing

All isolates identified as enterococci were tested by disk-diffusion method using Muller Hinton agar (DIFCO) as recommended by CLSI (2010). The selected antimicrobials included ciprofloxacin (10mcg), norfloxacin (10mcg), gentamycin (10mcg), lincocin (20mcg), streptomycin (30mcg), Rifampicin (20mcg), Erythromycin (30mcg), Ampiclox (20mcg), floxapen (10mcg) and chloramphenicol (30mcg) (Oxoid, UK). The sensitivity test was standardized using *E.faecalis* (ATCC 29212). Inhibition zone size was interpreted using standard recommendation of CLSI (4) as sensitive, intermediate resistance and resistance.

Plasmid isolation

The plasmid DNA of *Enterococcus* spp. were screened by the alkaline lysis method of Birnbom and Doly (2), modified by Maniatis (5). The products were then electrophoresed for 1 hour at 150V on a 0.8% agarose gel. After staining the gel with ethidium bromide ($0.5\mu g/ml$), the photograph was taken. Molecular mass of the plasmid was determined by approximate comparison with plasmid of known molecular weight, *E.coli* K-12 DHL that harboured 8 plasmid of 1.4 to 35.8 MDa (3).

Plasmid curing

Multiple resistant isolates were selected and submitted to plasmid curing according to Molina-Aja *et al* (7) with modifications. We use luria-Bertani broth (LB), supplemented with 0.85% Nacl and acridine orange at50$\mu g/ml$.Strains grown under constant shaking in LB medium for 24 hours at 30^{o}C were once again subjected to antimicrobial susceptibility testing against antimicrobials to which they were resistant. Resistance were classified as plasmid dependent when affected by plasmid curing

RESULTS

Table 1 shows the sensitivity ratings of *Enterococcus* before curing and transformation. This showed that the organisms were resistant to floxapen (90%), ciprofloxacin (70%) and norfloxacin (80%). It also showed that the organisms were sensitive to Lincocin (100%), chloramphenicol (85%) and gentamicin (75%).

In Table 2 there were significant differences (P<0.05) in some reactions of some *Enterococcus* isolates to certain antimicrobial agents especially to chloramphenicol, rifampicin and gentamicin. This indicates that some strains of *Enterococcus* contain multi-resistance plasmids. The *E.coli* k-12 that served as control was tested alongside with the *Enterococcus* isolates as shown in Table2. When Table 2 was compared with Table 1 it showed that some isolates that was sensitive to certain antimicrobial agents (gentamycin, rifampicin and chloramphenicol) during pre-curing were resistant after curing though not significant (P>0.05).

TABLE 1: RESISTANCE RATINGS OF *ENTEROCOCCUS* PRE-CURING AND PRE-TRANSFORMATION PROCESSES

Isolates/drugs	**Ciprofloxacin**	**Norfloxacin**	**Gentamycin**	**Lincocin**	**Streptomycin**	**Rifampicin**	**Erythromicin**	**Chloramphenicol**	**Ampicillin**	**Floxapen**
***Enterococcus* 1**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**R**	**R**
***Enterococcus* 2**	**SS**	**R**	**S**	**S**	**R**	**S**	**SS**	**S**	**SS**	**R**
***Enterococcus* 3**	**R**	**R**	**S**	**S**	**SS**	**S**	**S**	**S**	**SS**	**R**
***Enterococcus* 4**	**R**	**R**	**SS**	**S**	**R**	**SS**	**S**	**S**	**SS**	**R**
***Enterococcus* 5**	**R**	**R**	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**R**
***Enterococcus* 6**	**R**	**R**	**S**	**S**	**SS**	**S**	**S**	**S**	**R**	**R**
***Enterococcus* 7**	**R**	**R**	**S**	**S**	**R**	**S**	**S**	**S**	**R**	**R**
***Enterococcus* 8**	**SS**	**SS**	**S**	**S**	**R**	**R**	**R**	**S**	**SS**	**R**
***Enterococcus* 9**	**SS**	**SS**	**S**	**S**	**SS**	**SS**	**R**	**SS**	**R**	**SS**
***Enterococcus* 10**	**R**	**R**	**R**	**S**	**R**	**S**	**R**	**R**	**R**	**R**

Key: 1-14mm = Resistant (R), 15-19mm = slightly sensitive (SS); 20-35mm = Sensitive (S) (CLSL, 2005).

Table 3 showed resistance ratings of *Enterococcus* species post-transformation. The control organism *E.coli* k-12 was used in the transformation process. There was significant difference (P<0.05) among the isolates during pre-transformation and post transformation process. Plate 1 show the result of the plasmid profile of ten (10) representative *Enterococcus* isolates analyzed with 0.8% agarose gel. L is a DNA molecular ladder/control of size 100-1517 bp: E_t1, E_t6, and of E_t8 are samples which harbor plasmid with size of 800bp and 2000bp. In this study only small plasmids, which appeared as bright bands mostly below the band of chromosomal DNA on the gel, were used in the typing analysis because large plasmids tend to be lost during cell storage and sub culturing or plasmid extraction.

TABLE 2: RESISTANCE RATINGS OF ENTEROCOCCUS POST-CURING PROCESS

Isolates/drugs	Ciprofloxacin (10μm)	Norfloxacin (10μm)	Gentamycin (10μm)	Lincocin (20μm)	Streptomycin (30μm)	Rifampicin (20μm)	Erythromicin (30μm)	Chloramphenicol (30μm)	Ampicillin (20μm)	Floxapen (20μm)
Enterococcus 1	R	R	R	SS	R	R	S	R	R	R
Enterococcus 2	S	R	R	S	R	R	S	R	S	R
Enterococcus 3	S	R	R	S	R	R	S	R	SS	R
Enterococcus 4	S	R	R	SS	R	R	S	R	S	R
Enterococcus 5	R	R	R	S	R	R	S	R	R	SS
Enterococcus 6	R	R	R	S	R	R	S	R	R	R
Enterococcus 7	R	R	R	SS	R	R	S	R	R	R
Enterococcus 8	S	S	R	S	S	R	SS	R	S	S
Enterococcus 9	S	S	SS	S	S	R	SS	R	S	S
Enterococcus 10	R	R	R	S	S	S	R	R	R	R
E.coli K-12 (control)	S	S	S	S	S	S	S	S	S	S

Key: 1-14mm = Resistant (R), 15-19mm = slightly sensitive (SS); 20-35mm = Sensitive (S) (CLSL, 2005).

DISCUSSION

The study of the prevalence of antimicrobial resistance in indicator micro flora can be very useful in monitoring and understanding the process of antimicrobial-mediated selection in individual host as well as in the general population (14). Multiple antimicrobial resistances in bacteria pathogens which are common phenomenon in developing countries, including Nigeria is most likely related to the frequency use over-the-counter drugs without proper or no medical supervision.

Multiple antimicrobial resistances may be acquired through mobile genetic elements such as plasmids, transposons and class 1 integrons (17, 16). Integrons play an essential role in facilitating the transfer of the resistance genes contributing to the creation of multi drug-resistant phenotype (15).

In this study, all *Enterococcus* isolates tested were resistant to at least one antimicrobial class. The percentages of resistant isolates were generally significant and higher than or occasionally comparable to those previously reported for enterococci recovered from pig farms and slaughter houses (k18). Although a large proportion of isolates were susceptible to gentamicin, lincocin, chloramphenicol and erythromycin, high level resistance to amino-glycosides was detected in a significant percentage of isolates, in accordance with previous reports (19). The prevalence of high level resistance to floxapen and streptomycin was much higher than high level resistance to norfloxacin. Because aminoglycosides are antimicrobial of choice for treating enterococci infections, in combination with cell-wall inhibiting antimicrobials (20), the possibility of disseminating through the food chain of genes conferring a high level of resistance to aminoglycosides in enterococci is of much concern.

Another finding with mentioning is the frequency of isolates (40%) with plasmid-mediated resistance. These plasmid resistance expressions (phenotypic detection) were derived from poultry birds and pigs. For McBride *et al*; (21), the presence of this type of mobile genetic element is common in enterococci, as they make up a substantial fraction of their genome, and responsible for much of the horizontal gene

transfer. It is noted that the isolates from the same sample site showed different ratings before and after curing.

TABLE 3: RESISTANCE RATINGS OF *ENTEROCOCCUS* SPECIES POST-TRANSFORMATION

Isolates/drugs	**Ciprofloxacin (10μm)**	**Norfloxacin (10μm)**	**Gentamycin (10μm)**	**Lincocin (20μm)**	**Streptomycin (30μm)**	**Rifampicin (20μm)**	**Erythromicin (30μm)**	**Chloramphenicol (30μm)**	**Ampicillin (20μm)**	**Floxapen (20μm)**
***Enterococcus* 1**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**S**
***Enterococcus* 2**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
***Enterococcus* 3**	**S**	**S**	**R**	**R**	**R**	**S**	**S**	**S**	**S**	**S**
***Enterococcus* 4**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
***Enterococcus* 5**	**S**	**S**	**S**	**S**	**S**	**SS**	**S**	**S**	**S**	**S**
***Enterococcus* 6**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
***Enterococcus* 7**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**
***Enterococcus* 8**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**S**
***Enterococcus* 9**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**R**	**S**	**S**
***Enterococcus* 10**	**S**	**S**	**R**	**R**	**R**	**S**	**R**	**S**	**R**	**S**
***E.coli* K-12 (control)**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

Key: 1-14mm = Resistant (R), 15-19mm = slightly sensitive (SS); 20-35mm = Sensitive (S) (CLSL, 2005).

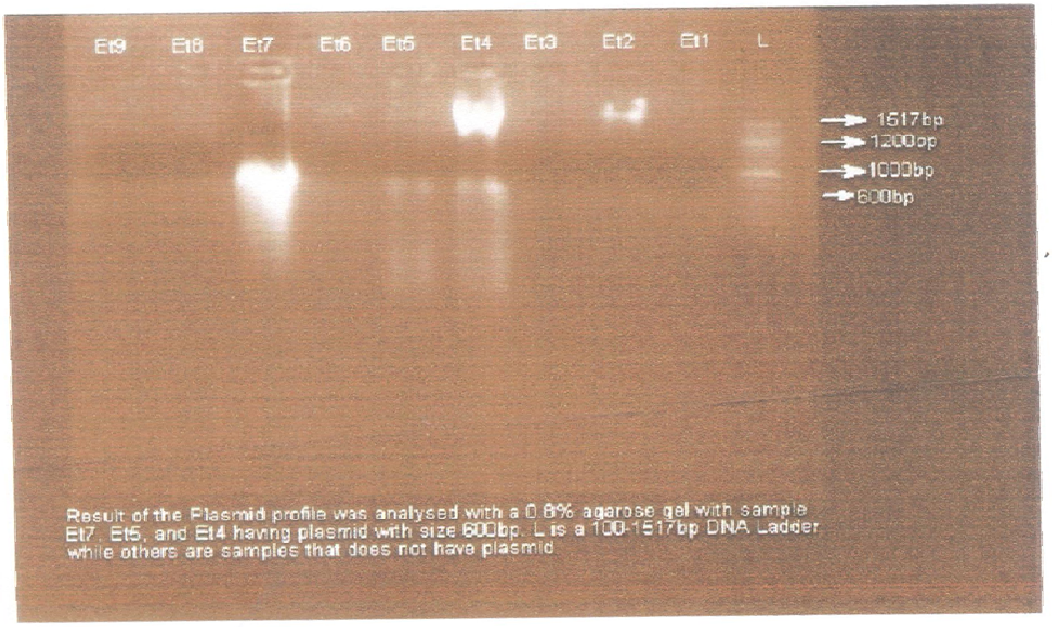

PLATE 1: PLASMID PROFILE OF *ENTEROCOCCUS* ISOLATES

Enterococci are prone to acquiring resistance to antimicrobials, either by mutation or by horizontal transfer of mobile genetic elements (Plasmids and transposons) (23). The reasons for the high number of plasmids in the resistance levels among several species of *Enterococcus* are still unknown (22).

Comparison of plasmid profiles could be a useful method for assessing relatedness of the clinical isolates with resistance to antimicrobial for epidemiological studies (16). In the present study, some of the plasmids were detected in most of the isolates, suggesting high rate of persistence of these plasmids in enterococci isolates. The maintenance of plasmids within the bacterial species is dictated by many environmental and genetic factors. Widespread usage of antimicrobials in Nigeria may cause significant variations in plasmids patterns amongst the enterococci isolates. Enterococci have intrinsic or acquired resistance to many commonly used antimicrobials. The resistances that cause the most severe therapeutic problems include high level resistance to floxapen, ceprofloxacine and norfloxacin. This, in turn, would limit the choice of antimicrobial (25). In the present study, the organisms were resistant to floxapen (90%), ciprofloxacin(70%) and norfloxcin (80%). On the other enterococci isolates were found to be sensitive to lincocin (100%), chloramphenicol (85%) and gentamycin (75%). The use of chloramphenicol in the treatment of Vancomycin Resistance Enterococci faecum (VREF) is limited due to side effects (24).

The resistance observed during post curing process showed that some isolates harboured resistance genes in their chromosomal DNA. The resistance ratings of *Enterococcus* during the pre-curing process when compared with the resistance ratings during post-curing indicate variations. The resistance observed during post-transformation showed that some antimicrobial resistance isolates were plasmid mediated when this tests was repeated during post-curing and post transformation, it showed that resistant isolates possess resistant genes in both plasmid and chromosal DNA. Thirty percent of *Enterococcus* isolates harboured plasmids with size between 0.8kbp to 1.6kbp.This study showed that some isolates of *Enterococcus* that possess antimicrobial resistance were seen to be harbouring plasmids which did not have correlation with the antimicrobial resistance pattern. These observations imply that plasmid does not respect any boundaries, either between animals and human or bacterial species and genera, demonstrating the strong capacity of plasmids to be horizontally transmitted.

REFERENCES

1. Vandamme, P., Vercauteren, E., Lammens, C. and Goossens, H. Survey of enterococcal susceptibility patterns in Belgium. Journal of Clinical Microbiology. 1996; 34: 2572-2576.

2. Birnboim, H.C. and Doly, J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nuclei Acid Res. 1979; 7: 1513-1523.

3. Macrina, F.L., Kopecko, D.J., Jones, K.R. A multiple-plasmid containing *Escherichia coli* strain: convenient source of size reference plasmid modules. Plasmid. 1978; 1: 417-420.

4. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; approved standard. 20th informational supplement. M100-520, vol. 31, No.1. Clinical and Laboratory Standards Institute . 2010; Wayne, P.A.

5. Maniatis, T., Fritsch, E.F. and Sambrook, J.C. Molecular cloning: A laboratory manual. 2nd edition cold spring harbor laboratory press. 1989; Cold spring Harbor, New York.

6. Eller-broek, L., Mac, K.N., Peters, J. and Hultquist, L. Hazard potential from antibiotic-resistant commensals like enterococci. Journal of Veterinary Medicine Series B. 2004; 51: 393-399.

7. Kuhn, I., Iverson, A., Burman, L.G. and Mollby, R. Epidemiology and ecology of enterococci, with special reference to antibiotic resistance strains in animals, humans and the environment. Example of an ongoing project within the European research programme. International Journal of Antimicrobial Agents. 2000; 14: 337 - 342.

8. Linden, P.K. and Miller, C.B. Vancomycin-resistant enterococci; the clinical effect of a common nosomocial pathogen. Diagnostic Microbiology of Infectious Diseases. 1999; 33: 113-120.

9. Mundy, L.M., Sahn, D.F. and Gilmore, M. Relationships between enterococcal virulence and antimicrobial resistance. Clinical Microbiology Review .2000;13: 513-522.

10. Eisner, A., Feierl, G., Goriewicz, G. and Kofer, J. High prevalence of Van A-type vancomycin-resistance enterorocci in Austrain poultry. Applied Environmental Microbiology. 2005; 71: 6407-6409.

11. Lester, C; Frimodt-Moller, N; Sorensen, T; Monnet, D. and Hammerum, A. In vivo transfer of the van A resistance gene from and *Enterococcus faecium* isolate of animals origin to an *E.faecium* isolate to human origin in the intestines of human volunteers. Antimicrobial Agents and Chemotherapy. 2006; 50: 596-599.

12. Ismaeel, N.A. Resistance of bacteria from human faecal flora to antimicrobial agents. Journal of Tropical Medicine Hygiene. 1993; 96: 51-55.

13. O'Brien, T.F. Emergence, spread and environmental effect of antimicrobial resistance: How use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clinical Infectious Diseases. 2002; 34(3 suppl): S78-S84.

14. Levin, B.R., Lipsitch, M., Perrot, V., Schrag, S., Antia, R. and Simonsen, L. The population genetics of antibiotic resistance. Clinical Infectious Diseases. 1997; 24: 59-66.

15. Shaheen, B.W., Oyarzabel, O.A. and Boothe, D.M. The role of class 1 and 2integrons in mediating antimicrobial resistance among canine and feline clinical *E.coli* isolates from the US: Veterinary Microbiology. 2010; vol. 144, No. 3-4. Pp. 363-370.

16. Singh, R; Schroeder, C.M. and Meng, J. Identification of antimicrobial resistance and class 1 intetrons in shiga toxin-producing *Escherichia coli* recovered from humans and food animals. Journal of Anti-microbial Chemotherapy. 2005; vol. 56, No.1, pp. 216-219.

17. Mora, A., Blanco, J.E. and Blanco, M. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* 0157: H7 and non-0157 strains isolated from humans, cattle, sheep and food in Spain. Research in Microbiology. 2005; vol. 156, No. 7, Pp. 793-806.

18. Mangalappali-Illathu, A; Duriez, P., Masson, L; Diarra, M.S. and Topp, E. (2010). Dynamics of antimicrobial resistance and virulence genes in *Enterococcus faecalis* during swine manure storage. Canadian Journal of Microbiology. 2010; 56: 683-691.

19. Jackson, C.R., Fedorka-Cray, P.J., Barett, J.B. and Ladely, S.R. High level aminoglycoside resistant enterococci isolated from swine. Epidemiol. Infect. 2005; 133: 367-371.

20. Davies, J. and Wright, G.D. Bacterial resistance to amino-glycoside antibiotic. Trends Microbiol. 1997; 5: 234-240.

21. McBride, S.M; Fischetti, V.A., Lablanc, D.J.,Moe Uering, R.C., Jr., and Gilmore, M.S. (2007). Genetic diversity among *EnterococcusFaecalis*. PLoS One. 2007; 2(7): e 582.

22. Jensen, L.B.,Garcia-migura, L, Valenzuela, A.J., Lorhr, M., Hasman, H. and Aarestrup, ,F.M. A classification system for plasmids from enterococci and other gram-positive bacteria. Journal of Microbiology Methods. 2010; 80:25-43.

23. Hasmann, H., Villadsen, A.G. and Aarestrup, F.M. Diversification and stability of plasmids from glycopeptide resistant *Enterococcus faecum* (GRE) isolated from pigs in Denmark. Microb. Drug Resistance. 2005; 11: 178-184.

24. Lim, S.M. and Webb, S.A.R. Nosocomial bacterial infections in intensive care units. Organisms and mechanism of antibiotic resistance. Anaesthesia 2005; 60: 887-902.

25. Nichol, A.K., Sill, M; Nancy, M.L. and Zhanel, G.G. Molecular epidemiology of urinary tract isolates of vancomycin-resistant *Enterococcus faecium* from North America. International Journal of Antimicrobial Agents. 2006; 27: 392-396.

26. Novick, R.P. Plasmid replicating system and plasmid behavior in *E.coli*. Microbiol. Rev. 1987; 51: 381-395.

27. Molina-Aja,A., Garcia-Gasca, A., Abreu-Grobois, A. and Gomez-Gil, B. Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. FEMS Microbiol. Let. 2002; 213: 7-12.

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IN-VITRO ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATES IN UMUAHIA, ABIA STATE, NIGERIA

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ABSTRACT

The antimicrobial susceptibility pattern of *Staphylococcusaureus* in Umuahia was investigated in this study.A total of 113 strains of *S. aureus* consisting of 30 isolates from clinical specimens obtained from 10 medical Laboratories and 83 isolates from nasal swabs of University students in Umuahia, were tested against 10 antimicrobial agents using the disc agar diffusion method. Resistance to penicillin, ampicillin, cloxacillin and tetracycline was 100% among strains isolated from clinical specimens. The clinical isolates also exhibited high rates of resistance to chloramphenicol (97%) and erythromycin (97%). Seven (23%) of these isolates were sensitive to Gentamicin and 6 (20%) to Streptomycin. Likewise, all the 83(100%) and 82 (98.8%)were resistant to penicillin and cloxacillin, respectively.Isolates from nasal passages appeared to be less resistant to chloramphenicol (54.2%), gentamicin (43.4), streptomycin (31.3%) and tetracycline (51.8%).Ampicillin did not produce any zone of inhibition against 29 (96.7%) isolates from clinical specimens and only slightly inhibited one with zone of inhibition of 8mm. Nineteen of the isolates manifested low to high level of resistance to chloramphenicol with mean zone of inhibition ranging from 15. 8± 0.7 mm to 9.8± 2.0 mm. All the isolates were completely resistant to penicillin and cloxacillin with no zone of inhibition at all. In the case of gentamicin, 5 (16.7%) had intermediate susceptibility (mean zone of inhibition 14±0 mm), 12(40%) had mean inhibition zone of 9.6±2.9 mm and 6(20%) were not inhibited. This study shows that *S. aureus* strains isolated from clinical specimens and healthy students in Umuahia are highly resistant to common antibiotics. This may not be unconnected with indiscriminate use of antibiotics and calls for more control and rational use of antibiotics to minimize the rate of development of resistance to other antibiotics.

Key words: *Staphylococcus aureus*, antimicrobial resistance, disc agar diffusion, antibiotic susceptibility

PROFIL DE SENSIBILITE ANTIMICROBIENNE IN-VITRO DE SOUCHES DE *STAPHYLOCOCCUS AUREUS* A UMUAHIA, Etat d'ABIA, NIGERIA

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Résumé

Le profil de sensibilité antimicrobienne de Staphylococcus aureus à Umuahia a été recherché dans cette étude. Au total, 113 souches de S. aureus composé de 30 souches provenant d'échantillons cliniques obtenus à partir de 10 laboratoires médicaux et 83 souches d'écouvillons nasaux des étudiants universitaires à Umuahia, ont été testées contre 10 agents antimicrobiens par la méthode de diffusion disque sur gélose. La résistance à la pénicilline, l'ampicilline, la cloxacilline et la tétracycline a été de 100% chez les souches isolées d'échantillons cliniques. Les souches cliniques ont également présenté des taux élevés de résistance au chloramphénicol (97%) et à l'érythromycine (97%). Sept (23%) de ces souches étaient sensibles à la gentamicine et 6 (20%) à la streptomycine. De même, tous les 83 (100%) et 82 (98,8%) étaient résistants respectivement à la pénicilline et à la cloxacilline. Les souches de voies nasales semblaient être moins résistantes au chloramphénicol (54,2%), à la gentamicine (43,4), à la streptomycine (31,3%) et à la tétracycline (51,8%). L'ampicilline n'a produit aucune zone d'inhibition contre 29 (96,7%) souches provenant d'échantillons cliniques et seulement légèrement inhibées avec une zone d'inhibition de 8mm. Dix-neuf souches ont manifesté un faible niveau élevé de résistance au chloramphénicol avec une zone d'inhibition moyenne allant de 15. 8 ± 0,7 mm à 9.8± 2,0mm. Toutes les souches étaient complètement résistantes à la pénicilline et à la cloxacilline sans zone d'inhibition. Dans le cas de la gentamicine, 5 (16,7%) souches avaient une sensibilité intermédiaire (zone d'inhibition moyenne de 14 ± 0 mm), 12 (40%) souches ont eu une zone d'inhibition moyenne de 9,6 ± 2,9 mm et 6 (20%) souches ne ont pas été inhibées. Cette étude montre que les souches de S.

aureus isolées à partir d'échantillons cliniques et des étudiants en bonne santé à Umuahia sont très résistantes aux antibiotiques courants. Cela peut ne pas être en rapport avec l'utilisation sans discernement des antibiotiques et des appels pour plus de contrôle et d'utilisation rationnelle des antibiotiques afin de minimiser le taux de développement d'une résistance à d'autres antibiotiques.

Mots clés: Staphylococcus aureus, résistance aux antimicrobiens, disque de diffusion sur gélose, sensibilité aux antibiotiques

INTRODUCTION

The reports of high level of antimicrobial drug resistance of *Staphylococcus aureus* in different parts of the world are generating serious public health concerns (1, 2,3). *Staphylococcus aureus* is one of the most successful and adaptable human pathogens that can exist as a commensal on human skin on one hand and become a pathogen capable of causing serious infections in both healthcare facilities and in the community (4). *Staphylococcus aureus* causes a plethora of human infections ranging from minor pus forming skin infections such as boils, styes, pustules, impetigo to wound infections, furuncles, ulcers, burns infections and to serious, sometimes life threatening infections like pleural empyema, pneumonia, meningitis, osteomyelitis and septicaemia(5 , 6). This organism is also frequently associated with surgical wound infections (3).

Antimicrobial agents have been used extensively to combat *S. aureus* infections but the increasing level of resistance of *S. aureus* to many antibiotics is complicating the treatment of serious infections caused by this pathogen (2).The knowledge of the antimicrobial susceptibility profile of this versatile pathogen in a particular area is important as this can contribute to rational choice and use of antimicrobial agents. The objective of this work was to survey the pattern of in-vitro antimicrobial resistance of *S. aureus* isolates to various antimicrobial drugs in Umuahia, Abia State.

MATERIALS AND METHODS

A total of 113 *S. aureus* isolates were tested for antimicrobial drug susceptibility profile. Thirty (30) of the isolates were obtained from ten (10) Medical Laboratories in Umuahia. Seventeen (17) isolates were from Urine samples, 5 from high vaginal swab (HVS) and 8 from wounds. The remaining 83 isolates were from culture of nasal swabs collected from 100 students of the Michael Okpara University of Agriculture, Umudike, between 2010 and 2012.

The primary isolation of the organisms from nasal swabs was done on Mannitol Salt agar, then sub-cultured on Nutrient agar for biochemical tests. The

30 isolates from Medical Laboratories were re-isolated on Nutrient agar and biochemically characterized like the nasal passage isolates. All the isolates were characterized and identified by Gram staining reaction, catalase and coagulase tests. The antimicrobial susceptibility testing was done using the Bauer-Kirby disc agar diffusion method on Mueller-Hinton agar with commercially available antibiotic sensitivity discs(AbtekBiologicals, Ltd, UK). The procedure of the antimicrobial susceptibility testing was done as described by Ekundayo and Omodamiro (7).

RESULTS

The antimicrobial susceptibility pattern of 113*S.aureus* strainsisolated from clinical specimens and nasal passages in Umuahia was investigated in this study. The pattern of susceptibility of the isolates to ten antimicrobial agents tested is presented in Table 1. All the 30 strains isolated from clinical specimens were resistant to ampicillin, cloxacillin, penicillin and tetracycline. Likewise, all the 83(100%) and 82(98.8%)strains isolated from nasal passages of students in Michael Okpara University of Agriculture, Umudike were resistant to penicillin and cloxacillin, respectively. Seven (23%) of these isolates were sensitive to Gentamicin and 6 (20%) to Streptomycin. Forty seven (56.6% and 57(68.7%) of the isolates from nasal passages were sensitive to Gentamicin and Streptomycin, respectively.

The zone of inhibition produced by the various antimicrobial agents against the *S.aureus* isolated from clinical specimens is presented in Table 2. Ampicillin did not produce any zone of inhibition against 29 (96.7%) isolates and only slightly inhibited one with zone of inhibition of 8mm. Nineteen of the isolates were inhibited by chloramphenicol with mean zone of inhibition ranging from 9.8± 2.0 mm to 15. 8± 0.7 mm. All the isolates were completely resistant to penicillin and cloxacillin with no zone of inhibition at all. In the case of gentamicin, 5

(16.7%) had intermediate susceptibility (mean zone of inhibition 14±0 mm), 12(40%) had mean inhibition zone of 9.6±2.9 mm and 6(20%) were not inhibited.

TABLE 1: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *S. AUREUS* ISOLATES FROM CLINICAL SPECIMENS AND NASAL PASSAGES IN UMUAHIA

	Clinical Isolates (n= 30)		**Nasal Passage isolates (n= 83)**		**Total (n=113)**	
Antimicrobial Agents	**№(%) Sensitive**	**№(%) Resistant**	**№(%) Sensitive**	**№(%) Resistant**	**№(%) Sensitive**	**№(%) Resistant**
Ampicillin	**0(0)**	**30(100)**	**NT**	**NT**	**0(0)**	**30(100)**
Chloramphenicol	**1(3)**	**29(97)**	**38(45.8)**	**45(54.2)**	**39(34.5)**	**74(65.5)**
Cloxacillin	**0(0)**	**30(100)**	**1(1.2)**	**82(98.8)**	**1(0.9)**	**112(99.1**
Erythromycin	**1(3)**	**29(97)**	**20(24.1)**	**63(85.9)**	**21(18.6)**	**92(81.4)**
Gentamicin	**7(23)**	**23(77)**	**47(56.6)**	**36(43.4)**	**54(47.8**	**59(52.2)**
Penicillin	**0(0)**	**30(100)**	**0(0)**	**83(100)**	**0(100)**	**113(100)**
Streptomycin	**6(20)**	**24(80)**	**57(68.7)**	**26(31.3)**	**63(55.8)**	**50(54.2)**
Tetracycline	**0(0)**	**30(100)**	**40(48.2)**	**43(51.8)**	**40(35.4)**	**73(64.6)**
Cotrimoxazole	**NT**	**NT**	**19(22.9)**	**64(77.1)**	**19(22.9)***	**64(77.1)***
Augmentin	**NT**	**NT**	**20(24.1)**	**63(85.9)**	**20(24.1)***	**63(85.9)***

NT= Not done; * Only isolates from nasal passage

TABLE 2: MEAN DIAMETER ZONE OF INHIBITION (MM) AND ANTIMICROBIAL SUSCEPTIBILITY STATUS OF *S. AUREUS* CLINICAL ISOLATES IN UMUAHIA

Antimicrobial agents	**Susceptibility status**			
	Sensitive	**Intermediate**	**Resistant**	**Resistant**
	№(Ẍ DZI± std)[a]	**№(Ẍ DZI± std)**	**№(Ẍ DZI± std)**	**№(%) with no zone of inhibition**
Ampicillin 10µg	**0(0)**	**0 (0)**	**1(8±0)**	**29(96.7)**
Chloramphenicol 30µg	**1 (18±0)**	**9 (15.8±0.7)**	**10(9.8±2.0)**	**10(33.3)**
Cloxacillin 5µg	**0(0)**	**0 (0)**	**0 (0)**	**30 (100)**
Erythromycin 5µg	**1 (18±0)**	**1(14±0)**	**3 (10±2.0)**	**25(83.3)**
Gentamicin 10µg	**7(16.1±0.9)**	**5(14±0)**	**12 (9.6±2.9)**	**6(20)**
Penicillin 10 IU	**0(0)**	**0(0)**	**0(0)**	**30(100)**
Streptomycin 10µg	**6(18±2.9)**	**5(12±0)**	**4(9.5±1.0**	**15(50)**
Tetracycline 30µg	**0(0)**	**2(17±1.4)**	**14(9.1±1.7)**	**14(46.7)**

[a]Ẍ DZI± std: Mean diameter of inhibition ± standard deviation; Interpretative reference range: Sensitive DZI 15≥20 mm, Intermediate DZI 13-14 mm, Resistant DZI ≤12 (Cheesbrough, 2002)

DISCUSSION

Staphylococcus aureus has a remarkable ability to develop resistance to antibiotics and has successfully developed resistance mechanisms to each new antibiotic introduced, beginning from the oldest penicillin, to methicillin and other newer antibiotics (8, 9). The strains of *S. aureus* in this study exhibited high level of resistance to multiple antibiotics and this suggests that the strains possess different or multiple resistance mechanisms. Resistance to antimicrobial agents in *S. aureus* has been mediated by genetic elements acquired either by spontaneous chromosomal mutation under drug pressure or acquisition of resistance genes through horizontal transfer from other microorganisms (9,10).

The prevalence of resistance to penicillin, ampicillin, cloxacillin and tetracycline was 100% among strains isolated from clinical specimens. The clinical isolates also exhibited high rates of resistance to chloramphenicol (97%) and erythromycin (97%). This may not be particularly surprising given the unrestricted access to and the indiscriminate use of antimicrobial agents in our study area.Isolates from nasal passages appeared to be less resistant to chloramphenicol (54.2%), gentamicin (43.4), streptomycin (31.3%) and tetracycline (51.8%). High rate of resistance to penicillin and other β-lactam antibiotics has been reported in parts of South-western Nigeria (11, 12). Researchers in other parts of the world have also reported high level of resistance to antibiotics by strains of *S. aureus*(13, 2, 3).

Analysis of the sizes of zone of inhibition produced by different antibiotics against strains of *S. aureus* from clinical specimensshows the degree of resistance to the drugs. Ampicillin did not produce any zone of inhibition against 29 (96.7%) and only slightly inhibited one with zone of inhibition of 8mm. All the isolates were completely resistant to penicillin and cloxacillin with no zone of inhibition at all. Although 29 of the 30 isolates were classified as resistant to chloramphenicol, 9(30%) of the isolates had intermediate susceptibility with mean zone of inhibition of 15.8±0.7 mm, 10(33.3%) had mean inhibition zone of 9.8±2.0 and 10(33.3%) were frankly resistant with no zone of inhibition. In the case of gentamicin, 5 (16.7%) had intermediate susceptibility (mean zone of inhibition 14±0 mm), 12(40%) had mean inhibition zone of 9.6±2.9 mm and 6(20%) were not inhibited. The isolates with zone of inhibition in the category of intermediate susceptibility might have been regarded as sensitive in routine laboratory practice where any reasonable size of inhibition is taken as evidence of susceptibility without the use of interpretative reference range.

In conclusion, this study shows that there is a very high level of resistance to commonly used antibiotics among strains of *S. aureus* in Umuahia. There is need to institute surveillance for drug susceptibility of important pathogen like *S. aureus*.There is a need for greater control and rational use of antibiotics in order to slow down the rate of resistance development and spread of resistant organisms in the community.

REFERENCES

1 ZetolaN, Francis JS, Nuermberger EL and BishaiWR. Community-acquired Methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infectious Disease* 2005; 5:275-786.

2 Wang SH, Khan Y, Hines L, Mediavilla JR, Zhang L, Chan L, Hoet A, Bannerman T, Panchohi P, Robinson DA, Kreiswirth BN and Stevenson KB. Methicillin-resistant *Staphylococcus aureus* sequence 239-III, Ohio, USA, 2007-2009. *Emerging Infectious Diseases* 2012; 18(10): 1557-1565.

3 Caraciolo FB, Santos JB, Magalhaus V, Maciel MAV and Rabelo MA. Antimicrobial resistance Profile of *Staphylococcus aureus* isolates obtained from skin and soft tissue infections of Outpatients from a University hospital in Recife-PE, Brazil. *AnasBrasilieros de Dermatologia* 2012; 87(6): 857-861.

4 Delorme T, Rose S, Senita J, Callahan C and Nasr P. Epidemiology and Susceptibility of Methicillin –Resistant *Staphylococcus aureus* in Northeastern Ohio*American Journal of Clinical Pathology* 2009; 132: 668-677.

5 Moore P and Broome C. Cerebrospinal meningitis epidemics *American Scientist* 1994; 271(5): 38-47.

6 Parsek MR and Sigh PK. Bacterial Biofilms: An Emerging Link to Disease Pathogenesis *Annual Review of Microbiology* 2003; 57:677-701

7 Ekundayo EO and Omodamiro OD. Evaluation of the quality of locally manufactured antimicrobial susceptibility testing discs in South

Eastern Nigeria *African Journal of Clinical and Experimental Microbiology* 2008; 9(3): 122-128

8 Pantosti A, Sanchini A and Monaco M. Mechanisms of antibiotic resistance in *Staphylococcus aureusFuture Microbiology* 2007; 2(3): 323-334

9 Jensen SO and Lyon BR. Genetics of antimicrobial resistance in *Staphylococcus aureusFuture Microbiology* 2009; 4(5):565-582

10 Espedido BA and Gosbell IB. Chromosomal mutations involved in antibiotic resistance in *Staphylococcus aureusFrontiers in Biosciences* (Schol Ed). 2012; 4:900-915

11 Esan CO, Famurewa O, Lin J and Shittu AO. Characterization of Staphylococcus aureus isolates obtained from healthcare institutions in Ekiti and Ondo States, South-Western Nigeria *African Journal of Microbiology Research* 2009; 3(12): 962-968

12 Shittu AO, Udo EE and Lin J. Phenotypic and molecular characterization of *Staphylococcus aureus* isolates expressing low-and-high-level mupirocin resistance in Nigeria and South Africa *BMC Infectious Diseases* 2009; 9:10, doi:10.1186/1471-2334-9-10

13 Turutoglu H, Ercelik S and Ozturk D. Antibiotic Resistance of *Staphylococcus aureus* and Coagulase-Negative staphylococci isolated from Bovine Mastitis *Bull Vet InstPulawy* 2006; 50: 41-45

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ANTIFUNGAL SUSCEPTIBILITY PROFILES AND RISK FACTORS OF VAGINAL CANDIDIASIS AMONGST FEMALE UNIVERSITY STUDENTS IN SOUTHWEST REGION, CAMEROON

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RUNNING TITLE: ANTIFUNGAL SUSCEPTIBILITY PROFILES AND RISK FACTORS OF VAGINAL CANDIDIASIS AMONGST FEMALE UNIVERSITY STUDENTS

ABSRACT

Vaginal candidiasis (VC) is second to bacterial vaginitis, as the most common opportunistic mucosal infection that affects large numbers of otherwise healthy women of childbearing age. The incidence of VC is significantly modified by dressing patterns and aberrant health-care practices. Contemporary young women often shift their preference from skirt to trousers and leggingswhich also coincides with a rise in auto-medication and over-the-counter drugs phenomena in our communities. These could result in increased occurrence of vaginal candidiasis infection and antifungal drug resistance. This was a cross-sectional study conducted between March 2011 and August 2011 among150 female students(aged 17-29 years) of the University of Buea.Socio-demographics information, risk factors and clinical symptoms were gotten through a standard questionnaire. Vaginal swabs were collected from each participant and cultured on Sabouraud'sdextrose agar supplemented with chloramphenicol (SDA-CAF). Identification and antifungal susceptibility testing was performed following standard microbiological procedures. Of the 150 participants who submitted vaginal swabs, yeasts was isolated in 98 (65.3%). Of the 98 yeasts isolates, 73.5% were *Candida* species, mainly *C. albicans* (65.3%). Overhalf (64.7%) ofstudyparticipantshadapreferencefortrousers,however, this attitude was not significantly associated (p = 0.559) with candidiasis.Previous episodes of vaginal infection and treatment for candidiasis were significantly associated with VC (p = 0.004). Antifungal susceptibility results showed a high resistance to fluconazole (82.0%), nystatin (80.0%) and ketoconazole (72.0%), while clotrimazole (50.0%) was the most activeantifungal drug. There was a high prevalence of VC in this study population with previous vaginal infectionbeing important risk factor for reoccurrence. Clotrimazole was the drug of choice in the treatment of VC in this population.

Key words: vaginal candidiasis, risk factors, antifungal susceptibility profiles

PROFILS DE SENSIBILITE AUX ANTIFONGIQUES ET FACTEURS DE RISQUE DE CANDIDOSE VAGINALE CHEZ LES ETUDIANTES UNIVERSITAIRES AU SUD-OUEST DU CAMEROON

TITRE COURANT:PROFILS DE SENSIBILITE AUX ANTIFONGIQUES ET FACTEURS DE RISQUE DE CANDIDOSE VAGINALE CHEZ LES ETUDIANTES UNIVERSITAIRES

La candidose vaginale (CV) est la deuxième infection opportuniste de la muqueuse la plus fréquente (après la vaginite bactérienne) qui affecte un grand nombre de femmes en âge de procréer. L'incidence de la CV est affectée de façon significative par certaines habitudes vestimentaires et pratiques de soins de santé « aberrante »s. Chez les jeunes femmes contemporaines, les pantalons et leggings sont de plus en plus préférés aux jupes, ce qui coïncide aussi avec une augmentation du phénomène d'automédication dans nos communautés. Ces deux facteurs pourraient entraîner une augmentation de la prévalence de l'infection à *Candida* vaginale et la résistance aux antifongiques. La présente étude transversale a été menée entre Mars et Août 2011 portait sur 150 étudiantes âgées de 17 à 29 ans, à l'Université de Buea (Cameroun). Elle avait pour objectifs majeurs d'évaluer les profils de sensibilité aux antifongiques ainsi que les facteurs de risque de candidose vaginale chez les étudiantes universitaires. Les données sociodémographiques, informations sur les facteurs de risque et les symptômes cliniques ont été explores à l'aide d'un questionnaire semi-structuré. Des spécimens vaginaux ont été prélevés dans chaque participante et soumis à une culture sur le dextrose gélose de Sabouraud supplémenté par le chloramphénicol (SDA-CAF). Les tests d'identification et de sensibilité antifongique ont été réalisés suivant des procédures microbiologiques standard. Parmi les 150 participants qui ont soumis des prélèvements vaginaux, des levures ont été isolées de 98 personnes (65,3%). Sur les 98 levures isolées, 73,5% étaient des espèces de *Candida*, principalement *C. albicans*(65,3%). Plus de la moitié des participants (64.7%) ont exprimé des préférences pour les pantalons et autres styles vestimentaires émergents. Cependant, de telles attitudes n'ont pas paru statistiquement associées à l'occurrence des candidoses au sein de la population ciblée (p = 0.559). Des précédents épisodes d'infection vaginale et le traitement de la candidose reportés par les participantes étaient significativement associés à CV (p = 0,004). Les résultats de sensibilité antifongiques ont montré une grande résistance au Fluconazole (82,0%), Nystatine (80,0%) et Kétoconazole (72,0%), tandis que le Clotrimazole (50,0%) était le médicament antifongique le plus actif. Il y avait une forte prévalence de CV dans cette population d'étude avec infection vaginale précédente étant facteur de risque important pour la répétition. Le Clotrimazole s'est avéré comme étant le médicament de choix dans le traitement des CV dans cette population, malgré la forte résistance.

Mots clés: Candidose Vaginale, facteurs de risque, les profils de sensibilité aux antifongiques

INTRODUCTION

Bacterialvaginitis, vaginal candidiasis and trichomoniasis are responsible for 90% of the cases of vaginal infections (1). Vaginal candidiasisissecond to bacterial vaginitis(2), as the most common opportunistic mucosal infection that affects large numbers of otherwise healthy women of childbearing age (3, 4). VC usually occurs when there is overgrowth of the fungus, *Candida*, present in the body as a normal commensal(5)whichis characterized by curd-like vaginal discharge and itches(6). Up to 75% of reproductive-age women are infected with VC at least once (6, 7) and about half of these women experience more than one recurrence, and 5%– 8% have multiple episodes each year(7). In addition to discomfort and the costs associated with medication and health care visits, several prospective studies have suggested that VC may increase a woman's risk of contracting other sexually transmitted diseases such ashuman immunodeficiency virus(9).

Although VC in pre-menarchal and post-menopausal women is rare, there are several endogenous and exogenousfactors that predispose menarchal women to acute VC; including several hormonal modulations associated with pregnancy, luteal phase of the menstrual cycle, oral contraceptive use, hormone replacement therapy and non-hormonal factors such as antibiotic use and uncontrolled diabetes mellitus(10).

Dressing pattern such as tight clothing and synthetic underwear have been reported to increase the risk of candidiasis(10, 11)although other investigatorshave contrary reports(12, 13). In recent times, young women often shift their preference from skirt to trousers/leggings or tight under wears and this also coincides with a rise in auto-medication and over-the counter drugs phenomena in our communities. Access to over-the-counter medications allows women to self-diagnose and treat vaginal symptoms (5). These and other possible risk behaviours could result in the increase prevalence of vaginal candidiasis and antifungal drug resistant.Consequently, the present study sought to investigate the prevalence of VC, determine the possible risk factors and antifungal sensitivity patternsamong female students of reproductive age at the University of Buea.Such data will provide important information in developing effective strategies for the prevention, control and possible treatment options for VC.

MATERIAL AND METHODS

Study design and population

This cross-sectional study was conducted between March 2011 and August 2011. The study population comprised female students of child-bearing age (17-29 years) enrolled in both postgraduate and undergraduate programs at the University of Buea, who gave their consent to participate in the study. Questionnaires were administered to obtain information on demographics, risk factors and medical history of VC.

Sample collection Samples were collected as previously reported (15). Briefly, each participant was given a sterile swab stick and instructed to self-collectan early morning vaginal swab before bath by introducing the sterile swab into vaginal area and gently moving the swab by rotatingand allowing for some time to absorbed vaginal discharge. The samples were delivered within 2 hours to Life Science teaching laboratory of the University of Buea for analysis.

Culture and Microscopy
Samples were inoculated on Sabouraud's dextrose agarsupplemented with chloramphenicol (SDA-CAF) (Plasmatec Laboratory Products LTD, UK). The plates were incubated aerobically at 37^{o}C for 24 – 48hours after which they were examined for raised, cream-coloured, opaque, yeast smelling colonies. Morphologically distinct colonies from each culture were sub-culturedand stored on SDA slant for subsequent identification.Vaginal swabs were rolled onto slides, air dried and Gram stained following standard microbiological procedures. The slides were viewed with light microscope at 100X for yeast morphology. Wet mount preparations were prepared by placing the swab stick into 0.5ml sterile normal saline and mixed vigorously. A drop of the suspension was then placed on a clean labelled slide, covered with a cover slip, and carefully examined under a microscope using the 40X objective to observe yeast morphology.

Identification and antifungal susceptibility testing

Positive cultures were further tested for germ tube formation (evidence for *C. albicans*). Discrete colonies were inoculated in bovine serum and incubated at 35-37^{o}C for 2-3hours. The serum preparations were then transferred on slides and viewed under the microscope (10X and 40X objectives) for the presence of germinating blastospores or germ tube formation. A complete identification of yeast isolates was carried out usingAnalytical Profile Index for *Candida* (API *Candida* kit, BioMerieux SA) according to manufacturer's instructions.Antifungal sensitivity testing was performed only on *Candida species* isolates, using the modified Kirby-Bauer disc diffusion method on SDA as previously reported(16). The antifungal discs [potency] used included nystatin [100 IU], fluconazole [100 µg], ketoconazole [10 µg] and clotrimazole [50 µg].

The data was recorded and analysed using descriptive analysis and Chi-square on SPSS Version 11.0 statistical software at 0.05 significance level. Ethical clearance for the study was obtained from the Regional Delegation of Public Health for the Southwest region.

RESULTS

Study population description and clinical presentation One hundred and fifty female students, mean age 20.5 year (Range 17-29) were enrolled for the

study, with 68% being symptomaticand 32% asymptomatic cases. The reported clinical symptoms included vaginal itches (57.3%), abnormal vaginal discharge (31.3%) and burning pain sensations(14%).Over half (64%) of the participants accepted to have had previous episodes of VC and among them half (48/96), sought for medical attention and VC was diagnosed by laboratory findings while the remainder were self-diagnosed. Of those who were self-diagnosed, 23 (47.9%) reported to have taken auto-medication. A total of 64.7% of the study participants acknowledged preference for trousers while 49.3% preferred tight under wares. Majority (70%) of the participants reported haverecently use antibiotics while 15.3% accepted have been on oral contraceptives.

Prevalence of *Candida species* in the study population

Microscopic examination of wet preparation showed presence of yeast cells in 48.7% (73/150) occurringmostly as single cells/bud (72.6%), while the rest (27.3%) presented as ovoid buds/pseudo-hyphae. A total of 85 of the samples (56.7%) were positive by Gram stained smears while 98 (65.3%) were identified by culture.Of the 98 positive cultures, 48% (47/98), 25.5% (25/98) and 26.5% (26/98) showed profuse, moderate and scanty growth respectively on SDA. Half (50%) of the isolates produced germ tubes and hence were presumptively considered *Candida species*. Isolates were further identified with Analytical Profile Index for *Candida species*(API candida). Overall, based on germ tube identification test and the API *candida* test, 73.5% (72/98) of the isolates were identified as *Candida species* while 26.5% (26/98) of the isolates were non-candida *species*. The *Candida species*,included*C. albicans*(42.7%),*C. tropicalis* (3.4%),*C. glabrata*(1.3%) and *C. krusei* (0.7). Other yeast species identified were *Saccharomyces cerevisiae* (12.7%) and *Trichosporon species* (4.7%).(Table 1).

TABLE 1: PREVALENCE OF *CANDIDA SPECIES* AND OTHER YEAST SPECIES IN THE STUDY POPULATION

Yeasts species	Germ tube test	API candida positive	Total (%)	Total prevalence (%)
C. albicans	49	15	64 (42.7)	*Candida spp* 72 (73.5)
C. tropicalis	/	05	05 (3.4)	
C. glabrata	/	02	02 (1.3)	
C. krusei	/	01	01 (0.7)	
Saccharomyces cerevisiae	/	19	19 (12.7)	Other yeast 26 (26.5)
Trichosporon species	/	07	07 (4.7)	
Total	49	49	98 (65.3)	

Antifungal susceptibility testing Antifungal susceptibility testing results was classified as susceptible, intermediateor resistant. Our results revealed that isolates were most susceptible to clotrimazole50.0% (25/50). On the other hand, none of the isolates were susceptible to fluconazole and nystatin, although some isolates showed intermediate to fluconazole (18.0%) and to and nystatin (20.0%). Resistance to fluconazole,nystatinand ketoconazole were (82%), (80%) and (72%) respectively (Figure 1).

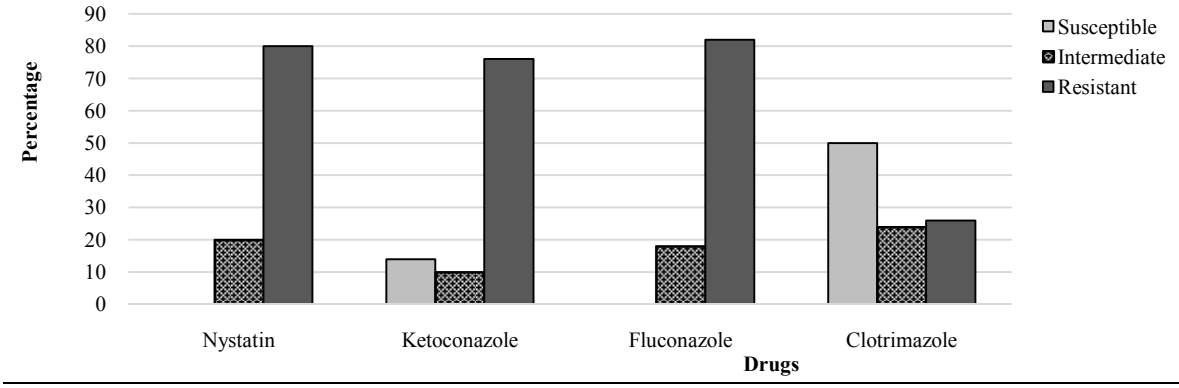

FIGURE 1: ANTIFUNGAL DRUG RESISTANT PROFILE OF *CANDIDA ALBICANS* ISOLATES

Risk factors associated with vaginal candidiasis
Although not significantly different, VC was higher among symptomatic (OR: 1.20, 95% confidence interval [CI]: 0.59-2.45) than non-symptomatic subjects, in participants who preferred tight (OR: 1.37, 95% CI: 0.69-2.69) than loose under wears, and also among those who had preference for trousers (OR: 1.23, 95% CI: 0.61-2.47) than skirt outfit. Similarly, use of oral contraceptives (OR: 1.25, 95% CI: 0.48-3.28) and recent use of antibiotics (OR: 1.44, 95% CI: 0.68-3.08) both increase the risk of vaginal candidiasis. Previous episodes of vaginal infection (OR: 2.79, 95% CI: 1.38-5.64) was significantly associated (p = 0.004) with vaginal candidiasis. On the other hand previous treatment for VC (OR: 0.36, 95% CI: 0.18-0.72) was protective against VC. (Table 2).

TABLE 2: PREVALENCE OF VAGINAL CANDIDIASIS AND ASSOCIATED RISK FACTORS

Parameters	Category	Overall prevalence (%)	Vaginal Candidiasis, n (%)		Odd ratios [95% CI]	P- value
			Yes	No		
Presence of clinical signs and symptoms	Yes	102 (68.0)	68 (66.7)	34 (33.3)	1.20 [0.59-2.45]	0.617
	No	48 (32.0)	30 (62.5)	18 (37.5)	1	
Previous episodes of vaginal infection	Yes	96 (64.0)	72 (75.0)	24 (25.0)	2.79 [1.38-5.64]*	**0.004**
	No	54 (36.0)	28 (51.9)	26 (48.1)	1	
Previously treated for vaginal candidiasis	Yes	72 (48.0)	37 (51.3)	35 (48.3)	0.36 [0.18-0.72]*	**0.004**
	No	78 (52.0)	58 (74.4)	20 (25.6)	1	
Under wares	Tight	74 (49.3)	51 (68.9)	23 (31.1)	1.37 [0.69-2.69]	0.363
	Loose	76 (50.7)	47 (61.8)	29 (38.2)	1	
Dressing outfit	Trousers	97 (64.7)	65 (67.0)	32 (33.0)	1.23 [0.61-2.47]	0.559
	Skirts	53 (35.3)	33 (62.3)	20 (37.7)	1	
Douching	Yes	79 (52.7)	48 (60.8)	31 (39.2)	0.65 [0.34-1.28]	0.216
	No	71 (47.3)	50 (70.4)	21 (29.6)	1	
Oral contraceptives	Yes	23 (15.3)	16 (69.6)	07 (30.4)	1.25 [0.48-3.28]	0.644
	No	127 (84.7)	82 (64.6)	45 (35.4)	1	
Recent use of antibiotics	Yes	105 (70.0)	78 (74.3)	27 (25.7)	1.44 [0.68-3.08]	0.342
	No	45 (30.0)	30 (66.7)	15 (31.1)	1	

* *Significant*

DISCUSSION
This study investigated the prevalence of VC, antifungal resistance profiles and possible risk factors among female students in the University of Buea. The overall prevalence of yeast species was 65.3%.However, the prevalence of *Candida species* in this study was 48% with *C. albicans* having the highest prevalence (42.7%) in conformity with prior studies(17, 4, 18).As previously demonstrated, *C. albicans*is the most predominant yeast species in the environment and hence occurs as a normal human vaginal flora. More so, its ability to form germ tube confers it survival abilities over other yeast species(19).

Generally, laboratory diagnosis of VC in resource-limited settings like ours is accomplished most often with less a sensitive microscopic examination of fresh and grams stained smears of vaginal specimens. In this study the prevalence of yeast by microscopic examination of wet preparation was 48.7%, which increased to 56.7% by microscopy of gram stained smears. The prevalence further increased to 65.3% by culture (20). Although culture

improves diagnosis of VC, this is not a common method of laboratory diagnosis in this setting and therefore, adequate characterisation of *Candida albicans* by microscopy remains challenging. Presumptive identification of*C. albicans*isolates can be achieved with germ tubes test and in this study up to 50% of the culture positive isolates were identified. Commercial identification test provide more sensitive and specific method for differentiating non-albicans*Candida species*. Three non-albicans species were identified (*C. tropicalis, C. glabrata, C. krusei*) and the two other yeast species (*Saccharomyces cerevisiae* and *Trichosporon species*) with API candida test kit.Unlike many previous findings which predominantly isolated *Candida species* and considered to be the main cause of VC (4, 18), our results revealed that *S. cerevisiae* and *Trichosporon species* could be responsible for causing VC since they were isolated from symptomatic subjects. In support of this findings, *S. cerevisiae* and rarely *Trichosporon species*(21)have also been isolated in vaginal infections and were assumed to be emerging pathogens isolated from fungal infections(22).

We also evaluated the antifungal susceptibility patterns of the *Candida* isolates to commonly prescribed and readily available drugs in the locality. Fifty per cent of Candida *species*were susceptible, while 24% were intermediate to a member of the azole group (clotrimazole), used in the study. Clotrimazoleis one of the reserved antifungal not frequently prescribed in the treatment of VC in this setting. Few (14%) of the isolates were susceptible to ketoconazole and showedno susceptibility to fluconazole. VC is treated effectively with azole-based antifungal drugs(23), however, most of the isolates were found to be resistant to ketoconazole (76%) andfluconazole (82%). On the other hand, the high resistance observed in nystatin (80%), (a polyene) could be blamed to the excessive use of this drug in the locality as topical ointment or suppository as a result of its availability and low cost.

As previously reported itching sensations, burning internal pain and cream white 'cheesy' discharge were the main reported symptoms with majority of the symptomatic participantshaving vaginal itches (57.3%)(20).Among those who reported previous vaginal infections, self-diagnosis (50%) and auto-medication (47.9%)were common practices. This behaviour is prompted by the availability of over-the-counter drugs. There was no statistically significant difference in the prevalence of yeast among symptomatic and asymptomatic subjects. A good number of women are infected with VC without any significant discomfortand this may be as results of the fact symptoms of VC, such as vaginal itches, abnormal vaginal discharge and burning vaginal pains are very nonspecific (8).

Even though, not statisticallysignificant in this study, tight fitting garments, trousers and synthetic underwear have been previously reported to increase the risk of VC (12) by increase temperature and humidity of the vaginal and hence may provide a more favourable environment for the growth of the organisms.However, other investigators have contrary reports (13, 14). Our studyshowed that previous vaginal infection was significantly associated (p =0.004) with the occurrence of VC.In line with prior studies (8, 22, 23, 24), recent use of antibioticsand oral contraceptives wereinsignificantly associated with VC. Nevertheless, antimicrobials tend todeplete the protective vaginal bacterial flora that normally keeps yeast in balance, hence the yeast overgrows and causes VC, meanwhile oral contraceptives are associated with increase oestrogen levels resulting in high glycogen in vaginal lining, a substrate on which *C. albicans* thrives (8, 23).Contrary to other studies douching (12)and previous treatment for VC were negatively correlated with the prevalence of VC but agrees with others reports(27)where no relationship existed between douching and VC.

In conclusion, this study demonstrated a high prevalence of VC among the study participants with previous vaginal infection being an important predictor of VC reoccurrence.*Candida albicans* continue to be the predominant species while the best antifungal treatment option in this locality is clotrimazole.

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REFERENCES

1. Adad SJ, de Lima R V., Sawan ZT, Silva ML, de Souza MA, Saldanha JC, et al. Frequency of Trichomonas vaginalis, Candida sp and Gardnerella vaginalis in cervical-vaginal smears in four different decades. Sao Paulo Med J. 2001;119(6):200–5.
2. Ilkit M, Guzel AB. The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: a mycological perspective. Crit Rev Microbiol. 2011;37(3):250–61.
3. Fidel PL, Cutright J, Steele C. Effects of reproductive hormones on experimental vaginal candidiasis. Infect Immun. 2000;68(2):651–7.
4. Nwadioha SI, Bako IA, Onwuezobe I, Egah DZ. Vaginal trichomoniasis among HIV

patients attending primary health care centers of Jos, Nigeria. Asian Pacific J Trop Dis. 2012;2(5):337–41.
5. Asticcioli S, Sacco L, Daturi R, Matti C, Nucleo E, Zara F, et al. Trends in frequency and in vitro antifungal susceptibility patterns of Candida isolates from women attending the STD outpatients clinic of a tertiary care hospital in Northern Italy during the years 2002-2007. New Microbiol. 2009;32:199–204.
6. Rathod SD, Klausner JD, Krupp K, Reingold AL, Madhivanan P. Epidemiologic features of vulvovaginal candidiasis among reproductive-age women in india. Infect Dis Obstet Gynecol. 2012;2012.
7. Sobel JD, Faro S, Force RW, Foxman B, Ledger WJ, Nyirjesy PR, et al. Vulvovaginal candidiasis: Epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol. 1998;178(2):203–11.
8. Faro S. Sexually transmitted diseases. Compr Ther. 1998;24(2):78–85.
9. Hester RA, Kennedy SB. Candida infection as a risk factor for HIV transmission. J Women's Heal. 2003;12(5):487–94.
10. Barousse MM, Van Der Pol BJ, Fortenberry D, Orr D, Fidel PL. Vaginal yeast colonisation, prevalence of vaginitis, and associated local immunity in adolescents. Sex Transm Infect. 2004;80(1):48–53.
11. Elegbe IA, Elegbe I. Quantitative relationships of Candida albicans infections and dressing patterns in Nigerian women. Am J Public Health. 1983;73(4):450–2.
12. Ekpenyong CE, Inyang-etoh EC, Ettebong EO, Akpan UP, Ibu JO, Daniel NE. Recurrent vulvovaginal candidosis among young women in south eastern Nigeria: the role of lifestyle and health-care practices. International Journal of STD & AIDS. 2012. p. 704–9.
13. Otero L, Palacio V, Carreño F, Méndez FJ, Vázquez F. Vulvovaginal candidiasis in female sex workers. Int J STD AIDS. 1998;9(9):526–30.
14. Mårdh P-A, Rodrigues AG, Genç M, Novikova N, Martinez-de-Oliveira J, Guaschino S. Facts and myths on recurrent vulvovaginal candidosis--a review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. International journal of STD & AIDS. 2002. p. 522–39.
15. Elegbe IA, Botu M. A preliminary study on dressing patterns and incidence of candidiasis. Am J Public Health. 1982;72(2):176–7.
16. Cheesbrough M. District laboratory practice in tropical countries [Internet]. 2nd editio. Vasa. Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo Cambridge: Cambridge University Press The Edinburgh Building, Cambridge CB2 8RU, UK Published; 2006 [cited 2013 Jul 31]. Available from: http://medcontent.metapress.com/index/A65RM03P4874243N.pdf
17. Pirotta M V., Garland SM. Genital candida species detected in samples from women in Melbourne, Australia, before and after treatment with antibiotics. J Clin Microbiol. 2006;44(9):3213–7.
18. Seddigheh Esmaeilzadeh, Saeid Mahdavi Omran ZR. Frequency and Etiology of Vulvovaginal Candidiasis in Women Referred to a Gynecological Center in Babol, Iran. Royan Institue Int J Fertil Steril. 2009;3(2):74–7.
19. Consolaro MEL, Albertoni TA, Svidzinski AE, Peralta RM, Svidzinski TIE. Vulvovaginal candidiasis is associated with the production of germ tubes by Candida albicans. Mycopathologia. 2005;159(4):501–7.
20. Sonnex C, Lefort W. Microscopic features of vaginal candidiasis and their relation to symptomatology. Sex Transm Infect. 1999;75(6):417–9.
21. Makela P, Leaman D, Sobel JD. Vulvovaginal trichosporonosis. Infect Dis Obstet Gynecol. 2003;11(2):131–3.
22. Hazen KC. New and emerging yeast pathogens. Clinical Microbiology Reviews. 1995. p. 462–78.
23. Mathema B, Cross E, Dun E, Park S, Bedell J, Slade B, et al. Prevalence of vaginal colonization by drug-resistant Candida species in college-age women with previous exposure to over-the-counter azole antifungals. Clin Infect Dis. 2001;33(5):E23–7.
24. Auger P, Joly J. Microbial flora associated with Candida albicans vulvovaginitis. Obstet Gynecol. 1980;55(3):397–401.
25. Spinillo A, Capuzzo E, Nicola S, Baltaro F, Ferrari A, Monaco A. The impact of oral contraception on vulvovaginal candidiasis. Contraception. 1995. p. 293–7.
26. Nwadioha S, Egah D, Banwat E, Egesie J, Onwuezobe I. Prevalence of bacterial vaginosis and its risk factors in HIV/AIDS patients with abnormal vaginal discharge. Asian Pac J Trop Med. Elsevier(Singapore) Pte Ltd.; 2011;4(2):156–8.
27. Martino JL, Youngpairoj S, Vermund SH. Vaginal douching: personal practices and public policies. J Womens Health (Larchmt). 2004;13(9):1048–65.

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EFFICACY OF HOUSEHOLD CLEANING AGENTS AGAINST SOME SELECTED PATHOGENIC BACTERIA

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ABSTRACT
The emergence and spread of microorganisms with reduced susceptibility to antimicrobial agents is a major public health problem. This study evaluated the antibacterial effect of household cleaning agents on selected bacterial isolates. Standard culture-based procedure was used to determine the efficacy of disinfectants on selected bacteria isolates. The activity assessed was against *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa.* Ariel was found to have more bactericidal effect on *Streptococcus pyogenes* being sensitive and *Staphylococcus aureus*. Dettol exhibited antibacterial effect against all tested isolates with zones of inhibition for *Streptococcus pyogenes* (24±0.12mm) and *Staphylococcus aureus* (9±0.01mm). Harpic revealed antibacterial activity against *Pseudomonas aeruginosa* and other tested isolates with average zones of inhibition of 20±0.20mm. Jik was active against *Klebsiella pneumonia* and *Escherichia coli* while Omo showed good inhibitory effect against all tested isolates except *Pseudomonas aeruginosa.* Based on the present study, the levels of decreased susceptibility to household cleaning agents seem to be increasing, regardless of whether these products used in the home or not. The eventual clinical implications of this decreased susceptibility need continue surveillance.

Key words: Antibacteria, Commensal flora, Disease, Disinfectant, Hygiene, Public health

L'EFFICACITE DES PRODUITS D'ENTRETIEN MENAGER CONTRE CERTAINES BACTERIES PATHOGENES SELECTIONEES

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RESUME
L'émergence et la propagation des micro-organismes d'une sensibilité réduite aux agents antimicrobiens est un problème majeur a la santé publique. Cette recherche a évalué l'effet antibactérien des agents d'entretien ménager sur isolats bactériens sélectionnés. Procédure fondée de culture Standard a été employé pour déterminer l'efficacité des désinfectants sur des isolats bactériens sélectionnés. L'activité évaluée était contre *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyogenes, Escherichia coli et Pseudomonas aeruginosa*. L'Ariel a été trouvé d'avoir plus effet bactéricide sur *Streptococcus pyogenes* étant sensible et *Staphylococcus aureus.* Le dettol a exposé l'effet antibactérien contre tous les isolats testés avec des zones d'inhibition pour *Streptococcus pyogenes* (24±0,12mm) et *Staphylococcus aureus*(9±0,01mm). Harpic a révélé l'activité antibactérienne contre *Pseudomonas aeruginosa* et les autres isolats testés avec des zones moyennes d'inhibition de 20±0,20mm. Jik a été actif contre *Klebsiella pneumonia* et *Escherichia coli* tandis que l'Omo a montré un bon effet inhibiteur contre tous les isolats testés sauf *Pseudomonas aeruginosa*. Fondée sur la recherche de ce moment, les niveaux de la diminution de la sensibilité aux agents d'entretien ménager paraissent être de plus en plus indépendamment du fait que ces produits sont utilisés à la maison. Finalement, les implications cliniques finales de cette diminution de la sensibilité ont besoin d'une surveillance continuelle.

Mots - clés: Antibactérien, Flore commensal, Maladie, Désinfectants Hygiène, Santé publique.

INTRODUCTION

Antibacterial products have been effectively used to prevent transmission of disease causing micro- organisms among patients, particularly in hospitals environment. They are now being added to products used in homes, schools (especially in day care centres), and veterinary settlings (1). The number of chemicals in antibacterial products are enormous, probably at least 10,000 with 1,000 commonly used in the hospitals and homes. Of the chemicals used to reduce or wipe out microbes important groups include halogens, phenols, ammonia compounds, alcohols,

heavy metals, acids and certain special compounds (2).

Hygiene has a measurable impact on reducing the burden of infections in the developing world, as well as in specialized populations. Homes, hospitals and other health care settings extensively use antiseptics and disinfectants on a variety of tropical and hard-surface applications to control the growth of microbes on both living tissues and inanimate objects (3). Over the years, antiseptics and disinfectants have generally played important roles in the control of infectious diseases, microbial food spoilage and unwanted microbes rather than the use of antimicrobial drugs (4). However, the antimicrobial activity of these agents may be influenced by their formation effects, level of organic load, synergy, temperature and dilution test method (5). Different pathogens vary in their response to different antiseptics or disinfectants (6) and they are continuously acquiring resistance to new antiseptics and disinfectants, as a result, no single antiseptic or disinfectant will be appropriate for all pathogen (7).

Jik, contains 3.5% sodium hypochlorite, it is used on a large scale for surface cleaning, bleaching, odour removal and water disinfection. Salvon is another disinfectant that is composed of 2.8% n-propyl alcohol, 0.3g chlorohexidine gluconate and 3.0g centrimole. Dettol is an antiseptic widely used in homes and healthcare settings for various purposes including disinfection of skin, objects and equipments, as well as environmental surfaces. With prior cleaning before application, the number of microorganisms colonizing the skin and surfaces are greatly reduced (8, 9). Omo and Ariel are detergents which are surfactants or a mixture of surfactants with cleaning properties in dilute solutions. They are used for laundry, fuel additives and dish washing. Detergents have been added into different disinfecting solutions to lower their surface tension and to enhance their antibacterial effects (10, 11).

Many household cleaners have been found to be effective against bacteria when used properly, but many times they are not properly used. These can cause mutation in the genetic make-up of the organisms making them to be resistant to that environment because of their high reproduction rate and transfer of resistant genes. Concern is growing over the use of household cleaning and hygiene products labelled as antibacterial as a result of laboratory data showing a link between exposure to ingredients in these products, particularly household agents, and emergence of antimicrobial drug resistance (1, 12, 13). This study aimed to determine the efficacy of some household cleaning agents against clinically relevant bacterial species.

MATERIALS AND METHODS

Collection of samples

Household cleaning agents were purchased from the market and local stores. The products were stored in the dark at room temperature and prepared at their recommended use dilution in sterile distilled water on the day of the evaluation. All products were tested within the specified shelf-life. The household cleaning agents used in this study include the following dettol, salvon, jik, harpic, omo and ariel.

Bacterial strains and culture conditions

Bacterial species were selected because they are specifically found in the home and hospital environments (*Staphylococcus aureus*, *Klebsiella pneumonia, Streptococcus pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa*). Prior to experimental use, cultures were initiated from single colonies and grown in trypticase soy broth for 48 h at 37^{o}C. Log-phase cultures, used as seed in disinfection studies, were obtained by inoculating 49mL of trypticase soy broth with 1.0mL of a 48 h culture, then incubating for 5 h at 37^{o}C.

Antibiotic susceptibility testing

All bacterial isolates were tested against a panel of antibiotics. Antibiotic susceptibility testing was done in accordance with the description of Bauer *et al*. (14) as recommended by the Clinical and Laboratory Standards Institute (15) using antibiotics discs Amoxicillin (30µg), Augmentin (30µg), Gentamycin (10µg), Pefloxacin (30µg), Tarivid (10µg), Streptomycin (30µg), Septrin (30µg), Chloram- phenicol (30µg), Sparfloxacin (30µg), Ciprofloxacin (10µg), Rifampin (30µg), Erythromycin (30µg), Ampiclox (30µg), Zinnacef (10µg). Determination of the resistance or susceptibility profile of the isolates was performed by measuring zones of inhibition and comparing with the interpretative chart to determine the sensitivity of the isolates to the antibiotics.

Determination of antibacterial activity on household cleaning agents

The antibacterial activity was determined by using a modified National Committee for Clinical Laboratory Standards (NCCLS) agar well dilution method (16). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (1.5×10^{8}cfu/mL). Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton

agar (Oxoid). Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 50µL of the respective household cleaning agents at 5.0mg/mL were inoculated into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C. The plates were observed for zones of inhibition after 24 h.

Determination of Minimum Inhibitory Concentrations (MICs)

MICs were assessed by using a modified NCCLS agar dilution method (16). Plates containing Mueller Hinton agar (Oxoid) were prepared by using twofold increasing concentrations of respective household cleaning agents (0.156-5.0mg/mL). Approximately 10^8 CFU of each logarithmically grown isolate was applied, and the inoculated plates were incubated aerobically for 24 h at 35°C. The lowest dilution that showed no visible growth indicated the MIC.

RESULTS

Omo and harpic exhibited zones of inhibition for *Staphylococcus aureus* of 23.7 ± 0.01 mm and 33.7±0.05 mm respectively (Figure 1). Zone of inhibition for both omo and harpic was 32±0.01 mm (*Escherichia coli*) and 21.3±0.04 mm (*Klebsiella pneumonia*) respectively (Figure 1). Ariel had zone of inhibition for *Staphylococcus aureus* (27±0.02 mm) and for *Streptococcus pyogenes* (42 ±0.01 mm) (Figure 1).

Minimum inhibitory concentrations (MIC) of the selected household cleaning agents were prepared at various concentrations 5.0, 2.5, 1.25, 0.625, 0.312 and 0.156 mg/mL. Results for salvon antiseptics revealed MIC of 0.312 mg/mL for *Streptococcus pyogenes, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Jik antiseptics, revealed an MIC result of 0.312 mg/mL in *Klebsiella pneumonia.* Detol antiseptic gave an MIC of 0.625 mg/mL and 0.312 mg/mL in the case of *Staphylococcus aureus* and *Klebsiella pneumonia* respectively. Ariel and omo revealed MICs of 0.312 mg/mL for *Staphylococcus aureus.*

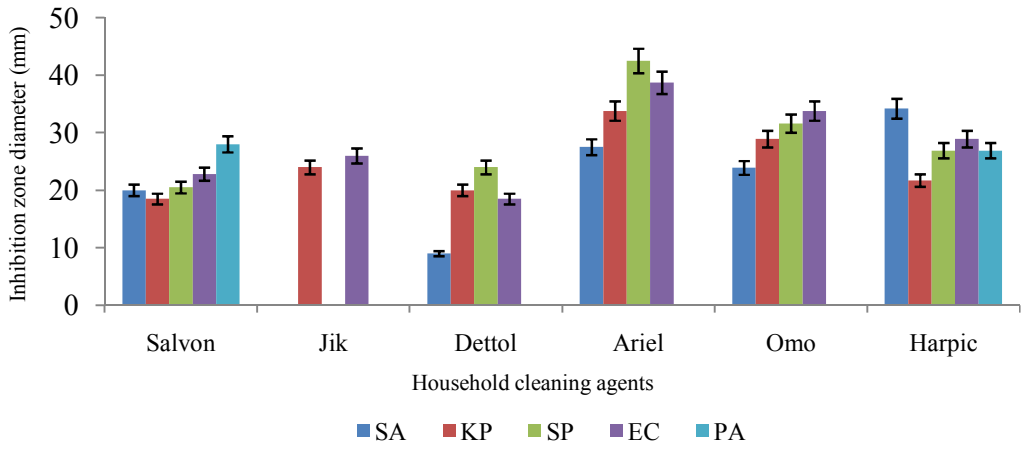

FIGURE 1: THE ANTIBACTERIAL ACTIVITY OF HOUSEHOLD CLEAING AGENTS ON SELECTED BACTERIA

Legend: SA-*Staphylococcus aureus*; KP- *Klebsiella pneumonia*; SP-*Streptococcus pyogenes*; EC-*Escherichia coli*; PA-*Pseudomonas aeruginosa*

TABLE 2: ANTIBIOTIC SUSCEPTIBILITY PROFILE OF BACTERIAL ISOLATES

Gram negative	AM	AU	CN	PEF	OFX	STR	SXT	CH	SP	CPX
K. pneumoniae	R	R	I	R	I	I	R	I	R	S
E. coli	R	R	S	S	I	R	R	R	R	I
P. aeruginosa	I	I	S	S	S	R	R	I	R	S
Gram positive	**AM**	**R**	**CPX**	**S**	**SXT**	**E**	**PEF**	**CN**	**APX**	**Z**
S. aureus	R	I	S	R	R	R	I	I	R	1
S. pyogenes	R	R	S	S	R	R	S	S	I	1

Legend: AM-Amoxacillin (30µg); AU-Augmentin (30µg); CN-Gentamycin (10µg); PEF-Pefloxacin (30µg); OFX-Tarivid (10µg); STR-Streptomycin (30µg); SXT-Septrin (30µg); CH-Chloramphenicol (30µg); SP-Sparfloxacin (30µg); CPX-Ciprofloxacin (10µg); R-Rifampin (30µg); E-Erythromycin (30µg); APX-Ampiclox (30µg); Z-Zinnacef (10µg)

DISCUSSION

Widespread contamination of environmental surfaces with commensal flora has been found in homes, hospital and child-care centres, especially in rooms housing diaper age children. These have been associated in person-to-person transmission of enteric pathogens (17). Results obtained for the antibacterial activities of the various household cleaning agents reveal several zones of inhibition obtained for each of the bacterial isolates employed in this study. The findings from the antibacterial activities is in accordance with the findings Ikegbunam *et al.* (10) where of all tested detergent, ariel was most effective against tested bacterial isolates. When bacteria are exposed to sub-lethal levels of biocides, only minor cell damage is caused.

The consequences of that may include changes in their phenotype and induction of gene expression, giving rise to a more resistant population. Resistance mechanisms are the means that living organisms have to respond to continuously changing environment in order to survive (18). Gram-negative bacteria are generally less susceptible to biocides than Gram-positive species. Such resistance is likely to be intrinsic, due to outer membrane that acts as a protective barrier. Due to

the capacity of surviving in unfavourable environmental conditions and to the high resistance to antibiotic agents, antiseptics and disinfectants, bacteria species continues to be an important pathogen in hospital acquired infections, mainly respiratory and urinary infections (19).

Scientific evidence supports the use of disinfectants as part of a program to control infectious disease by interrupting transmission through surface contamination. Their use in healthcare facilities is recommended by the Centres for Disease Control and Prevention (20), Occupational Safety and Health Administration and Professional Organizations such as the Association for Professionals in Infection Control and Epidemiology (8). Disinfectants are also used in child-care centres, extended-care facilities, restaurants, and the domestic home as part of an effort to control transmission of infectious diseases. The use of disinfectants on contaminated surfaces has been cited as a means to reduce or prevent the spread of gastrointestinal or respiratory pathogens.

The emergence of resistant microorganisms in hospitals and the community is causing problems for both the treatment of patients and infection control. Organisms of particular concern include methicillin-resistant *Staphylococcus aureus*, glycopeptide resistant enterococci and extended spectrum beta-lactamase producing *Klebsiella* (21). Environmental contamination has been demonstrated to play an important role in the transmission of certain nosocomial pathogens, including vancomycin resistant *Enterococcus* species, methicillin-resistant, *Staphylococcus aureus,* and especially the hospital associated *Clostridium difficile* (22). Careful studies using molecular analysis have suggested for these pathogens, environmental contamination has contributed to transmission between individuals.

Many human pathogenic viruses and bacteria may survive in a sufficient dose and for an appropriate duration to serve as a source of human exposure. In experimental trials, disinfection of environmental surfaces has been shown to decrease or eliminate potential pathogens and thereby decrease or eliminate acquisition of disease (23). Antibiotic sensitivity test demonstrated by using panel

standard antibiotics against bacterial isolates (Table 1). The alarming worldwide increase of bacterial resistance to antibiotics threatens their chemotherapeutic application leading to high mortality and morbidity in communities affected by epidemics or endemic infections. Since some of the resistant factors are also transferable to sensitive bacteria, frequent assessment of antimicrobial activity of commonly used antibiotic is desirable (24). Our results strongly suggest that the members of the bacterial isolates were significantly resistant and show multi-drug resistance with respect to antibiogram characteristics.

Our data demonstrate that currently available home disinfectants were moderately activity against potentially pathogenic bacteria likely to contaminate home environmental surfaces. Since the efficacy of commercial disinfectants for use in the home has been demonstrated, a controlled trial should be undertaken to determine if routine disinfection of home environmental surfaces will lead to decreased infection rates among household members.

REFERENCES

1) Levy SB. Antibacterial household products: cause for concern. *Emerging Infectious Diseases,* 2001; 7(Supplementary): 512-515.
2) Bhat, PR., Prajna, PS., Menezez, VP., Shetty P. Antimicrobial activities of soap and detergents. *Advances in Bioresearch,* 2011; 2: 52- 62.
3) Saha, AK., Haque, MF., Karmaker, S., Mohanta SK. Antibacterial effects of some antiseptics and disinfectants. *Journal of Life and Earth Science,* 2009; 3-4; 19-21.
4) Larson, EL., Morton HE. Alcohols in disinfection, sterilization and preservation, 4th edition. Block, S.S. (Ed.). Philadelphia. 1996: 191-203.
5) Russel, AD., Russel NJ. Biocides: activity, action and Resistance. *General Microbiology Journal,* 1995; 53: 327-365.
6) Russell AD. Activity of biocides against mycobacteria. *Journal of Applied Bacteriology Symposium Supplement,* 1996; 81: 87-101.
7) Tortora, GJ., Berdell, RF., Case CL. *Chemical methods of microbial control. In: Microbiology*. Benjamin Cumming Publishing Company, California, USA. 1998: 191.
8) Rutala WA. APIC guideline for selection and use of disinfectants. *American Journal of Infection Control,* 1996; 24: 313-342.
9) Gardener JS. Guideline for isolation precautions in hospitals. *Infections Control in Hospital and Epidemiology,* 2002; 17: 53-80.
10) Ikegbunam, MN., Metuh, RC., Anagu, LO., Awah NS. Antimicrobial activity of some cleaning products against selected bacteria. *International Research Journal of Pharmaceutical and Applied Sciences,* 2013; 3: 133-135.
11) Wang, Z., Shen, Y., Ma, J., Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. *Journal of Endodontics,* 2012; 38: 948-953.
12) Levy SB. Antibiotic and antiseptic resistance: impact on public health. *Pediatric Infectious Disease Journal*, 2000; 19(Supplementary): S120-122.
13) Aiello, AE., Larson E. Antibacterial cleaning and hygiene products as an emerging risk factor for antimicrobial drug resistance in the community. Lancet Infectious Disease, 2000; 3: 501-506.
14) Bauer, AW., Kirby, WMM., Sheris, JC., Turck M. Antibiotics susceptibility testing by standardized single disk method. *American Journal of Clinical Pathology,* 1966; 45: 493-496.
15) CLSI (Clinical Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing; 15th informational supplement, M100-S15, vol. 25, no. 1. Clinical and Laboratory Standards Institute Wayne, PA: CLSI 2005:
16) NCCLS (National Committee for Clinical Laboratory Standards). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa. 2003:
17) Laborde, DJ., Weigle, KA., Weber, DJ., Kotch JB. Effect of faecal contamination on diarrheal illness rates in day-care centers. *American Journal of Epidemiology,* 1993; 138: 243-255.
18) Araujo, P., Lemos, M., Mergulhao, F., Melo, L., Simoes M. Antimicrobial resisitance to disinfectants in biofilms. *Science against microbial pathogens.* Mendez-Vilas (Ed.). Porto, Portugal. 2011: 826-834.
19) Lotfipour, F., Nahaei, MR., Milani, M., Javaherzadeh, V., Omranic, A., Attar N. Antibacterial activity of Germicide-P: A Persulfate based detergent/disinfectant on dome hospital isolates. *Iranian Journal of Pharmaceutical Sciences*, 2006; 2: 225-230.
20) Anderson, RL., Carr, JH., Bond, WW., Favero MS. Susceptibility of vancomycin-resistant enterococci to environmental disinfectants. *Infections, Control, Hospital and Epidemiology,* 1997; 18: 195-199.
21) French, GL., Phillips I. Antimicrobial resistance in hospital flora and nosocomical infection. *Hospital Epidemiology and Infection Control*. Williams, B. and Wilkins, B. (Eds.). May-Hall. 1996: 980-990.
22) Rutala, WA., Barbee, SL., Aguiar, NC., Sobsey, MD., Weber DJ. Antimicrobial activity of home disinfectants and natural products against

23) potential human pathogens. *Infection Control and Hospital Epidemiology*, 2000; 21: 33-38.
24) Weber, DJ., Rutala WA. Role of environmental contamination in the transmission of vancomycin-resistant enterococci. *Infections Control in Hospital and Epidemiology*, 1997; 18: 306-309.
25) Igbinosa, EO., Obi, LC., Tom, M., Okoh AI. Detection of potential risk of wastewater effluents for transmission of antibiotic resistance from *Vibrio* species as a reservoir in a peri-urban community in South Africa. *International Journal of Environmental Health Research*, 2011; 21: 402-414.

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A COMPARATIVE ASSESSMENT OF PUBLIC AND PRIVATE DOTS LABORATORIES IN THE LAGOS STATE TB CONTROL PROGRAMME

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RUNNING TITLE: A COMPARATIVE ASSESSMENT OF MYCOBACTERIUM LABORATORIES IN LAGOS STATE

ABSTRACT

Background: The purpose of the laboratory services within the framework of the NTP is to provide bacteriologic evidence for the diagnosis, follow-up of TB patients and to document cure at the end of treatment. However to be fully functional, laboratory commodities should be available as needed. This study compared the laboratory hygiene practices and availability of laboratory equipment and other consumables for making diagnosis of TB in public and private DOTS laboratories in Lagos State.
Methods: A descriptive comparative cross sectional study comparing availability of commodities in public and private laboratories involved in TB services in Lagos State.
Results: Seventeen DOTS laboratories and 34 laboratory scientist/technician were recruited for this study. About three quarter and two thirds of the public and private DOTS laboratories respectively had reagents for smear microscopy. A significantly higher proportion of the public DOTS laboratories had separate area for TB work and separate table for smear preparation (p <0.05). A higher proportion (71.4%) of the laboratory scientist/ technicians at the public compared with 38.5% of those at the private DOTS laboratories had good knowledge of the laboratory diagnosis of TB.

Conclusion: Laboratories involved in TB service are not functioning optimally and need to be strengthened.

Key Words: Laboratory, DOTS, Consumables, Hygiene.

UNE EVALUATION COMPARATIVE DES LABORATOIRES DE DOTS PUBLICS ET PRIVES DANS LE PROGRAMME DE LUTTE CONTRE LA TUBERCULOSE DANS L'ETAT DE LAGOS.

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TITRE COURANT : UNE EVALUATION COMPARATIVE DES LABORATOIRES DE MYCOBACTERIUM DANS L'ETAT DE LAGOS.

RESUME
Contexte : L'objet des services des laboratoires dans le cadre du NTP est de fournir les preuves bactériologiques pour le diagnostic, de suivre les patients tuberculeux et de documenter la guérison a la fin du traitement. Toutefois, pour être pleinement fonctionnel, les produits de laboratoire devraient être disponibles au besoin. Cette recherche comparait les pratiques hygiènes de laboratoire et la disponibilité des équipements de laboratoire et autres consommables pour faire le diagnostic de la tuberculose aux laboratoires de DOTS publics et prives dans l'État de Lagos.
Méthodes : Une étude transversale comparative et transversale descriptive comparant la disponibilité des produits aux

laboratoires publics et prives concernes a fournir aux services tuberculeux dans l'état de Lagos. Résultats : Dix – sept laboratoires de DOTS et 34 scientifiques/techniciens ont été recrutés pour cette recherche. Environ trois quarts et deux tiers des laboratoires de DOT publics et prives ont eu réactif respectivement pour la microscopie des frottis. Une proportion significativement plus élevée des laboratoires de DOTS publics a eu un espace séparé pour les travaux de la tuberculose et une table séparée pour la préparation des frottis (p<0,05). Une proportion élevée(71,4%) des scientifiques/techniciens des laboratoires publics comparativement a 38,5% de ceux des laboratoires de DOTS prives avaient une bonne connaissance de diagnostic laboratoire de la tuberculose. Conclusion : Les laboratoires qui ont concerné a fournir les services tuberculeux ne fonctionnent pas de la façon optimale et doivent être renforcés.
Mots– clés : Laboratoire, DOTS, consommables, Hygiène.

INTRODUCTION

Sputum smear microscopy for acid-fast bacilli (AFB) is of vital clinical and epidemiological importance in the diagnostic process for tuberculosis (TB).(1) It is the most cost-effective method of diagnosing TB patients. The detection of AFB in pulmonary secretions identifies those patients with the greatest potential for transmission of mycobacterium tuberculosis.(2) Sputum smear microscopy offers the triple advantages of speed, simplicity and low cost.(3) The purpose of the bacteriological services within the framework of the National Tuberculosis Programme (NTP) is to diagnose and follow-up of TB patients as well as document cure at the end of treatment.(3) This makes the laboratory to be considered as one of the pillars of the NTP.(4) However to be fully functional, a laboratory service requires that laboratory commodities are available as needed. These commodities include equipment and supplies, such as laboratory reagents, diagnostic kits and other consumables.(5)

Studies from Ghana, India, Kinshasa and Uganda has shown that the laboratory services was the weakest component of the TB programme because of poor smear preparation, poor staining technique, poor documentation of smear results, lack of feedback from the NTP and poor availability of laboratory reagents and supplies (6-10) In addition some directly observed treatment short course (DOTS) laboratories in Ghana, Uganda and Iran were found to be in a deplorable and deteriorating conditions and laboratory hygiene requirements were poor.(6,10,11)

The Lagos State TB and Leprosy control programme (LSTBLCP) commenced in 2003 with the collaboration between the State Government, International Union against TB and Lung Diseases (IUATLD), World Health Organization (WHO), Canadian International Development. At the end of 2012, there were 218 DOTS centers and 55 microscopy centers in both the public and private DOTS facilities. One of the challenges of the LSTBLCP was the shortage of DOTS laboratories and personnel.(12) Given the important role laboratories play in the TB control programme, it is necessary to assess their functionality, therefore, this study compared the availability of laboratory equipment and other consumables for making diagnosis of TB and the laboratory hygiene requirements available at the public and private DOTS laboratories.

METHODS

Study Setting

Lagos State is located in South west Nigeria and it is the commercial nerve centre of the country. There are 20 Local Government Areas (LGAs), and 37 Local Council Development Area (LCDAs) in the state. A report showed that the current population of Lagos State is about 21 million.(13) DOTS laboratories could either be located in a DOTS facility or serve only as microscopy center where only smear microscopy is done. However not all DOTS center have a laboratory. TB patients managed in a DOTS facility without a laboratory were usually referred to the nearest DOTS laboratory for smear microscopy. Each DOTS facility is expected to have a list of the microscopy centers under the LSTBLCP. The Lagos State TB and Leprosy Control Programme supplies reagents and consumables for sputum AFB freely to microscopy centers under the programme.

Study design

A descriptive comparative cross sectional study was conducted to assess the availability of laboratory equipment and other consumables for laboratory diagnosis of TB in Lagos State between September 2011 and October 2012. A sampling frame of DOTS facilities provided by the Lagos State programme officer was used to select, 34 DOTS centers that were involved in the DOTS programme for at least 2 years using the systematic random sampling technique. All the seventeen DOTS laboratories in the selected DOTs facilities and all consenting laboratory scientists/ technicians working in the selected laboratories were recruited into the study.

A structured pretested questionnaire and a check list were used to obtain data. The questionnaire was administered on all consenting laboratory scientists/technicians working in the selected DOTS laboratories. It was used to collect data on knowledge of the staining method used in the laboratory identification of mycobacterium tuberculosis and knowledge of safety measures. A

check list was used to assess the availability of consumables and medical supplies in the laboratories and also the laboratory hygiene requirements put in place to protect the health workers working in the laboratory from TB infection.

Evaluation of the knowledge of laboratory scientists/technicians

A scale was formed from 20 questions assessing the knowledge of TB diagnosis and safety measures. Each correct answer to each question was scored 1 mark while each wrong answer was scored zero. Using the criteria established in a study conducted in Lagos among nurses,(14) laboratory scientist/technician who obtained scores of less than 50% were classified as having "Poor" knowledge, those who obtained scores of 50% - 74.9% were classified as having "Fair" knowledge while those whose scores were 75% and above were classified as having "Good" knowledge.

Ethical clearance was obtained from the health research and ethics committee of the Lagos State University Teaching Hospital. In addition permission was obtained from the Commissioner for Health of Lagos state to collect data from the DOTS facilities. Written consent was obtained from the respondents before administration of questionnaires.

Data analysis

Data was entered and analysis was done using the Statistical Package for Social Sciences (SPSS) version 19. Mean and standard deviation of numerical variables were determined while percentages of numerical and categorical data were determined. Chi square and Fisher's exact test were used to compare categorical variables where applicable. The confidence interval was set at 95% for the statistical tests.

RESULTS

Seventeen DOTS laboratories (11 public and 6 private) and 34 laboratory scientists/technicians (21 public and 13 private) were recruited for this study. Table 1 shows the laboratory equipment and consumables available at the public and private DOTs laboratories at the time of assessment. Although all the DOTS laboratories studied had functional microscopes, about three quarters and two thirds of the public and private DOTS laboratories respectively had reagents (carbol fuschin, acid alcohol and methelene blue) for smear microscopy.

Table 1: Laboratory equipment and consumables available at DOTS laboratories

Laboratory equipment and consumables available	DOTS laboratories		χ^2	p
	Public n = 11(%)	Private n = 6 (%)		
Binoculars Microscope	10 (90.9)	6 (100.0)	0.58	1.000[x]
Flourescent Microscope	1 (9.1)	0 (90.0)		
New Slides	4 (36.3)	4 (66.7)	1.43	0.3348[x]
Sputum containers	11 (100.0)	4 (66.7)	4.16	0.0415[x]
Carbol fushin	8 (72.7)	4 (66.7)	0.07	1.000[x]
Acid alcohol	8 (72.7)	4 (66.7)	0.07	1.007[x]
Methylene blue	8 (72.7)	4 (66.7)	0.07	1.007[x]
Immersion oil	7 (63.6)	4 (66.7)	0.02	1.000[x]
Cotton wool	8 (72.7)	6 (100.0)	1.99	0.5417[x]
Spirit lamps	10 (90.9)	6 (100.0)	0.58	1.000[x]
Water	8 (72.7)	6 (100.0)	1.99	0.5147[x]
Wooden rack	8 (72.7)	5 (83.3)	0.24	1.000[x]
Request form	9 (77.8)	4 (83.3)	0.50	0.5840[x]
Labeling materials	11 (100.0)	5 (83.3)	1.95	0.3529[x]
NTP lab register	11 (100.0)	5 (83.3)	1.95	0.3529[x]

Note: x = Fisher's Exact test

A higher proportion of the private DOTS laboratories (66.7%) compared with 36.3% of the public DOTS laboratories had new slides (p = 0.3348). About 83% of the private DOTS laboratories had labeling materials for the sputum cups. A significantly lower proportion of the private DOTS laboratories (66.7%) had sputum containers (p = 0.0415)

A significantly higher proportion of the public DOTS laboratories had a separate area for TB work and separate table for smear preparation (p <0.05). Less than half of the public and private DOTS laboratories decontaminated sputum cups before disposal. About 73% of the public and 67% of the private DOTS laboratories had waste bin with covers as shown in Table 2.

The majority of the laboratory scientists/technicians at the public and private DOTS laboratories were between 35 and 44 years. The mean age of the public laboratories scientists/technicians were significantly higher (39.0 ± 3.8 years) than those of the private laboratories (35.5 ± 5.2 years). A significantly higher proportion (76.9%) of the laboratory scientists/technicians at the private DOTS laboratories had no previous training (p < 0.05) as shown in Table 3

Table 4 shows that a higher proportion of the laboratory scientists/ technicians at the public DOTS laboratories had correct knowledge of the quickest diagnostic method for TB, number of sputum specimen required to make the diagnosis of TB, the mode of sputum collection for AFB, the uses of smear microscopy, the staining methods for AFB and the most appropriate place for the collection of sputum for AFB. About 14% of the laboratory scientists/technicians at the public and none at the private DOTS laboratories were aware that sputum container should be decontaminated before disposal. Table 5 shows that a higher proportion (71.4%) of the laboratory scientist/ technicians at the public compared with 38.5% of those at the private DOTS laboratories had good knowledge of the laboratory diagnosis of TB and disposal of sputa (p > 0.05).

TABLE LABORATORY HYGIENE REQUIREMENTS AVAILABLE AT DOTS LABORATORIES

Available laboratory hygiene	DOTS laboratories Public n=11 (%)	Private n = 6 (%)	χ^2	p
Separate area for TB work	10 (90.9)	2 (33.3)	6.20	0.028[x]
Separate table for smear preparation	11 (100.0)	1 (16.7)	12.99	<0.001[x]
Smear preparation done with the window opened	8 (72.7)	5 (83.5)	0.24	1.000[x]
Airflow in opposite direction to where smearing is performed	3 (27.3)	2 (33.3)	0.07	1.000[x]
Decontamination of used slides	0 (0.0)	1 (16.7)	1.95	0.3529[x]
Decontaminate used sputum cups	5 (45.5)	2 (33.3)	0.24	1.000[x]
Wearing of lab coats in the lab	11 (100.0)	1 (16.7)	12.99	0.0009[x]
Availability of Disinfectants	10 (90.9)	6 (100.0)	0.58	1.000[x]
Cleaning of work area after smearing	10 (90.9)	5 (83.3)	0.21	1.000[x]
Availability of waste bin with cover	8 (72.7)	4 (66.7)	0.07	1.000[x]

Key [x] Fisher's exact 2:

TABLE 3: SOCIO DEMOGRAPHIC CHARACTERISTICS OF LABORATORY PERSONNEL AT DOTS LABORATORIES

Variable	DOTS laboratories		χ2	p
	Public n = 21 (%)	Private n = 13 (%)		
Age group				
<40	15 (71.4)	11 (74.6)	0.78	0.4438ˣ
40 years and above	6 (28.6)	2 (15.4)		
Mean age	39.0 ± 3.8	35.5 ± 5.2		
Gender				
Male	4 (19.0)	6 (46.2)	2.84	0.1297ˣ
Female	17 (81.0)	7 (53.8)		
Cadre				
Laboratory scientist	11(52.4)	4 (30.8)	1.52	0.2174
Laboratory technician	10 (47.6)	9 (69.2)		
Had previous training				
Yes	16 (76.2)	3 (23.1)	9.19	0.002
No	5 (23.8)	10 (76.9)		

Note: ˣ = Fisher's exact

TABLE 4: KNOWLEDGE OF LABORATORY SCIENTISTS/TECHNICIANS ON THE LABORATORY DIAGNOSIS OF TB AND DISPOSAL OF SPUTA

Knowledge Parameters	DOTS laboratories		χ2	p
	Public n = 21 (%)	Private n = 13 (%)		
Fastest diagnostic method for TB				
Wrong response	2 (9.5)	5 (38.5)	4.11	0.057ˣ
Microscopy (c)	19 (90.5)	8 (61.5)		
Number of sputum specimens required to make diagnosis of TB				
Wrong response	0 (0.0)	1 (7.7)	1.66	0.3825ˣ
Two or three (c)	21 (100.0)	12 (92.3)		
Sputum collection schedule				
Wrong response	8 (38.1)	7 (53.8)	0.81	0.3687
SMS or SM (c)	13 (61.9)	6 (46.2)		
Uses of microscopic diagnosis in TB management				
Wrong response	3 (14.3)	2 (15.4)	0.01	1.000ˣ
Diagnosis and follow up (c)	18 (85.7)	11 (84.6)		
Method for Staining				
Wrong response	0 (0.0)	1 (7.7)	1.60	0.3825ˣ
ZN or AS	21 (100.0)	12 (92.3)		
Best area for sputum collection				
Wrong response	4 (19.0)	4 (30.8)	0.61	0.6795ˣ
Open air (c)	17(81.0)	9 (69.2)		
Best disposal method for sputum containers				
Wrong response	18 (85.7)	13 (100.0)	2.04	0.2701ˣ
Burning after Disinfection (c)	3 (14.3)	0 (0.0)		

Foot Note: ˣ = Fisher's Exact, C = correct, ZN = Ziehl Neelson, AS = Auramine Stain, SMS = Spot Morning Spot, SM = Spot Morning

TABLE 5: KNOWLEDGE OF LABORATORY SCIENTISTS/TECHNICIANS ON LABORATORY DIAGNOSIS OF TB AND DISPOSAL OF SPUTA

Variable	Laboratory scientist/personnel		x2	p
	Public DOTS n = 21 (%)	Private DOTS n = 13 (%)		
Good Knowledge	15 (71.4)	5 (38.5)	3.60	0.057
Fair knowledge	6 (28.6)	8 (61.5)		

DISCUSSION

Assessment of laboratories in the selected DOTS facilities showed that about a third of the laboratories assessed lacked reagents and slides for performing smear microscopy. In addition cotton wool, staining rack, spirit lamps and water were not available in a third of the public DOTS laboratories at the time of assessment. Although the LSTBLCP supplies the DOTS laboratories with reagents, proper coordination and consistency in the supply of these reagents cannot be ascertained. This could lead to the inability of some TB patients to do sputum test in some of the DOTS laboratories which may result in the spread of TB in the community and also deterioration in the health condition of undiagnosed TB patients. A study showed that TB laboratory services was the weakest component of the TB control strategy in Ghana.(6) Shortages of reagents and faulty microscopes in DOTS laboratories have also been reported in Ghana and India(7,8) which consequently had a negative effect on the quality of sputum microscopy.(8) In order to achieve the global targets of 85% cure rate,(15) a lot has to be put in place to make laboratory services functional and efficient.

This study showed that there were poor laboratory hygiene requirements at the public and private DOTS laboratories. Although the private DOTS laboratories were allowed to charge a token fee as service charge because reagents and consumables for sputum AFB are freely supplied to these facilities by the LSTBLCP, they may however compromise in provisions of the hygiene requirement in the laboratory in order to recover cost because they are profit driven. In addition poor funding of the health sector may also contribute to the poor laboratory hygiene practices observed in the public DOTS laboratories.

A higher proportion of the laboratory scientist/ technicians in the public DOTS laboratories assessed had good knowledge on the laboratory diagnosis of TB and disposal of sputa. Previous training by the LSTBLCP may be responsible for this finding. This study demonstrated that a significantly higher proportion of the laboratory scientists/technicians at public DOTS laboratories assessed had previous training compared with 23.1% of those at the private DOTS laboratories (p =0.002).

Limitations of study

This study was part of a study that assessed the tuberculosis management in public and private facilities providing DOTS treatment in Lagos state. The small number of the DOTS laboratories and laboratory scientist/technicians studied is a limitation. This was due to financial challenges. A large scale study assessing the laboratory services of the LSTBLCP in Lagos state is recommended.

Conclusion

There was suboptimal availability of laboratory reagents and consumables for making the diagnosis of TB and poor laboratory hygiene practices in both the public and private DOTS laboratories. The need to increase supplies of reagents and consumables for making the diagnosis of TB in Lagos State

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REFERENCES

1. American Thoracic Society/Centers for Disease Control and Prevention. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med 2000; 161: 1376–1395.

2. Behr M A, Warren S A, Salamon H. Transmission of Mycobacterium tuberculosis from patients smear- negative for acid-fast bacilli. Lancet 1999; 353:444–449.

3. World Health Organization. Laboratory services in tuberculosis control. Part II.Microscopy. WHO/TB/98.258. Geneva, Switzerland: WHO, 1998.
4. Mundy C J F, Harries A D, Banerjee A, Salaniponi F M, Gilks, C F, Squire S B. Quality assessment of sputum transportation, smear preparation and AFB microscopy in rural district in Malawi. Int J Tuberc Lung Dis 2002; 6: 47–54.
5. The TB coalition for technical assistance. Guidelines on specification for managing TB laboratory equipment and supplies. Available from www.tbcta.org/guidelinesandspecificationsforTBlab supplies.pdf (accessed 27th April 2012).
6. Ministry of Health. Review of the national tuberculosis control programme in Ghana. Report submitted by the Danish International Development Agency (DANIDA), 1998. Accra, Ghana: Ministry of Health, 1998.
7. Addo KK, Owusu-Darko K, Dan-Dzide M, Yeboah-Manu D, Ablordey A, Caulley P. Minamikawa M, Bonsu F, et al. Situation analysis of TB microscopy centres in Ghana. Int j tuberc lung dis.2006;10 (8):870–875.
8. Marija Joncevska. Laboratory assessment report Tajikistan. Project HOPE. Available from usaid.gov/pgf_docs/PNADP438.pdf (accessed 28 April 2011).
9. Van Rie A, Fitzgerald D, Kabuya G,. Van Deun A, Tabala M,. Jarret N, Behets F, and Bahati6 E. Sputum smear microscopy: Evaluation of Impact of training, microscope distribution, and use of external quality: Assessment Guidelines for Resource-Poor Settings. Journal of clinical microbiology 2008; 46(3):897–901.
10. Aziz M and Bretzel G. Use of a standardised checklist to assess peripheral sputum smear microscopy laboratories for tuberculosis diagnosis in Uganda. Int j tuberc lung dis 2002; 6 (4):340–349.
11. Parissa F, Mohammad R M, Foroozan M, Mahshid N, Mokhtar F, Salek S, Mohammad K, Mohammad A, Ahmad R B, Kiumars G, Mohammad F, Abol- Hassan Z Z, Moslem B and Ali AV. The results of three years surveillance on sputum smear microscopy in 285 district and regional tuberculosis laboratories of Iran. Tanaffos 2003; 2(5):29-36.
12. Lagos State Ministry of Health. Overview of Lagos State Tuberculosis control Programme. LSMOH 2012. (Unpublished)
13. Lagos State Government. Population of Lagos State. Available at www.lagosstate.gov.ng/pagelinks.php?p (Accessed 23rd November 2013.)
14. Odusanya OO, Tayo OO. Breast cancer knowledge, attitudes and practice among nurses in Lagos, Nigeria. Acta Oncol 2001; 40(7):844-8.
15. Resolution WHA 53.1. Stop Tuberculosis Initiative. Fifty-third World Health Assembly. Geneva. Resolution and decisions. World Health Organization 2000 (WHA53/2000/REC/1)