ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

MAY 2014 **VOLUME 15** **NUMBER 2**

Official Publication of the African Society for Clinical Microbiology

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY (ISSN 1595-689X)

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1409 http://www.ajol.info/journals/ajcem
COPYRIGHT 2014 http://dx.doi.org/10.4314/ajcem.v15i2.1
AFR. J. CLN. EXPER. MICROBIOL. 15(@): 51-59

ACUTE RESPIRATORY INFECTIONS IN THE MIDDLE-BELT REGION OF NIGERIA

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ABSTRACT

Background: ARI continues to be a leeding cause of death among children globally beyond the year 2000. Close 12 million children under the age of 5years die each year in the developing countries, mainly from preventable causes and approximately 2.28 million (19%) were due to acute respiratory infections (ARI). It therefore became necessary to assess the present status of the disease in Nigeria to mastermind workable plans for reducing the mortality and morbidity burden.

Methods: A designed pro-forma was used to collect and collate information from mothers or direct care givers of children at both hospital and community levels relating to family background, home setting, anthropometry, clinical presentation of ARI, previous medications, investigations, complications and outcomes of illness.

Results: A total of 163 children were recruited for the study. One hundred and six had moderate and severe form of ARI while 57 had mild form. The in-patients accounted for 15.2% of all the admission within the study period.All children were under 12 years of age with male preponderance. Fast breathing, Tarchypnoea, Cough and Fever were the leading ways of presentations. The immunization coverage of study population by various antigens in the EPI were poor. Majority of the hospital children had pre-consultation antibiotics while none of the children from the rural community had pre-recruitment antibiotics. Streptococcus pneumoniae and Staphylococcus aureus were the leading organisms isolated with good sensitivity to Quinolones, Gentamycin and Cephalosporins. Heart failure was the leading complications. Mortality was 12.3% among the hospitalized patient and none among the community children.

Conclusion: It was concluded that ARI is still a major cause of morbidity and mortality among children with opportunity for burden reduction.

Keywords: Acute Respiratory Infection, present outlook, burden

INFECTIONS RESPIRATOIRES AIGUËS DANS LA REGION DE MOYEN - CENTRE DU NIGERIA

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RÉSUMÉ

Contexte:Les infections respiratoires aiguës (IRA) continuent d'être une cause de décès chez les enfants dans le monde au-delà de l'année 2000. Près de 12 millions d'enfants de moins de cinq ans meurent chaque année dans les pays en voie de développement principalement des causes évitables et environ 2,28 millions (19%) sont dus à des IRA. Il devient donc nécessaire d'évaluer l'état actuel de la maladie au Nigeria pour orchestrer des plans réalisables visant à réduire la mortalité et la charge morbide.

Méthodes : Un pro - forma conçu a été utilisé pour recueillir et rassembler les informations de la mère ou soignants directe des enfants, tant au niveau de la communauté relatives aux antécédents familiaux, l'établissement d'accueil, l'anthropométrie, la présentation clinique des IRA, les médicaments précédents, les enquêtes, les complications et l'impact de la maladie. Résultats: Un total de 163 enfants ont été recrutés pour l'étude. Cent six avaient la forme modérée à sévère de l'IRA tandis que 57

avaient la forme bénigne. Les patients hospitalisés ont représenté 15,2 % de l'ensemble de l'admission pendant la période de l'étude. Tous étaient de moins de 12 ans avec une prédominance masculine. La respiration rapide, la tachypnée , la toux et la fièvre étaient les principaux moyens de présentations. La couverture vaccinale de la population étudiée par divers antigènes du PEV était très basse. La majorité des enfants de l'hôpital avait des antibiotiques pré- consultation alors qu'aucun des enfants de la communauté rurale n'avaitd'antibiotiques pré- recrutement. *Streptococcus pneumoniae* **et** *Staphylococcus aureus* **étaient les principaux organismes isolés avec une bonne sensibilité aux quinolones, gentamycin et céphalosporines. L'insuffisance cardiaque était la complication conséquente. La mortalité était de 12,3 % chez les patients hospitalisés et aucun parmi les enfants de la communauté.**

Conclusion : Il a été conclu que l'IRA est encore une cause majeure de morbidité et de mortalité chez les enfants avec possibilité de réduction de la charge.

Mots-clés: infection respiratoire aiguë, perspectives actuelles, la charge

INTRODUCTION

Nearly 12 million children under the age of 5years die each year in the developing countries, mainly from preventable causes. Of these approximately 2.28million (19%) were ascribe to acute respiratory infections (ARI) (1,2). A more recent 1997 report from the world health organization (WHO) indicated a rising trend with as high as17.2million potentially preventable deaths in developing countries .ARI especially that involving the lower respiratory tract was reportedly responsible for as many as 3.9million of these This figure is almost twice the number of death from malaria (3).

In the face of this death burden due to ARI, one major question that will need urgent answer is how optimally children with ARI were being treated or how appropriate is the empirical choice of antibiotics employed in ARI? If treatment is optimal and empirical antibiotic choice correct, then why this high ARI- associated mortality?

This present prospective survey was therefore designed to fill some of the gaps in our knowledge regarding the microbiological characteristics and anti-microbial sensitivity pattern of the bacterial pathogens associated with ARI / ALRI in the entire Paediatrics age spectrum and get data that will form a baseline at the beginning of a new millennium. It is expected that this will lead to better understanding of empirical antibiotics of choice in treatment of clinical cases, better understanding of areas of focus for the pharmaceutical company and drug-related Health Policy decisions, improvement in knowledge that will lead to better health education, increase in the knowledge of the causes, prevention and home-based treatment of diseases as contained in the Integrated Management of Childhood Illness and an overall reduction in ARI-related case fatality.

METHODS

A prospective study of ARI in children in both hospital and rural community was undertaken over a six consecutive month period. For the hospital-based study, the Emergency Paediatrics Unit (EPU) of the University of Ilorin Teaching Hospital was use where children were screened for ARI based on standard criteria. For community-based study Egbejila community, a rural community in Kwara State was used. A central place was used within the small community. Children were fished out from house-to-house after effective community mobilization was done. Also standard criteria were used to identify children with ARI standard criteria. A designed pro-forma was applied to collect and collate information from mothers or direct care givers of all children either at the Hospital or at the community levels relating to family background, home setting, anthropometry, clinical presentation of ARI, previous medications, complications and outcomes of illness All children who were diagnosed as having ARI had at least two of the following investigation carried out: throat swab, blood for cultures and plural aspirate. Those in which organism grew had their anti-microbial sensitivities determined. In those adjudged to require lung / pleural aspiration, a chest X-ray was requested as a prerequisite in addition to the usual investigations. Precautions to avoid procedure related complications as observed. Standard anti-microbial and supportive therapy was provided for all cases and those with complication were managed appropriately in a standard way.

Ethical considerations

Only selected patients with suspected pleural effusion had pleural aspirate. Also, blood culture was considered for only patient with evidence of systemic disease.

Patient recruitment

For the EPU patients had moderately severe and severe disease and needed hospitalization while those in the village were mild form of ARI. The criteria for entry included those with cough, fever, breathlessness, rhinorea or catarh, and chest-in-drawing. Specimen collected included Blood, Pleural aspirate, and Throat swab for culture.

Specimen collection

Blood cultures

Blood cultures were taken from peripheral veins in the upper extremities after alcohol skin disinfections. Samples were quickly transferred into bottles containing transport media for bacteria and transferred to the laboratory for processing (15).

Throat swabs

Sterile cotton tipped sticks were used to swab the pharynx. The cotton tips were then cut using a new

razor blade into a Stuart-medium containing culture bottles and transferred to the microbiology laboratory of the UITH Ilorin for processing.

Pleural aspirates

Skin was prepared using alcohol skin disinfectant. Pleural aspirates were taken using a 21G bore needle and 5mls syringes and transferred into sterile bottles for laboratory analysis.

Culturing of organisms

Standard laboratory procedures were followed (4).

Susceptibility testing

Plates were inoculated with the growing organisms. Antibiotics impregnated disk were then applied to the surface of the inoculated plates within 15minutes. This was done by hand using sterile forceps. The disks were then pressed down unto the agar with forceps giving 15mm space allowance from the edge of the plates and in between disks to forestall growth overlapping. Plates 150mm size had 12 disks while 100mm plates had 4 disks. Within 15minutes the plates were inverted and placed in incubator at 370c in 5% co2 atmosphere for 72hours. Standard laboratory procedures were followed for the microbiological analysis.

Additional Investigation

Chest radiographs and haematological screening were done in most hospitalized patients.

Data collection and handling

This was done using a questionnaire with 4 sections including the family background, a bio-data, home setting to evaluate predisposing factors, clinical situations that addressed presentations, interventions, compliance and outcome. The last section contains the antibiotics sensitivity. Data was entered in a computer, checked, cleaned and correct data before analysis was done using the EPI info version.

Intervention

All children found with ARI in the community were treated with an emperical antibiotics, sulphamethoxazole plus trimethoprim combination. Antipyretics and haematinics were given as necessary.

RESULTS

A total number of 163 children were recruited for the study comprising 106 from emergence pediatric unit (EPU) university of Ilorin teaching hospital (UITH) and 57 from Egbejila village, 55.8% were males 44.2% were females. Egbejila community has a total population of 735. Under –five years old children constituted 20% (147) of these 57(38.8%) developed acute respiratory infection (ARI) during the study period. EPU total admission for the study period was 699 out of which 106(15.2%) had ARI. Table II shows the monthly admission by sex and cases of ARI.

Severity of illness and diagnosis

Illness were mild in those recruited from the village but were moderately severe to very severe among those seen at the EPU who required hospitalization. The range of diagnosis was as on Table III.

The family characteristics of the study population

These are as shown in Table V. The fathers mean age for EPU was 33 years and 30.5 years for Egbejila village. Mothers mean ages were 28years in EPU and 25.5 years in Egbejila village. More than 94% of the respondents/ caregiver were married. Seventy-two (68%) in EPU were Moslems while 34(32%) were Christians whereas all (100%) were Moslem in the Egbejila village.

The fathers of the children in EPU were mostly civil servants 41(38.6%) but fathers were mostly farmers is the Egbejila village. Whereas, mothers in both EPU and Egbejila village were mostly traders. About 70% of the fathers in EPU had secondary or post – secondary level education while only about 9% had secondary and post-secondary level education Egbejila village.

Immunization coverage

The coverage of the following antigen were as follows BCG was, 51.8%, OPVo was 59.6%,while DPT1, DPT 2 and DPT3 coverage were 38.6%, 40.4% and 55.3%,respectively and Measles was 50.9%.

Pre-recruitment antibiotic use

For children admitted into the Emergence Paediatrics Unit (EPU) 90(85%) had taken one type of antibiotics or the other before cultured samples were taken. Twenty eight (31%) knew the specific antibiotics that was taken pre-recruitment of which 9 (32%) had Sultamycillin (sulbactam and ampicillin), 6 (21.4%) had Cotrimoxazole, 5 (17.8%) had Gentamycin, 2 (7.2%) had Ampiclox (ampicillin and cloxacillin), 2 (7.2%) had Chloramphenicol, 2 (7.2%) had Cefuroxime, 1 (3.6%) had Ampicillin and 1 (3.6%) Sulphathiazole. None of the children from Egbejila village had pre-recruitment antibiotics.

Microbiological investigations and bacterial causes of acute respiratory infection

Eighty blood samples were taken for cultures 10 (12.5%) grew microorganism. Ninety-five throat swabs were taken of which 18 (19%) grew microorganism. Pleural aspirate as taken for culture in 8 children out of which. 5 (62.5%) grew.

In all 183 specimens were cultured (Blood, throat swab and Pleural aspirate combined) of which 33 grew. This was a growth yield of 18%. The organisms were as follows: *Streptococcus pneumoniae* mainly from throat swabs were 9 (27.3%), *Staphylococcus aureus* mainly from blood culture were 4(12.1%), Coliforms mainly from blood culture were 4(12.1%), *Pseudomonas aeruginosa* mainly from throat swabs

were also 4(12.1%) *Streptococcus pyogenes* mainly from throat swabs were 3(9.1%). Others included *Staphylococcus epidermidis*, *Klebsiella pneumoniae* , Proteus vulgaris, *Escherichia coli* and *Acinetobacter*.

Clinical presentations of moderately severe and severe cases of ARI

Fast breathing was the leading presenting feature, present in all the subjects. Cough was present in 92 (86.8%), Fever in 77 (73.7%) and rhinorrhoea/ catarrh in 75 (70.2%).

Others were as on Table XII

Antibiotic susceptibility

The antibiotic susceptibility profile of the isolated organisms was a shown on Table XI. There were a total of 33 isolated tested against 17 different types of antimicrobial agents including; ofloxacin, ciprofloxacin, cefuroxime, ceftriazone, cefotaxime, ceftazidime, gentamycin, streptomycin, penicillin G, cloxacillin, Ampicillin, chloramphenicol, cotrimoxazole, collistin, tetracycline, erythromycin and azithromycin.

Complications

The complications of ARI were seen in 20 of 106 children with moderately severe and severe disease. Complications included Pleural effusion in 8(7.6%) patients, Febrile Convulsion also in 8(7.6%) patients and heart failure in 4(3.8%).

Outcome

Of the 106 cases of moderately severe and very severe cases of ARI admitted into the EPU, UITH, 13(12.3%) died. None of the 57 patients seen in Egbejila village with mild forms of ARI die.

TABLE I: DISTRIBUTION OF STUDY POPULATION BY SEX

SEX	EPU		EGBEJILA	
	No	%	No	%
Male	65	61.4	26	45.6
Female	41	38.6	31	54.4
Total	106	100	57	100

TABLE II: DISTRIBUTION OF STUDY POPULATION IN EPU BY MONTHLY ADMISSIONS, SEX AND MONTHLY ARI CASES

Month	Total No	Male		Female		ARI cases	
		No	%	No	%	No	%
February	101	60	59.4	41	40.6	23	22.8
March	102	59	57.8	43	42.2	24	23.5
April	99	66	66.7	33	33.3	6	6.1
May	108	63	58.3	45	41.7	11	10.2
June	124	61	49.2	63	50.8	19	15.3
July	165	84	50.9	81	49.1	23	13.9
Total	699	393	56.2	306	43.8	106	15.2

TABLE III: SPECIFIC DIAGNOSES AMONG ARI CASES IN BOTH EPU AND EGBEJILA VILLAGE

Diagnosis	No	%
Bronchopneumonia	50	28.8
Lobar pneumonia	5	3.1
Aspiration pneumonia	5	3.1
Bronchiolitis	5	3.1
URTI*	60	36.8
Measles	41	25.
Total	163	100

***57 cases from the rural community included here**

TABLE IV: THE FAMILY CHARACTERISTICS OF THE STUDY POPULATION

Characteristics		EPU		Egbejila	
Mean Age					
- Father		33years		30.5years	
- Mother		28years		25.5years	
Marital status		No	%	No	%
Mothers					
	Singles	6	5.7	1	1.8
	Married	100	94.3	54	94.7
	Widowed	0	0	2	3.5
Religion					
Christianity		34	32.0	0	0
Islam		72	68.0	57	100
Traditionalist		0	0	0	
Occupation					
Fathers					
Civil servants		41	38.6	3	5.3
Farming		6	5.7	30	52.6
Trading		20	18.9	4	7.0
Driving		8	7.6	3	5.3
Weaving		4	3.8	2	3.5
Others		27	25.5	10	17.5
Mothers					
Trading		45	42.1	32	56.1
Civil servant		28	26.3	0	0
Farming		4	3.5	12	21.1
Weaving		2	1.8	0	0
Others		26	24.6	13	22.8
Settlement type					
Urban		80	75.4	0	0
Peri-urban		22	21.1	0	0
Rural		4	3.5	57	100
Educational level		No	%	No	%
Father					
Nil		4	3.5	36	63.2
Primary	21	19.3	10	17.5	
Secondary		35	33.3	2	3.5
Post-secondary		39	36.8	3	5.3
Islamic		4	3.5	0	0
Mothers					
Nil		11	10.5	44	77.2
Primary	16	15.5	6	10.5	
Secondary		35	33.3	0	0
Post-secondary		37	35.1	1	1.8
Islamic		2	1.8	0	0

TABLE V: BACTERIAL ISOLATES FROM CASES OF ACUTE RESPIRATORY INFECTIONS

ORGANISMS	FREQUENCY	
	No	%
Streptococcus pneumonia	**9**	**27.27**
Staphylococcus aureus	**4**	**12.12**
Coliforms	**4**	**12.12**
Pseudomonas aeruginora	**4**	**12.12**
Streptococcus pyogenes	**3**	**9.09**
Staphylococcus epidermidis	**3**	**9.09**
Klebsiella pneumoniae	**2**	**6.06**
Proteus vulgaris	**2**	**6.06**
Escherichia coli	**1**	**3.03**
Acinetobacter	**1**	**3.03**
TOTAL	**33**	**100**

DISCUSSION

A total number of 163 children were recruited for the study comprising 106 from emergence pediatric unit (EPU) university of Ilorin teaching hospital (UITH) and 57 from Egbejila village, 55.8% males and 44.2% females. Egbejila community has a total population of 735. Under – five years old children constituted 20% (147) of these 57(38.8%) developed acute respiratory infection (ARI) during the study period. EPU total admission for the study period was 699 out of which 106(15.2%) had ARI. Table II shows the monthly admission by sex and cases of ARI. Thirty-eight percent of the Under-5 years in the rural community had mild ARI requiring hospitalization

Pre-recruitment antibiotic usage was common among our patients especially in the EPU. This may be due to easily available off-shelve purchases without doctors' prescription in Nigeria and the widespread of quarks and patent medicine store that do not stay within their certification limits. The rate of pre-recruitment antibiotics usage was more than reported from Ibadan, which showed 41% compared to our 85%(4). It has also been shown that pre-consultation antibiotic usage affects bacterial growth yield from cultures sixty-one (61%) percent of the blood culture negative case had pre-consultation antibiotics(4,5). This might have affected the yield in our cultures.

Majority of ARI cases would be due to viral infections. However majority of ARI-associated deaths have been attributed to the acute lower respiratory tract infection (ALRI) due to bacterial causes(6,7,8). It was therefore justifiable to make attempts to isolate bacterial agents from the blood because of the septicaemia it produce from the throat which serves as feeders to the lower lungs and the pleural which is the target for the organism. Blood culture is known for its specificity for identifying invasive pathogen(9) but it's not very sensitive when compared with lung aspirate(9,10).

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The 12.5% growth yield in this study was lower than the 33% yield in the Ibadan study. This may be the compensatory higher prevalence of pre-recruitment antibiotic usage in our series. Lung aspirate was not ethically justifiable because of the risk involved(9). However, pleural aspirate in the patient with effusion gave a high yield of 62.5%. This could be frequently employed but most patient may not have pleural effusion. The yield in our study was better than that reported by Johnson et al, Diakparomre et al and Aderele et al in Nigeria and other authors from other parts of the world9(5,11,12,13).

TABLE VI: CLINICAL PRESENTATION OF MODERATELY SEVERE AND SEVERE FORM OF ACUTE RESPIRATORY INFECTIONS AMONG 106 PATIENTS IN EPU

Presentation (symptom/ signs)	Frequency	
	No	%
Tarchypnoea	106	100
Cough	92	86.8
Fever	77	72.6
Catarrh	75	70.8
Breathlessness	30	28.3
Vomiting	26	24.5
Crepitations	23	21.7
Weight loss	21	19.8
Transmitted sounds	18	17.0
Chest-in-drawing	16	15.1
Diarrhoea	13	12.3
Abdominal pain	9	8.5
Pallor (conjunctiva)	8	7.6
Pleural effusion	8	7.6
Convulsion	8	7.6
Bronchial breathing	7	6.6
Inflamed throat/eardrum	7	6.6
Chest pain	7	6.6
Gallop rhythm	4	3.8
Others	3	2.8

TABLE VII: ANTIBIOTICS SENSITIVITIES OF BACTERIAL ISOLATES FROM CASES OF ACUTE RESPIRATORY INFECTION ANTIBIOTICS SENSITIVITY OF BACTERIAL ISOLATES (% SENSITIVITY) IN ACUTE RESPIRATORY INFECTIONS

Antimicrobial agents	S. pneumonia	Staph Aureus	PS aeruginosa	Kleb pneumoniae	Strept Pyogenes	E. coli	P. Vulgaris	Coliform	Acineto-Bacter	Staph epidremidis
1 Ofloaxacin	100	100	100	100	100	100	100	100	100	66.7
2 Ciprofloacin	100	100	100	100	100	100	100	100	100	100
3 Cefuroxime	100	100	100	100	100	100	100	66.67	____	100
4 Gentamicin	100	100	100	100	100	100	100	100	____	66.7
5. Cloxaillin	78	75	____	____	100	____	____	____	____	33.3
6. Ceftriaxone	67	75	50	100	100	100	100	66.7	0	33.3
7 Erythromycin	87	100	____	____	100	____	____	____	____	0
8 hloramphenicol	87	50	____	____	100	____	____	66.7	0	____
9. Co-trimoxazole	56	25	0	0	66.7	100	0	____	____	____
10 Colistin	___	___	100	100	____	100	100	____	____	____
11 Tetracycline	100	50	0	0	100	100	0	0	100	____
12 Ampicillin	67	25	0	50	66.7	100	0	0	____	0
13 Streptomycin	56	0	0	50	66.7	100	0	33.3	100	___
14 Penicillin	87	0	____	____	100	100	____	____	____	___
15 Cefotaxime	100	50	50	100	100	100	100	____	___	____
16 Ceftazidime	87	0	100	100	100	100	100	100	____	____
17 Azithromycin	____	____	____	____	____	____	____	____	100	100

Throat swabs culture growth yield in this study was also better than previously reported(5). However previous report, were not in favour of throat swab because it may not be of any therapeutic reassurance. A future case control study will be needed to ascertain the present advantage of throat swab in identifying bacterial agents from ARI. In this study there were some correlates in organism recovered from the throat and blood (*Pseudomonas aeruginosa*).

The commonest organism isolated was streptococcus pneumoniae all from the throat swabs majority fromthe village where non-use pre-recruitment antibiotic. Whereas staphylococcus aureus that is not common were mostly from blood and pleural aspirate among patients with pre-recruitment antibiotics. This agrees with other previous reports(5,7,14), except that H. influenzae was not isolated at all in our study. Therefore, the finding of staphylococcus aureus and gram negative bacterial may be related to the predominant use of antibiotic before recruitment which possibly wiped off all susceptible gram positive organisms . To truly ascertain pre-recruitment use of antibiotics, we needed facilities for measuring serum or urine level of antibiotic which could not be undertaken in our study due to limitations and hence parental responses were

studied as previously employed(15). The leading role of tarchypnea as a sign among children with ARI is understandable. Cough and Fever also were present in majority of our patient. However, crepitation was only found in few of our patient in contrast to previous reports by WHO and other authors(5,7). Bronchopneumonia was the leading diagnosis of ARI in this study. However, the significant role of measles in 38.7% of our in-patients was unparallel. Measles affected males more than females and especially in children who had previously received measles vaccines at nine months of age. Again the question of sero-conversion after a vaccination comes to mind again. The question is what was the quality of the measles vaccines or life span of the antigenecity or immunity it produces. Among those children with measles only 3 who were less than nine months has not previously received the vaccine. Generally in this study there was over 50% coverage for the measles antigen among all the children.

The complications observed in our patients were only among those with severe disease who needed hospitalization. The leading complication of ARI was pleural effusion and febrile convulsion. Heart failure was observed in some few others. This patter of complications agrees with previous reports except the infrequent occurrence of anaemia severe enough to require blood transfusion (5).

There were 13 deaths among patient with severe ARI. The major contributor to mortality was bronchopneumonia. The pattern of antibiotic sensitivities looks inconclusive. However, for now cotrimoxazole and most first line antibiotics may not be performing optimally. There is need to do trial works on the macrolides, the cephalosporins and quinolones.

We concluded that ARI is still common (15.2% of admission), its mortality still unacceptable (12.3%) due mainly to severe forms, a need for reviewing the present first line antibiotics. It was also concluded that pleural effusion, where present, are likely to yield bacterial agents than blood and throat swab. Measles contributed greatly to cases of ARI. A few cases of easles developed before 9 month of age when patients were due for the vaccine.

We recommend the use of Quinolones, Gentamycin and Cephalosporins as first line drugs in severe forms of ARI, Public education on physical management of fevers to reduce incidence of Febrile Convulsion, improvement on Immunization coverage for all antigens, the use of Edmondson-Zagreb measles vaccine that could be given before 9 months and to start a Community based training programmme to educate people on ARI prevention as contained in the IMCI.

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ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1410 http://www.ajol.info/journals/ajcem
COPYRIGHT 2014 http://dx.doi.org/10.4314/ajcem.v15i2.2
AFR. J. CLN. EXPER. MICROBIOL. 15(2): 60-68

NOSOCOMIAL WOUND INFECTION AMONGST POST OPERATIVE PATIENTS AND THEIR ANTIBIOGRAMS AT TERTIARY CARE HOSPITAL IN INDIA.

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ABSTRACT

Nosocomial infection constitutes a major public health problem worldwide. Increasing antibiotic resistance of pathogens associated with nosocomial infections also becomes a major therapeutic challenge for physicians. Thus, the aim of this study was to identify post operative bacterial infections in the patients developing surgical site infections at a tertiary University hospital in North India during July 2013 to Dec 2013.

Methods: One hundred and ninety six swabs/pus specimens from various types of surgical sites suspected to be infected on clinical grounds were processed, by standard methods and antibiotic susceptibility testing of all the isolates was done by using Kirby Baur disc diffusion technique.

Results: Of the one hundred and fifty-eight organisms isolated, the most common was *Staphylococcus aureus* **(27.8 %), followed by** *Escherchia coli* **(24.05 %),** *Klebsiella pneumoniae* **(13.29 %), Pseudomonas aeruginosa (6.32%),** *Klebsiella oxytoca* **(5%),** *Enterococcus* **(5.6%) and other miscellaneous gram negative rods (9.4%) and** *Streptococcus pyogenes* **(1.30%). About 50% of the Staphylococcus aureus isolates were found to be methicillin resistant. In case of** *Escherichia coli***, more than one-third of the isolates were found to be ESBL producers. The resistance to third generation cephalosporins and the quinolone ciprofloxacin was also quite high. Other isolates also showed a very high level of antibiotic resistance.**

Conclusion: In addition to the economic burden for antibiotic treatment, such infections for multi-resistant organisms are a serious threat to our surgical patients. To prevent these happenings, there is ar urgent need to adopt basic principles of asepsis and sterilization and to make judicious use of prophylactic and therapeutic antibiotics and determine current antimicrobial resistance to commonly prescribed drugs.

Keywords: Wound infection; microorganisms; anti-microbial sensitivity

INFECTIONS NOSOCOMIALES DE PLAIE PARMI LES PATIENTS POST-OPERATOIRES ET LEURS ANTIBIOGRAMMES A L'HOPITAL DU SOIN TERTIAIRE EN INDE

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Résumé

Contexte: les infections nosocomiales constituent un problème majeur de santé publique dans le monde. L'augmentation de la résistance aux antibiotiques de germes associés aux infections nosocomiales devient aussi un défi thérapeutique majeur pour les médecins. Ainsi, le but de cette étude était d'identifier les infections bactériennes post-opératoires chez les patients développant les infections au niveau du site chirurgical de centre hospitalier universitaire tertiaire au Nord de l'Inde de Juillet 2013 à Décembre 2013.

Méthodes: Cent quatre-vingt-seize (196) échantillons de prélèvements de pus de plusieurs types de sites chirurgicaux suspectés d'être infectés pour des raisons cliniques ont été traités par les méthodes classiques, et le test de sensibilité aux antibiotiques de toutes les souches a été fait selon la méthode de diffusion de disque de Kirby Baur.

Résultats: Sur cent cinquante-huit (158) germes isolés, le plus dominant était *Staphylococcus aureus* **(27,8 %), suivi de** *Escherichia coli* **(24,05%),** *Klebsiellapneumoniae***(13,29%),** *Pseudomonas aeruginosa* **(6,32%),** *Klebsiellaoxytoca* **(5%),** *Enterococcus* **(5,6%) et autre germes divers àGram négatif (9,4%) et***Streptococcus pyogenes* **(1,30%). Environ, 50% de souches de** *Staphylococcus aureus* **étaient résistantes à la méthillicine. Dans le cas de** *Escherichia coli***, plus d'un tiers (1/3) de souches étaient productrices de béta-lactamases (***BLSE***). La résistance aux céphalosporines de la troisième génération et à la ciprofloxacine était également assez élevée. Les autres souches ont aussi montré de résistance de haut niveau aux antibiotiques.**

Conclusion: En plus de charge économique de traitements aux antibiotiques, ces infections de souches multi-résistantes sont de menaces sérieuses pour nos patients opérés. Pour prévenir ces évènements, il y a un besoin urgent d'adopter les principes de base d'asepsies et destérilisation et de faire un usage judicieux des antibiotiques utilisés dans les traitements prophylactique et thérapeutique et déterminer la résistance antimicrobienne courante aux médicaments couramment prescrits.

Mots clés: infection de plaie post-opératoire; microorganismes; sensibilité aux antimicrobiens

INTRODUCTION

Despite an improved understanding of the patho-physiology, methods of prevention and prophylaxis and technological advances that have been made in surgery and wound management, surgical wound infections remain the most common cause of post operative morbidity and mortality1. A surgical wound may get infected by the exogenous bacterial flora which may be present in the environmental air of the operation theatre or by the endogenous flora2. Surgical wound infection remains one of the most important post-operative complications, accounting for 10 to 20% of the hospital costs. Although total elimination is not possible, a reduction in the infection rate to a minimal level could have significant benefits in terms of both the patient comfort and the medical resources which are used3. The rate of infection of the surgical wounds is influenced by the duration of the pre-operative hospitalization, administration of the prophylactic antibiotics, the duration of the surgery and by the fact as to whether the surgery was emergency or elective. Patient factors and environmental factors, both local and general, like age and nutritional status and preexisting illnesses also determine the final outcome. Postoperative wound infection can occur from first day onwards to many years after an operation but commonly occurs between the fifth and tenth days after surgery4. It may originate during the operation i.e. as a primary wound infection or may occur after the operation from sources in the ward or as a result of some complications i.e. secondary wound infection5.6 and can be characterized by various combinations of the signs of infection (e.g. pain, tenderness, warmth, erythema, swelling, drainage)4. Most post-operative wound infections are hospital acquired and vary from one hospital to the other and even within a given hospitals and they are associated with increased morbidity and mortality5.

The site of infection may be limited to the suture line or may become extensive in the operative site and the infecting microorganisms are variable, depending on the type and location of surgery, and antimicrobials. Surgical site infections (SSIs) which account17% of all health care-associated infections are the second most common HAIs next to urinary tract infections. They occur after approximately 3% of all operations and result in greater lengths of stay and additional costs6.

The emergence of poly antimicrobial resistant strains of hospital pathogens has also presented a challenge in the provision of good quality inpatient care7. The battle between bacteria and their susceptibility to drugs is yet problematic among public, researchers, clinicians and drug companies who are looking for effective drugs8. Therefore, the aim of this study was to isolate bacterial pathogens from hospital acquired surgical site infection and determine their current antimicrobial sensitivity patterns among patients who had clean and clean contaminated operations at MM Institute of Medical Sciences and Research Mullana, Ambala.

MATERIALS AND METHODS

Study Centre

The present study was conducted at a tertiary care university hospital and study centre in North India, between July 2013 and Dec 2013.The hospital has more than 5000 surgical patients in general surgery, orthopaedics and gynaecology wards per year and on average ten major operations are performed per day. In addition, the hospital accepts referred patients from different parts of the region.

*Clean Operations***:** a type of wound in which no inflammation is encountered and the respiratory, alimentary or genitourinary tracts are not entered and there is no break in aseptic operating procedure.

Clean-contaminated operations: a type of wound in which the respiratory, alimentary or genitourinary tracts are entered but without significant spillage (without visible contamination).

Contaminated operations: a type of wound where acute inflammation (without pus) is encountered, or where there is visible contamination of the wound. Examples include gross spillage from a hollow viscous during the operation or compound/open injuries operated within four hours.

Dirty Operations: a wound in the presence of pus, where there is a previously perforated hollow viscous or compound/open injury more than four hours old.

Postoperative nosocomial infection: a surgical site or blood stream infection occurring after 48 hours of operation until the time of discharge from hospital with clinical signs and symptoms and laboratory confirmation.

The Centre for Disease Control, (CDC), USA, classifies the surgical site infections into: (a) Superficial incisional SSI which involves only skin and subcutaneous tissue of incision, (b) Deep incisional SSI which involves deep soft tissues (e.g. fascia and muscle layer) of the incision, (c) Organ \Space SSI includes infection apparently related to the operative procedure and infection involves any part of the body, excluding skin incision, fascia, muscle layer that is operated or manipulated during operative procedure.

Patients

The study group included all the clean and clean contaminated surgeries which were conducted in this hospital during that period. Procedures in which healthy skin was not incised such as opening of an abscess, burn injuries and donor sites of split skin grafts and contaminated and dirty surgeries were excluded from the study. The samples were collected from those patients who showed an evidence of surgical wound infections like a serous, sanguineous or purulent discharge, soaked dressing or gaping wounds. Purulent materials were collected on sterile commercial cotton swabs aseptically and gently to avoid contamination of the specimens with normal microbial flora of the skin.

Specimens were collected before redressing and administration of antibiotic therapy. Specimens were labeled, kept in a vial and transferred immediately to the laboratory for bacteriological examination. Then, one of the wound swabs was inoculated on to Blood agar and MacConkey agar plate9. The inoculated agar plates were incubated aerobically at 37 °C overnight. The other wound swab was used for Gram staining smears to make presumptive diagnosis10.

The smear was screened for pus cells, the gram reaction, morphology, arrangement and number of types of the organisms and to select significant organism based on the quantitative measurements made on direct microscopy i.e. finding of bacteria on a given microscopic smear were taken as presence of 106 or more bacteria per swab which is reliably predicts a microbial load of >105 CFU/g of tissue11.

Antimicrobial susceptibility testing was performed using Kirby Bauer agar disc diffusion technique for the isolated pathogen12. A loop full of bacteria was taken from a pure culture colony and was transferred to a tube containing 5ml of phosphate buffer saline and mixed gently until it formed a homogenous suspension and the turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 standard in a tube. The standardized inoculums of each isolate were inoculated on to Mueller-Hinton antibiotic sensitivity medium. Finally, all the isolates were tested for the antibiotic discs as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Gram positive isolates were tested for drugs such as penicillin, oxacillin, linezolid, vancomycin, erythromycin, clindamycin, chloramphenicol, gentamycin, ciprofloxacin, tetracycline and cotrimoxazole. Gram negative isolates were tested for drugs such as gentamycin, ampicillin, chloramphenicol, amikacin, piperacillin-tazobactum, cefamandole, cefixime, ciprofloxacin, meropenem, imipenem, Cotrimoxazole, streptomycin, tobramycin, netilmycin ceftazidime and cefotaxime.

The plates were incubated aerobically at 370C for 18-24 hours and the interpretation of the results of the antimicrobial susceptibility was made based on the CLSI criteria as sensitive, intermediate and resistant by measuring diameter of inhibition the zone. All intermediate readings were taken as resistant during data entry. The standard reference strains, *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922 and *P. aeruginosa* (ATCC 27853) were used to assure testing performance of the potency of drug discs well as quality of culture media. Methicillin resistance was detected by disc diffusion technique using 1µg oxacillin discs.13 Test for ESBL production was done by double disc approximation test.14

RESULTS

A total of 1568 patients were undergone major operations and admitted in Surgical (n=860) and Gynecology (n=458) wards and Orthopedics (250) of which 314 (20.02%) had clean and 1254 (79.98%) clean

contaminated operations during the study period (Table1).

TABLE.1 INCIDENCE OF SSI ACCORDING TO RISK CLASS

Risk Class	Surgeries Performed No.	SSI	SSI %
Clean	**314**	**14**	**4.4%**
Clean Contaminated	**1254**	**134**	**10.6%**
Total	**1568**	**148**	**9.43%**

The sex profile of these patients showed that 678 (43.23%) were males and 890 (56.76%) were females making male to female ratio of 1:31.1. The mean age of patients was 32.2 years and1254 (80%) of them were older than 15 years. The overall culture confirmed nosocomial infection rate on these patients was 9.43 % (Table1). The infection rate was higher in males than females. The infection rate was relatively high (27.3%) in the age group of >51 years old followed by 21-30 years of age group (12.6%).

One hundred and ninety six cases were processed for bacteriological study, in 48 (24.5 %) cases there was no growth and 148 (75.5%) cases were culture positive and were considered definitive cases of SSI. Out of 148 positive cases, 138 cases showed single organism as causative factor and 10 showed two organisms. A total of 158 organisms were isolated.(Table2)

TABLE.2 DIRECT MICROSCOPY AND CULTURE POSITIVITY

Direct Microscopy	Microscopy Positive	Culture
Pus Cells+ GPC	**12**	**12**
Pus Cells + GNB	**40**	**37**
Pus Cells +GPC+GNB	**88**	**78**
Few Pus Cells + No Organisms	**20**	**11**
No Pus Cells + No Organisms	**36**	**10**

GPC – Gram positive cocci; GNB – Gram negative bacilli

TABLE.3 INCIDENCE OF MONOMICROBIAL / POLYMICROBIAL GROWTH

No. of organisms	No of cases
No Growth	**48**
One	**138**
Two	**10**
Total	**196**

On direct microscopy of samples collected from 196 clinically infected cases, 140 samples were positive for Gram staining for pus cells and organisms. In 20 samples, few pus cells and no organisms were seen and in 36 samples no pus and no organism was seen in direct microscopy. (Table3) *Staphylococcus aureus* (27.84%) and *E coli (24.05%*) were the commonest pathogens which were isolated, followed by Klebsiella pneumoniae (13.03%) and Pseudomonas aeruginosa (6.32%).(Table4).

S.aureus (n=44) was the commonest isolate of which 52% (n=23) isolates were MRSA; all sensitive to vancomyicn and linezolid followed by chloramphenicol, gentamicin, ciprofloxacin and, 48% (n=21) isolates were MSSA (Methicillin sensitive *Staphylococcus aureus*). *Enterococcus sp.*(n=9) was 100% sensitive to vancomycin and linezolid (Table5). Piperacillin-Tazobactum, Ceftazidime, Tobramycin and Gentamicin are the common antimicrobials used for surgical prophylaxis and also for empirical therapy of SSIs. Gram negative bacilli isolated in our study were highly sensitive to these antibiotics (Table-6). ESBL producers included *Klebsiella sp.* (50%) (n=10), E.coli (20%) (n=4), and *Pseudomonas sp.* (30%) (n=6). *Pseudomonas sp.* (n=14) were mostly sensitive to Piperacillin-Tazobactum combination, meropenem and imipenem and amikacn. Most of the Gram negative bacilli were resistant to cefamandole, cefixime & cotrimoxazole. (Table6)

DISCUSSION

Nosocomial infections, including surgical site infection, still form a large health problem and contribute substantially to patient morbidity, mortality, prolonged hospital stay, expensive hospitalization and prolonged therapy15,16. Emergence of poly antimicrobial resistant strains of

hospital pathogens has also presented a major challenge in the provision of good quality in patient care7. The overall infection rate was 9.43 % in our study. This was in agreement with the overall infection rate which ranged from 2.8% to 20.19% in other studies17,18,19,20.

Cruse and Foord observed that the rate of infection of clean wounds was more useful as an indicator of control of infection of surgical wounds than the overall incidence17. So a detailed analysis of Clean and clean contaminated cases was conducted in this study, which definitely are the most useful measures in microbiological surveillance and research17. Accordingly, contaminated and Dirty surgeries were excluded from the study.In our study surgical site infection was significantly associated with class of wounds. For clean contaminated operations, 10.6% presented with SSIs and 4.4% for clean operations. This high rate of infection among former wound type is probably because of profound influence of endogenous contamination during the time of operation. The present study confirms the understanding that there is a gradual rise in incidence of wound infection as age advances. The infection rate was relatively high (27.3%) in the age group of >51 years old followed by 21-30 years of age group (12.6%). The higher incidence in patients above 60 years in our study is perhaps due to decreased immune-competence and increased chances of co-morbid factors like Diabetes mellitus, Hypertension, other Chronic ailments and personal habits like Smoking and alcoholism.

In the present study, on direct microscopy of samples collected from 196 clinically infected cases 140, samples were positive for Gram staining for pus cells and organisms. In smears from 20 samples, few pus cells and no organisms were seen but 11 were culture positive. This may be probably due to low number of organisms which could not be detected by microscopy but, yielded growth on culture. *Staphylococcus aureus* (27.84%) and *E coli (24.05%*) were the commonest pathogens which were isolated. Similar findings were recorded by S.P. Srivastava et al21 and S.V.Bhatia et al22. The predominance *S. aureus* infection seen in this study is most likely associated with endogenous source as the organism is a member of the skin and nasal flora of the patients as it was explained by Isbori et al5 and Angu and Olila23. Infection with this organism may also be associated with contamination from the environment, surgical instruments or contaminated hands of the health professionals5,23. *E.coli* (24.05%) was the second most common isolated bacteria from SSI. This could be because of the profound influence of endogenous contamination from the bowel and hollow muscular organs of patients.

The present study has also indicated that most of *S. aureus* were resistant to penicillin and oxacillin. Most sensitive antibiotics in our study were: imipenem, meropenem, amikacin, piperacillin-tazobactum in *Klebsiella sp.*, *E.coli*, and *Pseudomonas sp.*, and vancomycin, linezolid in MRSA and MSSA. These drug combinations should be used for empirical therapy, though; the prophylaxis must be continued with lower drugs according to the available surgical prophylaxis guidelines to prevent selection pressure and spread of resistance.
Predominant role of MDR bacteria in nosocomial infections similar to our study has been proved by many previous workers19,20,22,23. Infection by Multi drug resistant bacteria enhances the need of antibiotic stewardship and also indicates the need of proper disinfection of hospital environment.

Conclusion

In conclusion, the rate of nosocomial infection obtained in this study was comparable to other similar studies carried out in other countries. However, the bacterial isolates detected from our patients were resistant for commonly available and prescribed antimicrobial drugs. Therefore, antibiotics such as Ampicillin, Amoxicillin, Penicillin, Trimethoprim-sulphamethoxazole, Chloramphenicol and Ceftriaxone are not the drug of choice for treating patients with nosocomial infections in the study area. Hospital also needs to make a concerted effort to minimize hospital acquired infections by following strict aseptic operation procedures, effective methods of sterilization and patient management.

TABLE.4 NUMBER OF ORGANISMS

Organism	Number	Percent
Staph aureus	44	27.84%
Esch coli	38	24.05%
Kleb Pneumoniae	21	13.30%
Pseudomonas sp	10	6.32%
Enterococcus sp.	9	5.70%
Kleb Oxytoca	8	5.05%
Coagulase negative staph	6	3.80%
Citrobacter sp.	6	3.80%
DiphtheroidesI	4	2.53%

Organism	Number	Percent
Enterobacter sp.	2	1.27%
Morganella morganii	2	1.27%
Acinetobacter sp	2	1.27%
Streptococcus sp	2	1.27%
Candida sp	1	0.63%
Total	158	100%

TABLE.5 GRAM +VE ORGANISM WITH ANTIBIOTIC SENSITIVITY (%)

Antibiotic	MRSA	MSSA	CONS	Enterococcus sp	Streptococcus Sp.
Pencillin	45.5	49	33.33	50	100
Oxacillin	0	100	50	0	-
Linezolid	100	100	100	100	
Vancomycin	100	100	100	100	-
Erythromycin	85.7		33.33	-	-
Clindamycin	15.3	70	-	-	
Gentamicin	70	70	13.33	100	100
Ciprofloxacin	64.3	60	33.33	100	50
Chloramphenicol	76.9	80	66.66	100	-
Tetracycline	60	50	50		-
Co-trimoxazole	15.3	20	50	-	-

TABLE.6 GRAM –VE ORGANISMS WITH ANTIBIOTIC SENSITIVITY (%)

Antibiotics	Esch. coli.	Kleb pneumonia	Kleb oxytoca	Citrobacter sp.	Enterobacter sp	Pseudomonas aeruginosa	Morganella morganii	Acinetobacter sp	Proteus sp.
Gentamicin	62.5	33.33	25	50	0	80	100	100	100
Ampicillin	5.2	2	0	0		10	0	50	0
Chloramphenicol	75	65	25	50	50	30	100	50	33.33
Amikacin	90	80	12.5	33.33	50	80	100	50	100
Tazobactam+ Pipracilin	87	88.6	0	50	0	70	100	100	33.33
Cefamandole	15	9	0	33.33	50	10	0	50	33.3
Cefixime	42.9	40	0	33.33	0	40	0	0	0
Ciprofloxacin	62.5	45	O	33.33		80	0	100	66.66
Metropenem	38	85	-	16.66	50	-	50	50	0
Imipenem	90	90	50	16.66	50	-	50	50	-
Co-trimoxazole	0	10.52	50	33.33	50	70	100	-	-
Tobramycin	100	80	25	50	100	90	50		100
Netilmycin	56		25	50	50	80	50	100	100
Ceftazidime	92.7	100	25	100	100	70	50	100	100
Cefotaxime	94.4	94.1	25	100	100	70	50	100	100

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ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1411 http://www.ajol.info/journals/ajcem
COPYRIGHT 2014 http://dx.doi.org/10.4314/ajcem.v15i2.3
AFR. J. CLN. EXPER. MICROBIOL. 15(2):69-75

PREVALENCE OF ANTIBIOTIC-RESISTANT STRAINS OF *ESCHERICHIA COLI* IN DRINKING WATER SAMPLES FROM MOWE METROPOLIS, OGUN STATE, NIGERIA

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ABSTRACT

A measured *Escherichia coli* level in drinking water is perhaps the most popular means of determining human health risks globally. Water samples from wells, boreholes and sachet water, the 3 predominant sources of drinking water in the study area were evaluated for the presence of bacteria, particularly *E coli*. Bacteria isolation was done using standard microbiological procedures while identification of isolates was done using cultural, morphological and biochemical characteristics. Enumeration of standard plate count was done by spread plate method on serially diluted water samples. The prevalence of *E coli* in the water samples and the activities of cefoxitin, fusidic acid, meticillin, penicillin and vancomycin against the *E coli* isolates and the susceptibility testing data were obtained using Kirby Bauer method. A total of six bacteria species *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Enterobacter aerogenes* were isolated from water samples obtained from borehole, well and sachet water samples in the study area. The mean bacteria counts ranged between 3.74 x 104 to 1.65 x 102 CFU/ml for well and borehole water and 0.81 to 5.1 x 102 CFU/ml for sachet water samples. Out of the 6 *E coli* strains representing 27.2% of the isolated bacteria species; two, representing 33.3% of the strains showed moderate to high resistance against meticillin. These findings are expected to motivate public health stakeholders in the study location to attempt reducing the growing resistance of pathogenic bacteria in the environment, and their ecotoxic effects.

Key words: antibiotic resistance, meticillin, water quality, *E. coli*

PREVALENCE DES SOUCHES RESISTANTES AUX ANTIBIOTIQUES D'*ESCHERICHIA COLI* DANS LES ECHANTILLONS D'EAU POTABLE DANS LA MUNICIPALITE DE MOWE, L'ETAT D'OGUN, NIGERIA

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RÉSUMÉ

Un niveau d'*Escherichia coli* mesurées dans l'eau potable est peut-être le moyen le plus populaire de la détermination des risques pour la santé humaine à l'échelle mondiale. Des échantillons d'eau de puits, de forages et de l'eau de sachet, les trois principales sources d'eau potable dans la zone d'étude ont été évalués pour la présence de bactéries, en particulier *E. coli*. L'isolement de bactéries a été effectué en utilisant des procédures microbiologiques standard tandis que l'identification des isolats a été effectuée à l'aide des caractéristiques culturelles, morphologiques et biochimiques. Énumération de nombre de plaque standard a été effectuée par la méthode de la plaque de propagation sur des échantillons d'eau dilués en série. La prévalence de *E. coli* dans les échantillons d'eau et les activités de la céfoxitine, l'acide fusidique, la méticilline, la pénicilline et de la vancomycine contre les isolats de *E. coli* et les données de tests de sensibilité ont été obtenus en utilisant la méthode de Kirby Bauer. Un total de six espèces de bactéries :*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterobacter aerogenes* ont été isolés à partir d'échantillons d'eau provenant de puits, de forage et des échantillons d'eau de sachet dans la zone d'étude. Les bactéries, les valeurs moyennes se situaient entre 3,74 x 104 à 1,65 x 102 UFC / ml pour le bien et l'eau de forage et de 0,81 à 5,1 x 102 UFC / ml pour les échantillons d'eau de sachet. Sur les 6 souches d'*E.coli* représentant 27,2% des espèces de bactéries isolées ; deux (33,3 %) des souches ont montré une résistance modéré à haute à la pénicilline. Ces résultats devraient inciter les intervenants en santé publique dans le lieu de l'étude de tenter de réduire la résistance croissante des bactéries pathogènes dans l'environnement et leurs effets écotoxiques.

Mots clés: **Résistance aux antibiotiques, pénicilline, qualité l'eau, *E. coli*.**

INTRODUCTION

About 2.5 billion people, roughly 40% of the world population lack access to safe drinking water (1). This teeming population of people are at risk of contacting water borne diseases, the most susceptible being children, the elderly, pregnant women and immunocompromised individuals. This makes water-borne illnesses one of the five leading causes of death among children under age five (2). 40% of deaths in developing nations occur due to infections from water related diseases and an estimated 500 million cases of diarrhoea, occurs every year in children below 5 years in parts of Asia, Africa and Latin America (3, 4). In Nigeria, drinking water pollution is further exacerbated due to the alarming rate of urbanization as major cities reportedly grow at rates between 10-15% per annum (5) and thus, human activities including soil fertility remediation, indiscriminate refuse and waste disposal, and the use of septic tanks, soak-away pits and pit latrines are on the increase. Groundwater pollution has been attributed to the process of industrialization and urbanization that has progressively developed over time without any regard for environmental consequences which eventually results in the deterioration of physical, chemical and biological properties of water (6, 7).

Microbial faecal contamination indicators of drinking water are *Escherichia coli*, *Clostridium spp.*, *Streptococci spp* (8) and other bacteria that could be of human or non-human origin. *Escherichia coli*, particularly those possessing virulence markers as; haemolysin, verocytotoxin and belonging to the enteropathogenic serotypes have been responsible for gastroenteritis in humans (9). Drinking water safety guidelines and water quality regulations throughout the world rely on measured *E. coli* levels to indicate human health risks (10).

Antimicrobial resistance in *Enterobacteriaceae* poses a critical public health threat, especially in the developing countries (4, 11). Much of the problem has been shown to be due to the presence of transferable plasmids encoding multidrug resistance and their dissemination among different enterobacterial species (12). *Escherichia coli* O157 is an important food-borne and water-borne pathogen with a worldwide distribution (13). The first reported outbreak of *E. coli* O157 infection in the developing world occurred in 1992 in Southern Africa (14). Outbreaks have also occurred in Central African Republic in 1996 and Cameroon, in 1997 (15). Such outbreaks have been linked to contaminated bovine food products, contaminated drinking water and flood irrigation with water contaminated by animal feces or surface runoff and cattle feces have been implicated as the major source of contamination (14).

The emergence of antimicrobial resistance is usually preceded by antimicrobial misuse; however, surveillance of the spread of antimicrobial-resistant pathogens is expected to play a very important role in reducing the rate of emergence and spread of antimicrobial-resistant pathogens since such early-warning signals make timely intervention possible. The present work examines the prevalence of antimicrobial-resistant strains of *E. coli* in the study area. Such monitoring can aid the infection-control community in reducing the emergence and spread of antimicrobial-resistant pathogens. Moreover, more work is being carried out to further characterize and to elucidate the mode of development of resistance in these isolated strains of *E. coli*.

MATERIALS AND METHODS

Water samples were obtained from 3 urban locations of Obafemi Owode Local Government, Ogun State namely, Mowe (N 06° 48. 220' E 003° 26. 167'), Imendu-Nla (N06° 48. 241 E003° 26. 303') and Loburo (N 06° 49. 240' E 003° 27. 033') communities between January and March, 2010. Bore-hole water collected at three different locations was designated B1, B2, and B3. Well water also collected at three different locations was designated W1, W2, and W3. The sachet water samples were purchased based on popularity at three different locations and were designated as S1, S2, S3, S4 and S5. Samples were labelled accordingly and taken to the laboratory for analyses within few hours after sampling. Physical characteristics such as specific odour and appearance and presence of extraneous materials and floating particles in the water samples were noted. Moreover, features external to the water itself such as the label and presence of certification number and other product information of the sachet water samples were also noted.

Total heterotrophic bacteria count of the drinking water samples was determined using pour plate method. The plates were inoculated aerobically at 37°C for 24hours. The total coliform bacteria were determined using the multiple tube fermentation tests and the calculated coliform density computed by the Most Probable Number (MPN) procedures (16). All measurements of parameters were made in triplicates. Results obtained were statistically analyzed using Analyse-it® v. 2.20, statistical software for Microsoft Excel. Variations were considered significant at $p \leq 0.05$.

5 Antibiotic disks were obtained from Oxoid (Oxoid Ltd., Cambridge, UK): fusidic acid (10µg), penicillin (10 units), cefoxitin (30µg), meticillin (10µg) and

vancomycin (30µg). Antimicrobial susceptibility testing was performed using a disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (17). Quality control was performed using test strain *E. coli* ATCC 25922.

RESULTS

Physical examination of the untreated water samples from boreholes and wells examined in the present study revealed that none of the well water samples met the WHO standards for physical appearance. The well water samples were turbid and/ or with odour, unlike the samples from the borehole that were colourless, odourless and with no particles as recommended by the WHO (Table 1). Moreover, a visual appraisal of the wells and boreholes reveals that these were located within less than an average of 30 meters from a septic tank or waste dump. On the other hand, apart from displaying the manufacturer's name, address and NAFDAC number, none of the treated sachet water brands showed other necessary information such as batch number, date of manufacture and best before date (Table 2).

TABLE 1: RESULTS OF PHYSICAL EXAMINATION OF WATER SAMPLES

Water Source	Colour/ Turbidity	Odour	Particles
B1	**Colourless**	**Odourless**	**None**
B2	**Colourless**	**Odourless**	**None**
W1	**Slightly turbid**	**Slight odour**	**Few particles**
W2	**Colourless**	**Odourless**	**Few particles**
W3	**Slightly turbid**	**Slight odour**	**Suspended solids**
S1	**Colourless**	**Odourless**	**None**
S2	**Colourless**	**Odourless**	**None**
S3	**Colourless**	**Odourless**	**None**
S4	**Colourless**	**Odourless**	**None**

Key: B1 = borehole water samples from Imedu Nla; B2 = borehole water samples from Mowe; W1 = well water samples from Imedu Nla; W2 = Well water samples from Mowe; W3 = well water samples from Loburo; S1 = sachet water brand no. 1; S2 = sachet water brand no. 2; S3 = sachet water brand no. 3; S4 = sachet water brand no. 4

TABLE 2: RESULTS OF PHYSICAL EXAMINATION FOR LABELLING COMPLIANCE OF SACHET WATER SAMPLES

Samples	NAFDAC number	Best before date	Manufacturing date	Nutritional information	Batch number	Producer's name & address
S1	**+**	**-**	**-**	**-**	**-**	**+**
S2	**+**	**-**	**-**	**-**	**-**	**+**
S3	**+**	**-**	**-**	**-**	**-**	**+**
S4	**+**	**-**	**-**	**-**	**-**	**+**
S5	**+**	**-**	**-**	**-**	**-**	**+**

Key: S1 = sachet water brand no.1; S2 = sachet water brand no. 2; S3 = sachet water brand no. 3; S4 = sachet water brand no. 4; S4 = sachet water brand no. 4; +: Displayed on sample label; - : Not displayed on sample label

Out of the 22 distinct bacteria isolates obtained from the water samples, the frequency of occurrence of *E. coli* was the highest (Table 3). 6 *E coli* isolates representing 27.27% of the total was recovered from the water samples from all sources except the treated sachet water samples S1, S2, S3 and S4. *E coli* was isolated from water samples from all the untreated water sources namely location B1, B2, W1, W2, W3 including a treated water source, sachet water S5. This was followed by a frequency of 5 out of 22 isolates representing 22.73% each for *Staphylococcus aureus* and *Enterobacter aerogenes* (Table 3). Other bacteria species isolated from the water samples include *Pseudomonas aeruginosa*, *Bacillus cereus* and *Klebsiella pneumonia* each with a frequency of occurrence of 2 out of the total of 22 isolates representing 9.09% (Table 3). The widest variety of bacterial organisms was isolated from well water W1, which also had some odour and was slightly turbid.

Water samples from location B1 displayed the highest bacterial load with a standard plate count of 3.74x102cfu/ml, water samples from this location was also found to contain coliform bacteria (Table 4). This is significantly higher than the bacterial load recorded for treated or untreated water samples from other locations (Table 4). Moreover, none of the treated sachet water samples contained coliforms, although a standard plate count revealed no significant difference in colony counts between the treated and untreated water sources with the exception of water samples from location B1 which displayed the highest standard plate count (Table 4).

With the exception of *Bacillus cereus* and the control organism (*E. Coli* ATC 25922), all the tested isolates were resistant to penicillin and vancomycin. On the other hand, with the exception of *Enterobacter aerogenes* which displayed resistance, all the organisms were highly susceptible to fusidic acid. Moreover, only *E. aerogenes* displayed resistance to cefoxitin, all the other assessed isolates were highly susceptible to both cefoxitin and fusidic acid. 33.3% of the *E. coli* isolates were meticilin resistant, while all the *B. cereus* isolates were assessed to be resistant to meticilin. All the isolates of the other organisms (regardless of whether they are gram negative or positive) namely, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *E. aerogenes* including the control were highly susceptible to meticilin (Tables 5a and 5b).

DISCUSSION

The absence of municipal piped water has forced residents of the Mowe metropolis to rely on untreated ground water sources such as wells and boreholes, although sachet water is also available as a treated water source. Results of the present study showing that the water samples failed to meet the WHO standards for microbial contamination is not surprising since the commonest causes of ground water pollution can be easily observed in these locations. These activities include exposure to contamination due to human activities. The wells are often not covered and residents could be found washing clothes and dirty kitchen wares and sometimes bathing around the wells.

Other possible damaging environmental factors attributable to the low bacteriological quality of drinking water in these communities include poor town planning, dilapidated infrastructure and indiscriminate citing of wells and boreholes near septic tanks. A clear positive correlation between location of wells and significant increases in bacterial counts is well documented (18, 19). Leachate from septic systems has been identified as a major potential source of groundwater contamination from pathogens such as bacteria, viruses, helminths, and protozoa, nutrients such as nitrogen and phosphorus (20, 21). The more advanced countries of the United States, Canada and the United Kingdom have set a minimum standard distance of 15.24 m (50 ft) between septic systems and ground water meant for human and livestock uses (22). The presence of coliforms in the untreated well and borehole water samples from the present study is a clear indication of faecal contamination. Although the treated sachet water samples from these communities contained no coliforms, the bacterial load in the water samples remains higher than the set WHO limits.

TABLE 3: FREQUENCY OF OCCURRENCE OF BACTERIAL ISOLATES FOUND IN THE WATER SAMPLES

Bacterial Isolates	Frequency	Frequency (%)	Location B1	Location B2	Location W1	Location W2	Location W3	Sachet 1	Sachet 2	Sachet 3	Sachet 4	Sachet 5
Escherichia coli	6	27.27	+	+	+	+	+	-	-	-	-	+
Pseudomonas aeruginosa	2	9.09	-	-	+	-	+	-	-	-	-	-
Bacillus cereus	2	9.09	+	-	-	+	-	-	-	-	-	-
Klebsiella pnuemoniae	2	9.09	-	-	+	+	-	-	-	-	-	-
Staphylococcus aureus	5	22.73	-	-	+	+	+	-	-	-	+	+
Enterobacter aerogenes	5	22.73	+	+	+	-	+	-	-	-	-	+
Total	22	100	3	2	5	4	4	0	0	0	1	3

Legend: B1 = borehole water samples from Imedu Nla; B2 = borehole water samples from Mowe; W1 = well water samples from Imedu Nla; W2 = Well water samples from Mowe; W3 = well water samples from Loburo; S1 = sachet water brand no. 1; S2 = sachet water brand no. 2; S3 = sachet water brand no. 3; S4 = sachet water brand no. 4

TABLE 4: TOTAL BACTERIA COUNT IS EXPRESSED IN COLONY FORMING UNITS (CFU) WHILE THE COLIFORM COUNT IS EXPRESSED IN MOST PROBABLE NUMBER (MPN) AS DESCRIBED IN CHEESEBROUGH (2000). VALUE WITH ASTERISK IS SIGNIFICANT AT P≥0.05, X2= 2.618, DF= 18.

SAMPLES	BACTERIAL LOAD (CFU/MPN)	
	Standard plate count (cfu/ml)	Total coliform count (MPN)
LOCATION B1	3.74x 102*	180
LOCATION B2	0.98x 102	160
LOCATION W1	1.51x 102	160
LOCATION W2	0.78x 102	160
LOCATION W3	1.65 x102	160
SACHET 1	0.81 x 102	Nil
SACHET 2	0.59 x102	Nil
SACHET 3	0.51x102	Nil
SACHET 4	0.85x102	Nil
SACHET 5	0.90x102	Nil

TABLE 5A: ANTIBIOTIC SUSCEPTIBILITY PATTERN (%) OF GRAM NEGATIVE BACTERIA ISOLATES

No. of Isolates tested	Organism isolated	Fusidic Acid	Penicillin	Cefoxitin	Meticilin	Vancomycin
6	*Escherichia coli*	**S 0.0**	**R 100**	**S 0.0**	**R 33.3**	**R 100**
2	*Pseudomonas aeruginosa*	**S 0.0**	**R 100**	**S 0.0**	**S 0.0**	**R 100**
2	*Klebsiella pnuemoniae*	**S 0.0**	**R 100**	**S 0.0**	**S 0.0**	**R 100**
5	*Enterobacter aerogenes*	**R 100**	**R 100**	**R 100**	**S 0.0**	**R 100**
Control	*E. coli* *(ATC 25922)*	**S 0.0**	**S 0.0**	**S 0.0**	**S 0.0**	**S 0.0**

Key: S=Sensitive/susceptible; R= Resistant

TABLE 5B: ANTIBIOTIC SUSCEPTIBILITY PATTERN (%) OF GRAM POSITIVE BACTERIA ISOLATES

No. of Isolates tested	Organism isolated	Fusidic Acid	Penicillin	Cefoxitin	Meticilin	Vancomycin
2	*Bacillus cereus*	**S** **0.0**	**S** **0.0**	**S** **0.0**	**R** **100**	**R** **100**
5	*Staphylococcus aureus*	**S** **0.0**	**R** **100**	**S** **0.0**	**S** **0.0**	**S** **0.0**
Control	*E. coli* *(ATC 25922)*	**S** **0.0**	**S** **0.0**	**S** **0.0**	**S** **0.0**	**S** **0.0**

Key: S=Sensitive/susceptible R= Resistant

Antibiotics are used extensively to prevent or treat microbial infections in human and veterinary medicine. Apart from their use in aquaculture, they are also employed to promote more rapid growth of livestock. Most of the compounds used in medicine are only partially metabolized by patients and are then discharged into the sewage system or end up in the environment, mainly in the groundwater compartment (23). There is increasing concern about the growing resistance of pathogenic bacteria in the environment, and their ecotoxic effects. Increasingly, antibiotic resistance is seen as an ecological problem. This includes both the ecology of resistance genes and that of the resistant bacteria themselves. Little is known about the effects of sub-inhibitory concentrations of antibiotics and disinfectants on environmental bacteria, especially with respect to resistance. The results of the present study showing that 33.3% of the *E. coli* isolates from well and borehole samples were meticilin resistant. The present results is a warning signal to all stakeholders in community health in the study location to direct action at protecting drinking water from faecal contamination and limiting persistence of antimicrobials in groundwater. Future studies will focus on determining the genetic source(s) of the observed antimicrobial resistance.

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ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1412 http://www.ajol.info/journals/ajcem
COPYRIGHT 2014 http://dx.doi.org/10.4314/ajcem.v15i2.4
AFR. J. CLN. EXPER. MICROBIOL. 15(2):76-83

MICROBIAL CONTAMINATION OF LOCALLY PRODUCED CHEESE AND DETERMINATION OF THEIR ANTIMICROBIAL POTENTIAL IN NIGERIA

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ABSTRACT

The high consumption rate of soft cheese and manner of cheese production in Nigeria prompted the need to determine the microbial quality and antimicrobial properties of locally produced cheese in Nigeria. A total of 20 cheese samples were obtained from different points in 4 cities in southern Nigeria, 5 cheeses per city. They were investigated for some physico-chemical properties, isolation and microbial counts and determination of antimicrobial potential. There was no significant variation in the composition of physic-chemical properties of cheese samples from various cities except for the acidity of cheese sample obtained from Ilorin. All the 20 samples (100%) yielded low level of lactic acid bacteria (LAB) with counts ~ 103. *Escherichia coli* or *Klebsiella* species were constantly isolated in all the cheese samples. Similarly, yeast and *Aspergillus* species were isolated either alone or in a mixed culture. The result showed increase in total bacteria count from the point of production to the hawkers. Antimicrobial potential was not found in cheese against the microorganisms used in the study. The study identified local cheese ('wara') as a high risk food in Nigeria due to the high rate of contamination since they are ready-to-eat food item and no antimicrobial property detected in the soft cheese.

RUNNING TITLE: MICROBIAL CONTAMINATION OF CHEESE

Key Words: Cheese; Bacteria; Fungi; Nigeria, Susceptibility

LA CONTAMINATION MICROBIENNE DES FROMAGE PRODUITS LOCALEMENT ET DETERMINATION DE LEUR POTENTIEL ANTIMICROBIEN AU NIGERIA

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RÉSUMÉ

Le taux de fromage à pâte molle et les modalités de production de fromage au Nigeria à forte consommation a incité la nécessité de déterminer la qualité microbienne et propriétés antimicrobiennes de fromage produit localement au Nigeria. Un total de 20 échantillons de fromage ont été obtenues à partir de différents points dans 4 villes au sud du Nigeria, 5 fromages par ville. Ils ont été étudiés pour certaines propriétés physico-chimiques, l'isolement et les numérations microbiennes et détermination du potentiel antimicrobien. Il n'y avait aucune variation significative dans la composition des propriétés physico- chimiques des échantillons de fromage à partir de différentes villes à l'exception de l'acidité de l'échantillon obtenu à partir de fromage de la ville d'Ilorin. Tous les 20 échantillons (100%) ont donné un faible niveau de bactéries lactiques (LAB) avec environs 103 espèces. *Escherichia coli* ou *Klebsiella* ont été constamment isolés dans tous les échantillons de fromage. De même, des espèces de levures et d'Aspergillus ont été isolés soit seuls, soit dans une culture mixte. Le résultat a montré l'augmentation des bactéries totales compté du point de production aux colporteurs. Potentiel antimicrobien n'a pas été trouvé dans le fromage contre les micro-organismes utilisés dans l'étude. L'étude a identifié fromage local (' wara ') comme un aliment à haut risque au Nigeria en raison du taux élevé de contamination, car ils sont prêts à consommer l'aliment et aucune propriété antimicrobienne détecté dans le fromage à pâte molle.

TITRE COURANT : LA CONTAMINATION MICROBIENNE DES FROMAGES

Mots clés: Fromage; bactéries; champignons; Nigeria, sensibilité

INTRODUCTION

Soft cheese ('wara'), in one of the ethnic language from Nigeria, (Yoruba) is a very nutritious food obtained from cow milk. Milk is an aqueous colloidal suspension of proteins, fats, and carbohydrates that contains numerous vitamins and minerals. Cheese is one of the numerous products obtained from the processing of milk (1). The production of cheese in African countries has increased and about one third of the total volume of milk is used for this purpose (2). This soft cheese produced in some parts of southern Nigeria and predominantly in the northern parts makes use of local ingredients. Cheese making began about 8000 years ago and now there are in excess of 1000 cheese varieties worldwide (3) each unique with respect to its flavour and form. Manufacture of most cheese varieties involves combining 4 ingredients; milk, rennet, microorganisms and salt. Variations in ingredient blends and subsequent processing have led to the evolution of all these cheese varieties. The manufacturing process of soft cheese in Nigeria is undeveloped and at its infancy left with the natives to manufacture locally, hence there is difficulty in extending shelf life and conserving the nutritious components of milk.

Microorganisms found in cheese can be classified based on their biochemical types, temperature, response and ability to cause infection and disease (4). Organisms associated with milk and milk products include streptococci, lactobacilli, coliform bacteria and some fungi. All of these are from various sources and act on different substrates in cheese thereby producing various end products (5). Soft cheese has also been found to have outstanding antimicrobial properties. It contains a variety of factors and compounds which have been reported to have health promoting effects and prevent disease (6-8). Several studies have found lactic acid bacteria (LAB) to occur naturally as an indigenous microflora in cheese (9, 10). The lactic acid fermentation that these bacteria carry out has long been known and applied by human for making different food stuffs (11). Lactic acid bacteria had the highest percentage occurrence (76%), followed by enterobacteria (17%), and *Staphylococci* (7%). Four genera of lactic acid bacilli have been isolated, they were: *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Pediococcus* (11). Survey of soft cheese have found *E. coli* in 40-50% of the products (12) demonstrating that a mode of contamination with *E. coli* exist during the production or processing.

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antimicrobial activities of cheese. Some of them have been seen to have antimicrobial effect which automatically has been conferred to the soft cheese (13,14). Lactic acid bacteria improve the quality of cheese and also play an important role in preventing the growth of undesirable bacteria like coliform (13). The lactic acid bacteria create an acidic environment conducive for proliferation of yeast while yeast provides growth factors such as vitamins and amino acid for lactic acid bacteria. Cheese however has been shown to have antimicrobial properties (6) and also prevent disease. It has been used as drug for certain infection when common antimicrobial agents failed. It has also been found that soft cheese has growth inhibitory activity against common bacteria that causes diarrhoea in South West Nigeria (15, 16).

The trend in manufacture of cheese all over the world is the production of flavoured, highly nutritive and good microbiological quality. Due to the high consumption rate of soft cheese and in view of manner of cheese production in Nigeria, there was need to determine the microbial quality which will be of utmost public health importance. Moreover, in order to continue in the search to developing natural novel antimicrobial agents that will be effective against a spectrum of commonly isolated microbial pathogens as done previously by the authors using various plant extracts with antimicrobial potentials, garlic (17); coconut oil (18). It was also important to determine the antimicrobial properties of cheese in view of multiple drug resistance nature of Gram negative bacteria pathogen in Nigeria (19) and presence of MRSA (20) and possible emergence of VRSA. This indicates a limited drug for use in such organisms. The study however investigates the extent of microbial contamination and antimicrobial properties of locally produced cheese in Nigeria.

MATERIALS AND METHODS

Cheese Samples

Twenty samples of soft cheese with the whey (water portion) were bought from hawkers and point of production at different locations of 4 cities in southern Nigeria; Osogbo, Ede, Ogbomosho and Ilorin. The soft cheese and the whey were put inside a sterile plastic universal container. The samples were transported immediately to the laboratory for processing or kept inside a refrigerator for few minutes if delay was envisaged.

Physico-chemical analysis

The moisture contents of cheese were determined by the oven drying method at 105ºC (21). About 5 g of the cheese sample was weighed and difference between the wet and dried weight of cheese represents the moisture content. The pH of cheese suspension at 2% strength was determined at room

temperature (29.7°C) using electrodes of a pH meter (Hanna instrument) placed directly into each suspension. The pH meter with accuracy of 0.1 was first standardised using buffer solution of pH 4 and 7. This was done in triplicate and the mean pH of each sample was determined.

Qualitative estimation of protein and glucose was done for all the samples using Combo 2 strips. The strip was dipped into the samples and allowed to stay for 45 sec; colour change was checked with the standard gradients of colour provided by the manufacturer. Sterile distilled water and standard protein or glucose solutions were used as controls.

Microbiological analysis

Viable microbial count analyses were performed on samples of cheese as follows; a 10-fold serial dilution of up to 10-10 for each sample was prepared in 0.1% peptone water. For viable bacterial count, each dilution was subsequently plated onto standard plate count agar (PCA). The PCA plates were incubated at 37°C for 48 hours. The colony forming units (CFU) were counted on plates having between 30 and 300 colonies using Quebec colony counter (22). The enumeration of viable bacteria count was carried out in duplicate on each sample and the isolated bacteria were identified using standard bacteriological procedures (23). Similarly, for fungi Saborauds dextrose agar (SDA) was used to plate the dilutions. The plates were incubated at room temperature in a moistened and dark environment for 3 to 5 days. The mean of colony forming unit per gram and log10 cfu were calculated and recorded. Middlebrook agar was also inoculated after previous treatment of the sample with NaOH to decontaminate the samples for possible isolation of *Mycobacterium* species. This was then incubated for 2 weeks.

Antimicrobial potential

In order to determine antimicrobial property of the cheese samples 10 g of each 20 samples of cheese was dissolved in 100 ml of whey. After extraction, filtration was done with the aid of sterile membrane filter of pore size 0.22 µm (Curringham, UK). The sterile extracts obtained were used to determine antimicrobial property using 45 microorganisms (*Escherichia coli*, 10; *Klebsiella pneumoniae*, 10; *Pseudomonas aeruginosa*, 10; *Staphylococcus aureus*, 10; *Candida albicans*, 5) from our collections. They were previously identified using API strips (bioMérieux, Marcy l'Etoile, France) accordingly and comprise of susceptible and multiresistant strains. These strains were from various clinical specimens (wound, urine, blood culture, sputum, catheter tip and ear swab). Inoculum obtained from overnight culture, suspended with sterile distilled water was adjusted to 0.5 MacFarland standards at 500 nm absorbance (24). Reference strains *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *Candida krusei* ATCC 6258 were included as control strains. The minimum inhibitory concentration of cheese for each isolate was determined using punch-hole agar diffusion method. With the aid of sterile swab sticks, the bacterial suspensions were inoculated separately on plates and wells of appropriate diameters were made on the seeded plates. Varying concentrations of each extracts 100, 80, 70, 50, 40, 30, 20 and 10% were introduced into the wells of different plates with the aid of sterile automatic pipettes. The plates were labelled and incubated at 37°C for 18 to 24 h. After incubation, clear zones of inhibition around the wells indicate sensitivity; diameters of the clear zones were measured as index of the degree of sensitivity. Tests were carried out in triplicates to ensure accuracy and reproducibility of results. Sensitivity pattern was compared with the control strains

Statistical analysis

Collation of data was carried out using Epi-info software from Centre for Disease control and prevention, USA. Data were analysed using statistical package within the Epi-info software. ANOVA was used to determine whether there were significant differences in moisture content and pH values. Similarly, for bacteria or fungi count between or within cheese samples collected from varying locations. The p value less than 0.05 was considered to be significant.

RESULTS

Composition of cheese

The moisture contents of the cheese expressed in terms of water availability aw were found to range between 3.3 and 4.6. The average aw for Ede, Ilorin, Osogbo and Ogbomoso were 3.9, 3.8, 4.0 and 3.9 respectively, there was no statistical significant difference between aw of these samples within the city or across the cities; $P > 0.05$. Similarly, pH ranged between 5.5 and 6.8. The average pH from each city was as follows; Ede, 6.5; Ilorin, 5.9, Osogbo, 6.4; and Ogbomoso, 6.6. Cheese samples from Ilorin are more acidic than rest of the cities, $P>0.05$. The glucose and protein were measured qualitatively and results ranged from trace to ++ as shown in Table 1.

Viable bacterial count

All the 20 samples (100%) yielded low level of lactic acid bacteria (LAB) with counts ~ 103 (data not shown).

TABLE 1: SOME PHYSICOCHEMICAL PROPERTIES OF CHEESE

Sample	Location	aw	pH	Glucose	Protein
P	Ede	3.7	6.5	+	+
1	Ede	4.0	6.5	+	+
2	Ede	4.2	6.5	+	Trace
3	Ede	3.9	6.5	+	+
4	Ede	3.8	6.5	+	+
P	Ilorin	3.3	6.0	-	Trace
1	Ilorin	3.8	5.5	-	-
2	Ilorin	4.0	6.0	-	Trace
3	Ilorin	3.8	5.5	-	-
4	Ilorin	3.9	6.5	-	-
P	Osogbo	4.6	6.5	Trace	++
1	Osogbo	3.9	6.5	-	+
2	Osogbo	4.1	6.5	-	+
3	Osogbo	3.7	6.0	+	+
4	Osogbo	3.7	6.5	+	+
P	Ogbomoso	3.6	6.5	+	+
1	Ogbomoso	3.7	6.5	+	+
2	Ogbomoso	4.1	6.8	-	+
3	Ogbomoso	4.1	6.5	Trace	+
4	Ogbomoso	3.8	6.8	Trace	++

P - point of production; 1 - 1st Hawker; 2 - 2nd Hawker 3 - 3rd Hawker; 4 - 4th Hawker; aw - water availability. + - Low; ++ - Moderate

Also, *Escherichia coli* or *Klebsiella* species were constantly isolated in all the cheese samples. On Sabouraud agar, yeast and *Aspergillus* species were isolated either alone or in a mixed culture. Nine of the samples yielded *Aspergillus* species while 12 yielded yeast and none of the samples yielded *Mycobacterium species*. There was high viable bacterial count in all the samples of soft cheese. The result showed increase in total bacteria count from the point of production to the hawkers (Table 2). Statistically, there was no significant difference in the proportion of bacteria or fungi count detected from the samples between locations ($P = 0.29$; $P > 0.05$) and within each of the 4 locations ($P = 0.19$; $P > 0.05$, a representative value for one of the within location).

Antimicrobial property

There was however no antimicrobial properties detected in the soft cheese to the isolates. All the isolates tested were resistant to the aqueous or whey extracts of cheese, no zone of inhibition (0 mm diameter) (Table 3). *E. coli*, ATCC 25922; *P. aeruginosa*, ATCC 27853; *S. aureus*, ATCC 25923; *Candida krusei*, ATCC 6258 were used as control strains.

DISCUSSION

The presence of Gram negative bacteria (lactose fermenters), yeast and mould indicate that the soft cheeses hawks in Nigeria are contaminated. Gram negative bacteria had the highest number of occurrence followed by yeast and mould. There was no difference in the microbial load of these cheese samples obtained from different locations. This is in line with the works of Sangoyomi et al. (25) that isolated some members of *Enterobacteriaceae* including *E. coli*, and yeast from soft cheese, and Abou Dawood et al. (26) who obtained high counts for aerobic bacteria, mould and yeast. In this study, the yeast was present in more than 50% of the 20 samples bought at these locations. It is evident that yeast strains which have activities of amylase, protease and lipase will have an impact on the textural and taste profile of soft cheese. The trend in cheese manufacture is production of nature flavoured cheese made in short time with highly nutritive value and good microbiological quality for human consumption (27, 28).

TABLE 2: VIABLE COUNTS OF ORGANISMS ISOLATED FROM CHEESE CONTAMINATION

Location	Sample	Bacteria	Count (cfu/ml)	Fungi	Count (cfu/ml)
Ede	P	*E. coli*	2.9 × 106	ND	-
	1	*E. coli/Klebsiella* spp	3.0 × 106/2.5 × 104	ND	-
	2	*E. coli*	3.0 × 106	Yeast	1.0 × 103
	3	*E. coli/Klebsiella* spp	3.0 × 108/3.0 × 106	Yeast/*Aspergillus* spp	1.0 × 102/1.0 × 103
	4	*E. coli*	3.0 × 108	Yeast/*Aspergillus* spp	1.5 × 103/1.5 × 102
Ilorin	P	*E. coli/Klebsiella* spp	8.0 × 103/5.0 × 102	Yeast	1.2 × 104
	1	*E. coli/Klebsiella* spp	9.0 × 103/5.0 × 103	Yeast	2.0 × 103
	2	*E. coli*	8.4 × 103	Yeast/*Aspergillus* spp	1.5 × 103/2.0 × 103
	3	*E. coli*	1.2 × 104	Yeast	1.0 × 105
	4	*E. coli*	1.4 × 104	Yeast/*Aspergillus* spp	1.5 × 103/1.0 × 103
Osogbo	P	*E. coli*	1.7 × 104	*Aspergillus* spp	1.2 × 103
	1	*E. coli*	1.9 × 106	ND	-
Location	Sample	Bacteria	Count (cfu/ml)	Fungi	Count (cfu/ml)
	3	*E. coli/Klebsiella* spp	3.0 × 106/1.0 × 103	ND	-
	4	*E. coli/Klebsiella* spp	2.0 × 108/2.0 × 104	Yeast/*Aspergillus* spp	1.0 × 104/1.8 × 102
Ogbomosho	P	*E. coli*	1.9 × 106	*Aspergillus* spp	1.0 × 103
	1	*E. coli*	2.0 × 106	*Aspergillus* spp	1.8 × 102
	2	*E. coli*	2.9 × 106	Yeast	2.0 × 105
	3	*E. coli*	2.7 × 106	Yeast/*Aspergillus* spp	1.0 × 104/1.5 × 103
	4	*E. coli/Klebsiella* spp	2.7 × 108/1.5 × 103	ND	-

ND- not detected

TABLE 3. NUMBER AND PERCENTAGES OF STRAINS INHIBITED WITH DIFFERENT CONCENTRATIONS OF CHEESE

Organisms	100% x (%)	80% x (%)	70% x (%)	60% x (%)	50% x (%)	40% x (%)	30% x (%)	20% x (%)	10% x (%)	5% x (%)
E. coli, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
K. pneumonia, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P. aeruginosa, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S. aureus, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C. albicans, n = 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

n = number of strains; x = number of strains that show inhibition

The samples consistently contained *E. coli* and *Klebsiella* species; this is similar to the study of Vigano et al. (29) where 98% of milk samples grew *E. coli*. Also, retail surveys by Aureli et al. (12) in Italy of soft and semi cheeses found *E. coli* in 40 - 50% of these cheeses demonstrating that a mode of contamination of *E. coli* exists during the chain of production or processing. *E. coli* is a consistent inhabitant of the human intestinal tracts and regular presence of the bacterium in the human intestine and faeces has led to tracking the bacterium in nature as an indicator of faecal pollution. Through this, it means wherever *E. coli* was found, there may be faecal contamination (30). The methods of transportation,

handling and sale of cheese or cheese products are not hygienic or sterile enough. One major observable problem is that the producers or vendors are really not educated and locals which consequentially affect the methods and handling of these products. Bhat et al. (31) had earlier opined that unclean hands of workers, poor quality of materials used and water supplied for washing utensils could be the source of accelerating the bacterial contamination of milk products. It has been reported that contamination of raw milk and cheese poses a significant risk to humans (32).

The high water availability and a neutral pH; these physical conditions favour the growth and survival of these bacteria, and these consequently caused the low level of bacteria count in LAB and vice versa. An increase in the moisture content of cheese could lead to increased susceptibility to spoilage. Similarly, the optimum pH for the growth of most common bacteria is around neutral, and growth is often poor at pH values <5.0. This explains in part the reasons why the samples were heavily contaminated.

It was observed from the present work that soft cheese did not have antimicrobial property against tested bacteria. This however is contrary to the work done by Olorunfemi et al. (33) who reported that soft cheese had antimicrobial properties against different organisms. There is a high level of contamination of soft cheese samples bought and this could account for the absence of antimicrobial properties in the sample. Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate antibacterial compounds in plants (14, 34) and food and food products (18).

In conclusion, local cheese ('wara') has been identified as a high risk food in Nigeria due to the high rate of contamination since they are ready-to-eat food item. They however do not have any antimicrobial activity. There is a need for standardising the production methods in order to set a benchmark for minimum standard of cheese quality.

Conflict of interest

We declare that we have no conflict of interest.

Authors Contributions

OAT contributed and advised DOO on concept and design of the experiment. DOO, AAO and ASO did preliminary work while the rest of the experiment was done by AAO and ASO under the supervision of OAT who also did the statistical analysis. The write-up was executed by DOO with contributions and proof-reading by other authors.

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ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1413 http://www.ajol.info/journals/ajcem
COPYRIGHT 2014 http://dx.doi.org/10.4314/ajcem.v15i2.5
AFR. J. CLN. EXPER. MICROBIOL. 15(2): 84-90

CANDIDURIA AMONG HIV- INFECTED PATIENTS ATTENDING A TERTIARY HOSPITAL IN BENIN CITY

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ABSTRACT

Background: Candiduria is a common finding. However, in immunocompromised patients like HIV-infected individuals, it has high risk of morbidity and mortality as it could be a pointer to systemic candidiasis. Unfortunately, there are no clear criteria for differentiating between colonization and infection or between upper or lower urinary tract infections.

Objective: This study focused on determining the spectrum of *Candida* species implicated in candiduria among HIV-infected individuals and their susceptibility to fluconazole and voriconazole in a tertiary hospital.Methods: A total of 300 subjects comprising of 200 HIV patients and 100 non-HIV individuals were used for this study. Clean catch midstream were collected from each individual and processed using standard microbiological techniques. Emergent *Candida* isolates were identified with CHROMagar Candida and sugar fermentation tests.Results: The overall prevalence of candiduria among HIV patients was 13.5%. HAART-naive patients had a significantly higher prevalence (OR=4.165, 95%CI=1.602, 10.828; P=0.0038) than their counterpart on highly active antiretroviral therapy (HAART). Female gender was a significant risk factor for acquiring candiduria. Age had no significant effect on the prevalence of candiduria in this study. A CD4+ count <200 cells/µl was a significant risk factor for acquiring candiduria only among HAART-naive patients (OR=11.711; 95%CI=3.943, 34.780; P= 0.0001). The three species of *Candida* recovered from this study were *C. albicans, C. krusei and C.parapsilosis. C. albicans* (64.52%, 83.36%) and *C. krusei* (66.67%, 100.00%) were resistant to fluconazole and voriconazole respectively.Conclusion: There is a significant relationship between antiretroviral therapy, CD4+ counts, and the prevalence of candiduria among the study population.

Keywords:HAART, HAART-naive, candiduria, CD4+ counts, Candida, prevalence.

CANDIDURIE CHEZ DES MALADES VIVANT AVEC LE VIH DANS UN HOPITAL TERTIAIRE DANS LA VILLE DE BENIN

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RÉSUMÉ

Contexte: La candidurie est un problème commun de sante publique. Cependant, chez les patients immunodéprimés comme les individus infectés par le VIH, elle présente un risque élevé de morbidité puisqu'elle peut évoluer vers la candidose systémique. Malheureusement, il n'existe pas de critères clairs permettant de distinguer la colonisation et l'infection de même que les infections des voies urinaires supérieures et inférieures.

Objectif: Cette étude a porté sur la détermination du spectre d'espèces de *Candida* impliqués dans la candidurie chez les personnes infectées par le VIH et leur sensibilité au fluconazole et voriconazole dans un hôpital tertiaire. Methodes : Un total de 300 sujets comprenant 200 patients atteints du VIH et 100 personnes non -VIH ont été utilisés dans cette étude. Les echantillons d'urine ont été collectées auprès de chaque personne par la methode de ''Clean catch midstream''et traitées en utilisant des techniques microbiologiques standard. Les isolats émergents de *Candida* ont été identifiés avec CHROMagar Candida et les tests de fermentation de sucre. Résultats : La prévalence globale du VIH chez les patients atteints de candidurie était de 13,5%. Les patients en naïfs de la multithérapie HAARTavaient une prévalence significativement plus élevée (OR = 4,165, IC à 95% =

1,602, 10,828, p = 0,0038) par rapport a leurs homologues sous traitement antirétroviral hautement actif (HAART) . Le sexe féminin était un facteur de risque important d'acquisition de candidurie. L'âge n'avait pas d'effet significatif sur la prévalence de candidurie dans cette étude. Un compte de CD4 + < 200 cellules / µl n'a été un facteur de risque important pour l'acquisition de candidurie que chez les patients en multithérapie naïfs (OR = 11,711 ; IC à 95% = 3, 943, 34, 780, p = 0,0001). Les trois espèces de Candida récupérés de cette étude étaient *C. albicans*, *C. krusei* **et** *C.parapsilosis***. *C. albicans*(64,52%, 83,36 %) et** *C. krusei* **(66,67%, 100,00 %) étaient résistants respectivement au fluconazole et voriconazole. Conclusion: Il existe une relation significative entre le traitement antirétroviral, CD4 +, et la prévalence de candidurie parmi la population de l'étude.**

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Mots-clés: multithérapie HAART , naïfs , candidurie , CD4 + , Candida , prévalence .

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) cause by human immunodeficiency virus (HIV) is the most important public health problem of modern times (1). HIV/AIDS continues to spread globally and remains a worldwide pandemic affecting about 40 million people (2). The pandemic is the leading cause of death in sub-Sahara Africa and the fourth leading cause of mortality worldwide and over 95% of these deaths have occurred among young adults in the developing world (3-4).

Fungal infections caused by yeast pathogens remain quite common in immunocompromised host, especially in HIV-infected individuals (5). These infections are playing an increasing important role in the morbidity and mortality of HIV/AIDS patients (6). Although the use of highly active antiretroviral therapy (HAART) has decreased the incidence of fungal infections (7-8), candidiasis continues to afflict HIV-infected individuals in HAART era (6, 9). Unfortunately, prolong use of antifungal among this population has led to increased incidence of resistance (10).

The healthy urinary tract is sterile so the presence of *Candidaspecies* in urine or candiduria represent a variety of clinical situations (11-13), such as contamination of urine specimen, colonization of bladder due to indwelling catheters, infection of upper or lower urinary tract, and primary or disseminated candidiasis (13-15).

Candidaspecies are the most commonly recovered fungi from urine (16) with *C. albicans* being the most frequently isolated, accounting for 50-70% of isolates in various studies (15, 17). Others non-albicans such as *C. glabrata* and *C. tropicalis* are the next most common species while *C. parapsilosis* is commonly found in urine of neonates and is usually associated with systemic infection in this population (18).

Conditions that predispose to candiduria includes immunosuppresion, use of broad spectrum antibiotic, gender, age, diabeties mellitus, chronic renal failure, malignancy, urinary tract abnormalities, pregnancy, and neutropenia (16, 19-23).

Asymptomatic candiduria is usually benign in most patients and do not require antifungal medication (24). However, in immunocompromised patients, it has a high risk of morbidity and mortality (25). There is little or no report on candiduria among HIV-infected individuals in our locality, thus this study focused on determining the spectrum of *Candida* species implicated in candiduria among HIV-infected individuals and their susceptibility to fluconazole and voriconazole.

MATERIALS AND METHODS

Study Area

The study was carried out in the University of Benin Teaching Hospital, Benin City, Nigeria. It is located in the South-South geopolitical zone of Nigeria. It serves as a referral hospital to about six to ten states in Nigeria. It is a centre for Institute of Human Virology, Nigeria and US President's Emergency Plan for AIDS Relief (PEPFAR) HIV/AIDS interventions in the zone.

Study Population

A total of 300 individuals consisting of 200 HIV patients and 100 (42 males and 58 females) apparently healthy, aged-matched, non-HIV individuals were recruited for this study. The patients consists of 100 (31 males and 69 females) HAART-naïve patients and 100 (22males and 78 females) HIV patients on HAART for 3-6 months. The HAART regimen included stavudine, zidovudine, and nevirapine. The HIV patients were out-patients and asymptomatic. Informed consent was obtained from all individuals prior to specimen collection. The Ethical Committee of the University of Benin Teaching Hospital approved the protocol for this study.

Specimen collection and processing

Venous blood (5ml) was collected into ethylene diamine tetraacetic acid (EDTA) container and mixed. Clean-catch mid-stream urine was collected into sterile universal container containing few crystals of boric acid as preservative (26).

The blood specimens were used for CD4 counts using flow cytometry (Partec, Germany) following

manufacturer's instruction. A loop-full (0.001ml) of well mixed un-centrifuged urine was streaked onto

the surface of Saboraund's Dextrose Agar (SDA) and Brain Heart Infusion Agar (BHIA) containing 5µg/ml gentamicin. The plates were incubated aerobically at 370C for 24-48 hours and counts were expressed in colony forming unit per ml. A count of ≥ 105 cfu/ml was considered significant to indicate asymptomatic candiduria. The urine specimens were centrifuged at 2000 g for 5minutes. The supernatant was discarded and a drop of the deposit was examined microscopically at high magnification for pus cells. Pus cells ≥5 per high power field were considered to indicate infection (26).

Emergent yeast colonies were stored for identification. All *Candida* isolates were identified with CHROMagarTM*Candida* (Paris, France) as previously described (27) and sugar fermentation tests as described by Forbes *et al.,* (28).Antifungal susceptibility test was performed using the CLSI (29) disc diffusion methods. Voriconazole disc (1µg)and fluconazole disc (25µg) (Oxoid, England) were used for this study.

RESULTS

The prevalence of candiduria among HIV and non-HIV individuals is shown in table 1. HIV status was a significant risk factor for acquiring candiduria (OR=3.746; 95%CI=1.273, 11.025; P=0.0189). Considering HIV status, the prevalence of candiduria among HAART-naïve patients was significantly higher than HIV patients on HAART (P= 0.0038). Female gender was significantly associated with candiduria.

TABLE 1: PREVALENCE OF CANDIDURIA AMONG HIV AND NON-HIV INDIVIDUALS.

	Male		Female		Total		
Status	**No. Sampled**	**No. Infected (%)**	**No. Sample**	**No. Infected (%)**	**No. Sampled**	**No Infected (%)**	**Fungal Isolates**
Non HIV	**42**	**-**	**58**	**4 (6.70)**	**100**	**4 (4.00)**	*C. albicans*
Mixed Infection HIV patients	**42**	**-**	**58**	**-**	**100**	**-**	**-**
HAAT naive×	**31**	**1 (3.23)**	**69**	**20 (28.99)**	**100**	**21 (21.00)**	*C. albicans, C. parapsilosis*
Mixed Infection	**31**	**-**	**69**	**3 (4.35)**	**100**	**3 (3.00)**	*C. krusei , C.albicans*
On HAART$^{\beta,\alpha}$	**22**	**-**	**78**	**6 (7.69)**	**100**	**6 (6.00)**	*C. albicans, C. krusei*
Mixed Infection	**22**	**-**	**78**	**1 (1.28)**	**100**	**1 (1.00)**	

HIV versus non-HIV: OR=3.746; 95%CI=1.273, 11.025; P=0.0189; βOn HAART versus non-HIV: OR=1.532; 95%CI=0.4187, 5.604; P=0.7475. ×HAART naive versus non-HIV: OR=6.380; 95%CI=2.102, 19.362; P=0.0006. αHAART naive versus on HAART: OR=4.165, 95%CI=1.602, 10.828; P=0.0038

Only one male among HAART-naive HIV patient had candiduria. The prevalence of candiduria did not differ significantly (P= 0.7475) between HIV patients on HAART and non-HIV individuals.

Table 2 show *Candida* isolates recovered from HIV and non-HIV individuals. *C. albicans* was still the most prevalent and the only isolate recovered from non-HIV individuals. *C. krusei* was recovered from HIV patients on HAART and HAART-naïve while *C. parapsilosis* were recovered only from HAART-naïve HIV patients.

TABLE 2: CANDIDA ISOLATES RECOVERED FROM URINE OF HIV AND NON-HIV INDIVIDUALS

Organisms	Non-HIV (%)	HAART-naïve (%)	On HAART (%)	Total (%)
C. albicans	4 (100.00)	20 (86.96)	7 (87.50)	31 (88.57)
C. krusei	-	2 (8.70)	1 (12.50)	3 (8.57)
C. Parapsilosis	-	1 (4.45)	-	1 (2.86)

TABLE 3: *CANDIDA* **ISOLATES RECOVERED FROM URINE OF HIV AND NON-HIV INDIVIDUALS IN RELATION TO GENDER**

Organisms	Non-HIV		HAART-naive		On HAART			
	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
C. albicans	-	4 (100.00)	1 (100.00)	19 (82.61)	4 (80.00)	7 (87.50)	1 (100.00)	30 (85.71)
C. krusei	-	-	-	2 (8.70)	-	1 (12.50)	-	3 (8.52)
C. parapsilosis	-	-	-	1 (4.35)	-	-	-	1 (2.86)

Table 3 show Candida isolates recovered from HIV and non-HIV individuals in relation to gender.

All *C. albicans* recovered from non-HIV individuals were from female. Similarly, *C. krusei*, and *C. parapsilosis* were recovered from female HIV patients.

TABLE 4: PREVALENCE OF CANDIDURIA AMONG HIV AND NON-HIV INDIVIDUALS IN RELATION TO AGE

Age	Non-HIV		HAART-naive		On HAART	
Years	No. sampled	No. infected (%)	No. sampled	No. infected (%)	No. sampled	No. infected (%)
11-20	5	-	-	-	-	-
21-30	40	2 (5.0)	15	2 (13.3)	14	-
31-40	34	1 (2.9)	48	15 (31.3)	47	4 (8.5)
41-50	17	1 (5.9)	32	5 (15.6)	29	2 (6.9)
51-60	3	-	4	1 (25.0)	7	1 (14.3)
61-70	1	-	1	-	3	-

Non-HIV; P=0.9810; HAART-naïve; P=0.4160; On HAART; P=0.7329

TABLE 5. EFFECT OF CD4 COUNTS ON PREVALENCE OF CANDIDURIA AMONG HIV INDIVIDUALS

CD4 Counts (Cells/μl)	No. sampled	No. infected (%)	OR	95%CI	P. value	*Candida* isolates
HAART naïve						
<200	32	17(53.13)	11.711	3.943, 34.780	0.0001	*C. albicans*
≥200	68	6(8.82)	0.085	0.029, 0.254		*C. albicans*, *C. parapsilosis*
Mixed Infection						
<200	32	4(12.50)	21.632	1.127, 415.35	0.0152	*C. albicans*, *C. krusei*,
≥200	68	-	0.046	0.002, 0.888		
On HAART						
<200	12	2(16.67)	3.320	0.567, 19.426	0.4260	*C. albicans*
≥200	88	5(5.68)	0.301	0.052, 1.762		*C. albicans*
Mixed Infection						
<200	12	1(8.33)	23.087	0.886, 601.310	0.2399	*C. albicans*, *C. krusei*
≥200	88	-	0.043	0.002, 1.128		

Age had no significant effect on the prevalence of candiduria in this study (Table 4).

CD4 <200cells/µl was significantly associated with candiduria. However, among HIV patients on HAART, CD4<200cells/µl did not significantly affect the prevalence of candiduria (Table 5).

Among the yeasts recovered, more *C. albicans* and *C. krusei* were resistant to fluconazole and voriconazole. The only isolate of *C. parapsilosis* was susceptible to both antifungal agents (Table 6).

TABLE 6: ANTIFUNGAL SUSCEPTIBILITY PROFILE OF *CANDIDA* ISOLATES

Organisms	FLUCONAZOLE			VORICONAZOLE		
	S (%)	S-DD (%)	R (%)	S (%)	S-DD (%)	R (%)
C. albicans	9(29.03)	2(6.45)	20(64.52)	10(90.91)	2(100.00)	19(83.36)
C. krusei	1(33.33)	-	2(66.67)	-	-	3(100.00)
C. parapsilosis	1(100.00)	-	-	1(100.00)	-	-

DISCUSSION

Fungal infections are playing an increasing important role in the morbidity and mortality of HIV/AIDS patients (6). Although the use of highly active antiretroviral therapy (HAART) has decreased the incidence of fungal infections (7-8), candidiasis continues to afflict HIV-infected individuals in HAART era (6, 9).

Recent studies have shown that candiduria is getting increased due to immunocompromised patients; prolong hospitalization, uncontrolled use of antibiotic, prophylaxis by antifungal agents, urinary tract surgeries (11, 30-31). Candiduria accounted for up to 10% of UTIs and has resulted in increased rate of mortality during the last decades due to use of new treatments, surgery and transplantation (14, 32-33).

In this study, the overall prevalence of candiduria among HIV-infected individual was 13.5%. This is lower than 22% reported in Brazil among hospitalized patients (35). HIV status was significantly associated with candiduria (OR=3.746; 95%CI=1.273, 11.025; P=0.0189). HIV results in immunosuppression which has been reported as a risk factor for acquiring candiduria (11). However, in regard to treatment status, the prevalence of candiduria among HIV patients on HAART (6%) have no significant different with non-HIV (4%) individuals. This indicates that candiduria in HAART-naïve HIV patients contributed to the significant difference in prevalence among HIV and non-HIV individuals. Thus as, HAART improves immunity, candiduria decreases to almost the same prevalence with that of non-HIV individuals.

Candida albicans (88.57%) was the most predominant isolate recovered. This agrees with the report of previous investigators (11, 16, 24). Other species of Candida recovered includes *C.krusei* (8.57%) and *C. parapsilosis* (2.86%). These species of *Candida* have previously been reported as causes of candiduria (16, 35).Irrespective of HIV status and treatment status, the female gender was associated with candiduria in this study. This is in agreement with the report of previous investigators (14, 19, 22).In this study, increased age had no significant effect on the prevalence of candiduria. This does not agree with the report of Kauffman, (14). The reason for this is unclear.

Among HAART-naïve HIV patients, CD4<200cells/µl was associated with candiduria (Table 5). It has been reported that fungal agents such as *Candida* takes advantage of the immune suppression seen in HIV among HIV patients as a result of CD4 T cells depletion (35). This may explain the findings in this study. It has also been reported from a number of experimental studies that acquired resistant to *Candida* infection is dependent upon the participation of T-lymphocytes (35). HAART causes a decline in the incidence of some opportunistic infection in AIDS and this decline is currently attributed to restoration of immunity and anti *Candida* activity of protease inhibitors among the HAART regimen (35). This may explain the non significant difference in the prevalence of candiduria among HIV patients on HAART with CD4<200cells/µl and CD4≥200cells/µl. However, protease inhibitors were not among the HAART regimen for our HIV patients on HAART. This may therefore indicate that immune

reconstitution may account for the observed results among HIV patients on HAART in relation to candiduria.Most of *C. albicans* (64.52%, 83.36%) and *C. krusei* (66.67%, 100.00%) were resistant to fluconazole and voriconazole respectively. It has been reported that *Candida* species from HIV patients are more resistant to antifungal agents (36-37). Prolong used of antifungal among HIV patients has been reported as predisposing factor (10).

In conclusion, there is a significant relationship between antiretroviral therapy, CD4+ counts, and the prevalence of candiduria among HIV patients. Therefore candiduria should be verified by obtaining a second specimen and appropriate therapy instituted after antifungal susceptibility teststo prevent systemic candidiasis amongthis population.

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ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1414 http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL. 15(2): 91-96

SEROLOGICAL SCREENING FOR ANTE-NATAL TOXOPLASMOSIS IN MAIDUGURI MUNICIPAL COUNCIL, BORNO STATE, NIGERIA

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ABSTRACT

Toxoplasma gondii infection causes devastating defects including, blindness, neurological impairment and mental retardation in congenitally infected children. Congenital infection occurs when a woman becomes infected during pregnancy; and the severity of the illness is related to the trimester period. This research was designed to evaluate the seroprevalence of toxoplasmosis amongst pregnant women (n=90) using enzyme linked immunosorbent assay (ELISA) kit (Cortez Diagnostics Inc. USA). A structured questionnaire was used to collect socio-demographic data. A significant (x2 Cal 233.0/ x2 tab 124.1, p=0.01) overall prevalence of 22.2% (20/90) was obtained. Pregnant women within 25-29years had the highest prevalence of 33.3% (3/9); this was significant (x2 Cal 35.85/ x2 tab 9.21, p=0.01). An association between high levels of toxoplasma IgG and miscarriage was established in four of twenty five women who had suffered miscarriage (t cal 5.3/t tab 2.81, p=0.01). The results presented indicate that toxoplasmosis is a significant public health burden in the area of study, which requires drastic remedial measures.

Key words: Toxoplasmosis, pregnant women, miscarriage, Nigeria

DEPISTAGE SEROLOGIQUE POUR TOXOPLASMOSE PRENATALE DANS LA MUNIPACILITE DE MAIDUGURI, L'ETAT DE BORNO, NIGERIA

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RÉSUMÉ

L'infection de *Toxoplasma gondii* provoque des malformations dévastatrices, y compris, la cécité, des troubles neurologiques et un retard mental chez les enfants infectés congénitalement. L'infection congénitale se produit quand une femme est infectée au cours de la grossesse, et la gravité de la maladie est liée à la période de trimestre. Cette recherche a été conçue pour évaluer la séroprévalence de la toxoplasmose chez les femmes enceintes (n = 90) à l'aide de dosage immunoenzymatique (ELISA) (Cortez Diagnostics Inc. USA). Un questionnaire structuré a été utilisé pour recueillir des données socio- démographiques. Une prévalence importante globale de 22,2 % (20/ 90) a été obtenu (p = 0,01). Les femmes enceintes dans les tranche d'âge25- 29 ans ont eu la plus forte prévalence de 33,3 % (3/9)qui était significative (p = 0,01). Une association entre des niveaux élevés de Toxoplasme IgG et fausse couche a été établie dans quatre des vingt-cinq femmes qui avaient subi une fausse couche (p = 0,01). Les résultats présentés indiquent que la toxoplasmose est un problème de santé publique dans cette localité, ce qui nécessite des mesures correctives drastiques.

Mots clés: Toxoplasmose, femmes enceintes, fausses couches, Nigeria

INTRODUCTION

Toxoplasmosis infection is caused by the parasite, *Toxoplasma gondii (T. gondii)*, an obligate intracellular protozoan parasite found in many species of animals throughout the world and may cause a variety of clinical syndromes in humans and animals, which leads to many serious health complications. Although

toxoplasmosis is a cosmopolitan infection, the disease appears to be overshadowed in the tropics by other endemic diseases such as malaria and HIV (1) It has been estimated that one third of the world population has been infected by *T. gondii* (2). Transmission of *T. gondii* is through food-borne, with cat playing the major role of transmission (3), through faecal contamination of soil and water (4) and human acquired infection by consumption of improperly cooked meat, unpasteurized goat milk and eating unwashed fruits/vegetables (5). Infection is characterized by headache, cough, fever, body weakness, infection of the eyes, and muscle fatigue (2).
Primary infection may be mild and asymptomatic, but when transmitted transplacentally can cause congenital toxoplasmosis. Congenital toxoplasmosis leads to wide range of manifestation including mild chorioretinitis to mental retardation, microcephaly, hydrocephalus, epilepsy and seizures. It can also cause some repeated abortion, still birth and fetal loss in infected pregnant women (6).

The prevalence of toxoplasma gondii in human population varies among different countries and geographical area (7). In Asia, seroprevalence rate of 52.1% anti-toxoplasma IgG in Southern Turkey, 55.7% in Malaysia, 55.3% in India and 19.4% among Chinese population have been reported. While in Africa, 40.2% from Senegal, 34.1% from pregnant women in Sudan (7), 63.1% in Sao Tome and Principe (1) and 27% in Mali (8) have been reported.
However, in Nigeria, statistics on seroprevalence of toxoplasmosis reveals 29.1% in Zaria, 26.1% in Sokoto, 44.4% in Calabar and 40.8% in Lagos (9, 5, 2, 6).

During pregnancy, the clinical implication of this infection is tremendously dangerous which necessitate the importance of evaluating the immunological status of pregnant women regarding toxoplasmosis. Yet toxoplasmosis is a neglected parasitic infection although it is extremely important economically, medically and epidemiologically (2). Compared with other parasitic infections such as malaria and filariasis, it is grossly underreported. Information on research data on toxoplasmosis in pregnant women in North East Nigeria and Maiduguri in particular is largely unavailable.

In this study, we examined the immunological status of pregnant women attending ante natal care in a secondary health facility in Maiduguri, Borno State, Nigeria.

MATERIALS AND METHODS

Specimen collection

The aim of the research was explained to antenatal attendees and there informed consent and that of the relevant hospital authority was obtained. Therefore ninety blood samples were collected from volunteer pregnant women attending antenatal care at the secondary health facility in Maiduguri, Borno State by venipuncture. Serum was obtained by centrifuging at 3000rpm for 5 minutes (9). Serum was kept frozen at -20C in the Department of Immunology, University of Maiduguri Teaching Hospital until analysis was done.

Specimen Analysis

The following reagents and materials were provided preparatory for the assay: Microwell strips: purified Toxoplasma antigen coated wells (12x8wells), Sample diluents: Blue color solution (22ml), washing concentrate 10x bottles (100ml), TMB chromogenic substrate: Amber (12ml), Enzymes conjugate: red color solution (12ml), Negative calibrator: 0Iμ/ml. Natural cap (150ul/via), Cutoff calibrator: 8Iμ/ml. Yellow cap. Toxo G index=1.0 (150uI/vial) Positive calibrator: 50Iμ/ml. (150μI/Vial), Positive calibrator: 150Iμ/ml. (150μI/vial), Negative control: (150μI/vial), Positive control: (150μl/vial), Stop solution: 2N HCL (12ml). The Enzyme linked immunosorbent assay technique was employed. Abiding strictly by manufacturer's instruction, one in forty (1:40) dilutions of specimen, negative control, positive control and calibrator were prepared by adding 5μl of the aforementioned to 200μl of sample diluent and mixed well. One hundred microliter (100μl) of diluted sera, calibrator and controls were dispensed into the appropriate wells. One hundred microliter (100μl) of absorbent solution was dispensed in 1A well position for the reagent blank. The holder was tapped to remove air bubbles from the liquid and was mixed well and incubated for 30 minutes at room temperature. Liquids from all wells were removed and washed three times repeatedly with washing buffer. One hundred microliter (100μl) of enzyme conjugate was dispensed into each well and incubated for 30 minutes at room temperature. The enzyme conjugate was then removed from all wells and washed repeatedly three times with washing buffer. One hundred microliter (100μl) of TMB Chromogenic Substrate was dispensed to each well and incubated for 15 minutes at room temperature. Then 100μl of 2 N HCl was added

to stop reaction. While ensuring there were no air bubbles in each well, O.D. at 450 nm was read with a microwell reader.

Interpretation of Result

Based on manufacturer's instruction, the mean value of Toxo- G Index for each specimen was calculated by dividing the mean absorbance value of each sample by the cut off calibrator mean value. A sample was then considered positive for anti- Toxoplasma IgG antibody whenever a Toxo G Index value is equal or greater than 1.0 (>8Iu/ml), and considered negative whenever a Toxo G Index value is equal or less than 0.90 (<7Iu/ml).

RESULTS

Out of 90 pregnant women screened, 20 were positive for anti-toxoplasma IgG. Therefore this gives an overall 22.2% seroprevalence (Table 1). We found pregnant women within 25-29 and ≥35 years age bracket to have the highest prevalence of 33.3% (Table 1). Based on trimester, we found prevalence of 18.1%, 29.6% and 19.4% for pregnant women in first, second and third trimester respectively (Table 2). History of miscarriage shows that 16.6% (4/24), and 24.2% (16/66) of pregnant women who have suffered and those who have not had miscarriage respectively were positive for anti-toxoplasma IgG (Table 3) while 33.3% and 20.9% of those who have had and those who have not had blood transfusion respectively were positive for anti-toxoplasma IgG (Table 3).

TABLE 1: SEROPREVALENCE OF *TOXOPLASMA GONDII* IgG IN PREGNANT WOMEN IN RELATION TO AGE

Age (Years)	n	*T. gondii* IgG +ve (%)
15-19	16	4 (25.0)
20-24	45	8 (17.7)
25-29	09	3 (33.3)*
30-34	17	4 (23.5)
≥35	03	1 (33.3)
Total	90	20 (22.2)**

*** X2 cal35.85, p=0.05; ** X2 cal 233.0, p=0.05**

TABLE 2: SEROPREVALENCE OF TOXOPLASMA GONDII IgG IN PREGNANT WOMEN IN RELATION TO TRIMESTER

Trimester	n	*T. gondii* IgG positive (%)
First	11	02 (18.1)
Second	27	08 (29.6)
Third	52	10 (19.4)
Total	90	70 (22.2)

TABLE 3: DISTRIBUTION OF *TOXOPLASMA GONDII* IgG ANTIBODY IN PREGNANT WOMEN BASED ON HISTORY OF BLOOD TRANSFUSION (B/T) AND MISCARRIAGE

T. gondii IgG profile	B/T History		Miscarriage history	
	Yes	No	Yes	No
N	09	81	24	66
Positive	03	17	04	16
(%)	33.3	20.9	16.6	24.2

DISCUSSION

The study showed that overall prevalence of *T. gondii* IgG antibodies in pregnant women in Maiduguri was statistically significant 22.2% (X2 cal 233.0/ X2 tab 124.0, p=0.05). This agrees with the finding of (10) who reported 22% prevalence among pregnant women from the Swansea area of the UK. It is however higher than 19.4% prevalence among Chinese population (7). Comparison of the prevalence obtained in this study (22.2%) with those of other studies in

Nigeria show that while it is slightly lower but within the same range with prevalence from other northern states: 29.1% and 26.1% in Zaria and Sokoto respectively (9, 5), it is much lower than the prevalence from the southern states: 44.4% and 40.8% in Calabar and Lagos respectively (2, 6). This notable difference may be attributable to the extreme temperature in the north which might be inimical to the survival of the oocyst shed in the feaces of cats. The foregoing buttress the report by (11), that the distribution of this parasite depends on regions and weather condition where the oocysts survive in environment. Put together, the prevalence of *Toxoplasma gondii* in Nigeria could be said to have declined if the finding of (12) which reported that seroprevalence rates for pregnant women in a Nigerian population ranged from 72.5% to 88.8% with an overall rate of 75.4% is compared with seroprevalence from various states in Nigeria in recent past. This might not however portend reduction in the sequelae of the infection.

There was no definite age-related pattern of increase in seroprevalence. This is contrary to previous works which reported that seroprevalence was found to increase with age (2, 9). Pregnant women within 25-29 years group (14), vertical transmission occurs causing mental retardation, blindness, epilepsy, and death (15), and that "one of the late sequelae of congenital toxoplasmosis is chorioretinitis" (16), it therefore mean that these women are prone to the aforementioned consequences of toxoplasma infection.

We also found that all four women were in the third trimester of pregnancy which further increases the possibility of the fetuses being affected as "the risk of the infection being passed on to the fetus increases to between 60% and 90% in the third trimester" (17). Worthy of note also are fourteen (14) pregnant women who have never suffered miscarriage but were found to have high concentration of anti-toxoplasma IgG in there sera. In this case IgG-avidity index would be required to establish whether the IgG antibody indicate evidence of chronic or acute infection before allusion can be made the danger such high level of antibody portends. Should these pregnant women be proven to be undergoing acute infection, their fate would be earlier discussed.

Transmission may occur transplacentally, or through organ transplantation (18). Some animals including humans serve as intermediate hosts in which the parasite may cause systemic infection that result in the formation of tissue cysts (2). Since transmission can occur transplacentally and parasite can cause systemic infection, we posit that it might therefore not be impossible that this infection could be transmitted through blood transfusion. This because, if blood donated was "immediately" transfused to a recipient while the parasite is yet blood-borne before it establishes itself in any tissue, then it might be transmitted. Therefore in this study, we present 33.3% (3/9) seroprevalence among pregnant women who have undergone blood transfusion, although without an assessment of test of association between infection and blood transfusion. However, we recommend that these assertions be investigated in future researches.

had the highest prevalence of 33.3% (3/9).This was significant (x2 Cal 35.85/ x2 tab 9.21, p=0.01). However, summing those within 20-24 years and 25-29 years together, we have the highest population of pregnant women in this study. This is indicative of the early marriageable age in the study area. This statistic which reveals the population at risk becomes significant when any remedial measure (such as health education, possible pre or ante natal screening) to forestall the sequelae of toxoplasmosis in the study area is to be planned by relevant authority.

In this study, an association between high level (Toxo G Index > 1.5) of toxoplasma IgG and miscarriage was established in four of twenty five women who had suffered miscarriage (t cal 5.3/t tab 2.81, p=0.01). Precisely, the Toxo G indices of the four pregnant women were 5.56, 5.35, 2.98 and 2.89. Since Toxo G index ratio between paired samples greater than 1.5 is highly suggestive of a significant rise in antibody and it may be considered as indicative of acute toxoplasmosis infection (13) and as it is reported that toxoplasmosis could be severe and life-threatening during pregnancy, and to fetuses, and new born babies

By and large, there are factors such as level of education, rearing of cat and consumption of 'suya' that could have influenced the prevalence obtained in this study. Illiteracy is generally rampant in the study area. About 70% of those positive for anti-toxoplasma IgG in this study were illiterate. Transmission of *Toxoplasma gondii* is possible by containers, knives or cutting boards or other preparation surfaces contaminated with infected raw meat. This category of people might

be less likely to wash cutting boards, knives e.t.c, with soap after cutting of raw meat due unhygienic habits. This agrees with the findings of other workers who reported that lower levels of education were associated with increased risk for toxoplasmosis (4). The culture/habit of cat rearing or cohabiting with cats in the study area may well have influenced the outcome of this work as it makes cats to come in close proximity with humans. This agrees with (19) who reported that cohabiting with cats increases the chances of getting infected. It is worthy of note to state that indigenes in the study area favoured the consumption of 'suya', i.e. roasted meat. This may be a veritable source of infection where the temperature of roasting is not enough to, during processing, eliminate meat parasites (20).

In conclusion, this first report on *T. gondii* IgG immune status of pregnant women in Maiduguri shows 22.2% seroprevalence but does not indicate immunity, rather it shows that 77.8% of the population of the study area is susceptible to *T. gondii* infection. Comprehensive research should be conducted not only in Maiduguri but in whole of north-east region of Nigeria to ensure adequate surveillance and representative result.

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ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1415 http://www.ajol.info/journals/ajcem
COPYRIGHT 2014 http://dx.doi.org/10.4314/ajcem.v15i2.7
AFR. J. CLN. EXPER. MICROBIOL. 15(2): 97-102

TOXOPLASMA GONDII INFECTION IN HIV/AIDS: PREVALENCE AND RISK FACTORS

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ABSTRACT
BACKGROUND: Toxoplasmosis is an infection caused by the protozoan *Toxoplasma gondii*. It is common in severely immune-compromised persons.
OBJECTIVE: To determine the seroprevalence of *T. gondii* infection and the risk factors associated with the infection and to investigate the association between *T. gondii* infection and CD4 cell count.
METHODS: Sera collected from 242 HIV positive HAART- naïve patients were tested for *T. gondii* specific immunoglobulin G antibodies. Information was obtained using a structured questionnaire. Baseline CD4 cell counts were obtained from patients' case files. Data was managed using SPSS version 20 software and Microsoft Excel worksheet.

RESULTS: One hundred and sixty eight (69.4%) subjects were females while 74(30.6%) were males. One hundred (41.3%) of study participants were *Toxoplasma* IgG antibody positive. Thirty two(32) HIV positive pregnant women were among this group studied with 12(37.5%) being Toxo IgG antibody positive. *Toxoplasma* seropositivity was higher in females (42.8%) than in males (39.2%), P= 0.60. CD4 cell count level of < 200 was negatively associated with *Toxoplasma* seropositivity than CD4 count ≥ 200 by logistic regression (OR= 0.6; 95% CI 0.3- 1.0). Living in proximity with cat was positively associated with *T. gondii* infection (P= 0.01).

CONCLUSION: *T. gondii* infection is common in pregnant women indicating greater probability of congenital transmission of *T. gondii*. This could form a basis for recommending intensifying health education and prophylactic treatment for all HIV positive pregnant women. Measures should be taken to prevent stray cats from entering homes.

Keywords: *Toxoplasma gondii* , IgG, Seroprevalence, HIV positive, CD4 cells.

INFECTION A *TOXOPLASMA GONDII* CHEZ LES PERSONNES VIVANT AVEC LE VIH / SIDA : PREVALENCE ET FACTEURS DE RISQUE

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RÉSUMÉ
Contexte: La toxoplasmose est une infection causée par le protozoaire *Toxoplasma gondii* . Il est fréquent chez les personnes gravement immunodéprimées. Objectif: Déterminer la séroprévalence de l'infection à *T. gondii* et les facteurs de risque associés à l'infection et à étudier l'association entre l'infection par *T. gondii* et la numération des CD4. Méthodes: Les sérums prélevés à partir de 242 sujets séropositifs au VIH et naïfs au HAART ont été testés pour des anticorps immunoglobulines G spécifiques de *T. gondii*. L'information a été obtenue à l'aide d'un questionnaire structuré. Le nombre de cellules CD4 de base ont été obtenus à partir des dossiers des patients. Les données ont été analysées avec les logiciels SPSS version 20 et Microsoft Excel.
Résultats : Cent soixante-huit (69,4%) sujets étaient des femmes alors que 74 (30,6 %) étaient des hommes. Cent (41,3 %) des participants à l'étude étaient positif à des anticorps IgG de Toxoplasma. Trente deux (32) femmes enceintes séropositives ont été parmi ce groupe étudié avec 12 (37,5%) étant anticorps IgG Toxo positive. La séropositivité de Toxoplasma était plus élevé chez

les femmes (42,8%) que chez les hommes (39,2%), P = 0,60. Le niveau de CD4 < 200 a été négativement associé à la séropositivité de Toxoplasma que le comptage de CD4 ≥ 200 par régression logistique (OR = 0,6, IC 95% 0,3-1,0). Vivre à proximité du chat était positivement associée à l'infection par *T. gondii* (P=0,01).

Conclusion: L'infection à *T. gondii* est commune chez les femmes enceintes indiquant une plus grande probabilité de la transmission congénitale de *T. gondii*. Cela pourrait constituer une base pour recommander l'intensification de l'éducation sanitaire et le traitement prophylactique pour toutes les femmes enceintes séropositives. Des mesures devraient être prises pour empêcher les chats errants de pénétrer dans les habitations.

Mots-clés: *Toxoplasma gondii* **, IgG , séroprévalence , VIH positif , les cellules CD4**

INTRODUCTION

Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii*, which affects humans in the brain causing *Toxoplasma encephalitis* and other organs like eyes and lungs. *T. gondii* encephalitis could be via acute infection or reactivation of latent infection among immune suppressed persons including those with acquired immuno-deficiency syndrome, those with immunosuppressive cancer and transplant recipients on immunosuppressive drugs. Toxoplasmosis occurs in 3-10% patients in USA and up to 50% of patients in Europe and Africa (1). In patients with AIDS, CNS involvement is the most common manifestation, ranging from nonspecific, generalized symptoms to focal findings such as headache, altered levels of consciousness, motor impairment, and seizures. Seizures was reported as an early manifestation of cerebral toxoplasmosis (2). Various studies have revealed different incidences; 12-25% toxoplasmosis in HIV infected persons (2, 3). Adults with normal immune function are usually asymptomatic or might have symptoms such as fever, malaise and lymphadenopathy that resolve spontaneously. The disease is rare among HIV positive people with T- cell counts of above 200 and most common among HIV positive people with T-cell count below 50(4). Similar study by Jacques *et al.*, 2006 reported a strong association of *T. gondii* seroprevalence with HIV serostatus. Nahlen *et al.*, (1992) (6) also associated very low CD4+ cell counts with CNS toxoplasmosis in HIV/AIDS.

Congenital Toxoplasmosis is the most serious form of human infection. Intra-uterine infection with *T. gondii* due to active parasitaemia during pregnancy can cause severe and fatal damage to a foetus. Fetal infection with *T gondii* may result in stillbirth or abortion. Infection could also lead to fetal brain damage or mental retardation, blindness, and epilepsy in infancy or much later in life. CNS involvement with *Toxoplasma gondii* is uncommon in HIV infected children. Rate of transplacental transmission has been reported to be 55% for untreated mothers and 25% for treated mothers (7). Approximately 10-20% of pregnant women infected with *T. gondii* show clinical signs. Congenital infection is most severe if acquired in the first or, in some cases, second trimester. Infection during the second or third trimesters tends to be asymptomatic. Seventy-five percent of infants born with congenital toxoplasmosis infection are asymptomatic. Eight percent show severe CNS impairment, which might not manifest for several years.

Serologic prevalence data indicate toxoplasmosis as one of the most common human infections throughout the world, more common in warm climates. Prevalence of 25.3% was reported in pregnant women in Burkina Faso (5), 22.5% in USA with 15% seroprevalence found among women of child bearing age (8). Seroprevalence in Nigeria was reported as 78% among pregnant women in Ibadan (9) and 83% in the population of South Delta in Nigeria (10).

Common sources of this infections are: eating of raw or undercooked meat containing *T. gondii* tissue cysts or eating food that has been cross- contaminated; ingesting of oocysts from soil through gardening, handling and eating unwashed vegetables or changing a cat litter box, through placenta (congenital infection), and through sexual contact as suggested by Jacques *et al.*, 2006 in the study of HIV, HBV, HCV and *T. gondii* co-infection. Studies revealed presence of *T. gondii* antibodies in the animal populations and suggested that toxoplasmosis is a widely spread zoonotic infection(11, 12, 13).

Evidence has shown *T. gondii* infection to cause complication in children born of HIV infected pregnant woman, with latent infection reactivated when immunity is suppressed. This poses a serious health threat especially in our country Nigeria with high prevalence of HIV/AIDS. Hence the need for the study in HIV/AIDS patients in our clinic and this could form a basis for primary prophylaxis.

Objectives

1. Determine the prevalence of toxoplasmosis in HIV patients including pregnant women.
2. Identify risk factors to toxoplasmosis in HIV patients.

3. To find association between toxoplasmosis and T cell counts.

MATERIALS AND METHODS

Study centre

This study was conducted at the HIV counseling and testing (HCT) unit of the outpatient clinic, Nigerian Institute of Medical Research, Yaba, Lagos. It is one of the clinics implementing the federal government of Nigeria anti-retroviral (ARV) access programme with support from Havard School of public health. HIV care, treatment and support is given at the center.

Sample size

242 consenting individuals attending the clinic for the first time was recruited in the study. This was estimated using the standard cross-sectional sample size formula where P is the expected prevalence rate, Z the value of the reference normal distribution for the desired confidence level (1.96) for 95% confidence level. D is the highest acceptable error in the estimate.

$n = \frac{Z2\,[\,P\,(1-P)\,];}{D2}$; P = 83%(0.83); Z = 1.96 ; D = 5% (0.05)

Inclusion criteria and Exclusion criteria
All new HIV positive males and females including pregnant women were included in this study, however non- consenting HIV positive patients and infants of < 18 months were excluded.

Ethical clearance
Nigerian Institute of Medical Research ethical review board approved the study.

Study design
A cross-sectional study of 242 participants was conducted from April, 2009 to April, 2012 in Anti-retroviral (ARV) clinic, N.I.M.R Yaba, Lagos. Data was collected from individuals on first visit to assess anti-retroviral treatment from ARV clinic, NIMR.

Data Collection
A structured questionnaire was administered to each consenting participant to assess the socio-demographic characteristics, variable risk factors to *Toxoplasma gondii* infection by the individual. T- Cell count of each individual was extracted from the patient's clinic folder in order to evaluate its association with laboratory findings.

Laboratory Procedure
2mls blood sample was collected at the HCT centre of NIMR. This was followed by laboratory analysis of the samples; the serum was tested for IgG antibody to *Toxoplasma gondii* using Toxoplasma ELISA- based test kit (TECO Diagnostics, USA).

Data management
Data was entered and analysed using SPSS 20 software.

RESULTS

Of the 242 HIV positive patients tested between 2009 and 2012, 100 (41.5%) were positive for *T. gondii* IgG antibody. Toxoplasmosis prevalence by sex, age group, ethnic group and level of education is shown in table 1. *Toxoplasma gondii* IgG seroprevalence decreased across the group with age group < 21-30 being the highest (58.9%). The prevalence was also higher in females (42.8%) than in males (39.2%). Among 32 HIV-positive pregnant women, 12(37.5%) were positive for *T. gondii* IgG antibody. Only the factor, 'living in proximity with cats' was significantly associated with *T. gondii* seropositivity (Table 2). However, Mean CD4 count was 273 cells/µl; Min- 6 cells/µl, Max- 1407cells/µl. CD4 level of <200 was a greater predictor of seropositivity than ≥ 200 by logistic regression analysis (OR = 0.6; 95% C.I 0.3 – 1.0).

TABLE 1: PREVALENCE OF TOXOPLASMOSIS BY DEMOGRAPHY

Demographic characteristics	Prevalence N (%)	P- value
Sex Male	29 (39.2)	0.603
Female	71 (42.8)	
Ethnic group Hausa		
Igbo	4(50)	0.885
Yoruba	48(43.2)	
Others	23(39)	
	24(39.3)	
Level of Education None		
Primary	9(64.3)	0.475
Secondary	21(42.5)	
Tertiary	47(41.6)	
	23(38.3)	

TABLE 2: RISK FACTORS OF TOXOPLASMOSIS

Risk factors		Frequency (%)	P- value
Sex partners	Faithfulness	74 (40.2)	0.103
	> 1 partner	17 (45.0)	
	None	8 (36.4)	
Cat ownership	Yes	4 (66.7)	0.208
	No	96 (41.0)	
Living in proximity with cat	Yes	29 (56.9)	0.013
	No	71 (37.6)	
Eat raw pork/meat	Yes	10 (52.6)	0.290
	No	88 (40.2)	
Drink unpasteurised milk	Yes	1 (33.3)	0.768
	No	99 (41.8)	
Indulge in gardening	Yes	6 (40)	0.881
	No	94 (42)	
Soil related occupation	Yes	6 (30)	0.258
	No	93 (43.1)	
Eat unwashed vegetables and fruits	Yes	12 (40)	0.843
	No	88 (41.9)	
Eat unwashed veg. and fruits outside home	Yes	38 (41.3)	0.991
	No	60 (41.1)	
Wash kitchen knife after use	Yes	91 (41.4)	0.917
	No	6 (40)	
Wash hand before eating	Yes	99 (41.9)	0.396
	No	0 (0)	
Source of drinking water	Pipe borne water	5 (23.8)	0.228
	Borehole	84 (43.5)	
	Pure water	11 (50)	
Boil, filter or use water guard	Yes	42 (38.2)	0.314
	No	58 (44.6)	

DISCUSSION

From the result, the prevalence was higher in females than in males; with majority (89%) of the females from this study being of childbearing age. This suggests that pregnant women should take appropriate precautions to protect themselves against

this infection. Such precautions include cooking meat, especially lamb and pork, until it is well done; thorough washing of cutting boards used to prepare meat; wearing gloves when gardening; rigorous hand washing after handling raw meat or working in the soil; and avoiding contact with cat feces. From this study, 37.5% of the pregnant women were positive to *T. gondii* IgG antibody. We were not able to confirm recent infection in the women using anti- *T. gondii* IgM for possible congenital transmission. However, IgG avidity testing is the preferred method to confirm recent infection (14), because IgM antibodies can persist for months after initial infection in some individuals. It have been shown that 43 cases of toxoplasmosis acquired during pregnancy would be expected to result in 11–21 cases of congenital toxoplasmosis, assuming a 25–50% probability of transmission to the fetus *in the uterus (15)*.

The *T.gondii* IgG seroprevalence (41.5%) from this study was similar to that (38.8%) obtained in Jos, Nigeria (16) among HIV positive individuals. Prevalence of 37.5% observed in pregnant women was higher than what was obtained in pregnant women (25.3%) in Burkina Faso (5) and 22.5% in USA (8). However, our result is lower than what was obtained (78%) among pregnant women in Ibadan, Nigeria (9) and 83% in the population of South Delta in Nigeria (10). It could be an improvement in the control strategies against *Toxoplasma gondii* infection that brought about the reduction in prevalence in the high risk group. Highest prevalence obtained in the lower age group as was highlighted by Papoz *et al*., (1986) probably reflected soil exposure to *T. gondii* oocysts. Living in proximity with cat was positively associated with *T. gondii* seropositivity. Others studies have associated eating of rodents, having soil related occupation (16), cat ownership etc. with seropositivity. This is understandable because in our environment, most people do not own cats (table 2) but we see cats gaining access to people's homes especially in the night where they look for leftover food leaving their droppings to contaminate the surfaces. It is encouraging to note the hygienic practice of the study participants and these goes to show that infection in this group would have been from eating of improperly cooked meat/ meat products, vegetables and fruits, though it was not significant, especially outside home. Earlier studies have acclaimed that the disease is rare among HIV positive people with T- cell counts of above 200 and is most common among HIV positive people with T-cell count below 50(4). T- cell count group < 200 from this study is associated to *T. gondii* seropositivity than above or equal to 200.

In conclusion, *T. gondii* infection is common in pregnant women indicating greater probability of congenital transmission of *T. gondii*. This could form a basis for recommending intensifying health education and prophylactic treatment for all HIV positive pregnant women. Screening of newborns over a specified time period could elucidate the true burden of congenital toxoplasmosis. Measures should also be taken to prevent stray cats from entering homes.

ACKNOWLEDGEMENT

We sincerely express our profound gratitude to the following staff of Human virology laboratory of Nigerian Institute of Medical Research, Yaba- Azuka Okwuraiwe, Muyiwa Salu and Chika Onwuamah; for their assistance in reading of the ELISA plates.

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ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1416 http://www.ajol.info/journals/ajcem
COPYRIGHT 2014 http://dx.doi.org/10.4314/ajcem.v15i2.8
AFR. J. CLN. EXPER. MICROBIOL. 15(2): 103-108

PREVALENCE OF ACID-ALCOHOL-FAST BACILLI AMONG PATIENTS WITH SUSPECTED CASES OF PULMONARY TUBERCULOSIS IN JOS, NIGERIA

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ABSTRACT

Mycobacterium tuberculosis is a major public health problem in globally due to its high tendency of person-person transmission, morbidity, and mortality. This study aimed at determining the prevalence of AAFB within the study area. Sputum samples were collected from three hundred and three (303) patients with suspected cases of pulmonary tuberculosis attending Plateau State Specialist Hospital and Faith Alive Foundation. The samples were examined using Ziehl Neelsen method. Structured questionnaires were administered to obtain some demographic data from patients that consented. Results were tested statistically for significance at $p < 0.05$ using Chi-square test. Out of the samples examined,29(9.57.0%) were positive for AAFB .The study showed that the prevalence of smear-positive increased with age between 15 and 45 and then decreased from age groups 46 and above. The study also revealed that males had a higher prevalence with 19(12.34%)than females who had 10(6.71%).Marital status showed that divorced individuals had the highest prevalence of 2(12.50%) followed by married ,singles and the widowed with 18(11.76%),8(6.34%),and 1(5.90%) respectively. Statistically the study reveals that age groups, sex, hospital (location) does not have any effects on the prevalence ($p > 0.05$) while marital status showed a significant effect on the prevalence ($p < 0.05$).There is need for a more collaborative efforts and political will by the government and non-governmental agencies in order to fast track prevention and control measures aimed at eliminating the infection in the nearest future.

Key words: AFB, Tuberculosis, Jos, Nigeria.

PREVALENCE DE BACILLES ACIDO- ALCOOLO-RESISTANTS CHEZ LES PATIENTS AVEC DES CAS PRESUMES DE TUBERCULOSE PULMONAIRE A JOS, AU NIGERIA

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RÉSUMÉ

***Mycobacterium tuberculosis* est un problème majeur de santé publique dans le monde en raison de sa forte tendance de transmission de personne en personne, la morbidité et la mortalité. Cette étude vise à déterminer la prévalence de BAAR dans la zone d'étude. Les échantillons d'expectorations ont été recueillis à partir de trois cent trois (303) patients avec suspicion de tuberculose pulmonaire qui fréquentent l'hôpital Spécialiste de l'Etat du Plateau et la Fondation Foi Vivante. Les échantillons ont été examinés en utilisant la méthode de Ziehl Neelsen. Des questionnaires structurés ont été administrés pour obtenir des données démographiques des patients ayant consenti. Les résultats ont été testés statistiquement à $p < 0,05$ au test du chi - carré. Sur les échantillons examinés, 29 (9.57.0%) étaient positifs pour BAAR. L'étude a montré que la prévalence des frottis positif augmente avec l'âge entre 15 et 45, puis a diminué de groupes d'âge 46 et plus. L'étude a également révélé que les mâles avaient une prévalence plus élevée de 19 (12,34%) que les femmes qui avaient 10 (6,71%). L'état civil a montré que les personnes divorcées ont la plus forte prévalence de 2 (12,50%), suivie par les mariés , les célibataires et les veuves avec les valeurs de 18 (11,76%), 8 (6,34%) , et 1(5,90 %) respectivement . Statistiquement l'étude révèle que les groupes d'âge, le sexe, l'hôpital (lieu) n'a pas d'effets sur la prévalence ($p > 0,05$), tandis que l'état matrimonial a montré un effet significatif sur la prévalence ($p < 0,05$). Il est nécessaire d'implémenter d'efforts pour une grande collaboration entre la volonté politique du gouvernement et des organismes non gouvernementaux afin de prendre des mesures de prévention de la voie rapide et de contrôle visant à éliminer l'infection dans un proche avenir.**

Mots clés: **AFB , la tuberculose , Jos , Nigeria.**

INTRODUCTION

Tuberculosis (TB) remains a major cause of illness and death worldwide, especially in Asia and Africa. Globally, there were an estimated 9.2 million new cases and 1.7 million deaths from TB in 2006 (1).Although the control of TB has improved dramatically in most industrialized countries during the century ,the disease continues to be a major cause of morbidity and mortality in developing countries (2).The World Health Organization (WHO)estimates that 22 countries account for 80% of all new cases and 98% of all deaths from TB, a situation that is exacerbated in countries with high rates of HIV infection(1).

The causative agent of this dreaded infectious disease is called *Mycobacterium tuberculosis* discovered in 1882 by Robert Koch (3).Tuberculosis most often affect the lungs , it is curable, preventable and is spread from person to person through the air. When people with lung TB cough, sneeze or spit, they propel the TB germs into the air. A person needs to inhale only a few of these germs to become infected. Common symptoms of active lung TB are cough with sputum and blood at times, chest pains, weakness, weight loss, fever and night sweats (4).

About one-third of the world's population has latent TB, which means people have been infected by TB bacteria but are not (yet) ill with disease and cannot transmit the disease. When a person develops active TB (disease), the symptoms (cough, fever, night sweats, weight loss etc.) may be mild for many months. This can lead to delays in seeking care, and results in transmission of the bacteria to others. People ill with TB can infect up to 10-15 other people through close contact over the course of a year. Without proper treatment up to two thirds of people ill with TB will die.TB occurs in every part of the world. In 2010, the largest number of new TB cases occurred in Asia, accounting for 60% of new cases globally. However, Sub-Saharan Africa carried the greatest proportion of new cases per population with over 270 cases per 100 000 population in 2010 (4).

Nigeria ranks fifth among the world's high-burden countries, with a prevalence of tuberculosis (TB) cases of 280,000. The TB prevalence is at 171/100,000 and the incidence rate of sputum smear positive disease is approximately 118/100,000 (5)

Tuberculosis (TB) is a serious public health concern globally, and almost half of new infections are undetected(*1*).Tuberculosis bacteriology is one of the fundamental aspects of a national tuberculosis control programme and a key component of the DOTS strategy, yet the tuberculosis laboratory service is often the most neglected component of these programmes (6).The use of smear microscopy in patients suspected of tuberculosis presenting to health services is of great value in case detection and in reducing the spread of the infection throughout the population by treatment of such cases (7).

Rapid and accurate diagnosis of symptomatic patients is the cornerstone of global TB control strategies. Remarkable progress has recently been made upgrading the speed and quality of TB diagnostic services in developed countries but for most of the world where TB is a large public health burden, these gains are still unrealized (8). Thus, the primary laboratory tool supporting case detection in vast majority of cases in disease endemic countries remains microscopic examination of the stained sputum smear. The shortcomings of this method seriously limit the extent and quality of its application, and ultimately, its impact in TB control (9)

This study aimed at determining the prevalence of AAFB in patients with suspected cases of *Mycobacterium tuberculosis* in the study area.

MATERIALS AND METHODS
STUDY AREA

Plateau State is the twelfth largest state of Nigeria, and is roughly located in the center of the country. It is geographically unique in Nigeria because its boundaries totally surround the Jos Plateau, having the Jos Plateau totally in its central and northern part. Its capital is Jos. Plateau State is located in Nigeria's middle belt. With an area of 26,899 square kilometres, the State has a population of 3,178,712 people according to 2006 census. It is located between latitude 80°24'N and longitude 80°32' and 100°38' east. Though situated in the tropical zone, a higher altitude means that Plateau State has a near temperate climate with an average temperature of between 18 and 22°C. Harmattan winds cause the coldest weather between December and February. The mean annual rainfall varies from 131.75 cm (52 in) in the southern part to 146 cm (57 in) on the Plateau (10).

The main occupation in the rural areas is farming, while those in urban areas are civil servants, traders and students. The two tertiary hospitals Jos University Teaching Hospital (JUTH) and Plateau Specialist Hospital (PSSH) serve as referral centers for the primary and secondary health care facilities in the area. Plateau State Specialist Hospital and Faith Alive Foundation (FAF) are situated within Jos city and they serve patients mostly within the state.

ETHICAL CONSIDERATION
Ethical clearances were obtained from Jos University

Teaching Hospital, Jos, Plateau Specialist Hospital, and Faith Alive Foundation before the commencement of the work.

STUDY POPULATION

The study population focused on all Patients within the age range 15 years and above with suspected cases of pulmonary tuberculosis attending Plateau Specialist Hospital, and Faith Alive Foundation.

SAMPLING METHODOLOGY

A structured questionnaire was administered randomly to patients who gave their consent in other to obtain some useful bio-data.

SAMPLE COLLECTION

The sample collection were done with the assistance of Medical personels.The patients were given sterile universal bottles in which they produced three samples (one spot, one early morning and one spot).

SAMPLE ANALYSIS

Three consecutive sputum samples were collected in leak proof universal plastic containers and stained using Ziehl-Neelsen's method and examine in accordance with standard methods (11). Three smears were prepared from each patient, heat fixed and stained using Ziehl-Neelsen technique as follows: Strong carbol fuchsin was flooded on the slides and steamed, it was then allowed to stain for 5 minutes followed by decolourization with 3% acid-alcohol and washed with water and then counterstained with 0.3% methylene blue for 1 minute, washed with water and allowed to air-dry before examination for AAFB.

MICROSCOPIC EXAMINATION OF SLIDES

The stained smears were examined with the Olympus light, binocular microscope under the oil immersion objective, scanning all fields at high power field for the presence of bright red slender rods, the presence of which signified positive AAFB and the absence, negative. The microscopy revealed the tubercle bacilli being bright red on a blue background; straight or slightly curved, quite short (1-4μm) often granular, arranged in groups of 3-10 bacilli close together like bits of string.

GRADING OF MICROSCOPY RESULTS

This was done in accordance to standard method as shown below:

1-9/100 fields – 1+

1-9/10 fields – 2+

1-9/1 field – 3+

9/1 field – 4+

Negative – 0.

RESULTS

Three hundred and three (303) sputum samples were examined for AAFB from the study area. Out of the 303 sputa, 29 (9.57%) were positive for AAFB. The prevalence of AAFB in relation age groups as shown in table 1.The age group 36-45 had the highest prevalence with 9(13.85%),followed by age group 46-55 with a prevalence of 4(11.43%) ,while the age groups 56 and above had the least. Statistical analysis reveals that age does not have a significant relationship with the prevalence ($p > 0.05$).

Table 2 shows the prevalence of AAFB in relation to Gender. The males had a prevalence of 19(12.34%) while females had 10(6.71%).This showed that gender does not have a significant effect in this association ($p > 0.05$).

The prevalence of AAFB in relation to marital status as shown in Table 3 reveals that divorced people had the highest prevalence with 2(12.50%),followed by the married, single and widowed with 18(11.76%), 8(6.34%) and 1(5.90%) respectively. This result showed a significant association ($p < 0.05$). The prevalence of AAFB in relation to hospital location as shown in Table 4 indicated that Plateau State Specialist Hospital(PSSH) had the highest with 17(11.56%) while Faith Alive Foundation(FAF) had 12(7.69%).The result showed that patients attending different hospital in the same area does not have any effects on the prevalence rate ($p > 0.05$).

TABLE 1: PREVALENCE OF ACID-ALCOHOL-FAST BACILLI IN RELATION TO AGE

Age	No. Screened	No. positive (%)	X2/P-Values
15 -25	2	6(9.70)	8.10; DF=4
26 -35	111	9(8.11)	P > 0.05
36- 45	65	9(13.85)	
46- 55	35	4(11.43)	
≥ 56	30	1(3.33)	
Total	303	29(9.57)	

TABLE 2: PREVALENCE OF ACID-ALCOHOL-FAST BACILLI IN RELATION TO SEX

Sex	No. Screened	No. positive (%)	X2/P-Values
Male	154	19(12.34)	X2=2.83; DF=1
Female	149	10(6.71)	P > 0.05
Total	303	29(9.57)	

TABLE 3: PREVALENCE OF ACID-ALCOHOL-FAST BACILLI IN RELATION TO MARITAL STATUS

Marital status	No.Screened	No.positive(%)	X2/P-Values
Single	117	8(6.34)	X2=25.21; DF=3
Married	153	18(11.76)	P < 0.05
Divorced	16	2(12.50)	
Widowed	17	1(5.90)	
Total	303	29(9.57)	

TABLE 4: PREVALENCE OF ACID-ALCOHOL-FAST BACILLI IN RELATION TO HOSPITALS

Hospital	No. Screened	No. positive (%)	X2/P-Values
FAF	156	12(7.69)	X2=0.89; DF=1
PSSH	147	17(11.56)	P > 0.05
Total	303	29(9.57)	

DISCUSSION

The result obtained in this study reveals that out of the three hundred and three (303) sputum samples examined for AAFB using Ziehl Neelsen staining technique, 29(9.57%) were positive for AAFB. Considering the fact that sputum smear microscopy is less sensitive to sputum culture in tuberculosis diagnosis, and with the number of positivity obtained, it is obvious that the study area is endemic to tuberculosis. The result of this study agreed with earlier findings of a prevalence of 10.5% in Osogbo, Southwestern Nigeria(12) , a prevalence of 12% in Zaria, North western Nigeria(13), a prevalence of 16.83% in some parts of Abia ,Southeastern Nigeria(14) and a prevalence of 7.1% was reported among suspected new tuberculosis patients attending University College Hospital Ibadan, Southwestern Nigeria(15) while a higher prevalence of 31.7% was reported in Southeastern Nigeria(16) which is far higher than what was obtained in this study.

This study indicates that most of the smear positive cases were within the age groups between 15 and 45years. This may be associated with the demands associated with these age groups, since these age groups constitute the most productive part of national development and their engagement in several activities may predisposes them to the infection. This study was in agreement with an earlier findings in Osogbo, Southwestern Nigeria which reported a highest prevalence among age group 16-30 while the age group 75 and above had the least (12), and a report from Southeastern Nigeria which stated that the prevalence of smear-positive pulmonary TB increased with age, up to the 41-50 years age category but decreased among patients who are 50 years and above (17).

The study shows that males had a higher prevalence 19(12.34%), while females had 10(6.71%).The results showed that there was no significant statistical association with the prevalence ($p > 0.05$).This study agreed with some earlier findings which reported a higher prevalence in males than females (12, 17, 18). The result may be attributed to a higher exposure of males to some risks factors associated with the transmission of the infection than in females.

The prevalence of AAFB in relation to marital status as seen in this study reveals that married people had the highest prevalence of 18(11.76%),followed by the divorced, singles and widowed with, 2(12%), 8(6.34%) and 1(5.90) respectively. The result of this finding indicates that people who are always in close contact or staying together in a household especially in endemic areas have a higher chances of contracting the infection.

The prevalence of AAFB in relation to hospital location as shown in this study indicated that Plateau State Specialist Hospital (PSSH) had the highest with 17(11.56%) while Faith Alive Foundation (FAF) had 12(7.69%).The result showed that patients attending different hospitals within the study area does not have any effects on the prevalence of the infection.

CONCLUSION

The 9.57% prevalence of AAFB in the study area obtained using the Ziehl Neelsen method is an indication that the area is endemic to the spread and transmission of tuberculosis, although the method is less sensitive when compared with the cultural method, the method is still the main stay of diagnosis of AAFB among patients with suspected cases of pulmonary tuberculosis in the area. The need for provision of standard tuberculosis laboratories with modern facilities for cultures can not be over emphasized; this can further compliment the on going work in the Direct Observed Therapy Short course (DOTS) centres in the study area and hence improve health care delivery.

RECOMMENDATIONS

In order to curtail the spread of AAFB, there is need:

1. For the establishment of standard tuberculosis laboratories with all the required equipments, reagents and well trained personnel for both microscopy and culture
2. To create more awareness through campaigns on mode of transmission, risk factors and the prevention and control of the infection
3. There should be a political will and commitment at the different levels of government and NGOs on adequate funding of research, campaigns and implementation of the research findings with the aim of eliminating tuberculosis in Nigeria.

ACKNOWLEDGEMENTS

We thank the authorities of Plateau State Specialist Hospital and Faith Alive Foundation, for granting the permission to carryout the study among the study population in their facilities and also we appreciate the assistance rendered to us by the staffs of the facilities.

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ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1417 http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER.MICROBIOL. 15(1): 109-116

CASE-DETECTION RATE OF DIRECT SPUTUM SMEAR MICROSCOPY FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS IN ABIA STATE, NIGERIA

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ABSTRACT

The accuracy of sputum smear microscopy, the tuberculosis case-finding method in the Abia State TB Control Programme has never been assessed due to lack of culture facilities. To assess the accuracy of sputum smear microscopy in routine control programme conditions in Abia State, sputum samples from patients undergoing investigation for tuberculosis were analyzed using Ziehl-Neelsen staining technique for sputum smear microscopy and culture on Löwenstein-Jensen medium as reference standard. Out of 150 participants tested, 51 were smear –positive for acid fast bacilli (positivity rate, 34.0 %, 51/150) while 79 were culture positive for *Mycobacterium tuberculosis* complex and 12 for non-tuberculous mycobacteria (NTM). Thirty-seven of the 79 culture positive for *M. tuberculosis* were smear positive giving a ratio of smear to culture positivity of 46.84%. Forty-two (42.4%) of the 99 smear negative cases were culture positive for *M. tuberculosis*. The sensitivity of smear microscopy was 50.0% (95%CI=39.0-61.0) and specificity was 92.3% (95% CI=86.4-98.2). The prevalence of HIV/TB coinfection among the study participants was 48% (12/25). Although the case- detection rate of smear microscopy was moderate in this study, the large proportion of TB patients missed by smear microscopy is a cause for concern and requires concerted effort to improve the sensitivity of smear microscopy. Introduction of more sensitive diagnostic methods like culture also need to be considered.

TAUX DE DETECTION DIRECTE FROTTIS POUR LE DIAGNOSTIC DE TUBERCULOSE PULMONAIRE EN ÉTAT D'ABIA, NIGERIA

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RÉSUMÉ

La précision des frottis, la méthode la recherche de cas de tuberculose dans le programme de lutte contre la tuberculose dans l'Etat d'Abia n'a jamais été évalué en raison du manque d'installations de culture. Pour évaluer la précision des frottis dans les conditions du programme de contrôle de routine dans cet Etat , les expectorations de patients subissant une enquête de la tuberculose ont été analysées à l'aide de technique de coloration de Ziehl-Neelsen pour la microscopie des frottis de crachats et de la culture sur milieu de Löwenstein- Jensen en tant que norme de référence . Sur les 150 participants testés, 51 étaient à frottis positif pour les bacilles acido rapide (taux de positivité, 34,0%, 51/150), tandis que 79 étaient positifs à la culture pour complexe *Mycobacterium tuberculosis* et 12 pour les mycobactéries non tuberculeuses (NTM). Trente-sept de la culture 79 positive pour *M. tuberculosis* étaient à frottis positif donnant un ratio de frottis de positivité de la culture de 46,84 %. Quarante-deux (42,4%) des 99 cas à frottis négatifs étaient positifs à la culture pour *M. tuberculosis*. La sensibilité de la microscopie des frottis était de 50,0 % (IC à 95% = 39,0 à 61,0) et la spécificité était de 92,3 % (IC à 95% = 86,4 à 98,2). La prévalence du VIH / TB coïnfection parmi les participants à l'étude était de 48% (12 /25). Bien que le taux de microscopique des frottis de dépistage des cas ait été modéré dans cette étude, la forte proportion de patients atteints de tuberculose manqués par examen microscopique des frottis est un sujet préoccupant et exige un effort concerté pour améliorer la sensibilité de la microscopie des frottis. La présentation des méthodes de diagnostic plus sensibles comme la culture doivent aussi être pris en considération.

INTRODUCTION

In most of the high-prevalence countries, rapid laboratory diagnosis of TB relies on the microscopic examination of direct sputum smear stained by the Ziehl Neelsen technique (1, 2). It is considered the most appropriate method available for case-finding in TB Control Programmes in resource-limited countries as it is good in detecting the most infectious cases excreting large number of bacilli (3). Culture is a more sensitive method than smear microscopy and is regarded as the gold standard for definitive diagnosis of TB but due to technical constraints, culture of *M. tuberculosis* is not done routinely in developing countries.

The sensitivity of Sputum smear microscopy (SSM) may be low and variable depending on several factors, both technical and epidemiological, which include the proficiency and diligence of the laboratory personnel, the quality of reagents, condition of microscope, workload of the laboratory, the disease stage at presentation as well as the prevalence of HIV/AIDS in the population of TB suspects (4, 5). To minimize the effects of these factors, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) have produced guidelines which recommend quality control of SSM as an essential part of an effective national TB control Programme (6). The goal of quality control of SSM is to optimize the technical and operational components of the test so as to minimize diagnostic errors such as false positives and false negatives (7).

Assessing the case-detection rate of SSM in a particular TB Control Programme is of interest as it can indicate how effective the quality control measures have been observed as well as the proportion and the category of TB patients the laboratory is detecting. The ratio of smear to culture positivity is commonly used as a measure of the overall performance of SSM. In developed countries with well functioning laboratories and lower prevalence of smear positive cases, the ratio of smear to culture positivity on the same specimen was estimated to be around 40% to 60% (8, 9). In high-prevalence countries such as South Africa, Kenya or India, maximum values of 57% to 75% were recorded (10). In routine laboratory diagnosis in African laboratories, the sensitivity of the direct sputum smear microscopy ranged from 8.8%-46.6% (11). More recently, Mfinanga *et al*., (12), reported a sensitivity of 36.9% among the peripheral diagnostic centres in Dar es Salam, Tanzania.

The assessment of the case-detection rate of SSM has never been done in the Abia State TB Control Programme. The availability of such data will inform the Programme managers on the current status and the appropriate course of action to improve the diagnostic performance of the programme. The aim of the study reported here was therefore to assess the case detection rate of direct sputum smear microscopy for diagnosis of TB in the Abia State TB Control Programme.

MATERIALS AND METHODS

Study Participants. Study participants consisted of 150 patients with high clinical index of suspicion for tuberculosis, defined as patients with cough of ≥3 weeks' duration. The patients were undergoing investigation for TB at the Leprosy and Tuberculosis Referral Hospital, Uzuakoli, Bende Local Government Area (LGA) and the Sputum Smear Microscopy Centre, Aba South LGA Health Office, Aba, all in Abia State, between November 2008 and February 2010. Eligible participants were individuals ≥ 15 years of age newly referred to the study centres to undergo SSM examination for acid-fast bacilli (AFB). Those already on anti-tuberculosis treatment at the time of recruitment were excluded from the study. Sputum collection was done according to routine procedure using spot-morning-spot (SMS) scheme. Informed consent was obtained from the study participants and the study protocol was approved by the research Ethical Committee of the Federal Medical Centre, Umuahia, Abia State.

Acid-fast staining

Direct smear was made from each sputum specimen, stained by the ZN method, and read at the study centres by experienced laboratory technicians. A subject was diagnosed as a smear-positive TB patient if at least one of the three smears was positive for AFB.

Sputum culture

The sputum culture was performed on the morning sputum specimen, or if not available, on a spot specimen from each study participantat the Microbiology Laboratory, Michael Okpara University of Agriculture, Umudike (MOUAU). The specimens were decontaminated and concentrated using modified Petroff's alkali method and inoculated unto slants of Lowenstein-Jensen (LJ) medium. The inoculated LJ slants were incubated at 37OC and examined for growth daily for the first 1 week and once weekly thereafter up to 8 weeks. Cultures that showed no growth after 8 weeks were recorded as negative. A patient was defined as true TB patient if the culture produced *Mycobacterium tuberculosis* and as a non-TB case if the culture showed no growth. The mycobacterial isolates were identified according to

criteria based on the rate of growth, colonial characteristics such as roughness and pigment production and nitrate reductase test. Representative suspected NTM isolates were sent to a reference laboratory for further identification.

HIV Screening

The study participants were screened for HIV using two rapid HIV tests according to the National HIV Screening guidelines (13). A patient was screened first with DetermineTM HIV 1& 2 (manufactured by Abbot Japan Co., Ltd for Inverness Medical, Japan Co., Ltd). If positive, the specimen was retested with DoublecheckGoldTM Ultra HIV 1& 2 (Orgenics Ltd, Israel) for confirmation. The two tests must be positive for a patient to be regarded as HIV positive.

RESULTS

A total of 150 participants were enrolled in the study. The demographic and clinical characteristics of the study participants are presented in Table 1. There were 67 (44.7%) males and 83 (55.33%) females, median age was 37 years (range, 17-85 years) and the mean age was 41.66 years. All the study participants submitted the first sputum specimens, 136 (90.7%) submitted 3 complete sputum specimens, 2 submitted two specimens and 12 (8%) submitted one sputum specimen each. The age bracket of 25 to 34 years contained the highest number of study participants and 42.6% (20/47) of them were positive for AFB.

TABLE 1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS

	Overall	Male	Female
No. of study participants	**150**	**67**	**84**
Age group (yr)			
15-24	**19**	**4**	**15**
25-34	**47**	**18**	**29**
35-44	**26**	**16**	**10**
45-54	**21**	**11**	**10**
55-64	**15**	**8**	**7**
>65	**22**	**10**	**12**
Age range (Min.-Max.)	**17-85**	**17-84**	**17-85**
Median age(yr)	**37.00**	**34.00**	**33.00**
Mean age(yr)	**41.66**	**44.31**	**39.52**
Sputum smear microscopy			
No. smear Positive	**51**	**29**	**22**
No. smear Negative	**99**	**38**	**61**
Sputum culture			
No. culture positive (Mtb)	**79**	**36**	**43**
No. culture negative	**41**	**17**	**24**
No. culture positive (NTM)	**12**	**7**	**5**
No. Contaminated(CTN)	**18**	**7**	**11**
HIV status			
No. tested	**140**	**64**	**76**
No. positive	**25**	**11**	**14**
No. negative	**115**	**53**	**62**
HIV/TB coinfection	**12**	**6**	**6**

Mtb : *Mycobacterium tuberculosis* **complex; NTM: Non-tuberculous mycobacteria**

Human immunodeficiency virus (HIV) screening was done in 140(93.3%) of the study participants who gave consent, 25 (17.9%) were sero-positive for HIV and 48% (12/25) were co-infected with TB. The detection rates of pulmonary tuberculosis (PTB) by SSM and culture among the study population is presented in

Table 2. The sputum smear positivity rate was 34.0% (51/150).Of the 51 smear positive subjects, 44 (86.27%) had all the three specimens positive for AFB, 6(11.76%) had two positive specimens and 1(1.96%) had one positive specimen. A total of 91 participants were culture positive for mycobacteria, 79(86.8%) for *Mycobacterium tuberculosis* complex and 12(13.19 %) for non-tuberculous mycobacteria (NTM). Eighteen (18) [12%] of the 150 sputum cultures were contaminated. The culture positivity rate for *M. tuberculosis* was 52.67% (79/150).

The smear positive cases and pattern of culture positivity according to the AFB grading is presented in Table 3. Thirty-seven (46.84%) of the 79 cases culture positive for *M. tuberculosis* were smear positive. However, culture revealed that 8 of the smear positive cases were due to NTM. Forty- two (95.45%) of the 44 subjects with all three specimens positive for AFB were culture positive; the remaining two were contaminated. Forty-two (42.4%) of the 99 smear negative cases were culture positive for *M. tuberculosis*. In 136 subjects who gave three sputum specimens, 47(34.56%) were smear positive for AFB while 80 (58.82%) were culture positive for mycobacteria. The sensitivity of SSM with reference to culture was 50.0% (95%CI=39.0-61.0) and the specificity was 92.3% (95% CI=86.4-98.2).

DISCUSSION

Sputum microscopy remains the sole diagnostic test used for diagnosis of TB in most areas where TB is endemic because there is no alternative method that is technically feasible and can be implemented affordably (14, 1). In this study, the case detection rate of sputum microscopy was assessed using culture as the reference standard. The sensitivity and specificity of SSM in this study was 50 % (95% CI: 39.0%-61.0%) and 92.3% (95% CI: 86.4% - 98.2%) respectively. This suggests that the proficiency of our laboratory system was moderate and falls within the acceptable range of diagnostic performance of smear microscopy commonly reported (9, 10). In a study among peripheral diagnostic centres in Dar es Salaam, Tanzania, a sensitivity of 36.9% was reported for SSM (12).

TABLE 2 DETECTION RATES OF PTB IN THE STUDY POPULATION BY SSM AND CULTURE

Sex	№ (%) tested	№.(%) Positive for AFB № (%) aCx +ve (Mtb)	№ (%) bCx+ve (NTM)
Male	**67**	**29(43.28)**	**36(53.73)**	**7(10.45)**
Female	**83**	**22 (26.51)**	**43(51.81)**	**5(6.02)**
Total	**150**	**51 (34.00)**	**79(52.67)***	**12(8.00)**

*** Not excluding contaminated cultures (n=18); aCx Mtb- Culture positive** *Mycobacterium tuberculosis* **complex bCx NTM- Nontuberculous mycobacteria**

Our study also showed a high level of agreement between the culture and SSM results among the patients with high bacillary load (3+ and 2+ categories). The discrepancy was highest among the paucibacillary patients (1+ and scanty categories) and AFB smear negative patients. This indicates that the performance of the laboratory was acceptable as per the diagnostic objective of DOTS which is to detect the most infectious cases with high bacillary load. However, a sensitivity of 50% implies that SSM failed to detect half of true PTB cases in this setting. On the basis of microscopy results alone, the 42 culture positive but smear negative PTB cases would have been missed for treatment. Majority of these cases would continue to transmit the disease in the community and delay in detection would result in increased risk of greater morbidity and mortality. This emphasizes the inadequacy of SSM as the case-finding method in the long term and overall success of controlling TB.

TABLE 3 DIAGNOSTIC PERFORMANCE OF SSM IN ABIA STATE IN RELATION TO CULTURE

Sputum smear microscopy		Culture			
AFB+ve Grading	№ of cases AFB positive	№ culture positive Mtb (% agreement)	№ culture Positive NTM	№ culture Negative	№ contaminated
3+	15	12 (80.0)	3	0	0
2+	16	15 (93.8)	0	0	1
1+	14	8 (57.1)	2	3	1
Sct	6	2 (33.3)	3	1	0
AFB+ve	51	37 (72.5)	8	4	2
AFB-ve	99	42 (42.4)	4	37	16

a 1-9 AFB/100HPF (High power field); b 10-99 AFB/100HPF; c 1-10 AFB/HPF; d >10 AFB/HPF; Mtb: *M. tuberculosis* **complex**

To our knowledge, this study is the first ever attempt to assess the diagnostic performance of SSM against culture in the Abia State TB Control Programme. The study has thrown light on important aspects of laboratory diagnosis of TB in the Abia State TB Control Programme. First, although the sensitivity of SSM during the period of this study was moderate, it is clear that a high proportion of smear negative TB patients were not being detected by the routine smear microscopy in use. Furthermore, the use of culture also revealed that 12(13.19%) of the subjects were infected with NTM.

Culture of *M. tuberculosis* from clinical specimens is more sensitive and specific than smear microscopy and is considered the gold standard for definitive diagnosis and bacteriological confirmation of tuberculosis. However, culture has a major disadvantage of being too slow for immediate clinical utility. The growth of mycobacteria may take from 4-8 weeks on conventional solid media. In addition, culture requires high technical skill, it is labour intensive and there is need for expensive biosafety system. Despite these shortcomings and challenges associated with utilizing culture, culture is indispensable for addressing the increasing incidence of sputum smear negative TB, particularly in Human Immunodeficiency Virus (HIV) associated TB in Africa (15). Culture is also needed for drug susceptibility testing (DST) in the face of emerging multidrug-resistant TB (MDR-TB). Although culture cannot replace sputum smear microscopy as a routine laboratory diagnostic method in developing countries, international authorities and experts are of the view that culture and DST capacity need to be strengthened at country level to address smear – negative TB and multidrug resistant TB (16, 17).

The specificity of SSM for detection of AFB is widely believed to be very high (4, 17,). The specificity of SSM of 92.3 % (95% CI: 86.4%-98.2%) found in this study is in agreement with this view. However, the inclusion of nontuberculous mycobacteria (NTM) isolates in the analysis reduced the specificity to 77.36% and the sensitivity was reduced to 46.8%. The significance of this is that in the absence of culture, all the acid fast bacilli (AFB) detected by smear microscopy would be treated as *M. tuberculosis* infections. The 12 patients with NTM would have been put on anti-TB treatment wrongly on the basis of the SSM results. This fact and the low sensitivity of SSM underscore the necessity to implement culture for diagnosis of pulmonary tuberculosis in developing countries despite the great technical challenges (18).

The incidence of NTM in patients undergoing investigation for TB has not been fully investigated in developing countries. This may be due to the low level of research involving culture of mycobacteria in developing countries. The incidence of NTM in this study was 12% which appears to be high but it is comparable with the prevalence rates reported by others. In a study in Plateau State in the north central part of Nigeria, a prevalence rate of 23.08% was reported (Mavak *et al* (19). Petersen *et al* (20) reported a prevalence rate of 20% for *M. avium-M-intracellulare* complex and 15% for other mycobacteria and Srisuwanvilai *et al*., (21) reported a prevalence of 11%.

The rate of contamination of classical LJ falls within 2-5%. The contamination rate in this study was well above this. This is a reflection of the technical difficulties encountered in the course of establishing the culture facility used in this study. The LJ medium was prepared locally and did not include a cocktail of antibiotic supplement commonly included in commercial mycobacterial culture media. Furthermore, we employed the Petroff decontamination and digestion method which might not have been as effective as the N-acetyl-L-cysteine-NaOH (NALC-NaOH) employed in most advanced laboratories conducting culture. However, contamination figures of 10.1%, 11% and 14.7% have been reported by others even in well established laboratories (21, 22, 23).

Sputum smear negative TB is a real challenge to laboratory based diagnosis of TB. About 5,000 to 10,000 AFB per milliliter of sputum is required for AFB to be detected in a sputum specimen (24). In early disease, disease outside the lungs, in the elderly and in children and HIV associated TB, the extent of cavitations is minimal and as a result, lower number of bacilli are present in the airways (25).This often results in undetectable number of bacilli in the specimen leading to false negative smear result. Other factors that may lead to false negative smear results include inadequate specimen quality, inadequate number of specimens, heavy workload, poorly prepared smears and poor staining technique (25). To ensure the reliability of laboratory results, external quality assurance (EQA) systems for smear microscopy should be implemented (3, 26). Adequate training and supervision of the laboratory personnel are also important in optimizing the performance of sputum smear microscopy (10, 12). Recent innovations such as the use of bleach for sputum concentration (27, 28) and the use of light emitting diode (LED) fluorescence microscopy (29, 30, 31) have potential to improve the sensitivity of sputum smear microscopy.

About half of the HIV-positive subjects were coinfected with *M. tuberculosis* but non with NTM. This may mean that NTM is not a common cause of TB in our setting but this should be taken with caution in few of the small number of HIV-positive cases.

In conclusion, the proficiency of our laboratory system in detecting PTB cases with high bacillary load was acceptable but SSM is inadequate as the method for case-detection of PTB for long term success of our TB Control Programme. There is a need to implement a more sensitive method like sputum culture for diagnosis of sputum smear negative pulmonary tuberculosis in our TB Control Programme.

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