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PREVALENCE OF SEPTICAEMIA AND ANTIBIOTIC SENSITIVITY PATTERN OF BACTERIAL ISOLATES AT THE UNIVERSITY TEACHING HOSPITAL, YAOUNDÉ, CAMEROON

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Abstract

Bloodstream infections are important causes of mortality and morbidity. Rapid empiric antibiotic therapy is often needed. Knowledge of epidemiological data of common pathogens and their antibiotic sensitivity pattern is needed for rapid therapy. This study was aimed at determining the common causes of septicaemia and their antibiotic sensitivity pattern from the University Teaching Hospital, Yaoundé. Blood samples were collected and cultured aerobically. Isolates were identified using bacteriological and biochemical methods and antibiotic sensitivity was done using the Kirby-Bauer disc diffusion method. Results showed that of the 396 patients examined 112 (28.3%) had septicaemia. Children below the age of 15 years constituted the greatest percentage of infected subjects (63.4%) followed by patients aged between 16-30 years (10.7%) ($P < 0.05$). The highest incidence of septicaemia were from medicine (8.95‰), followed by paediatrics (7.04‰), surgery (6.46 ‰), out-patients (5.79‰), neonatology (5.12‰), obstetrics and gynaecology (5.05‰) and emergency (2.05‰) wards. The overall incidence of septicaemia was 5.79 per 1000 admissions. Gram-positive bacteria were encountered more often than gram negative bacteria (56.2% versus 43.8%, $P < 0.05$). Among the gram-positive bacteria, 52 (82.5%) were *Staphylococci*; 6 (9.5%) were *Streptococcus species*; while 5 (7.9%) were unidentified gram-positive bacteria. Among gram-negative bacteria, *Enterobacteriaceae* 39 (79.6%) and non-fermenting bacteria 10 (20.1 %) were more frequent. *Staphylococci* were generally sensitive to Minocycline and Rifampin (90%) while *Enterobacteriaceae* were most sensitive to Cefoxitin (71%) and Aztreonam (74%). *Staphylococcus epidermidis*, *S. aureus* and *Salmonella typhi* are the leading causes of bacteraemia among patients attending the University Teaching Hospital, Yaoundé

Keywords: Septicaemia, antibiotic sensitivity, Cameroon

Introduction

Septicaemias are important causes of mortality and morbidity and are among the most common healthcare associated infections [1]. Illnesses associated with bloodstream infections range from self-limiting infections to life threatening sepsis that require rapid and aggressive antimicrobial treatment [2]. A wide spectrum of organisms has been described and this spectrum is subject to geographical alteration. Patients who are granulocytopenic or inappropriately treated may have a mortality rate that approaches 100% [1]. Moreover, fatalities among patients infected with Gram-negative bacilli are higher than those among patients who have Gram-positive cocci as causative agents of their bacteraemia [3]. Worldwide, emergence of antibiotic resistance in all kinds of pathogenic bacteria is a serious

public health issue. It is associated with greater hospital mortality and longer duration of hospital stay, thereby increasing health care costs [4]. Also, colonization and infection with antibiotic-resistant bacteria has made the therapeutic options for infection treatment extremely difficult or virtually impossible in some instances [5]. There are many reasons for this alarming phenomenon, including increasing antibiotic use and misuse in humans, animals and agriculture, clustering and overcrowding and poor infection control [6].

Due to the high mortality and morbidity associated with septicaemia, antimicrobial therapy in most cases is initiated empirically before the results of blood culture and antimicrobial susceptibility pattern of the isolates are available [1]. Knowledge of local antimicrobial resistance

patterns from accurate bacteriological records of blood culture results is needed to provide guidance towards an empirical therapy before sensitivity patterns are available. There is large excess mortality in Sub-Saharan Africa particularly in children. The mortality rate among five-year-old children is about 25-100 per 1000 compared with 10-30 per 1000 in developed countries [7]. Bacteraemia is usually caused by a wide spectrum of bacteria with varying antimicrobial susceptibility pattern. However, there is a paucity of information about the relative contribution of different bacteria to infections in Sub-Saharan Africa and how this varies across the full range age groups [7]. Bacteraemia often require prompt diagnosis and effective treatment to prevent death and complications from septicaemia. Physical signs and symptoms are usually useful in identifying patients with septicaemia and other non-localized infections but these have limited specificity [8]. Bacteriological culture to isolate the offending pathogen and determine its antimicrobial sensitivity pattern has remained the mainstay of definitive diagnosis of septicaemia [9]. In most cases of suspected All the subjects were patients suspected clinically for septicaemia and sent to the bacteriology laboratory for blood culture by physicians. There was no formal definition and recruitment was at the discretion of the attending physicians. However, prior to blood collection, patients were explained the purpose of the study and made to understand that it was not a hospital obligation to participate in the research and neither was it a pre-requisite to accessing any hospital services publicly available. Written informed consent forms were got. 5-10 mls of blood was collected from adults and 1-2ml from children and inoculated into the biphasic culture medium (brain-heart infusion (BHI) agar and trypticase soya agar (TSA) with slopes. The blood culture bottles were immediately incubated aerobically at 35°C for 24 hrs.

After 24hrs, bottles were checked for positive cultures (growth on the agar slope and/or turbidity in the broth). Negative cultures were re-incubated and checked daily for up to three weeks unless growth occurred. Before re-incubation, the slope was re-inoculated by tipping the bottle. Blind culture was done on all bottles without visible growth at the end of the three weeks

septicaemia antimicrobial therapy is always initiated empirically because bacteriological culture results take about a week to be available. Epidemiological data on common blood stream pathogens and their antimicrobial sensitivity pattern is thus very important to make the right choice of empiric therapy.

In Cameroon, such data are scarce in most healthcare settings due to dwindling resources [4]. Data used is usually from developed countries. This study was therefore carried out to determine the common causes of bacteraemia and their antibiotic susceptibility pattern in Yaoundé to help guide healthcare providers initiating empirical therapy on the choice of antibiotics to be used.

Materials and Methods

This study was conducted in the University Teaching Hospital of Yaoundé, Cameroon, a tertiary health care hospital providing a full range of surgical, medical and super speciality facilities. The study was carried out from January to June 2010.

Gram staining was done using both the broth and the colonies on the slope. The gram stain reaction of the bacteria guided on which medium to be used for subculture. Gram positive bacteria were inoculated onto chocolate agar supplemented with polyvitex and blood agar and incubated at 35°C in a candle jar for about 24hrs depending on the growth rate of the bacteria. Gram-negative bacilli were subcultured on Eosine Methylene Blue (EMB) aerobically at 35°C and gram positive cocci subcultured aerobically at 35°C on Chapman agar for 24 hrs.

Routine laboratory techniques were used to identify the bacteria [10]. SLIDEX Strepto PLUS® reagent was used for Lancefield grouping of Streptococci. Api20E® was used following the manufacturer's instructions for the *Enterobacteriaceae*. Non-fermenting bacilli were tested as described above but Api20nE® system was used instead of Api20E®.

Antibiotic susceptibility testing was done on MH using the Kirby-Bauer disc diffusion technique [11]. Antibigram for *Streptococcus* species was done on blood agar. Susceptibility testing of *Staphylococcus* species to oxacillin, vancomycin and teicoplanin was done on salted MH. Antibiotic discs used included penicillin, amoxicillin (25µg),

gentamycin(15µg), streptomycin(30µg),
 cefoxitime,(30µg), fuscidicacid(30µg),
 cotrimoxazole(1.25/23.7µg), vancomycin(30µg),
 erythromycin(15µg), lincomycin(15µg),
 spiramycin, (100µg), rifampicin(30µg),
 tetracycline(30µg), perfloxacin(30µg),
 oxacillin(30µg), imipeneme(10µg),
 ceftazidime(30µg), aztreonam (30µg), Cefalotine
 (30µg), Cefixime (30µg) Cefuroxime (30µg),
 Cefotaxime (30µg), eftriaxone(30µg), Ceftazidine
 (30µg), Aztreonam (30µg), Amikacin
 (30µg), Netilmicin(30µg), Nalidixic acid (30µg),
 Norfloxacin (5µg), Ciprofloxacin (5µg) and
 Cefoxitime (30µg). Quality control for
 antibiogram was done weekly using *E. coli* ATCC

25922, *S. aureus* ATCC 25923 and *P.aeruginosa*
 ATCC 13048. Each batch of culture media
 prepared was controlled using *S. aureus*
 ATCC25923 (Chapman), *S. pyogenase* (Trypticase
 soja) and *E. coli* ATCC 25922(EMB).

Results

Of the 396 patients examined for septicaemia,
 positive culture was found in 112 (28.3%). Age
 distribution of the patients is shown in Table 1.
 Children below the age of 15 years constituted the
 greatest percentage of infected subjects (63.4%),
 followed by patients aged between 16-30 years
 (10.7%)($P<0.05$).

TABLE 1: AGE DISTRIBUTION OF PATIENTS WITH SEPTICAEMIA

Age ranges (years)	No clinically examined	No (%) of positive culture
0-15	224	71 (63.4)
16-30	76	12 (10.7)
31-45	32	9(8.0)
46-60	36	11(9.8)
61-75	18	5 (4.5)
>76	10	4(3.6)
Total	396	112 (100)

Throughout the study period the hospital
 admitted a total of 19348 patients. Table 2 shows
 septicaemia cases/1000 admissions in the various
 wards and clinics. The highest incidence of
 septicaemia were from medicine (8.95%),
 followed by paediatrics (7.04%), surgery (6.46%),
 out-patients (5.79%), neonatology (5.12%),
 obstetrics and gynaecology (5.05%) and

emergency (2.05%) wards. The overall incidence
 of septicaemia was 5.79 per 1000 admissions.
 Septicaemia was very high among children less
 than one month old (34.9%). The overall rate of
 isolation reduced with increasing age but the type
 of bacteria isolated did not vary with age except
 for *Staphylococci*.

TABLE 2: SEPTICAEMIA CASES/1000 ADMISSIONS IN THE VARIOUS WARDS AND CLINICS

Ward/Clinic	No of admissions During study period =(a)	Cases of septicaemia during the period= (b)	Cases per 1000 admissions= b/a x 1000
Surgery	2785	18	6.46
Emergency	1466	3	2.05
Neonatology	3909	20	5.12
Paediatrics	3127	22	7.04
Medicine	782	7	8.95
Obstetrics and Gynaecology	198	1	5.05
Out-Patients	7082	41	5.79
Total	19348	112	5.79

The type and pattern of bacteria isolates in the various age groups is shown in Table 3. Gram-positive bacteria were encountered more often than gram negative bacteria (56.2% versus. 43.8%, $P < 0.05$). Among the gram- positive bacteria, *Staphylococci* constituted 52 (82.5 %), *Streptococci* species 6 (9.5%) and unidentified gram-positive bacteria 5 (7.9%). Among gram-negative bacteria, enterobacteriaceae 39 (79.6 %) and non-fermenting bacteria 10 (20.1 %) were more frequent

Table 4 shows the *in-vitro* sensitivity pattern of *Staphylococci* to selected antibiotics. *Staphylococcus epidermidis* was most sensitive to minocycline (91%) followed by rifampin (88%). *S. aureus* was mostly sensitive to rifampin (95%) and *S. saprophyticus* to perfloxacin (100%). The sensitivity pattern of gram-negative bacilli to selected antibiotics is shown in Table 5 Among the enterobacteriaceae *Salmonella typhi* showed high sensitivity to cefoxitin (71%) and Aztreonam (74%).

TABLE 3: THE TYPE AND DISTRIBUTION OF BACTERIA ISOLATES ACCORDING TO AGE

Bacteria Isolates	Age ranges (years)						Total (%)
	0-15 n(%)	16 -30 n(%)	31- 45 n(%)	46-60 n (%)	61-75 n (%)	>76 n (%)	
<i>S.aureus</i>	12 (10.9)	2 (1.8)	4 (3.6)	3 (2.7)	1 (0.9)	1 (0.9)	23 (20.9)
<i>S. epidermidis</i>	18 (16.4)	1 (0.9)	2 (1.8)	3 (2.7)	1 (0.9)	0	25 (22.7)
<i>S. saprophyticus</i>	3 (2.7)	0	0	1 (0.9)	0	0	4 (3.6)
<i>Streptococcus sp.</i>	5 (4.5)	0	0	1 (0.9)	0	0	6 (5.5)
<i>S. typhi</i>	3 (2.7)	6 (5.4)	0	1 (0.9)	0	0	10 (9.1)
<i>Salmonella species</i>	3 (2.7)	1 (0.9)	1 (0.9)	2 (1.8)	0	0	7 (6.4)
<i>K. pneumonia</i>	3 (2.7)	1 (0.9)	1 (0.9)	2 (1.8)	0	0	7 (6.4)
<i>E. coli</i>	3 (2.7)	0	2 (1.8)	0	1 (0.9)	0	6 (5.5)
<i>Enterobacter sp.</i>	6 (5.5)	1 (0.9)	0	0	0	0	7 (6.4)
<i>Pseudomonas sp.</i>	2 (1.8)	1 (0.9)	0	0	0	0	3 (2.7)
<i>Acinetobacter sp.</i>	2 (1.8)	0	0	0	0	0	2 (1.8)
<i>Proteus mirabilis</i>	1 (0.9)	0	0	0	0	0	1 (0.9)
Non enterobacteriaceae	2 (1.8)	0	0	0	1 (0.9)	0	3 (2.7)
Gram positive bacilli	3 (2.7)	1 (0.9)	1 (0.9)	0	0	0	5 (4.5)
<i>Klebseilla oxytoca</i>	1 (0.9)	0	0	0	0	0	1 (0.9)
Total	67 (60.9)	14 (12.7)	11(10.0)	13 (11. 8)	4 (3.6)	1 (0.9)	110(100)

Discussion

This study is a record of septicaemia in patients attending the University Teaching Hospital in Yaoundé, Cameroon. We included patients of all age groups. Results showed that septicaemia was

present in 28.3% of patients examined. Gram positive bacteria were encountered more than gram-negative bacteria, and the most frequent invasive bacteria were *Staphylococcus epiderimidis*, *S. aureus*, *Salmonella typhi* and *Klebseilla* species.

These results are similar to those obtained in some previous studies [9]: Bacteriamia was identified in 552 (45.9%) of 1201 children in Nigeria; 53.4% of the infections were due to gram positive bacteria and 46.6% due to gram negative bacteria. The most frequent isolate was *S. aureus* (47.7%) followed by coliforms (23.4%), unidentified gram negative rods (8.0%), *Pseudomonas aeruginosa* (5.8%), Streptococcal species (4.7%) and Chromobacteria species (4.5%). Hill et al [7] also reported an incidence of 34% (297) out of 871 patients studied. The isolates were dominated by gram-positive bacteria. *Streptococcus pneumoniae* study. The high rate of isolation from children may be due to their weak immune system as compared to adults, and most children often take in medications by means of intravenous devices that may easily introduce bacteria into their bloodstream when proper hygiene is not ensured.

The *in-vitro* susceptibility test of most common isolates showed very high resistance to commonly used antibiotics as penicillin, cotrimoxazole, amoxicillin, and amoxicillin/clavulanic acid. Staphylococci were generally sensitive to minocycline and rifampin (90%). Also noted was high rate of resistance to glycopeptides (Teicoplanin-29% and vancomycin-32%) by Staphylococci. Cotrimoxazole, amoxiciline and

accounted for 45.2%, *S. aureus* -18.3%, *E. coli*-9.7% and non-typhoidal *Salmonella* (8.6%) of the isolates. Results have also shown a very high incidence of septicaemia among children below the age of 15 years. This is similar to the results obtained from Nigeria [9] where 44.4% suspected cases were neonates and the rate of isolation among newborn was 22.6% out of the overall 45.9% incidence cases. It is also in accordance with results from Laos in which 69.2% of Staphylococci were from infants [12]. The rate of isolation also reduced with increasing age as seen in this

penicillin are extensively used in Africa due to their spectrum of activities [9]. This may account for the high resistance observed. Among the enterobacteriaceae, there was high rate of resistance to cephalosporins and other classes of antibiotics except for few *Salmonella typhi* that showed 100% sensitivity to cefotaxim, ceftriaxone and aztreonam. There was a high rate of resistance among the few *Klebsiella* species to all antibiotics used. This is probably due to production of penicillinase and extended spectrum beta lactamases [13]. Among the antibiotics used for all isolates, Staphylococci had a sensitivity of 70% and 66% to cefoxitin and gentamycin. Gram negative bacilli had sensitivity of 67% and 65% to cefoxitin and gentamycin respectively.

TABLE 4: SENSITIVITY PATTERN OF STAPHYLOCOCCI TO SELECTED ANTIBIOTICS

Bacteria Isolates	P n(%)	AMC n(%)	GN n(%)	S n(%)	FA n(%)	SXT n(%)	VA n(%)	E n(%)	L n(%)	SP n(%)	RA n(%)	TE n(%)	MI n(%)	PEF n(%)	OX n(%)	TEC n(%)	FOX n(%)
<i>S. Epidermidis</i>	5 (20)	20 (80)	15 (60)	15 (75)	18 (78)	5 (22)	18 (72)	15 (60)	18 (72)	13 (65)	22 (88)	13 (52)	21 (91)	17 (68)	14 (70)	17 (68)	14 (56)
<i>S. aureus</i>	5 (23)	17 (77)	16 (80)	15 (68)	17 (85)	5 (25)	15 (68)	13 (62)	11 (50)	16 (84)	21 (95)	11 (52)	19 (90)	15 (68)	18 (82)	17 (77)	21 (95)
<i>S. Saprophiticus</i>	2 (50)	2 (66)	2 (50)	2 (50)	2 (50)	2 (50)	1 (33)	1 (25)	1 (25)	2 (66)	3 (75)	2 (50)	1 (33)	4 (100)	1 (50)	1 (25)	1 (50)

P= penicillin, AMC amoxicillin, GN=gentamycin S=streptomycin, FA=fuscidicacid, SXT= ceftriaxone, A= vancomycin, E= erythromycin, L=lincomycin, SP=spiramycin, RA=rifampicin, TE=tetracycline, PEF=perfloxacin, OX=oxacillin, TEC=ceftazidime, FOX= cefoxime

Our results agree with the work of Meremikwu et al [9] who found that *S. aureus* and coliforms were highly resistant to amoxicillin, penicillin and cotrimoxazole. Sensitivity of *S. aureus* to gentamycin was 86.6% and 61.6% for coliforms. In India, Atul et al [1] found that 80% of *S. aureus*

strains were penicillin resistant. Resistance to erythromycin ciprofloxacin and gentamycin were above 45%. No strains showed resistance to vancomycin. Among enterobacteriaceae ceftriaxone was very effective and amikacin was very effective for gram-negative non-fermenters

like *Pseudomonas* and *Acinetobacter* species. This work also agrees with previous findings [13] in negative bacilli to amoxicillin (87%), piperacillin (74%) and cotrimoxazole (73%). Imipenem (98%) was the most active antibiotic followed by

which there were high rates of resistance among gram-ofloxacin (88%). Susceptibility to all isolates was 67%.

TABLE 5: SENSITIVITY PATTERN OF GRAM-NEGATIVE BACILLI TO SELECTED ANTIBIOTICS

Bacteria Isolates	AMX n (%)	AMC n (%)	CF n (%)	CFM n (%)	CTX n (%)	CRO n (%)	CAZ n (%)	AZN n (%)	AN n (%)	CIP n (%)	SXT n (%)	FOX n (%)	NET n (%)	GN n (%)	NO n (%)
<i>S. typhi</i>	1 (10)	7 (70)	6 (60)	9 (100)	7 (100)	9 (100)	9 (90)	10 (100)	9 (90)	9 (90)	2 (20)	8 (80)	6 (60)	8 (89)	9 (90)
<i>Salmonella</i> species	2 (28)	3 (42)	5 (71)	6 (85)	5 (7)	4 (80)	7 (100)	6 (85)	5 (83)	6 (85)	3 (42)	5 (71)	6 (85)	5 (71)	5 (83)
<i>Klebsiella</i> <i>pneumonia</i>	0	0	3 (37)	4 (50)	4 (50)	1 (14)	4 (50)	3 (37)	3 (37)	1 (12)	4 (50)	2 (40)	2 (40)	2 (50)	3 (37)
<i>E.coli</i>	2 (40)	2 (40)	2 (40)	2 (80)	4 (100)	4 (100)	3/5	5 (100)	3/5	4 (80)	3 (75)	6 (100)	2 (50)	1 (33)	3 (60)
<i>Enterobacter</i> species	1 (16)	1 (16)	2 (33)	2 (33)	2/5	1 (16)	2 (33)	2 (33)	3 (50)	1 (25)	1 (16)	6 (100)	2 (50)	1 (33)	3 (50)
<i>K. okytoca</i>	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)	0	0	0
Overall Enterobacteria ceae	6 (16)	13 (34)	18 (47)	25 (68)	23 (66)	21 (68)	25 (66)	28 (74)	23 (62)	25 (65)	11 (32)	27 (71)	17 (53)	25 (65)	23 (62)

AMX=amoxicillin, AMC=amikacin, CF= Cefuroxime CFM=, CTX= cotrimoxazole CRO=Cefuroxime CAZ= Ceftazidime AZN= aztreonam SXT=cotrimoxazole, AN= Nalidixic acid, CIP= Ciprofloxacin, SXT=ceftriaxone, FOX= Cefoxitine, NET=Netilmicin, GN=gentamycin, NO= Norfloxacin

However, while this study represents real life clinical practice in the hospital in which it was conducted, our approach had some limitations. The primary reason for requesting the blood culture from patients is still not clear.

Conclusion

This study shows that *Staphylococcus epidermidis*, *S. aureus* and *Salmonella typhi* are the living cause of bacteraemia among patients in the Yaoundé locality. In general Staphylococci were most sensitive to minocycline and rifampin while gram-

negative bacilli were more sensitive to cefoxitine. Observed decline in susceptibility of these common pathogens (especially gram-negative bacilli) to common antibiotics calls for increase effort to ensure more rational use of drugs. None of the antibiotics used singly showed high sensitivity to all the gram-negative bacteria, so a combination of two or more drugs (such as gentamicin, cefoxitine and ciprofloxacin) is needed to cover the broad range of gram-negative bacilli.

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HAND HYGIENE AMONGST DENTAL PROFESSIONALS IN A TERTIARY DENTAL CLINIC

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Running title: Hand hygiene among dental professionals.

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ABSTRACT

Objective: To evaluate hand washing attitude and practices among Dentists and Dental Students treating patients in a Nigerian Tertiary Dental Clinic.

Materials and Methods: A cross-sectional survey of Dentists and Dental Students treating patients in University of Benin Teaching Hospital was conducted between February and March 2010. Data collection tool was a 28-item, self-administered questionnaire, which elicited information on demography, handwashing practices, material used for handwashing, methods of drying hands after washing, attitudes towards prevention of spread of infection by handwashing measures in dental practice, barrier to regular hand washing, information need on handwashing and mode in which they would desire to receive the needed information.

Results: One-quarter (25.7%) of the respondents washes their hands before wearing gloves and 98.1% wash their hands when they are visibly soiled. Less than half (46.7%) washed their hand when the worn gloves are torn. Majority strongly agreed that hand washing helps to prevent transmission of infection to patients (91.4%), health workers (92.4%) and health workers family members (89.5%). The main barriers to regular hand hygiene were inadequate facilities, forgetfulness and lack of time. About 69.5% desired more information on hand hygiene with the most indicated area of information needs being the indications and steps in hand washing in form of seminars and pamphlets.

Conclusion: This study revealed positive attitude to hand washing, inadequate hand washing practices and poor monitoring of hand hygiene in the health institution. The studied dental professionals however know that hand washing plays an important role in the prevention of cross infection.

Keywords: hand hygiene, dental professionals, tertiary, dental clinic, infection control

INTRODUCTION

Direct contact transmission is one of the most frequent means of transmission of infectious diseases in healthcare setting worldwide (1). The role of a health worker's contaminated hand in this form of infection transmission was recognized since the mid-1800s by Ignaz Semmelweis in Vienna, Austria and Oliver Wendell Holmes in Boston, USA (2, 3). Health care workers' hands get contaminated by touching body secretions, excretions, wounds of patients, intact skin of patients and environmental surfaces in the immediate vicinity of the patients (4). To avoid prolonged hand contamination, it is important to perform hand hygiene.

Hand hygiene is the single, most critical measure for reducing the risk of transmitting organisms to patients and health care providers. It has been cited as the most effective measure for preventing healthcare associated infections, and its impact on the reduction of these infections is estimated at 50% (5). The value of hand hygiene extends beyond health care setting as it helps in preventing chemically related occupational hazards and up to 80% of infections, including influenza, in the community setting (6). Hand hygiene compliance among health care workers is low

despite the fact that hand hygiene is one of the simplest and most important aspects of infection control⁷. Noncompliance with hand hygiene practices is associated with health care-associated infections, the spread of multi-resistant organisms, and has been a major contributor to outbreaks of infectious diseases.

Hand hygiene is very important in dentistry because of the ease of contamination of hands by blood, body fluids and saliva. The Centers for Disease Control (CDC) in 2003 guidelines for infection control in dentistry listed the specific instances when hand hygiene should take place (8). Studies on hand hygiene have been conducted on different groups of health workers including dental professionals in different parts of the world (9-18). To the best knowledge of researchers, none of such study on Nigerian dental professional exists in indexed literature.

The objective of the study was to evaluate attitude to and practices of hand washing among Dentists and Dental Students treating patients in a Nigerian Tertiary Dental Clinic.

MATERIAL AND METHODS

This survey was conducted between February and March 2010 at the Dental clinic of the University of Benin Teaching Hospital. University of Benin Teaching Hospital is located in Ugbowo, Benin City, capital of Edo State, Nigeria. It was established in 1973 as the sixth 1st generation Teaching Hospitals in Nigeria. The study population was Dentists and Dental Students treating patients who attend the University of Benin Teaching Hospital dental clinic. Data collection tool was a 28-item, self-administered questionnaire, which elicited information on demography, hand washing practices, materials used for hand washing, methods of drying hands after washing, attitudes towards prevention of spread of infection by hand hygiene measures in dental practice, barrier to regular hand washing, information need on hand washing and mode in which they would desire to receive the needed information. Informed consent was obtained from participants after educating them on the study and its objectives. Ethical approval for the study was obtained from the University of Benin Teaching Hospital Ethics and Research Committee. The collated data was analyzed with SPSS version 15.0 and chi square was used to test for statistical significance which was set at $p < 0.05$.

RESULTS

Majority (87.6%) of the respondents were in 21-30 year age group. Two-thirds of the respondents were male. Only 16.2% were married. About one-third (32.4%) of the respondents were of Bini tribe. Three-tenth (30.5%) of the respondents were Dentists. A total of 16 (15.2%) of the respondents wear ring of which 11 (10.5%) were smooth in nature while the remaining 5 (4.8%) were serrated in nature (**Table 1**).

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF THE RESPONDENTS

Characteristics	Frequency	Percent
Age (years)		
21-30	92	87.6
31-40	12	11.4
>50	1	1.0
Sex		
Male	70	66.7
Female	35	33.3
Marital Status		
Single	88	83.8
Married	17	16.2
Religion		
Christianity	101	96.2
Islam	2	1.9
Trad religion	2	1.9
Tribe		
Bini	34	32.4
Esan	18	17.1
Igbo	16	15.2
Yoruba	9	8.6
Urhobo	9	8.6
Etsako	6	5.7
Isoko	4	3.8
Igala	3	2.9
Others	6	5.7
Status		
Dentist	32	30.5
Dental student	73	69.5
Ring		
Smooth ring	11	10.5
Serrated ring	5	4.8
No ring	89	84.8
Total	105	100

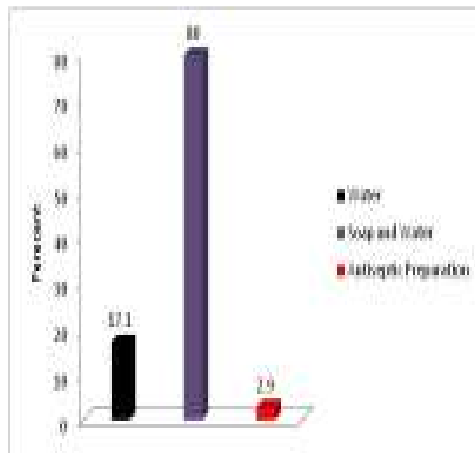
Majority (93.3%) of the respondents reported that hand washing is important in dentistry. One-quarter (25.7%) of the respondents wash their hands before wearing gloves. Almost all the respondents (98.1%) wash their hands when they are visibly soiled. Less than half (46.7%) wash their hand, after removing torn gloves, before re-gloving (**Table 2**). There was no significant difference in the handwashing practices of dentists and dental students in this study ($P > 0.05$).

Majority (80%) of the respondents wash their hand regularly with soap and water (**Figure 1**).

TABLE 2: HAND WASHING PRACTICES AMONG THE RESPONDENTS

Practice	Yes	No	No response
Before gloving	27 (25.7%)	78 (74.3%)	0 (0%)
After gloving	91 (86.7%)	13 (12.4%)	1 (1.0%)
Torn glove	49 (46.7%)	53 (50.5%)	3 (2.9%)
Before leaving Operatory	69 (65.7%)	36 (34.3%)	0 (0%)
Contaminated hand	93 (88.6%)	12 (11.4%)	0 (0%)
Visibly soiled hand	103 (98.1%)	2 (1.9%)	0 (0%)
Before lunch	57 (54.3%)	44 (41.9%)	4 (3.8%)
After using restroom	83 (79.0%)	20 (19.0%)	2 (1.9%)

FIGURE 1: MATERIALS UTILIZED BY RESPONDENTS FOR HAND WASHING



Drying of washed hands is done using hand towel by 42.9% of the respondents. One-fifth (20%) of the respondents dry their hand using a personal handkerchief. More than one-quarter (26.7%) do not dry their hands after washing (Figure 2).

Majority (91.4%, 92.4% and 89.5%) of the respondents strongly agreed that hand washing helps to prevent spread of infection to the patients, health workers and family of health worker respectively (Table 3).

The identified barriers to regular hand hygiene among the respondent were inadequate facilities, forgetfulness and lack of time (Figure 3).

FIGURE 2: WAYS OF DRYING HANDS AFTER WASHING AMONG THE RESPONDENTS

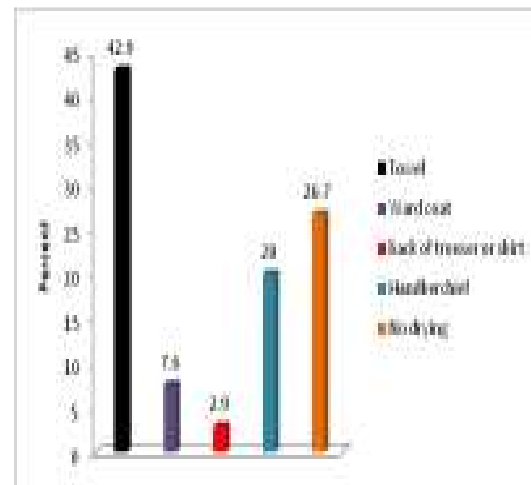


FIGURE 3: BARRIERS TO REGULAR HAND HYGIENE AMONG ESPONDENTS

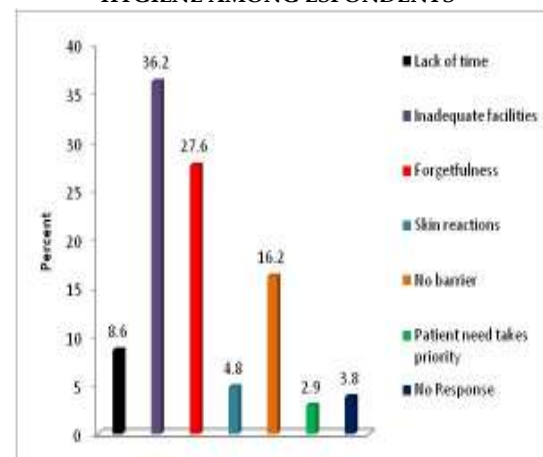
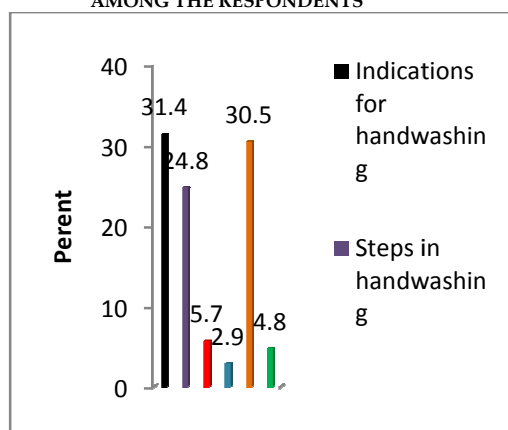


TABLE 3: ATTITUDE OF THE RESPONDENTS ON THE PREVENTION OF SPREAD OF INFECTION BY HAND WASHING

Questions	STA	SOA	UNS	SOD	STD	NOR
Hand washing helps to prevent the spread of infection to patients?	96(91.4%)	8(7.6%)	0(0%)	0(0%)	0(0%)	1(1.0%)
Hand washing helps to prevent the spread of infection to family of health worker?	94(89.5%)	8(7.6%)	1(1.0%)	0(0%)	1(1.0%)	1(1.0%)
Hand washing helps to prevent the spread of infection to health worker?	7(92.4%)	5(4.8%)	2(1.9%)	0(0%)	0(0%)	1(1.0%)
My institution monitors hand washing?	7(6.7%)	9(8.6%)	28(26.7%)	16(15.2%)	43(41%)	2(1.9%)

Key to the table: STA-Strongly Agree, SOA - Somewhat Agree, UNS-Unsure, SOD - Somewhat Disagree, STD-Strongly Disagree, NOR- No Response

FIGURE 4: INFORMATION NEEDS ON HAND HYGIENE AMONG THE RESPONDENTS



DISCUSSION

Hand hygiene is critical in the prevention of hospital-acquired infections which contribute to the death of nearly 90,000 hospital patients per year and \$4.5 billion in medical expenses (19). Hand washing is also the single most effective way to reduce the spread of microorganisms in dentistry (8). In this study, majority of the respondents (93.3%) knew that hand washing is important in dentistry. This high percentage is encouraging and it may imply that many of the respondents will be willing to carry out hand hygiene in their practice. Wearing of rings can increase the possibility for hand contamination in dental and medical settings. The skin of hospital personnel underneath rings is more heavily colonized than comparable areas of skin on fingers without rings (8,20,21). The presence of ring impedes the removal of microorganisms by routine hand hygiene, resulting in prolonged and persistent periods of microbial contamination (8,22). A total of 16 respondents (15.2%) wear rings at work, 10.5% of which are smooth nature and 4.8% serrated in nature. These group of people may not be able to achieve optimal hand hygiene since difficulty of gloving and the high rate of glove tear associated with rings, especially serrated ones, result in contamination of the skin around the ring area²³

The study revealed that many of the respondents will not wash hands before putting on gloves or before changing gloves in the event of torn gloves. The drive to deliver dental care as quickly as possible, despite inadequate facilities, is a possible reason for not carrying out the recommended hand decontamination before gloving and in the event of torn glove. It may also be that the respondents are ignorant of the fact that high microbial load on the hand is significant enough to cause cross-infection.

A total of 73 (69.5%) indicated their need for more information on hand hygiene. The indicated areas of information needed by the respondents were indications for hand washing and steps in hand washing (Figure 4). Most of the respondents will prefer the information to be given in form of Seminars.

A total of 65.7% usually leave dental operatory without washing their hands and little more than half (54.3%) of the respondents would not wash hand before lunch. It may be proper to say that the respondents have deficient personal hygiene, as hand washing stands out prominently as a measure of personal hygiene.

Hand hygiene can be performed by washing hand with plain soap and water, and this has been the standard practice in dentistry. The use of a persistent-level antimicrobial hand wash or an alcohol-based hand rub is also acceptable. In this study, 80% of the respondents wash their hands with soap and water and only 2.9% of the respondents wash their hands with antiseptic preparations. Unacceptable practice of washing hands with only water was reported among 17.1% of the respondents. This further reflects bad practice among the studied health professionals. Drying of hands is an important aspect of Hand hygiene. The use of electric hand dryers is not so common in a developing country like Nigeria with epileptic power supply. Respondents mostly use hand towels provided by the clinic, personal handkerchiefs or they allow the hands to dry up naturally. The hand towels provided are usually not disposable and so there is a possibility of reuse and therefore contamination. The use of personal handkerchief for drying or leaving the hands to dry up naturally may also result in unexpected contamination.

Hand washing is an important indicator of safety and quality of care delivered in any health-care setting, because there is a substantial evidence to demonstrate the correlation between good hand hygiene practices and low health care associated infection rates (24). It substantially reduces the number of microbes that may be shared between patients and health care

personnel or between health care personnel and contaminated surfaces. In this study, majority strongly agreed that hand washing helps to prevent transmission of infection to patients, health worker and health workers family members. However, a reasonable number of respondents strongly disagreed that there exists any monitoring of hand washing to ensure compliance in the health institution. Formulation of policy geared at monitoring hand hygiene compliance in this institution is therefore desirable. There are a number of known factors affecting compliance with hand hygiene such as lack of time, high patient workload, patients' need taking priority, forgetfulness, lack of knowledge of importance of hand hygiene in preventing cross infection, poor access to handwashing facilities, lack of institutional commitment and skin irritation to hand hygiene products⁹⁻¹¹. In this study, the main barriers to regular hand hygiene in descending order were lack of adequate facilities, forgetfulness and lack of time. Lack of time has also been cited as a barrier to hand hygiene among nursing students (11). Many of

the respondents realize that there is a need for more information on hand hygiene. This signifies that there is a self perception of deficiency in hand hygiene among the respondents. The indicated areas of information needed were the indications for hand washing and steps in hand washing. These are two key areas and if properly taught, a lot of ground would have been covered in hand hygiene.

CONCLUSION

This study revealed high knowledge of the role of hand washing in the prevention of cross infection but inadequate hand washing practices and poor monitoring of hand hygiene in the health institution. There is an important need for educational and motivational intervention targeted at Dentist and Dental Students mostly in form of seminars and pamphlets. There is also a need for Institutional reforms which would facilitate the procurement of hand hygiene facilities, eliminate barriers to handwashing, formulate and implement policy to monitor hand hygiene compliance.

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BIOFILM, DENTAL UNIT WATER LINE AND ITS CONTROL

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Abstract

Biofilms are well-organized communities of cooperating microorganisms that can include bacteria, algae, fungi and diatoms. Dental unit waterlines (DUWL) are an integral part of dental surgery equipment, supplying water as a coolant, primarily for air turbine and ultrasonic scalers. Surveys of dental unit waterlines (DUWLs) indicate that biofilm formation is a universal problem and great majority of bacteria that have been identified from DUWL are ubiquitous, although present in only low numbers in domestic water distribution systems, but can flourish as biofilms on the lumen surfaces of narrow-bore waterlines in dental units. DUWL contamination and its significance as a factor in nosocomial infection of patients and health care workers has stressed the risk to immunocompromized persons. Not only patients but also dentists and dental personnel are at risk of being infected with opportunistic pathogens such as *Pseudomonas* or *Legionella* species by means of cross-infection or after aerosol formation from water emanating from DUWL. Several methods of decreasing the level of contamination in DUWL have been proposed. At present, the goal of this review is to discuss various aspects of biofilm formation and effective standardized disinfecting methods to maintain low bacterial counts in dental water line. This will increase the awareness of potential health risks posed by biofilm formation and provide information on techniques and devices designed to control the microbial contamination of DUWLs.

Introduction

Bacteria exist in two forms i.e., planktonic (free swimming) and attached forms (in communities). Traditional studies of bacterial cells in planktonic (free-swimming) phase have focussed on pure culture physiology, a model for major microbiological studies today. However, the study of planktonic bacteria does not accurately reflect the growth of bacteria in nature because different microbial life style exists when bacteria live in association with different microorganisms and with different surfaces (1). Historically, Antonie van Leeuwenhoek was the first to examine bacteria from plaque on his teeth in the 17th Century followed by the observations of thus leading to the theory of biofilms (2).

A biofilm may be defined as a community of micro-organisms irreversibly attached to a surface, producing extracellular polymeric substances (EPS) (2). Bacteria in biofilm mode have an altered phenotype compared to their corresponding planktonic counterparts, particularly with regard to gene transcription, and in interaction with each other (2, 3). The conversion from a relatively simple planktonic cell to a complex, highly differentiated multi-cultural community is monitored by a close genetic regulation. In addition to bacteria, fungi, yeasts, algae, protozoa and viruses have also been isolated from biofilms in industrial and medical

settings but bacteria as microorganisms provide the best-studied model with regard to colonization of surfaces and subsequent biofilm formation (2). Furthermore, different biofilms are formed in different environments because of different hydrogeochemical properties. Depending upon the environment, in which biofilm formed, non living components also varies. Monocellular materials such as mineral crystals, corrosion particles, clay and silt particles, or blood components, from different environments may act as physical components of biofilms (2). Other important variables involved in cell-cell attachment and biofilm formation are: (i) properties of the substratum (texture or roughness, hydrophobicity, conditioning of film); (ii) properties of the bulk fluid (flow velocity, pH, temperature, cations, presence of antimicrobial agents); (iii) properties of the cell (cell surface hydrophobicity, fimbriae, flagella, EPS) (2).

BIOFILMS

Biofilms are heterogeneous and complex in structure, function and metabolism. The microbes in biofilm mode exhibit coordinate behaviour and live in cooperative consortia which is identical to higher multicellular organisms (3). There are number of reasons, due to which bacteria like to live in the form of biofilms; (1) Genetic material can be easily exchanged between microorganisms;

(2) Nutritive substances can be accessed much easily from the water phase; (3) Shelter can be provided by other organisms against excess of nutritive substances, toxic substances, drying and dessication (2). Due to this joint relationship, biofilm bacteria are found to be more resistant to surfactants, biocides and antimicrobials. The various mechanisms conferring resistance to biofilms are: (1) Some of the cells of biofilm suffer from nutrient limitation and undergoes slow-growing or starved state. Thus, many antimicrobial agents are unable to target these slow-growing or starved cells (2) Another mechanism explores that 90% of dry weight mass of biofilm is comprised of exopolysaccharides (EPS). EPS protect the biofilms against deep penetration of antimicrobials in them. As a result, the cells present deeper in biofilms remain protected against bactericidal or bacteriostatic action of various antimicrobials. (3) Some of the cells have unique phenotypes in biofilms. Because they have anionic and hydrophobic nature, thus repel the biocides/disinfectants and protect the dessication of biofilms. (4) Certain kinds of deposits are also present in the underlying surfaces of biofilms, acting as diffusion barriers. These diffusion barriers deactivate various antimicrobials and disinfectants and prevent their entry into biofilms (2). In one study, Xu *et al.* (4), using fluorescent probe and gene technology reported that only top one-fifth of the biofilm is metabolically active. Spatial heterogeneity due to physiological activity of biofilm is responsible for resistance against antimicrobial agents. Mechanisms like nutrient limitation and cell-cell signalling may switch cells into inactive non-growing protected phenotypes (4). Biofilms are playing important roles in industries, medical settings, waste water treatments, terrestrial and aquatic ecosystems. One important aspect of biofilm is in detoxification of heavy metals. Bacteria present in biofilms either alone or in combined form with other microorganisms and in the presence of EPS components form an association which favours the detoxification and consequently removal of the heavy metals. EPS has negatively charged functional groups like pyruvate, phosphate, hydroxyl, succinyl and uronic acid (5). Various genetics mechanisms play an important role in formation of biofilm. The planktonic bacteria which harbour plasmids, form healthy and thick biofilms as compared to the plasmid lacking strains. Strains without plasmids form only microcolonies without any further development and conversion into fully matured biofilm (6). If plasmids carry genes for resistance

to antimicrobial agents, then biofilm association will provide a mean of spreading bacterial resistance against various antimicrobial agents. One of the mechanisms responsible for transfer of resistance genes in bacterial biofilms is via natural horizontal gene transfer (conjugation). Conjugation occurs at greater rate between the cells present in biofilms than free swimming planktonic cells (6). This may be due to the reason that biofilm environment provides less shear force and better cell to cell contact resulting in greater conjugation ability. It has been reported previously that F conjugation pilus acts as a part in adhesion for both cell-surface and cell-cell interactions, resulting in development of a three-dimensional biofilm (7).

In humans, bacterial biofilms also play an important role with reference to both beneficial and harmful aspects. Among harmful aspects, one reported example is of caries, the result of a chronic undermining demineralisation of the teeth by organic acids that are produced by the bacteria of the dental biofilm while fermenting carbohydrates from the human diet (8). Another harmful aspect is catheter associated biofilm infections. The port of catheters is placed surgically or percutaneously in patients for long term effect. But it often leads to considerable morbidity, occasional mortality, and an increase in medical costs derived from its diagnosis, treatment, and mainly, prolongation of the patient's in-hospital stay due to development of biofilms in such devices (9). In contrast, in another study, human gut epithelial cells are a port for the development of mixed consortia of commensal bacteria. These mixed consortia of commensal bacteria provide a barrier against food borne pathogens. Other experimental studies and results from various repeated trials under controlled conditions have shown that certain gut bacteria, particularly species of *Lactobacillus* and *Bifidobacterium*, may exert beneficial effects in the oral cavity by inhibiting cariogenic *streptococci* and *Candida* sp. (10). Formation of dental plaque on teeth is also a good example of biofilm formation in both healthy and diseased mouths (11). Similar to plaque which is omnipresent, biofilm formation within the small bore plastic tubing in dental unit water lines (DUWL) is quite common. Dental units, in general, are equipped with different types of plastic tubings. The tubings are of different diameter and are most important surfaces for the development of biofilms. Biofilms develop within various tubing samples differ from one another in their size, texture and resistant to antimicrobials/biocides (12).

DUWL provide a particularly favourable environment for biofilm formation (13). Water at the tubing walls is almost stagnant, allowing bacteria to adhere and colonize the tubing surfaces. In DUWL, biofilm formation starts by presence of conditioned layer. Molecules of water may adhere to lumen surfaces by utilizing physical adsorption and chemisorption mechanisms. Once the conditioned substratum is formed, it can attract other molecules. The van der Waal's forces, electrostatic forces, hydrophobic forces, or chemisorption of bacterial fimbriae, pili or adhesions are few means which are helpful in attachment of different molecules (14). After adherence, the molecules enter the second phase i.e., quiet phase of surface associated lag time. In this phase bacteria prepare themselves for different types of adaptations. Some changes/changes in gene expression can be accomplished in this phase (15). After division and making phenotypic shifts, bacteria enter into the rapid phase of growth. During this phase, they secrete the cemented material (EPS), which binds the cells and protect them from shearing force of the fluid. Different microcolonies grow within the matrix, thus coaggregation of different microbes with each other and matrix increase the depth of the biofilms. Once bacteria adhered irreversibly, they increase their density enormously compared to the planktonic bacteria and it is at this stage that they secrete certain autoinducer signal molecule (16).

The risk of acquiring infections through DUWL supplies are known to be not very uncommon. Often potential pathogenesis can spread through surgical procedures, local mucosal contact, ingestion and inhalation (17). Different standards and strategies have been adapted to control DUWL transmitted infections. According to American Dental Association (ADA) (18), dental water should not have more than 200 colony-forming units per millilitre (CFU/ml) of aerobic, mesophilic, heterotrophic bacteria. Different methods like (1) antiretraction valves and retrograde aspiration of oral fluid; (2) filtration; (3) drying; (4) flushing of biocides; (5) Sterile water delivery systems (6) use of biocides/chemical disinfectants have been evaluated previously. Various authors have reported the use of biocides/disinfectants as effective decontamination methods to control DUWL contamination (13). Biocides are non-antibiotic, antiseptic, disinfecting chemical compounds, having both bactericidal and bacteriostatic properties (19). Other properties include that these should be effective at low concentrations, should be non toxic and biodegradable (19). The biocidal

action depends on (i) chemical properties (e.g. optimum pH and temperature of activity, reactivity), (ii) micro-organism (e.g. tolerance/resistance, metabolic status, number of organisms in the population), (ii) environment (e.g. surface type, water activity, presence of other reactive compounds). The biocide should therefore have a wide range of activity, both in terms of type of microorganisms susceptible and conditions of action (19).

Despite their extensive use and long history, the mode of action of a number of biocides has not been clearly established. Biocides affect a number of different target sites in microorganisms, which appears cumulatively to result in a loss of microbial viability. The effect of biocides on multiple target sites in microorganisms is probably the principal reason for the lack of development of bacterial resistance to biocides. Several biocides have been utilized as oral care antiseptics for decades without any adverse microbiological reports (20).

Different biocides are in use in DUWL including sodium hypochlorite, chlorhexidine gluconate, povidine iodine, peroxide (13), peracetic acid (21), ethanol, and glutaraldehyde. Integral automated flush systems in DUWL are commercially available. They employed glutaraldehyde flush systems in dentistry (22). Application of biocides to control DUWL biofilm contamination may be either as periodic shock treatments or by continuous treatment system (23). In these two treatments, different biocides not only act on biofilms differently but also effectively at varying concentrations. Furthermore, biocides behave differently against free planktonic forms and biofilms attached to various surfaces. For example, diluted solutions of sodium hypochlorite (NaOCl) effectively removes planktonic cells, but biofilm shows 150-3,000 times more tolerance against diluted solutions of NaOCl. Sims *et al.* (24) reported the effects of using varying concentrations (0.5%-5.25%) of bleach in dental settings. According to him, although bleach is effective in biofilms from tubing samples but it also causes (i) slow corrosion of metal fitting in dental units (ii) compliance problems in private practise dental settings (iii) reacts with matrix to create chlorinated by products (24).

CHLORINE DIOXIDE

Chlorine dioxide is another biocide which effectively removes biofilms, prevents metal corrosion and fouling of reverse osmosis membranes. In dental settings, 0.1% stabilized chlorine dioxide is also used as mouth rinse. It

reduced the bacterial counts in effluents of four stimulated DUWL to less than 200 CFU/ml. Stabilized chlorine dioxide used in private practice setting as well as its application as a lavage with ultrasonic scalers, result in significant ($p < 0.05$) reduction heterotrophic plate count (HPC) (25). Ethylene-diamine-tetra-acetic acid (EDTA), a divalent cation chelating agent, has been proved to be very effective agent against biofilms. It prevents catheter related infections by medically important microorganisms (26).

Since bacteria from the biofilm are shed continuously while the film is in contact with water. Use of compounds like UV, hydrogen peroxide and ozone are advantageous in this situation. They can be continuously added into the water lines during patient treatment. Thus maintaining low levels of planktonic counts throughout the working day. Hydrogen peroxide has been used in dentistry as a bleaching agent, root canal irrigant, in dentrifices and mouth rinses. It has been used as a disinfectant (7% solution) for flexible endoscopes (27).

OTHER PRODUCTS

The other products, including dialox, sanosil, sporklenz, sterilex ultra would require evaluation in terms of materials compatibility before they could be recommended for routine use in DU waters (DUWS). A number of the other products, including alpron (a three-part component cleaner containing sodium hypochlorite, citric acid, and sodium- toluolsulfonechloramide), sterilex ultra (alkaline peroxide), and oxigenal (hydrogen peroxide), were reported to be effective in DUWS and resulted in a complete kill of planktonic cells as well as removal of biofilms (28).

Other oral antiseptics or chemical agents that have antimicrobial properties which are commonly used include: quaternary ammonium compounds, phenolic compounds, halogens, alcohols and heavy metals. These agents are chosen to be active ingredients of oral health care products because of their antimicrobial properties. They are safe to use in their normal working doses and stable over reasonably long shelf-life. Chlorhexidine and Bio2000 (active agents ethanol and chlorhexidine) achieved a complete kill of the total viable count (TVC) (13) but did not completely remove the biofilm. Likewise, the aldehyde-containing products tegodor and gigasept rapid eliminated the biofilm TVC (i.e., no viable cells were detected) but were unable to completely remove biofilm from the surface. However, the use of aldehyde-containing products may require occupational exposure monitoring for dental staff (28).

CHLORHEXIDINE

Chlorhexidine is among one of the most tested Compounds. At high concentration it is bactericidal and in regular concentration (0.12-0.2%) it is bacteriostatic. It also has good substantivity in the mouth. The chlorhexidine mouthrinse is also commonly used for symptomatic treatment of recurrent aphthous stomatitis/ulcers (29).

The emergence of bacterial resistance following biocidal exposure is not novel and has been described since the introduction of biocides in clinical practice. Bacterial isolates from clinical settings showing increased tolerance due to natural evolution, adaptations or lateral gene transfer and mutations have been documented in several studies (19). In addition there have been many reports highlighting the failure of disinfectants used for clinical applications (19). Biocides resistance in bacteria have been studied *in vitro*. Several mechanisms like efflux systems, intracellular traps, extrachromosomal precipitation at cell wall and degradation of biocides are important with reference to the biocides resistances in free planktonic microorganisms as well as in biofilms (19). The exact mechanisms of resistance in various strains are still being studied but it is clear that biocide resistance is an important clinical phenomenon (13).

Bacterial mechanisms are dependent upon the interaction of the bacterial cell wall, outer membrane or the spore outer layers with the biocides. They may act as permeability barriers to the intracellular uptake of antibiotics and biocides (30). Depending upon the type of biocide alongwith used concentration, it may damage DNA, proteins or enzymes, cell wall, cytoplasmic membrane resulting in death of microbes. Additionally, action of biocides on microorganisms also depend on the environmental conditions and the type of microorganism itself. The bacterial cell wall plays an integral role in relation to inactivation or insusceptibility to biocides (31).

The cell wall of Gram-positive bacteria has been recently studied (31). It consists essentially of highly cross-linked peptidoglycan, which can provide about 90% of the wall structure, together with 'secondary' wall polymers (teichoic acids, polysaccharides and proteins), which are covalently linked to peptidoglycan. The peptidoglycan is made up of amino sugars (N-acetylglucosamine and N-acetylmuramic acid) and various amino acids, some of which are in the unnatural D-form. The peptidoglycan and associated anionic polymers permit the entry of

large molecular weight polymers. The teichoic acids are major cell wall components of most Gram positive bacteria (31). Mostly, they are polymers of ribitol or glycerolphosphates attached to glycosyl and D-alanine ester residues.

The 20th century initially offered the use of antibiotics to fight against bacterial infections, but ended with the gloomy scenario of emerging multi-resistant bacteria. But 21st century emerges as a post-antibiotic era, highlighting the importance of novel strategies to control bacterial diseases. One of the novel strategies in use is to target the quorum sensing (QS) system of bacteria (16).

Quorum sensing (QS) is not only important for intraspecies survival and differentiation in bacterial communities, but also relates interspecies information between symbionts and competitors (16). It regulates gene expression by producing and responding to secreted autoinducers (AIs) whose concentrations reflect the population density, commonly exists in bacteria. Gram-negative bacteria use acylated homoserine lactones as autoinducers AIs, and gram-positive bacteria use oligopeptides (16). Among the gram-negative bacteria, two quorum-signaling mechanisms have been identified i.e., the *LuxI/LuxR* system and the *LuxS* system. In first system, bacteria use an acetylated homoserine lactone signal molecule. When the cell density is high, the binding of AIs to cell receptors regulates gene expression for a variety of phenotypes, such as production of specific virulence factors, protein production, bioluminescence and biofilm formation (16). Generally, each bacterial species uses its own signal; however, a common AI-2 signal has been discovered for interspecies communication (32).

Autoinducer-2 (AI-2) is the only species-nonspecific autoinducer known in bacteria and is produced by both Gram-negative and Gram-positive organisms. Consequently, it is proposed to function as a universal quorum-sensing signal for interaction between bacterial species possessing the characteristic *luxS* gene. The *luxS* gene is highly conserved among many species of gram-negative and gram-positive bacteria. AI-2 is able to regulate a range of genes and cellular processes. The extent to which AI-2-based signaling represents true quorum sensing or is dependent to some degree on the metabolic status of the bacterial cells remains to be determined. AI-2 is involved in mixed-species biofilm formation and interspecies gene regulation (16).

The chemical structure of the actual signal is still under investigation; however, crystallographic studies of the AI-2 receptor in *V. harveyi* seem to

suggest that AI-2 is a furanosyl borate diester formed from the metabolite 4,5-dihydroxy-2, 3-pentadione (33). Unlike to *luxL* and *luxM* genes which are AIs for *V. harveyi* system 1 autoinducer (AI-1). These are important for the synthesis of hydroxybutanoyl-L-homoserine lactone, an important signalling molecules identified by purification of AI-1 QS system. Whereas, the ecological role of *luxS* in bacteria is still poorly understood, but it functions to allow bacteria to optimize gene expression in response to the density of all *luxS*-containing species occupying the same niche. *LuxS* converts S-ribosylhomocysteine to 4,5-dihydroxyl-2, 3-pentanedione, catalysing AI-2 formation (32). Whereas type II QS in the regulation of expression of virulence-related factors, motility, secretion systems, regulatory proteins, and polypeptides involved in the acquisition of hemin (32). Certain environmental conditions have been reported to regulate the AI-2 by bacteria. For example AI-2 activity in dental unit water line biofilm isolates is influenced by the presence of certain preferred carbon sources i.e., glucose (16).

Conventional methods used to control bacterial infection have been resulted in the development of resistant isolates (13). However, one novel method is to fight against bacteria by interfering with their command language and disrupting their virulence at non growth inhibitory concentration, thus without increasing their resistance profile (34). Number of studies have identified several molecules that function as QS inhibitors (16,34, 35). Identification of such inhibitors could present us with new opportunities for the development of novel nonantibiotic drugs for treating bacterial diseases in humans as well as in other animals and plants. As compared to conventional antibiotics, QS inhibitory compounds (QSIs) that do not kill or inhibit microbial growth are less likely to impose a selective pressure for the development of resistant bacteria (16). Furthermore, QSIs are not expected to cause harm to beneficial flora (34). Rasmussen *et al.* (35) identified two QSIs i.e., patulin and penicillic acid during screening a selection of *Penicillium* sp., Using DNA microarray-based transcriptomics, patulin (PAT) and penicillic acid (PA) were found to downregulate 45% and 60%, respectively, of the QS-regulated genes in *P. aeruginosa*, thus indicating specificity for QS-regulated gene expression. These approaches, also known as 'quorum quenching', 'anti-pathogenic', or 'signal interference', have been considered as feasible ways to prevent and combat bacterial infection (16,34).

Conclusion

Microbial biofilms is proceeding on many fronts. One important aspect is the elucidation of the genes specifically expressed by biofilm-associated organisms, evaluation of various control strategies for either preventing or remediating biofilm colonization of medical devices. Role of biofilms in antimicrobial resistance including treatment of medical devices through the use of antimicrobial agents and antimicrobial locks as well as development of new methods for assessing the efficacy of these treatments are in progress. Biofilm in dental unit waterlines, once established, has proven hard to remove by applying disinfectants/biocides. There is a clear requirement for a reliable, relevant laboratory method to prevent microbial contamination within DUWLs, thereby permitting the objective evaluation of antimicrobial and antibiofilm products to control such contamination. The method must be economical and require minimal effort to use on the part of dental staff. There are currently no rational, evidence-based guidelines available to dentists for the control of DUWL contamination. The prevention strategies which are designed to reduce the impact of the biofilm in DUWLs are a real and continuing problem. Education should stress the need for improvement in the quality of water delivered to patients during treatment.

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AN EVALUATION STUDY OF THE SPUTUM SMEAR CONCENTRATION TECHNIQUE FOR THE LABORATORY DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

The microbial diagnosis of Pulmonary Tuberculosis plays a key role in routine treatment and Tuberculosis control Programmes in developing countries. Many patients have presented with signs and symptoms of pulmonary tuberculosis, of which consecutive direct sputum smear microscopy have given negative results for Acid-Fast Bacilli (AFB). Microscopy of smears made directly from sputum has a low sensitivity and there is an urgent need for improved methods. This study was carried out at the Yaoundé University Teaching Hospital and is aimed at evaluating the sputum smear concentration technique in the laboratory diagnosis of pulmonary TB. Sputum samples were collected in screw-cap tight containers and evaluated by both the direct and concentrated methods. Microscopy of direct smears of sputum after liquefaction with 5% sodium hypochlorite (NaOCl) solution; and concentration of the organisms by centrifugation were compared and evaluated. Results showed an increase in sensitivity from 18.27% to 25% with a specificity of 90.95%. The tuberculosis prevalence was 25%. 13.33% belongs to the age range 20-40 years and 11.67% to the age range >40 years. The positive predictive value was 73%. We concluded that the use of sodium hypochlorite (NaOCl) in the concentration of acid-fast bacilli (AFB) in sputum significantly improves the laboratory diagnosis of pulmonary tuberculosis

Keywords: Sputum smear concentration, Laboratory diagnosis, Pulmonary tuberculosis

INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* (Koch's bacillus) which kills many victims than any infectious disease. It continues to be the major cause of disability and death worldwide [1]. The human host serves as the only natural reservoir for *M. tuberculosis*, but the ability of the organism to effectively establish latent infection has enabled it to spread to nearly one-third of the world's population. From this reservoir, eight million new TB cases occur each year and about three million die from it. In 1993, because of the serious public health threat posed by TB, the World Health Organization (WHO) declared it a "Global Emergency" [2].

The emergence of HIV/AIDS has had grave consequences for TB [3]. HIV infection markedly increases the susceptibility for new TB infection to develop into active disease, which can be rapidly progressive. Consequently, a large number of epidemics of TB have been reported from facilities in which HIV infected people are concentrated. In one out break, the entry of an index case into an HIV residence facility was followed by

11 patients developing TB over a five months period [4]. Since these are epidemiologically linked, they must be tackled in tandem [5]. The very large increase in the number of tuberculosis cases yearly and the reactivation of latent infection worldwide is a called for concern for early diagnosis and initiation of effective chemotherapy. Bacteriological diagnosis of tuberculosis is largely dependent on Ziehl Neelsen (ZN) microscopy.

Many patients continue to present with signs and symptoms of pulmonary tuberculosis of which consecutive direct sputum smear technique have proven negative for acid-fast bacilli (AFB) by Ziehl Neelsen staining. As such more sensitive diagnostic techniques are needed which can be readily available and affordable especially in less developed countries. The diagnosis of tuberculosis in some cases is simply based upon the clinical presentation; including the history and the patient's physical signs and symptoms that relate to the site of the disease. In many other cases, diagnosis relies on the bacteriological examination of sputum. However microscopy of smears made

directly from sputum has a low sensitivity and there is an urgent need for improved methods [6], hence this study was carried out to find out whether the concentration sputum smear technique could significantly improve the laboratory diagnosis of pulmonary tuberculosis over the direct sputum smear technique.

Materials and Methods

Study Area

This study was carried out at the University teaching hospital, Yaoundé from March to July, 2009. Yaoundé is the administrative capital of Cameroon with a population of 143,000,000 inhabitants. It is centrally located and made up of immigrants from all the regions of Cameroon. The outpatient clinic of the Yaoundé Central Hospital is in the heart of the city.

Study Subjects and Sampling:

The study population consisted of in and out patients who came to receive tuberculosis treatments or those who were hospitalized for tuberculosis at the university teaching hospital Yaoundé.

Study Type .

The study consisted of one part which is prospective study of patient's samples that were hospitalized or coming for consultation for tuberculosis or to complete their tuberculosis treatment. This was a cross sectional study with an analytical component. Consecutive adult patients with a productive cough of more than three weeks participated in the study. One spot and two early morning sputa were screened for AFBs.

Specimen Analysis The specimens were all analyzed using two techniques Direct sputum smears technique. Concentrated sputum smears technique.

Macroscopy

Analysis was done with sputum samples which appear purulent, mucopurulent, mucoid, or mucosalivery.

Purulent: green looking, mostly pus.

Mucopurulent: green looking with pus and mucus.

Mucoid: mostly mucus.

Mucosalivery: mucus with a small amount of saliva.

Salivary specimens were rejected as this will be of no diagnostic significance of pulmonary tuberculosis.

Laboratory Procedure The first, second and third sputum samples of patients who had consented were pooled and divided into two. The first of this pooled sample was smeared directly and stained by the ZN technique. The second of the pooled samples was bleach digested, centrifuged, supernatant discarded. Sediment fixed and stained by the Ziehl Neelsen technique.

Sodium Hypochloride (NaOCl) Concentration Technique.

Reagents

Sodium hypochlorite (NaOCl), 5% Distilled water
Procedure 2ml of sputum was transferred to a test tube (15-20ml capacity). An equal volume (2ml) of concentrated sodium hypochloride (bleach) solution was added and mixed well. And this was left at room temperature for 10 to 15 min, shaking at intervals to break down the mucus in the sputum. 8ml of distilled water was added and mixed well. It was then centrifuged at 3000g for 15 minutes. A plastic bulb pipette was used to discard the supernatant fluid and the sediment was then transferred to a clean glass slide, spread to make a thin preparation and allowed to air dry. The preparation was heat fixed and stained using the Ziehl Neelsen technique as described elsewhere Acid-Fast Bacilli (AFB) appears red, straight or slightly curved rods.

REPORTING SPUTUM SMEARS FOR AFB.

Number of AFB objective x 100	Note	Answer	Conclusion of bacillus
0/100 fields	Negative	No AFB	
1-9/100 fields	Nb/100 fields	Nb/100 fields	Rare AFB
10-99/100 fields	Nb/100 fields	1+	Fairly numerous AFB
1-10/100 fields	Nb/100 fields	2+	Numerous AFB
>10/100	Nb/100 fields	3+	Numerous AFB

*WHO (2000), if less than 3AFB/100 fields are seen, this must be considered as Rare AFB

CALCULATION OF ANALYTICAL SENSITIVITY

The Analytical sensitivity was calculated using the formula: *Analytical sensitivity (Direct technique)* = Total number of positive result / total number of specimens examined

Analytical sensitivity result(Concentrated technique) = Total number of positive / total number of specimens examined

The ability of a diagnostic test to indicate when a disease is present or absent is dependent on its quality and is described in terms of sensitivity, specificity and the predictive value.

Predictive value of positive test = $\frac{\text{True Positive}}{\text{All Positive tests}} \times 100$

Statistical analysis

Chi Square(χ^2) statistical test was used to test for the significance of results obtained

RESULTS

A total of 104 samples were processed by the sodium hypochloride (NaOCl) concentration technique and the direct sputum smear technique for the Ziehl Neelsen staining method. This constituted of 67 males and 37 females. The ages of the patients ranged from 17 to 73 with the mean age being 39 years. The ages were further divided into three groups of; < 20, 20-40, and > 40. Evaluation was also done based on the nature of sputum sample into purulent, mucopurulent, mucoid and mucosalivary.

Table 2: Distribution by Age group

Age range	Direct technique	Concentrated technique	Percentage
20-40	9	16	55.56%
> 40	10	10	44.44%

Table 3: Distribution by Nature of Specimen

Technique	Purulent	Mucopurulent	Mucoid	Mucosalivary
Direct	8	8	0	3
Concentration	9	8	3	6
Percentage	37.78%	35.56%	6.67%	20%

$\alpha=0.01$ $d.f = 3$ $\chi^2 = 5.25$ $P < 0.05$

The Ziehl Neelsen staining had a specificity of 90.59%. The analytical sensitivities were 18.27% for the direct smear technique and 25% for the concentration sputum smear technique. The prevalence of pulmonary tuberculosis was calculated to be 25%. The positive predictive value was 73%. Based on the ages, there was no positive subject for the age range less than 20 years. 55.56% of the study population was positive for the age range 20 to 40 years while a total of 44.44% was positive for the age range greater than 40 years (Table.2). Based on sex, the males were more infected than the females.

DISCUSSION Proper identification of tuberculosis cases is the pillar of tuberculosis control programs

[7]. Currently, this is accomplished mostly by microscopic examination of stained sputum. Thus, technical accuracy and proficiency are of paramount importance [8]. In this study, the specificity of the Ziehl Neelsen technique was 90.59%. The analytical sensitivity of the direct technique was lower than that of the concentrated technique. The sensitivity was increased from 18.27% to 25%.

This is in accordance with the conclusion of Ghazisaeedi *et al.*[9] who found that the concentration smear technique could improve the sensitivity of the direct sputum smear technique. was also done based on the nature of sputum sample into purulent, mucopurulent, mucoid and mucosalivary. The Ziehl Neelsen staining had a specificity of 90.59%. The analytical sensitivities were 18.27% for the direct smear technique and 25% for the concentration sputum smear technique. The prevalence of pulmonary tuberculosis was calculated to be 25%. The positive predictive value was 73%.Based on the ages, there was no positive subject for the age range less than 20 years. 55.56% of the study population was positive for the age range 20 to 40 years while a total of 44.44% was positive for the age range greater than 40 years (Table.2). Based on sex, the males were more infected than the females.

The prevalence of pulmonary tuberculosis was 25%. The positive predictive value was 73%. Since the positive predictive value is high, then the higher is the probability that a positive result means the patient is infected. Based on the nature of specimen being analysed, 37.78% was purulent, 35.56% was mucopurulent, 6.67% was mucoid and 20% was mucosalivary. There was a statistically significant difference between the nature of the specimen and the diagnostic technique being used. Results indicate that age is a risk factor for the prevalence of pulmonary tuberculosis. Therefore the increased incidence of tuberculosis in this age group can be attributed to low accumulated wealth, consumption of unpasteurized milk, diabetes, being unemployed, living in overcrowded conditions, illicit drug use and a history of incarceration, HIV/AIDS infection which are significantly associated with the development of tuberculosis. Among the methods suggested for smear preparation, the N-acetyl-L-cysteine method has been shown to be a sensitive (28 to 87%) and reliable method for microscopy and

culture [7]. However, due to limitations in funds and equipment, this technique is not being performed in TB laboratories of countries with limited resources [10]. In most parts of these countries direct smear microscopy with low sensitivity (25 to 50%) is the only available method for the diagnosis of tuberculosis[11]. The concentration sputum smear technique is appropriate for developing countries and its application would increase the efficiency of tuberculosis control programs. As a potent disinfectant, NaOCl also has the advantage of lowering the risk of laboratory infection. However, in developing countries where most health units lack culture facilities this method could still be utilized to accelerate initiation of treatment since tuberculosis is curable and the drugs are readily available.

CONCLUSION

Within the limits of this study where there was a small sample size we could conclude that: the use of sodium hypochloride (NaOCl) in the concentration of acid-fast bacilli (AFB) in sputum significantly improves the laboratory diagnosis of pulmonary tuberculosis. We therefore recommended that all sputum samples should undergo the concentration sputum smear technique for the laboratory diagnosis of pulmonary tuberculosis, that more sensitive and rapid tests should be adopted and used and education of laboratory personnel on the need to concentrate sputum specimens for tuberculosis diagnosis.

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**INFLUENCE OF PHENOTYPES ON IMMUNITY TO *PLASMODIUM FALCIPARUM*
MALARIA AMONG WOMEN IN PARTS OF THE IMO RIVER BASIN, NIGERIA.**

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ABSTRACT

This study analyses the association between ABO blood group phenotypes in relation to placental malaria pathology and birth outcomes in the lower Imo river Basin, Nigeria. A cross-sectional study of 647 mother/child pairs delivering in Aboh Mbaise General Hospital, Aboh Mbaise Local Government Area between February-June 2007 and January-July 2008 was undertaken. Maternal peripheral and cord blood samples were obtained at delivery. Placental tissue was obtained and malaria histology classified as active, past or no malaria infection. Birth anthropometry was recorded. ABO blood group was measured by agglutination. Results showed that in primiparae, blood group O was significantly associated with increased risk of active placental infection (OR 2.18, 95% CI 1.15–4.6, $p = 0.02$) and an increased foetal-placental weight ratio compared to non-O phenotypes (5.68 versus 5.45, $p = 0.03$). In multiparae blood group O was significantly associated with less frequent active placental infection (OR 0.59, 95% CI 0.36–0.98, $p = 0.04$), and a higher newborn ponderal index compared to non-O phenotypes (2.65 versus 2.55, $p = 0.007$). In multivariate regression parity was independently associated with increased risk of placental malaria (active and past infection) in primiparae with blood group O ($p = 0.034$) and reduced risk in multiparae with the same phenotype ($p = 0.015$). Parity related susceptibility to placental malaria is associated with the mothers ABO phenotype. This interaction influences foetal and placental growth and could be an important modifying factor for pregnancy outcomes. The biological explanation could relate to sialic acid dependent placental membrane differences which vary with ABO blood group.

KEYWORDS: Malaria, Parity, Immunity, Phenotype, Ponderal index, Placenta.

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SYMPTOMATIC VULVOVAGINAL CANDIDIASIS: KNOWLEDGE, PERCEPTIONS AND TREATMENT MODALITIES AMONG PREGNANT WOMEN OF AN URBAN SETTLEMENT IN WEST AFRICA

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ABSTRACT

Background The use of oral contraceptive pills are increasingly becoming popular among women in both urban and rural Nigerian settings, its perceived association with gynaecologic infections notwithstanding.

Aim: To ascertain the rate of urogenital candidiasis among women on oral contraceptive pills (OCP) in Gboko town.

Methods: All the willing women on OCP attending family planning clinic and Comprehensive health centre in Gboko were consecutively recruited in the month of September, 2009. Questionnaires were used to obtain relevant data such as age, marital status, occupation and urogenital symptoms. Urine, High vaginal swab and Endocervical swab specimens were subsequently collected, transported and processed for isolation of microorganisms using standard laboratory procedures.

Results The rate of urogenital candidiasis among the 153 women on OCP was significantly higher 36.5% compared to the control 20.3% ($P < 0.05$) in as much as their general knowledge about the disease was poor; similarly, genitourinary symptoms were recorded in 22.2% of the women on OCP as compared to the 5.2% in the control group ($P < 0.001$) and was significantly higher among the singles, separated, widowed and separated (64.4-64.7%) compared to the married 27% ($P < 0.05$). 79.4% of the symptomatic infections were caused by *Candida spp*.

Conclusion Women should be properly counselled and health educated on the need for prompt and adequate treatment of vulvovaginal candidiasis while facilities for appropriate treatment and proper laboratory diagnosis provided.

Key Words: Oral contraceptive pills, Urogenital candidiasis, women

INTRODUCTION

Vulvovaginal candidiasis is not a rare gynaecological finding among women from both temperate and tropical parts of the world (1-3). These infections, when asymptomatic may persist for long periods of time with out obvious sequelae. The disease however poses serious health risks to pregnant women and the unborn children. In Poland, treatment of recurrent candidiasis in pregnant women was accompanied by increased episodes of bacterial vaginosis with risks of preterm labour (4); in Minnesota USA, development of candida chorioamnionitis with preterm labour and subsequent development of congenital candidiasis of the new born was reported (5); and in Canada, pregnant women recorded significantly higher rates of candidal urinary tract infections with obvious risks to the mother and the unborn child (6).

Since the advent of human immunodeficiency virus (HIV) infection in 1982 to date, the clinical relevance of vaginal candidiasis has proportionately deepened along with the course of other sexually transmitted disease (STDs) (7,8). The disease was found to contribute significantly to the spread of HIV infections at the onset of this disease's global epidemic and proper treatment of the disease along with other STDs was strongly recommended for effective of control of HIV AIDS in communities (9,10).

At present HIV AIDS is still a major problem in Nigeria as well as other parts of sub-Saharan Africa, a region believed to harbour at least 70% of the global burden of the disease (11,12). Proper and efficient control of the disease still appears to be a major challenge among African communities especially in the treatment and control of STDs. It is in this regard that this

study was set up to assess the level of vulvovaginal candidiasis among pregnant women in an urban community and their attitudes towards its control. The findings would be useful as one of the tools to assess maternal and child health care services in the community towards attainment of the millennium development goals (MDG) (13,14).

MATERIALS AND METHODS

Setting

The study was carried out in September 2009 in Gboko town, arguably the second largest town in Benues state situated about 82 kilometres north-east of Makurdi the state Capital. Based on 2006 population census, the town has an estimated population of 300,000 people; over 95% of the population is made up of Tiv people who are predominantly farmers by occupation and Christians by religion. Two major health centres- General hospital (GH) and Government comprehensive health centre (GCH) centre are located in the town which serves the health needs of most of her inhabitants.

Procedure

Pregnant women attending ante-natal clinic at GH and GCH centre were recruited into the study. Pre-tested questionnaires were administered either self or interviewer to the respondents to obtain relevant information. These include age, marital status, occupation, and educational level, knowledge about candidiasis and presence or absence of genital symptoms.

All the women who volunteered to enrol in the study were consecutively recruited into the study throughout the study period. Pre-enrolment briefing about the study was carried out for each participant then informed written or oral consent obtained from them. A control group, age matched was drawn from apparently healthy women attending the GCH centre for other reasons other than family planning and who were not on oral contraceptive pills.

Sample collection and Processing

High vaginal swab (HVS), Endocervical swab (ECS) and urine specimens were collected from both the test and control subjects using standard laboratory procedures and transported to the GH Gboko laboratory within one hour of collection. Wet preparations were carried out on the specimens and examined microscopically using X40 objective lens (15). The specimens were inoculated on Sabouraud's dextrose agar, Chocolate, Blood agar, Macconkey agar and Cystin lactose electrolyte deficient (CLED) agar and incubated overnight at 36.5°C. *Candida species* were identified based on cultural characteristics, gram stain and cell

morphology, and biochemical properties using germ tube test, chlamydospore formation test and carbohydrate fermentation (15,16). Bacteria were identified using relevant and appropriate biochemical tests such as catalase test, oxidase test, coagulase test, citrate utilization, urea hydrolysis, motility, sugar fermentations and sulphide production (15,16).

Analysis of Results Results obtained were analysed using Epi Info 6 statistical software, P values ≤ 0.05 were considered significant.

RESULTS

One Hundred and fifty three pregnant women were interviewed and urogenital samples subsequently collected and processed during the study period, and a corresponding 153 samples were collected from age matched females who were not pregnant. The rate of urogenital colonization by *Candida species* among pregnant women was 47.7% (73/153) while 20.3% (31/153) was recorded among the control (95% CI, OR=1.28-4.53, RR=1.95-3.09, $P < 0.01$). Over all, the rate of genitourinary symptoms were recorded in 28.8% (44/153) and 5.2% (8/153) of the test and control subjects respectively (95% CI, OR=2.03-17.84, RR= 1.89-11.77, $P < 0.001$). *Candida spp.* were recovered from 84.1% (37/44) of pregnant women with urogenital symptoms; other microbial isolates recovered were *Trichomonas vaginalis* 6.8% (3/44), *Proteus mirabilis* 4.5% (2/44), and both *Enterococcus spp.* and Others 2.3% (1/34) each. Also 87.5% (7/8) of the isolates from the control group with urogenital symptoms were *Candida spp.* while the only other single isolate recovered was *Trichomonas vaginalis* (12.5%) ($P > 0.05$).

TABLE 1: AGE DISTRIBUTION PATTERN AND RATE OF VULVOVAGINAL CANDIDIASIS AMONG PREGNANT WOMEN IN GBOKO, NORTH CENTRAL NIGERIA (N=153).

Age Interval (Years)	Candida Present (%)	Candida Absent (%)	Total (%)
≤ 20	5 (3.3)	12 (7.8)	17 (11.1)
21-25	18 (11.8)	15 (9.8)	33 (21.6)
26-30	9 (5.9)	19 (12.4)	28 (18.3)
31-35	24 (15.6)	15 (9.8)	39 (25.5)
36-40	11 (7.2)	10 (6.5)	21 (13.7)
≥ 41	6 (3.9)	9 (5.9)	15 (9.8)
Total	73 (47.7)	80 (52.3)	153 (100)

χ^2 (Yates corrected)= 0.41, OR= 0.50, RR= 0.51, P (Fisher Exact)= 0.49

Those who did not know Candidiasis as a disease were 94.1% (144/153) and 95.4% (7/153) could not list at least one urogenital symptom unique to candidiasis; 83.6% (134/153) of the respondents felt there was no compelling need for treatment while 86.3% (132/153) did not consider the disease of any serious clinical significance. Also, 94.1% (144/153) could not mention at least one valid health risk associated with candidiasis.

A review of the age distribution pattern of vulvovaginal candidiasis among the respondents showed that those aged 31-35, 21-25 and 36-40 years had 15.6% (24), 11.8% (18) and 7.2% (11) infections respectively with no significant age difference ($P > 0.05$). (Table 1) Analysis of clinical features associated with vulvovaginal candidiasis among the respondents with candidal colonization showed that 39.7% (29) had no symptoms, 15.1% (11), 17.8% (13), 9.6% (7) and 2.7% (2) had itching, discharge, rashes and waist pain respectively as the only complaint while others presented these feature in varying combinations. (Table 2)

TABLE 2: CLINICAL FEATURES ASSOCIATED WITH VULVOVAGINAL CANDIDIASIS AMONG PREGNANT WOMEN IN GBOKO, NORTH CENTRAL NIGERIA.

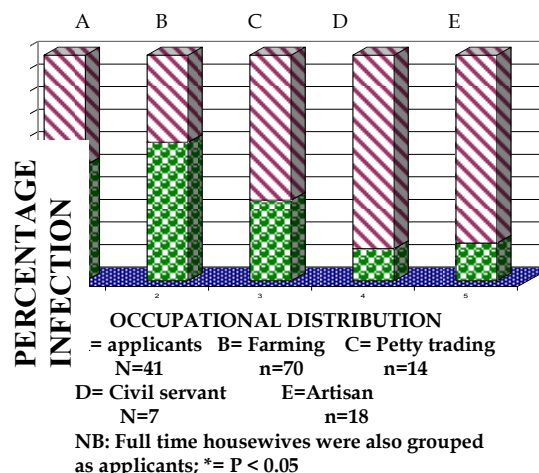
Clinical Features	Number (%)
Nil	29 (39.7)
Itching only	11 (15.1)
Discharge only	13 (17.8)
Discharge + Itching	5 (6.8)
Urinary Frequency	1 (1.4)
Painful Micturition	1 (1.4)
Waist Pain	2 (2.7)
Rashes only	7 (9.6)
Itching + Rashes	4 (5.5)
Total	73 (100)

Based on occupational distribution of the respondents, 51.2% (21), 61.4% (43/70) and 35.7% (5/14) of the applicants, farmers and petty traders were respectively infected while 14.3% (1/7) civil servants and 16.7% (3/18) were respectively infected ($RR=3.0$, $CI=3.3-4.2$). (Figure 1)

Among the *Candida species* recovered, *C. albicans* 64.4% (47), *C. glabrata* 15.1% (11), *C. stellatoidea* and *C. tropicalis* 6.8% each (5) were the four most common species. All the *Candida species* were recovered from genital specimens and none from urine specimens. Urinary tract

infection was detected in 1.3% (2) of the subjects with *Proteus mirabilis* and *Enterococcus species* respectively being responsible. (Table 3).

FIGURE 1 OCCUPATIONAL DISTRIBUTION AND RATE OF VULVOVAGINAL CANDIDIASIS AMONG PREGNANT WOMEN IN GBOKO, NORTH CENTRAL NIGERIA (N=153)



Analysis of the modes of transmission of candidiasis among the respondents showed that 58.2% (89), 36.6% (56) and 23.5% (36) attributed acquisition from toilet, dirty water and insects respectively; 12.4% (19) and 8.5% (13) attributed it to cold and sexual means respectively; 17.6% (27) had no idea while 2.0% (3) attributed the infections to other factors such as dirty hands and seats. (Table 4)

A review of the modes of treatment showed that 76.5% (117), 12.4% (19) and 11.1% (17) respectively usually sought no treatment, visit hospital or clinic, and take traditional herbs. Those who would mention correctly at least one antifungal drug for its treatment were 17 (11.1%). (Figure 2)

TABLE 3 SPECIES OF CANDIDA RECOVERED FROM UROGENITAL SPECIMENS OF PREGNANT WOMEN IN GBOKO, NORTH CENTRAL NIGERIA (N=73).

Candida Species	Number (%)
<i>Candida albicans</i>	47 (64.4)
<i>Candida glabrata</i>	11 (15.1)
<i>Candida tropicalis</i>	5 (6.8)
<i>Candida stellatoidea</i>	5 (6.8)
<i>Candida pseudotropicalis</i>	2 (2.7)
<i>Candida guilliermondi</i>	2 (2.7)
Unspecified	1 (1.4)
Total	73 (100)

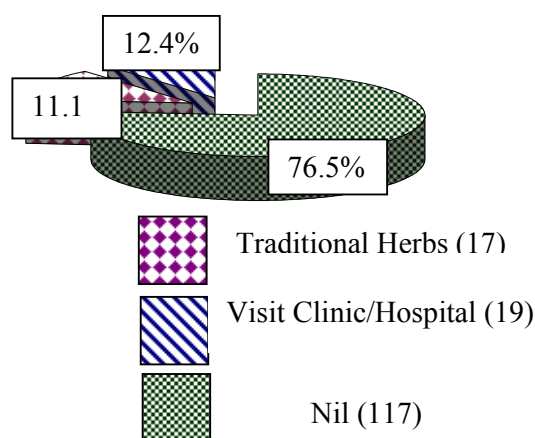
TABLE 4 MODES OF TRANSMISSION OF CANDIDA SPECIES ADVANCED BY PREGNANT WOMEN IN GBOKO, NORTH CENTRAL NIGERIA (N= 153).

Modes of Transmission	Number (%)
Toilet	89 (58.2)
Dirty Water	56 (36.6)
Insects	36 (23.5)
Cold	19 (12.4)
Sexual Means	13 (8.5)
Others	3 (2.0)
No Idea	27 (17.6)

NB: Respondents were allowed to identify more than one option.

Based on educational levels, 40.0% (61), 30.7% (47), 20.5% (31) and 9.0% (14) of the respondents had nil, primary, secondary and tertiary education respectively with no significant difference in the rate of infection ($P > 0.05$). Also, 147 (96.0%), 3 (2.0%) and 3 (2.0%) of the respondents were married, separated or widowed of divorced, and singles respectively with no significant marital difference ($P > 0.05$).

FIGURE 2 MODES OF TREATMENT OF VULVOVAGINAL CANDIDIASIS AMONG PREGNANT WOMEN IN GBOKO, NORTH CENTRAL NIGERIA (N=153).



DISCUSSION

The incidence of asymptomatic vulvovaginal candidiasis among pregnant women in Gboko of 47.7% was significantly higher than that of the control (20.3%) ($P < 0.001$); similarly the rate of symptomatic candidiasis among the pregnant women significantly higher (28.8% vs 5.2%) ($P < 0.001$). The general knowledge about the disease among the women was poor especially as concerning modes of transmission, treatment attitudes, and most especially health implications. The level of care

by the respondents was similarly low as none of them with candidal symptoms came to the clinic primarily for it. Only 12.4% would visit clinic for its treatment however, none of the women found with symptomatic candidiasis visited clinic with the aim to seek medical attention for it. The infections were found to be strongly associated with farmers, applicants and petty traders ($CI=3.3-4.2$).

The findings from the present study partly agrees with findings from similar studies in: Italy where candidiasis was detected in 53.3% of the women studied and was the single most common infection (17); Israel where *Candida species* constituted the highest number of microorganisms recovered and were detected in 35.5% of the women (18); Poland where *Candida* was recovered from 20.7% of the genital specimens of women in labour and was the commonest organism (19); and in Bulgaria where a much higher figure of recurrent vaginal candidiasis of 85.72% was documented among pregnant women (20). The correlation of the disease with certain groups of people such as farmers, petty traders and applicants is most probably a reflection of the general trend of the disease in the community which strongly points towards socioeconomic factors^{11,12,19}.

Candida albicans was the single most common specie recovered from the genital specimens followed by *Candida glabrata*. This pattern of *Candida species* recovered corroborates fairly with findings from Tanzania (21), Sweden²², Italy²³ and Nigeria²⁴ where *Candida albicans* was the single most common specie recovered from the genital specimens processed. The relatively higher rate of *C. glabrata* recovered in Germany²⁵ from a similar study could be attributed to, most probably regional or geographic specie variations, although, the influence of genetic factors may need to be explored.

The species of *Candida* recovered from the present study are slightly different from that of Nikolov, *et al* in Bulgaria where *Candida parapsilosis* was isolated²⁰, and in Spain where *Candida krusei* was recovered (26). Besides confirming the world wide distribution of infectious *Candida species*, the lack of adequate laboratory facilities in the present study could have influenced the scope of speciation of the *Candida* isolates. Paucity of laboratory facilities and competent laboratory personnel was noted as a general problem by the research team in most of the health centres in the community.

RECOMMENDATIONS

In view of the low level of understanding and awareness about the effect of candidiasis

among pregnant women in Gboko, health education should be instituted at the ante-natal clinics as well as other clinics where pregnant women seek similar services. This would raise their level of awareness and correct their perception towards the need for its prompt treatment as well as other STDs. These health talks should be extended by appointed health educators or voluntary health educators to other social and informal gatherings of women in the community.

Due to the high prevalence of HIV/AIDS presently in the country as a whole (11.1%), safe and protective sex should be strongly advocated with proper sex education to the people. This campaign should involve both pregnant and non-pregnant women so as to reduce the risk posed by symptomatic *Candida* infections towards the transmission of other STDs including HIV.

Towards improving maternal and child health in the community and with the present high level of candidiasis among pregnant women, routine screening for symptomatic vulvovaginal candidiasis should be instituted at hospitals and clinics in the locality. This would protect both the mother and child from both the primary and secondary effects of the disease (27-29).

Government in collaboration with her regulatory authorities should review guidelines for setting up hospitals and clinics in the country. This should include, among others, a minimum basic requirement for effective laboratory diagnosis of STDs as well as other infectious diseases since these diseases constitute a major segment of all the hospital or clinic attendees.

CONCLUSION

The rate of symptomatic vulvovaginal candidiasis among pregnant women in Gboko is high with a corresponding low level of awareness and care about the disease. Health education should be instituted at ante-natal clinics so as to raise the knowledge and level of awareness of the people towards seeking prompt and appropriate treatment.

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HIV INFECTION AND MYCOBACTERIUM TUBERCULOSIS DRUG-RESISTANCE AMONG TUBERCULOSIS PATIENTS IN BURKINA FASO, WEST AFRICA

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RUNNING TITLE: CO-INFECTION WITH HIV AND MDR-TB IN BURKINA FASO

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ABSTRACT

The aim of this study was to compare the drug-resistance patterns of *Mycobacterium tuberculosis* strains among pulmonary tuberculosis patients, according to their HIV serostatus, in Burkina Faso. Tuberculosis (TB) patients were classified in new and previously treated cases by using a structured questionnaire. Susceptibility testing to isoniazid, streptomycin, rifampicin and ethambutol was done by the proportion method. Association between HIV-serostatus and drug-resistant TB was assessed with χ^2 tests, and the statistical significance was set to $P < 0.05$. Of 316 (249 new, 67 previously treated) patients included in the study, 68.7% were males and 28.8% were HIV-positive; females (36.4%) were more infected than males (25.3%), ($P = 0.04$). The average age of the patients was 37.24 ± 12.76 years [11-75 years]. Most of the patients infected with HIV were aged from 15 to 44 years and were females, ($P = 0.01$). In the new cases of TB, the difference between HIV-positive and HIV-negative patients was not statistically significant neither for the MDR-TB ($P = 0.40$), nor for the resistant-TB to any drug ($P = 0.26$). However, the difference was significant for the resistance to isoniazid and streptomycin alone ($P = 0.04$). Among the previously treated patients, although there was more MDR-TB and more resistance to any drug in HIV-negative patients than among HIV-positive patients, these differences also were not statistically significant ($P = 0.54$ and $P = 0.63$, respectively). This study found no significant difference between TB/HIV-negative and TB/HIV-positive patients according to the resistance patterns to anti-TB medications, excepted the resistance to isoniazid in new cases and to isoniazid and streptomycin in all patients took globally. These results encourage the collaboration between the programs against TB and HIV to prevent rapid diffusion of drug-resistant TB and high mortality in patients living with HIV/AIDS as recommended by the World Health Organization.

Keywords: Tuberculosis, HIV, Drug resistance, Burkina Faso

INTRODUCTION

Tuberculosis (TB) is one of the most important opportunistic infections in people living with the human immunodeficiency virus and AIDS (PLHIV/AIDS). The increase of TB cases worldwide was attested by the World Health Organization (1); every second, one new person in the world is infected with *Mycobacterium tuberculosis* and nearly 1% of the world population is newly infected annually. Almost 9 million people develop the disease while 3.4 million die of it. Overall nowadays, one third of the world population is infected and 22 countries account for 80% of cases worldwide. Nearly 2 million annual cases of TB occur in sub-Saharan Africa (1, 2). Since 2009, this region is the most affected by the epidemic of HIV infection in the world. Despite the efforts, the epidemic is stable overall and the number of new infections continues to exceed the number of people put on antiretroviral treatment (3). The epidemic of AIDS contributes to worsen the impact of TB but the extent of the increase varies in different studies (4-

6). TB and HIV infection are in fact a complex co-infection; the PLHIV/AIDS are 50 times more likely to develop TB during their lifetime than HIV-negative patients since TB is an early manifestation of infection by HIV (5, 7). TB is the leading cause of death in people infected with HIV. Every 3 minutes, a person with HIV dies of TB worldwide. The negative relationship between TB bacilli resistant to antibiotics and HIV infection has been reported in some parts of the world. Given the poor adherence to TB treatment and the weakness of health systems, the growing epidemic of multidrug-resistant TB (MDR-TB) in HIV-positive patients in the world is a challenge that requires immediate action. PLHIV/AIDS are in fact more likely to be infected by mycobacteria including drug-resistant strains. In addition, HIV infection greatly increases the fatality rate of MDR-TB and ultra drug resistance (XDR) (8, 9). Several countries have reported increasing rates of drug resistance, but in most of low-income countries, the magnitude of the problem remains unknown.

In Burkina Faso, 33,437 new cases of TB including all forms, were diagnosed in 2007, with an incidence of 226 cases per 100 000 habitants. This represents 60% of acid fast bacilli (AFB) smear-positive (10). Recent studies have revealed the prevalence of co-infection with *Mycobacterium tuberculosis* and HIV to 31.6% (11). Few studies have been devoted to assessing the resistance of *M. tuberculosis* in TB patients infected with HIV and it is only recently that research on drug-resistant TB began to take a reasonable size.

The aim of this study was to compare the patterns of resistance to anti-TB drugs according to the HIV serostatus of the patients in Burkina Faso, a country where the prevalence of HIV infection is decreasing.

PATIENTS AND METHODS

Patients and study setting

Between April 2005 and September 2006, 316 TB patients who had accepted to be tested for antibodies to HIV, were identified by smears sputa microscopic and cultures. They were included consecutively in two great public centers, "Centre National de Lutte Antituberculeuse" (CNLAT) in Ouagadougou, "Centre Regional de Lutte Antituberculeuse" (CRLAT) in Bobo-Dioulasso, and in two other centers, Dori Hospital Centre and Gorom-Gorom Medical Centre. The careful examination using a structured questionnaire was used to classify the patients into "new" and "previously treated" tuberculosis cases. The study was carried out under anonymous conditions and both Review Board of the University Hospital "CHU Yalgado Ouedraogo" and the National Ethics Committee approvals were obtained.

General laboratory procedures

TB was confirmed by culture; drug susceptibility testing (DST) of *Mycobacterium tuberculosis* complex (MTC) strains as well as HIV-serostatus testing were performed. The non-consenting patients, those with a history of TB treatment has failed to classify them as "never treated" or "already treated", those infected with non-tuberculous mycobacteria (NTM) and patients with bacilli with unknown bacteriological profile were not included in the study.

Bacteriological investigations

Standard procedures were used according to the practice in the country. AFB sputum microscopy, cultures, identification and first-line drugs susceptibility testing (DST) in complex *M. tuberculosis* strains were performed at the CNLAT laboratory. After microscopic analysis, specimens were sent by other Centres to CNLAT laboratory for cultures and DST. They were homogenized and decontaminated using the method of Petroff. The pellet was then inoculated into four media of Lowenstein-Jensen (LJ), including one with 0.4% sodium pyruvate (LJ+Pyruvate) and another one with thiophene-2-carboxylic acid (LJ+TCH). The

tubes were incubated and examined after 3 days and then weekly during three months. The isolates were identified by acid-fast stain, growth rate, morphology, resistance to TCH, growth on LJ+Pyruvate and reactions to usual biochemistry tests (niacin, nitrate reductase, catalase activity at 22°C and 70°C). Drug susceptibility testing (DST) was performed by a simplified version of the proportion method described elsewhere (12, 13). The following drugs have been tested: isoniazid (0.2µg/ml), streptomycin (4µg/ml), rifampicin (40µg/ml), ethambutol (2µg/ml). The *M. tuberculosis* H37 ATCC 27294 strain was used for the quality control in DST. Proficiency testing for culture and identification were done in collaboration with the National Reference Center for Mycobacteria in Borstel (Parkallee Borstel, Germany): 10 strains of *M. tuberculosis* Complex strains were used for this control.

HIV testing

A blood sample was collected for HIV testing after counselling and consent had been obtained from the patients. All patients were reassured of the confidentiality of their results. Two rapid tests were performed according to the UNAIDS/WHO recommendations (14): the blood sample was first tested using the Determine™HIV-1/2 (Inverness Medical, Courbevoie Cedex, France). Afterwards, the positive samples were tested with ImmunoComb®II BiSpot HIV-1&2 (PBS Orogenics, Courbevoie Cedex, France) to discriminate among HIV-1, HIV-2, and dual-reactive. Any sample that was nonreactive on the first assay was considered HIV negative.

Statistical analysis

The standard chi-square tests (χ^2) were used to assess statistical relationships between HIV-serostatus and drug-resistant TB by using SPSS 15.0 (SPSS Inc., Paris, France). Linear-by-linear association or likelihood ratio was used to interpret the values. The statistical significance was set at $P < 0.05$.

RESULTS

Patient Characteristics

This study did not include patients with negative or uninterpretable cultures, unknown HIV-serostatus in non-tested patients, or infected by nontuberculous mycobacteria. Thus, the population of study comprised 316 patients infected with strains of *M. tuberculosis* complex (MTC) and having DST results. Of the 316 patients examined, 249 were new cases and 67 were previously treated patients. In the latter group of patients, 14 (20.9%) were relapses, 8 (11.9%) returned to treatment after default and 45 (67.2%) were failures. These failures included 31 chronic cases and 14 failures in the 5th and 7/8th months. Among the 316 patients, 217 (68.7%) were male and 99 (31.3%) female. The average age was 37.24±12.76 years [range: 11-75

years]; 25 (14.9) patients were less than 25 years old, 190 (60.1%) were aged from 11 to 44 years and 79 (25%) were more than 44 years old (Table 1).

HIV serostatus of the patients

HIV-serostatus of the patients according to their sex and group age is shown in table 1. Of 316 patients, 91 (28.8%) were HIV-positive, including 78 of the 249 new cases and 13 of the 67 previously treated

patients. Globally, female patients (36.4%) were more infected by HIV than male (25.3%), and this difference according to genders was statistically significant ($P=0.04$). The rate of HIV infection was higher in patients being less than 44 years than in those aged of 45 years old and more.

TABLE 1 HIV SEROSTATUS OF PATIENTS ACCORDING TO THEIR GENDER AND AGE GROUPS

Gende	Age	New cases (N=249)			Previously treated (N=67)			All patients (N=316)		
		HIV+ (%)	Total	P valu	HIV+ (%)	Total	P	HIV+ (%)	Total	P
Male	≤25	4 (16.7)	24		0	6		4 (13.3)	30	
	25-44	31(31)	100	0.33	4 (14.8)	27	0.34	35 (27.6)	127	0.223
	>44	14(28.6)	49		2 (18.2)	11		16 (26.7)	60	
	Total	49(28.3)	173		6 (13.6)	44		55 (25.3)	217	
Female	≤25	3 (21.4)	14		0	3		3 (17.6)	17	
	25-44	24 (47.1)	51	0.06	7 (58.3)	12		31 (49.2)		0.001
	>44	2 (18.2)	11		0	8		2 (10.5)	19	
	Total	29 (38.2)	76		7 (30.4)	23		36 (36.4)	99	

Drug resistance according to HIV-serostatus

The drug-resistance rate in TB patients according to their HIV-serostatus is shown in Table 2. Seventy-eight (31.3%) patients among the new cases were HIV-positive: among them, 0.8% was MDR versus 2% in TB/VIH-negative ($P=0.40$). Ten (4%) TB/HIV-positive and 17 (6.8%) TB/HIV-negative were resistant to any drug ($P=0.26$); 6 (2.4%) TB patients HIV-positive and 4 (1.6%) HIV negative were resistant to isoniazid and streptomycin alone; the rates of resistance to isoniazid was statistically significant ($P=0.046$).

Thirteen (19.4%) of the 67 patients previously treated were HIV-positive. There was more MDR-TB in HIV-negative patients ($n=30$; 44.8%) than in HIV-positive patients ($n=6$; 8.9%), and this difference was not statistically significant ($P=0.54$). Eight (11.9%) TB/HIV-negative were resistant to all or any drug versus 37 (55.2%) in HIV-negative patients ($P=0.63$).

According to genders, 4.6% of male and 8.1% of female, all HIV-positive, were resistant to any drug ($P=0.27$ and $P=0.85$, respectively).

DISCUSSION

According to the UNAIDS' report on the global HIV/AIDS epidemic in 2007, women were more infected than men: 61% of adults living with HIV in sub-Saharan Africa were women, while this rate was 58% in 2004 and 37% in 2001(15). In this study realized in Burkina Faso, females TB patients also were more infected than males, particularly those aged from 15 to 44 years old.

Concerning the DST results in Table 1, the most notable results and most disturbing are the high

resistance to any anti-TB and multidrug resistance (MDR) observed in previously treated cases, although the resistance rates were not significantly different in both groups of HIV-positive and HIV-negative. The resistance in HIV-positive new cases is lower than in HIV-negative patients. The high rates of resistance to INH and streptomycin STM are frequent within the strains of MTC. These strains were subjected for a long time to the pressure of antibiotics, what resulted in a more important resistance to INH and STM (16). In Burkina Faso, the combinations of anti-TB drugs include these antibiotics; the health workers have to remind themselves that 10% of the TB patients are spontaneously resistant to INH. As it is retained in standard treatments, patients and health workers must take greater control of all treatments started to avoid the maximum loss of generators and irregular treatment resistance. Resistance to streptomycin was also high but this drug is replaced in the second phase by ethambutol. In this study, the resistance rate to anti-TB drugs in HIV-negative and HIV-positive patients was not statistically different according to genders and age ranges, suggesting that the epidemiology of resistance at the phenotypic level in West Africa is similar. With or without HIV, the treatment success of patients with MDR tuberculosis is lower than that of drug-susceptible cases, but nevertheless it reached 70% (17).

Although the rates of TB/HIV co-infection and resistance strains to anti-TB were high in our patients (28.8%), MDR-TB was not significantly associated with HIV infection; this corroborates the

results of other African studies (18-20). It is possible that many cases are not detected in patients with HIV because all AFB-positive identified patients were not included in the study; more particularly those who had refused HIV serological testing, which is always done in patients who accept it after their counselling. The TB patients included in the study were not under antiretroviral treatment when they had been diagnosed as HIV-positive. Contrary to our results, those obtained in industrialized

countries had found a significant association between HIV infection and MDR-TB (21-24). The association between HIV infection and drug-resistant tuberculosis is complex and multifaceted (25, 26). HIV co-infection in TB patients is not believed to increase the rate at which spontaneous resistance-conferring mutations occur. However, it might increase the number of mutants that arise overall by enlarging the pool of individuals with active tuberculosis disease (27).

TABLE 2: COMPARISON OF TB PATIENTS ACCORDING TO HIV SEROSTATUS AND THE RESISTANCE TO FIRST-LINE ANTI-TB THERAPY

Resistance	Resistance in New cases			Resistance in Previously treated cases			Resistance in All patients		
	HIV+ (n=78)	All cases (N=249)	P value	HIV+ (n=13)	All cases (N=67)	P value	HIV+ (n=91)	All cases (N=316)	P value
To all drugs	10 (4)	29 (11.6)	-	8 (11.9)	45(67)	-	18 (5.7)	74 (23.4)	-
To each drug									
H	8 (3.2)	20 (8)	0.39	8 (11.9)	37(55.2)	0.63	16 (5.1)	65 (20.6)	0.39
R	1 (0.4)	7 (2.8)	0.29	6 (8.9)	30(44.8)	0.54	7 (2.2)	43 (13.6)	0.04
E	1 (0.4)	7 (2.8)	0.29	6 (8.9)	30(44.8)	0.54	7 (2.2)	43 (13.6)	0.04
S	4 (1.6)	13 (5.2)	0.98	7 (10.4)	24(35.8)	0.54	11 (3.5)	44 (14)	0.56
Monoresistance									
H only	6 (2.4)	12 (4.8)	0.09	0	2(2.9)	-	6 (1.9)	12 (3.8)	0.99
R only	0	0	-	0	0	-	0	0	-
E only	0	1 (0.4)	-	0	0	-	0	1 (0.3)	-
S only	2 (0.8)	6 (2.4)	0.90	0	0	-	2 (0.6)	6 (1.9)	0.797
Total	8 (3.2)	19 (7.6)	0.14	0	2(2.9)	-	8 (2.5)	19 (6)	0.18
MDR									
RH	0	2 (0.8)	-	0	5 (7.5)	-	0	7 (2.2)	-
RHE	0	0	-	1 (1.5)	4 (5.9)	0.53	1 (0.3)	4 (1.3)	0.80
RHS	0	0	-	0	0	-	0	0	-
RHES	1 (0.4)	4 (1.6)	0.94	5 (7.5)	27(40.3)	0.50	6 (1.6)	31 (9.8)	0.13
Total	1 (0.4)	6 (2.4)	0.4	6 (8.9)	36(53.7)	0.54	7 (2.2)	42 (13.3)	0.05
Other patterns									
HE	0	2 (0.8)	-	0	3 (4.5)	-	0	5 (1.6)	-
HS	1 (0.4)	2 (0.8)	0.58	2 (2.9)	2 (2.9)	-	3 (0.9)	4 (1.3)	0.040
HSE	0	0	-	0	2 (2.9)	-	0	2 (0.6)	-
RE	0	0	-	0	0	-	0	0	-
RS	0	0	-	0	0	-	0	0	-
RES	0	0	-	0	0	-	0	0	-
ES	0	0	-	0	0	-	0	0	-
Total	1 (0.4)	4 (1.6)	-	2 (2.9)	7 (10.4)	-	3 (0.9)	11(3.9)	-
Resistance to									
1 drug	8 (3.2)	19 (7.6)		0	2 (2.9)		8 (2.5)	21 (6.6)	
2 drugs	1 (0.4)	6 (2.4)		2 (2.9)	10(14.9)		3 (0.9)	16 (5.1)	
3 drugs	0	0		1 (1.5)	6 (8.9)		1 (0.3)	6 (1.9)	
4 drugs	1 (0.4)	4 (1.6)		5 (7.5)	27(40.3)		6 (1.6)	31 (9.8)	
Total	10 (4)	29 (11.6)	0.26	8 (11.9)	45(67)	0.63	18 (5.7)	74 (23.4)	0.60

H: isoniazid; R: rifampicin; E: ethambutol; S: streptomycin; MDR: multidrug-resistance;

If immunosuppression is not very advanced, then the response to anti-TB treatment is usually similar in HIV-negative and HIV-positive patients, whereas HIV-positive patients have greater risk of suffering from drug toxicity and to die during the treatment (20, 28). This is well illustrated by the study conducted in KwaZulu-Natal in South Africa (29). The possible association between HIV and drug-resistant MTC strains can be mainly explained by the fact that antiretroviral treatment could interfere with anti-TB drugs and make them less effective, or lead to resistance to these drugs (30). Patients with HIV-associated TB could have diminished adherence to treatment due to increased pill burden,

overlapping toxic effects (31, 32). There is also the problem of malabsorption; therefore, they are prone to having subtherapeutic concentrations of anti-TB drugs (33).

Often there is a dilemma when the treatment of TB/HIV-positive cases was not well codified: treat TB first or treat TB and HIV together, knowing that some antiretroviral drugs interfere with TB drugs (34). The protease inhibitors and non-nucleoside reverse transcriptase inhibitors are known to interact with rifampicin, a drug included in many major associations for the treatment of TB.

In this work in Burkina Faso, the HIV testing was done during the study without knowledge on the patients' serostatus before. Moreover, it was possible that HIV-positive patients and those having drug-resistant TB shared similar risk factors, such as the environmental conditions. Indeed, many outbreaks of drug-resistant TB were triggered in areas where relatively large numbers of HIV infected people were in close contact with each other, such as hospitals or prisons. However, information regarding the transmission of tuberculosis in these settings can be used to predict the spread of drug resistant tuberculosis in the general population. HIV infection and TB form a lethal combination when the degree of immunosuppression is advanced (5, 35). Studies of outbreaks in the early 1990s could show that patients with the acquired immunodeficiency syndrome (AIDS) and multidrug-resistant tuberculosis had a median survival of 4 to 16 weeks (36, 37).

This study had not found a significant difference between TB/HIV-negative and TB/HIV-positive patients according to the resistance patterns to anti-TB medications, excepted the resistance to INH in new cases and to INH and STM in all patients took globally. Indeed HIV infection increases TB epidemiology, but it cannot be responsible for the MDR-TB resurgence. Other serious factors may be implicated.

To reduce the number of people with this dual infection, it is important to diagnosis contagious pulmonary TB patients early and to prevent transmission in the places where infected people are in close contact with each other, such as hospitals and prisons. Collaboration between the better and stronger programs against TB and HIV is needed to prevent rapid transmission of drug-resistant tuberculosis and high mortality in communities heavily affected by HIV. To this end, the WHO has recommended expanding the scope of collaborative TB/HIV (10).

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IMPORTANCE OF THE CONFIRMATORY ASSAY FOR THE DETECTION OF THE HBsAg IN THE EPIDEMIOLOGICAL STUDIES AND IN THE DIAGNOSIS OF THE VIRAL HEPATITIS B

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RUNNING TITLE: CONFIRMATORY ASSAY FOR HBSAG DETECTION

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ABSTRACT

Several epidemiological studies have reported high prevalence of HBsAg among pregnant women in Burkina Faso. They used various algorithms, as it is also done for the routine diagnostic. Knowing this antigen carriage rate in such a population or in other clinic attendees is important for the implementation of a national immunisation programme and the monitoring of patients with hepatitis B. Often, the screening tests were not confirmed in spite of the existence of known false positive and false negative results. The aim of this study was to determine a more accurate prevalence of HBsAg, among the pregnant women in Burkina Faso. From October 2006 to January 2007, blood samples were collected from 1139 pregnant women. Each sample was analyzed for HBsAg, using two assays and according to manufacturers' instructions vis, Hepanostika®HBsAg Uniform II B9 (Bio-Mérieux; France) and HBsAg (V2) Abbott AxSYM® system (Abbott Diagnostics). All the positive samples were tested with a confirmatory neutralization assay- Hepanostika®HBsAg Uniform II B9 Confirmatory (Bio-Mérieux). The mean age of the pregnant women was 24.85years [range: 15-45years] and the age range of 20-24 (37%) and 25-29 (25.4%) years were the most represented. The overall rate of HBsAg-positive pregnant women with the two screening assays was 20.9%. The HBsAg detection rate was significantly higher with Hepanostika® UniformII B9 (16.9%) than with HBsAg (V2) AxSYM system assay (12.1%), with $P < 0.0001$. The general seroprevalence of HBsAg was 9% after the confirmatory neutralization testing, with 56.7% of false positive results: this difference was statistically significant ($P < 0.0001$). The rate of HBsAg positive pregnant women was higher in the age range of 25-29years than in the others; however, this difference was not statistically significant. In an epidemiological approach, the results found in this study confirmed the Burkina Faso belonging to the high endemic carriage area for HBsAg. The results showed that in an individual approach, the confirmatory assay is necessary and there is a need to implement more accurate algorithm for the routine diagnostic in patients.

Key words: HBsAg, confirmatory assay, prevalence, pregnant women, Burkina Faso.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem. The World Health Organization (WHO) estimates that more than 2 billion people worldwide have been infected with HBV. Of these, approximately 360 million individuals are chronically infected, and each year acute and chronic infections cause 500,000 to 1.2 million deaths (1). Among these 360 million people in the world chronically infected with HBV, 65 million reside in Africa and of the 1.2 million deaths due to HBV related diseases recorded annually throughout the world, approximately 250,000 occur in Africa (2- 4).

The endemicity of hepatitis B is defined according to the prevalence of the hepatitis B surface antigen (HBsAg) in the general population of geographical areas, and it varies considerably globally: HBsAg prevalences of >8% are typical of highly endemic areas, prevalences of 2-8% are found in areas of intermediate endemicity, whereas in areas with low endemicity less than 2% of the population is HBsAg-positive (1). In Africa, the rate of HBsAg carriage in the general population ranges up to 20% (2), and three levels of endemicity exist also, as measured by

the prevalence of HBsAg: hepatitis B virus is hyperendemic in sub-Saharan Africa (>8%), with the exception of a few countries which constitute areas of intermediate endemicity (2-8%), and regions of low endemicity (<2%) in the northern African countries. However, in the late area, pockets of high endemicity can occur within these countries (4).

The HBsAg is also used for the screening of hepatitis B in pregnant women. Currently, in the countries having national programmes against hepatitis B, notably in pregnant women, HBsAg is the main serologic marker recommended by the guidelines for the detection of maternal HBV infection (5-8). HBsAg can be assessed by various assays in clinical specimens; those which are recommended must have the highest relative sensitivities and specificities. They include mostly enzyme immunoassay (EIA), particularly the late generation of enzyme-linked immunosorbent assays (9, 10). Commercially available tests are based on the detection of wild-type and mutant HBsAg. The specificity of HBsAg enzyme immunoassays is over 99%: false positive results occur with inappropriate samples (heparinised

samples, with haemoglobin or bilirubin). Higher rates of false positive results are observed during pregnancy than in the general population. False negative or atypical results are also observed under several circumstances, including S (surface) gene mutants and variants in HBV, and HCV co-infection which may interfere with HBV replication and/or HBsAg expression (10). To avoid such situations, two different assays have been used in certain studies for HBsAg screening in pregnant women, the second test to “affirm” the results found with the first (7, 11). However, confirmation assays were not used more often. These immunoassays failure to detect HBsAg could impact as well the epidemiological studies as the diagnosis of HBV infection, and therefore compromise national programs against viral hepatitis B if an appropriate screening algorithm is not used. The aim of this study was to determine the current prevalence of HBV infection and to re-examine the epidemiology of HBV infection among pregnant women in Burkina Faso.

MATERIALS AND METHODS

Study population and settings

The population of study was constituted of pregnant women from whom the annual seroprevalence of HIV is determined in Burkina Faso, according to the UNAIDS/WHO guidelines. The Review Board of the University teaching Hospital “CHU Yalgado Ouedraogo” and the National Ethics Committee approved the study. It was carried out under anonymous conditions. The Demographic data were recorded using a structured questionnaire, and the blood samples were collected with the voluntary consent of each pregnant woman.

Sample collection and laboratory procedures

Blood sample were collected from 1139 pregnant women in three medical centres, CMA Saint Camille, CMA Kossodo and CMA Schiphra in Ouagadougou, from October 2006 to January 2007. After centrifugation, sera were aliquoted and stored at -20°C until assays were performed. Each sample was tested for HBsAg by two enzyme linked immunoassays: Hepanostika®HBsAg Uniform II B9 (Bio-Mérieux; France) and Abbott HBsAg (V2) AxSYM® system (Abbott Diagnostics; Germany), according to their manufacturer instructions. Then, all the positive samples were tested with a confirmatory neutralization assay, Hepanostika®HBsAg Uniform II B9 Confirmatory (Bio-Mérieux).

Statistical analysis

The Epi Info 2004 version 3.3 software was used to record all the sociodemographic data and the results of the serological assays. Comparisons between variables were done using the Chi-2 test. Statistically significant difference between variables was set at $P < 0.05$.

RESULTS

Characteristics of pregnant women

A total of 1139 pregnant women aged from 15 to 45 years, on prenatal visits, were enrolled from three centres in Ouagadougou. Their mean age was 24.8 ± 5.7 years [15-45 years of age]. Among them, 54.1% were less than 25 years old, 79.5% were less than 30 years old, 13% were and only 1.7% were more than 40 years old (Table 1).

HBsAg prevalence without neutralization confirmatory assay

Among the 1139 non repetitive samples which were tested, 238 (20.9%) were HBsAg+ with one or with both screening tests simultaneously (Figure 1). Of these 1139 samples, the Hepanostika® HBsAg Uniform II B9 assay detected 193 (16.9%) HBsAg-positive versus 138 (12.1%) with Abbott HBsAg (V2) AxSYM system assay and this difference was statistically significant ($P = 0.0001$; $OR = 18.621$; 95% CI: 12.33-28.10).

The distribution of the pregnant women HBsAg-positive according to the age ranges is reported in table 1. The results showed that all the age ranges were affected; the rates were higher with both assays in the age ranges of 25-29 years than in the other age groups. However, the differences between the age ranges were not statistically significant neither with Hepanostika® HBsAg Uniform II B9 assay ($P = 0.31$), nor with Abbott HBsAg (V2) AxSYM system assay ($P = 0.19$).

HBsAg+ rate in pregnant women after neutralization confirmatory assay

Hundred and three (9%) samples were HBsAg-positive after the confirmatory assay. In comparison with the rate found with the two screening assays (20.9%), this difference represented 56.7% of false negative results, globally. Discordant results were found as well between the confirmation assay and Hepanostika®HBsAg Uniform II B9 with 90 samples (54 false positives and 19 false negatives), as with Abbott HBsAg (V2) AxSYM system (90 false positives). After confirmation, the rate of HBsAg-positive samples was lower than those found with Hepanostika®HBsAg Uniform II B9 or HBsAg (V2) AxSYM system ($P < 0.0001$; $OR: 80.398$; 95% CI: 45.54-141.92). The analysis of the results after confirmation, according to the age, showed also that the rates of HBsAg-positive pregnant women were also higher in the age range of 25-29 years (11.1%) than in the others (Table 1).

DISCUSSION

Hepatitis B surface antigen (HBsAg) in the serum is the most commonly used marker to indicate ongoing infection with HBV which may be completely asymptomatic. In this study, it was detected in pregnant women with two assays at the same time, but without physical exams to detect clinical hepatitis. The rate of 20.9% obtained with one or with both screening tests simultaneously, but without confirmation assay, showed that Burkina Faso belongs to the hyperendemic area for HBsAg. Comparable high prevalences were reported by

previous studies in Burkina Faso, as well in Ouagadougou (11-14), as in other urban or rural areas (12, 15). Two different tests were used in some of these studies to screen HBsAg (11, 13, 14), while only one test was used in the others (12, 15). In the studies

in which used a single screening test, the reported prevalences seemed higher (8.3%-24.1%) than those reported in the works which used two different tests to screen the HBsAg (9.3%-11.5%). Only one of these studies used a confirmatory assay, with a final

TABLE 1: RATE OF HBS-POSITIVE PREGNANT WOMEN BEFORE AND AFTER CONFIRMATION ASSAY ACCORDING TO THEIR AGE

Age (years)	Total tested		Hepanostika® HBsAg Uniform II B9 (Bio-Mérieux)		HBs Ag (V2) AxSYM system (Abbott Diagnostics)		Hepanostika®HBsAg UniformII Confirmatory (Bio-Merieux)	
	Number	%	HBsAg+ (%)	HBsAg- (%)	HBsAg+ (%)	HBsAg- (%)	HBsAg+ (%)	HBsAg- (%)
15-19	195	(17.1)	33(16.9)	162 (83.1)	23 (11.8)	172 (88.2)	17 (8.7)	178 (91.3)
20-24	421	(37)	68 (16.2)	353 (83.8)	44 (10.5)	377 (89.5)	34 (8.1)	387 (91.9)
25-29	289	(25.4)	60 (20.8)	229 (79.2)	48 (16.6)	241 (83.4)	32 (11.1)	257 (88.9)
30-34	148	(13)	18 (12.2)	130 (87.8)	15 (10.1)	133 (89.9)	11 (7.4)	137 (92.6)
35-39	66	(5.8)	10 (15.2)	56 (84.8)	6 (9.1)	60 (90.9)	7 (10.6)	59 (89.4)
≥40	20	(1.8)	4 (20)	16 (80)	2 (10)	18 (90)	2 (10)	18 (90)
Total	1139	(100)	193 (16.9)	946 (83.1)	138 (12.1)	1001 (87.9)	103 (9)	1036 (91)

prevalence of 11.5% (14). All these studies could suffer from bias in the definition of study populations. Besides, the used commercial kits were not identical in all these studies, and the algorithms were different.

Despite the high performance of the HBsAg screening assays, “false” results are still reported (10). After the confirmation assay in this study in Burkina Faso, the rate of HBsAg-positive pregnant women decreased from 20.9% to 9%. Nineteen false positive samples were found with Abbott HBsAg (V2) AxSYM system assay. The causes of false-negative might be various, including the HBsAg level below the detection limit in chronic HBV carriers, either or not combined with mutations, the presence of variants that are not recognized by the antibodies used in the screening assay, or immune complexes masking HBsAg epitopes (10, 16). No false negative sample was found with Hepanostika®HBsAg Uniform II B9 assay. However, there were 90 false positive samples with Hepanostika®HBsAg Uniform II B9 assay and 54 with HBs Ag (V2) AxSYM system. Nevertheless, according to an epidemiological approach, the various results obtained had no impact on the belonging of Burkina Faso to a hyperendemic area of hepatitis B virus infections.

In acute viral hepatitis B, HBsAg is present during less than 6 months in the blood of infected persons. In chronic forms of the infection, HBsAg remains detectable in the serum for period of time longer than 6 months, and sometimes indefinitely. However, HBsAg can become undetectable when natural or

acquired mutations occur in the genes which code for HBsAg (10, 17, 18): these cases include the viral hepatitis B occult which is defined by the presence of HBV DNA in the serum or liver of people without HBsAg (19). Former studies had reported that selection of HBsAg mutants were a rare event and their prevalence in the population of HBV-infected patients remained relatively low, even in highly endemic areas, despite extensive immunization which had not favored the emergence of HBsAg variant viruses (20). However Chemin and Trepo (21) showed that the prevalence of cryptogenic hepatitis varies widely among the published studies and that evidence is accumulating that occult HBV infections are widespread in many geographic areas. How such forms can impact the epidemiology of HBV in Burkina Faso is unknown to our knowledge, because no data is available on occult hepatitis in the country. In a clinical approach of the disease in individuals, the accuracy and the reliability of assays are fundamental since the results are used either to monitor the disease in patients, or/and to protect non-infected people. In such cases, false results must be excluded as much as possible. Using only one and even two assays to screen HBsAg, without a confirmation assay could appear insufficient. Confirmatory assays are necessary to enhance the overall quality of HBsAg screening assay (22). Taking in account the cases of occult hepatitis, HBsAg detection alone could be insufficient also, as highly accurate the assay(s) used can be. An HBV screening algorithm that includes anti-HBc testing in combination with HBsAg testing is

able to reveal occult hepatitis, especially in situations where HBV DNA detection by nucleic acid amplification technology is not implemented (6, 10, 17).

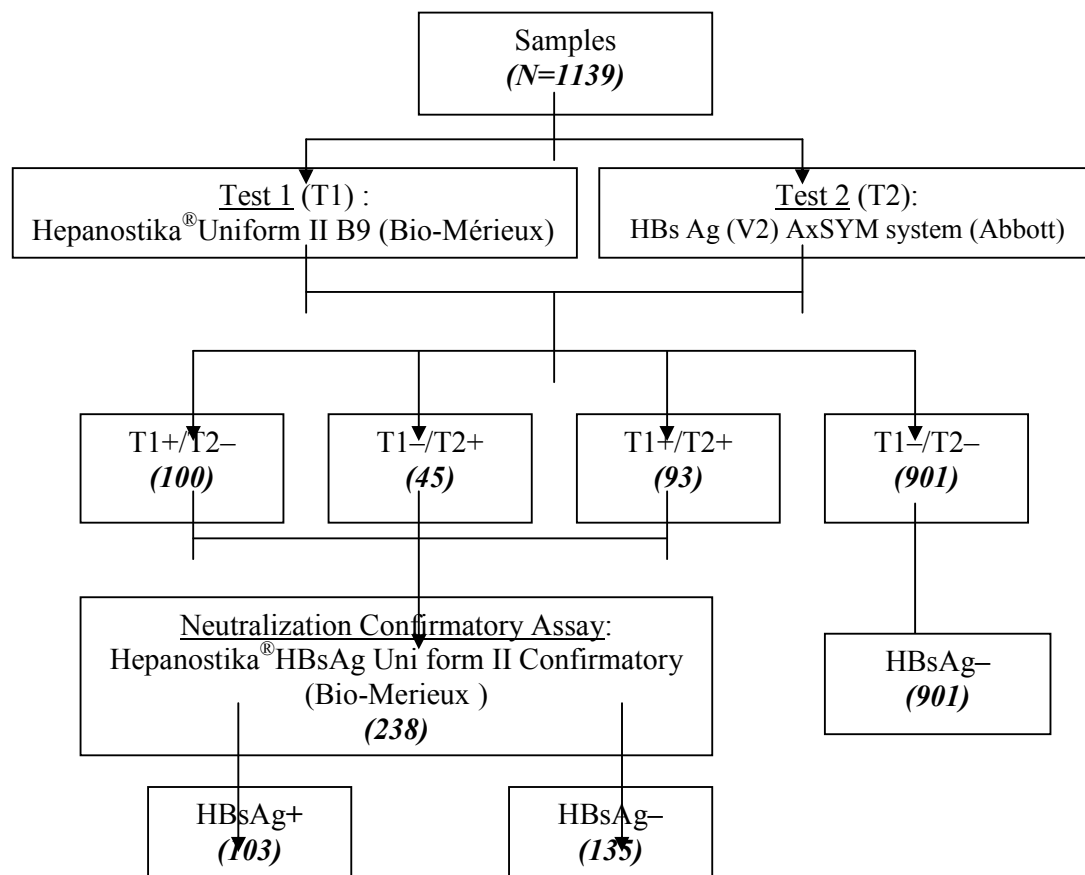
The results found in this study in Burkina Faso showed that the difference between the HBsAg screening assays and the confirmation assay was statistically significant. This difference could not impact data used to classify countries in epidemic areas of HBsAg carriage. However, in clinical and the immunization assessment contexts, it could be important to confirm the results of HBsAg screening testing by a neutralization assay. To take the cryptic hepatitis as occult hepatitis B cases in account, it appears essential to detect other markers like anti-HBc, mainly in settings where HBV nucleic acid amplification assays are not available. The

immunization of newborns against hepatitis B was implemented in Burkina Faso on January 2006. In the future, the detection of HBsAg alone should be insufficient to assess the HBV prevalence in the country. But already, an accurate screening or diagnostic of the viral hepatitis B according to the actual algorithms require a confirmation assay.

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FIGURE 1: ALGORITHM OF THE HBSAG DETECTION IN PREGNANT WOMEN (THE RESULTS ARE IN BRACKETS)



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EDITORIAL

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THE INCREASING DANGERS OF INFECTIOUS DISEASES

Infectious Diseases are becoming more and more important as causes of morbidity and mortality worldwide. This is exemplified by the recent outbreak of cholera in the northern parts of the Cameroons and Nigeria that claimed over 400 lives. Bacterial sepsis also occurs both as epidemics and endemically. Bloodstream infections are common in Africa and are associated with high mortality. Non-malaria bloodstream infections in Africa are due to *Salmonella enterica* (58.4% of these are non-typhoidal *Salmonella*), the most prevalent isolate overall and in adults; and (18.3% overall) are due to *Streptococcus pneumoniae*, the most common isolate in children. Other common isolates include *Staphylococcus aureus* and *Escherichia coli* (1)

The work by Kanga and associates in this edition showed that septicaemia had an overall incidence of 5.79 per 1000 admissions in a Cameroonian Hospital; with Gram positive cocci, being the commonest aetiological agents. The importance of Gram-positive organisms as pathogens is increasing, and toxic shock syndrome (TSS) is likely to be under diagnosed as a complication of septicaemia in patients with staphylococcal or group A streptococcal infection who present with shock (2).

The articles by Omogbai and associates, and Liaqat and associates show that more needs to be done by promoting aseptic procedures, so as to prevent nosocomial infections, such as endocarditis, meningitis, septicaemia, osteomyelitis, nephritis, etc, in dental practice. Such complications could be life-threatening, particularly in these days of multidrug resistant bacterial infections.

A common cause of bloodstream infection is food poisoning by salmonella as was widely reported in the United States of America last year. It resulted from faecal contaminated animal feeds of poultry layers; and was transmitted to man via their eggs. The Centers for Disease Control and Prevention estimate that Salmonella infections alone sicken 40,000 people each year in the United States, though the actual number of infections is likely much higher because many cases are mild and not diagnosed or reported. Currently, Salmonella is the focus of an ongoing U.S. public health investigation into contaminated chicken eggs.

There is a need to improve personal hygiene, environmental sanitation, adequate disposal of household and human waste, and general hygiene and antisepsis in health centers. Food handlers need to be investigated annually to see whether or not they are carriers of pathogenic microbes

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