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NON-ATTENUATION OF HIGHLY PATHOGENIC AVIAN INFLUENZA H_5N_1 BY LABORATORY EXPOSURE TO ULTRAVIOLET RAYS

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Abstract

Avian influenza H5N1 represents one of the most researched viruses in laboratories world-wide in recent times with regards to its epidemiology, ecology, biology and geography. The virus has caused 409 human cases and 256 human fatalities to date. Some laboratory activities and other lab related works predispose certain workers to exposure to this virus. In this work, we assessed the effect of exposure of HPAI infective allantoic fluid to ultraviolet rays for between 15 and 180 minutes. No significant difference was found between the unexposed and exposed viruses. The ability of the virus to haemagglutinate chicken red blood cells, the haemagglutination titre and its pathogenicity in embryonating eggs did not change despite this prolong exposure to UV-light. We call for caution in the handling of HPAI viruses in laboratory inside the microbiological safety cabinet despite sterilization using UV-light.

INTRODUCTION

Avian influenza is a very important zoonotic and trans-boundary animal disease. In recent times, the highly pathogenic avian influenza H5N1 (HPAI H5N1) has been in the centre of attention and activities of most viral research laboratories world widely. This is not unconnected with the recent spread (1996 to date) of the virus in many countries and the trans-boundary cum zoonotic potentials held by the avian influenza

H5N1 virus. The virus has spread in over fifty countries and linked to the death of more than 500 million birds of different species. To date, four hundred and nine (409) number of human infections and two hundred and fifty-six (256) human fatalities has been recorded (1).

While the majority of the countries that has been affected from western countries have got the capacity to easily manage and control the infection using standardized laboratory procedures, achievable policies and management practices, affected countries in developing economies and those country in transition still battle with the ability to cope with effective diagnoses, management and control (2, 3). These latter groups of countries sometimes grapple with inadequate laboratory facilities/expertise and often improvise for equipment and materials.

Ultra-violet (UV) irradiation is a proven germicidal widely used in many research laboratories. A survey of literatures showed that although UV irradiation has been assessed in its ability to inactivate viral, protozoan and bacteria organisms including *Escherichia coli* (4), Sendai virus (5), Polio virus and Adenovirus (6), *Mycobacterium avium* subspp. *paratuberculosis* (7), and protozoans (8), none has been documented with regards to avian influenza viruses.

The manufacturers of most microbiological safety cabinet instruct that the regular assessment of the UV efficiency on work surface be carried out using UV light meter, and several scientists are aware of the need to replace the UV tube should the intensity falls below the adequate requirement (for example 40 microwatts per square centimeter at a wavelength of 253.5 x 10^{-9}m) (The Baker Company, Sanford ME, www.bakerco.com), however, a number of researchers in low income food deficient countries (LIFDC) tend to assume that a relatively new cabinet should have an efficient UV system.

A recent assessment of a set of final year Medical Laboratory Science (virology option) students and other spectrum of research scientists revealed that over 80% believed that the UV rays are able to attenuate/kill any virus after some long period of exposure. Such believe is carried into the work environment and this sometimes unwittingly predispose many researchers to potential hazards associated with undue/unprotected exposure to HPAI H5N1 virus through working in the cabinet, since the aerosolized virus may remain for some time in the cabinet. Technicians, service personnel and laboratory cleaning staff are at similar risk in the course of performing their routine duties.

This work therefore aims at investigating the potential dangers held by the residual avian influenza H5N1 virus following its manipulation in the biological safety cabinets despite extended periods of exposure to ultra-violet radiation.

Materials and Methods

Three candidate H5N1 HPAI viruses were selected from the isolate bank (-70^{o}C) and allow to thaw on wet ice inside a microbiological safety cabinet (SterilGARD® III Advance, The Baker Company, Sanford ME,

www.bakerco.com). The isolates have been characterized using standardized methods as previously reported (9). Briefly described, all un-contaminated allantoic fluids (ALF) arising from inoculation of 20% tissue samples were spot tested by haemagglutination test; the chorio-allantoic membrane (CAM) of positive harvest were further prepared and tested by agar-gel immuno-diffusion (AGID) to detect influenza A group antigen. α-haemagglutination test was conducted to determine the virus subtype. For further confirmation, a cascade-type molecular analysis was performed starting with the M-gene. Every positive result for M-gene was subjected to an RT-PCR for haemagglutinin gene of subtypes H5 and H7. Every positive HA result was confirmed for N1 by RT-PCR. The primers used are listed in Table 1.

The haemagglutination titres of all the viruses were determined afresh using standardized protocol (9). 200µl of each virus sample was inoculated into five 9-day-old embryonating chicken eggs (ECE) and incubated at 37^{o}C. The chicken embryos were monitored for mortality through candling. All dead eggs were chilled at 4^{o}C, aseptically opened and the ALFs tested for haemagglutinating activities and plated on blood agar to exclude bacteria contamination. Fresh ALF was harvested from each of the sample for experimental purposes.

Exposure to UV-light and inoculation

Five pieces each of a new set of 9-day-old ECE were grouped based on assigned timing (0minute, 15 minutes, 30 minutes, 60 minutes, 120 minutes and 180 minutes). All eggs were properly marked and disinfected using 70% ethanol. Freshly harvested ALF were aliquoted and stored at -20^{o}C. Portions of aliquots were placed on wet ice and exposed to UV- irradiation for the time previously assigned. Another portion was left unexposed to UV-light and kept at -20^{o}C.

At the end of each assigned time, 200µl of exposed and unexposed aliquots with the same timing were inoculated through the allantoic route into the marked 9-day-old ECE and sealed with wax. All eggs were incubated at 37^{o}C and monitored for mortality as previously described above. Haemagglutinating ability of the exposed and unexposed ALF arising from the experiment was tested for using the appropriate procedure. Simply described, about 10-20µl of the ALF was mixed with about 20µl of c-RBC on a sterile white porcelain, gently rocked and observed for haemagglutination after about 2-3 minutes.

Portions of all the ALFs were taken for the determination of haemagglutinating titre post- exposure to UV-light. All ALF titres

were determined using standardized procedure (9).

Diagnostic PCR was conducted to determine whether the exposure to ultraviolet rays has had significant effect on the amplicon sizes of the virus samples.

Results

No significant difference exists between the viruses exposed to ultraviolet irradiation at 95% CI (P value =0.3118). The exposure to UV-light does not seem to have any effect on the HPAI H5N1 virus ability to haemagglutinate c-RBC, pathogenicity in eggs and haemagglutination titre (Tables 2-5). However, the unexposed inoculum appears to have increasing titre with longer period of maintenance in the -20^{o}C freezer. The exposure to UV-light does not seem to have any effect on the amplicon sizes of the exposed samples.

TABLE 1: H-GENE AND M-GENE PRIMERS USED IN THE STUDY

H forward	**5′-CCT CCA GAR TAT GCM TAY AAA ATT GTC-3′**
H reverse	**5′-TAC CAA CCG TCT ACC ATK CCY-3′**
M forward	**5′-AGA TGA GTC TTC TAA CCG AGG TCG-3′**
M reverse	**5′-TGC AAA AAC ATC TTC AAG TCT CTG-3′**

TABLE 2: PRE-EXPERIMENTATION VIRUS ISOLATES CHARACTERISTICS.

Passage	Ability to haemagglutinate 10% c-RBC	HA Titre at passage level 1 (Log_2)	Pathogenicity in embryonating eggs (48 hours)	Isolates Designation based on characterization
07/415	100%	5	100%	HPAI (H5N1)
07/456B	100%	4	100%	HPAI (H5N1)
07/439B	100%	5	100%	HPAI (H5N1)

TABLE 3: PATHOGENICITY OF INOCULUM (P2) IN EMBRYONATING CHICKEN EGGS POST EXPOSURE TO UV-LIGHT.

Result of pathogenicity in chicken embryo 48 hours after inoculation					
Virus isolate	15minutes	30minutes	60 minutes	120 minutes	180 minutes
07/456B exposed	100%	100%	100%	100%	100%
07/415 exposed	100%	100%	100%	100%	100%
07/439B exposed	100%	100%	100%	100%	100%
Result of pathogenicity (Unexposed)					
07/456B unexposed	100%	100%	100%	100%	100%
07/415 unexposed	100%	100%	100%	100%	100%
07/439B unexposed	100%	100%	100%	100%	100%

Between 50 and 100% of all embryo die within 24 hours and all embryo die within 48 hours. P2= Passage level 2.

TABLE 4: HAEMAGGLUTINATION TEST OF ALLANTOIC FLUIDS FROM EMBRYONATING CHICKEN EGGS POST-EXPOSURE OF INOCULUM TO UV-LIGHT.

Result of HA after Exposure						
Virus	Unexposed	15minutes	30 minutes	60 minutes	120 minutes	180 minutes
07/456B	3/3 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
07/415	3/3 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)

07/439B	3/3 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)

Results indicate complete haemagglutination of all tested and control samples

TABLE 5: HAEMAGGLUTINATION TITRE OF ALLANTOIC FLUIDS FROM EMBRYONATING CHICKEN EGGS POST-EXPOSURE OF INOCULUM TO UV-LIGHT.

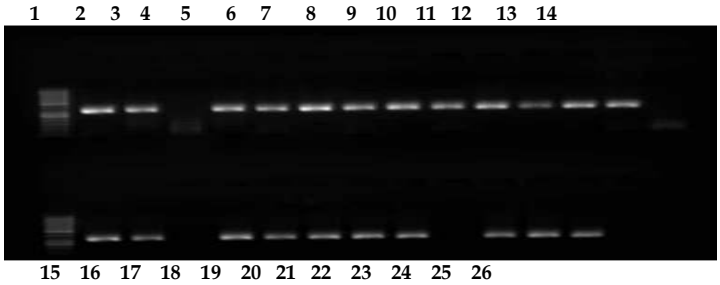
Result of HA titre after UV Exposure P3 (all results in Log_2)					
Virus isolate	15minutes	30 minutes	60 minutes	120 minutes	180 minutes
07/456B	7	6	5	7	6
07/415	4	4	4	8	5
07/439B	6	7	7	7	5
Result of HA titres (Unexposed) P3					
07/456B	4	5	6		
07/415	4	5	6		
07/439B	5	5	8		

P3= Passage level 3

Discussion

Our assessment of the effect of the UV-light rays on virus haemagglutinability, haemagglutination titre, pathogenicity in embryonating chicken eggs and amplicon sizes has shown that the virus seem unaffected by UV-rays. This conflicts with the report of other workers with regards to other viruses affected by UV-light (5, 6). Lowy and co-workers (10) have similarly agreed that gamma irradiation rather that UV irradiation is more effective in penetrating through most biological and non biological agents for purposes of inactivation.

FIGURE 1. AMPLICON SIZE OF THE DIFFERENT SAMPLES ANALYZED FOLLOWING EXPOSURE OF THE INFECTIVE ALLANTOIC FLUIDS TO UV RAYS.



1= 07/456B (60mins, E), 2= 07/456B (P2), 3= 07/149 (Newcastle isolate), 4= 07/439B (60mins, U), 5= 07/439B (15mins, E), 6= 07/456B (15mins, E), 7=07/456B (15mins, U), 8= 07/439B (P2), 9= 07/456B (30mins, U), 10= 07/456B (60mins, U), 11= 07/439B (30mins, E), 12= 07/439B (15mins, U), 13= 07/439B (180mins, E), 14= 07/640 (Newcastle isolate), 15= 07/415 (15mins, E), 16= 07/415 (120mins, E), 17= 07/156 (Newcastle isolate), 18= 07/415 (60mins, E), 19= 07/456B (120mins, E), 20= 07/415 (30mins, E), 21= 07/415 (180mins, E), 22= 07/456B (180mins, E), 23= negative control, 24= 07/456B (30mins, E), 25= 07/439B (120mins, E), 26= 07/415 (60mins, U).
E=Exposed to UV-light; U =Unexposed to UV-Light.

The lack of penetrating power of UV light through the virus may therefore explain why the virus are not inactivated by the UV-rays despite prolong exposure.

Although, we are aware that the degree of thickness of the glass container holding the aliquots may to some extent serve as barrier to penetration of UV light, we ensure the usage of containers with thin

walls (≈1mm thickness) as it will be unethical to expose the naked virus to the environment for such long time in the safety cabinet. However, there is no difference in virus characteristics despite the wide disparity in exposure time (30 minutes up to 180 minutes).

Despite our inability to carry out animal infection assessment study using the exposed virus due to limitations of animal experimentation facilities, it is our strong opinion that the virus may still be able to cause pathogenicity in live chicken comparable to the initial field isolates.
This area of study will need further investigation.

The observed increasing titre recorded in the unexposed inoculum (Table 4) may be as a result of on-going virus activity. Webster and co-workers (9) has indicated that the virus is unstable and may have increasing activity if kept at -20^{o}C for a relatively long time. We therefore affirmed the call for caution in the handling of H5N1 influenza viruses especially on wet ice.

Our opinion survey of virology students and other laboratory staff suggested that 43% have good knowledge, 52% have fair knowledge while 5% have poor knowledge of UV rays. Although 93% agreed that it will attenuate/kill bacteria, only 80% believed that it will attenuate/kill viruses, however two individuals believed that encapsulated bacteria may not be affected and four persons agreed that not all viruses may be affected by UV rays. 74% of the respondents claimed to have knowledge of depreciation in the effectiveness of UV rays over long period of usage time. However, none seem to be sure of whether the UV-rays will inactivate the H5N1 virus. This revealed that virology staff is at high risk of infection with agent like avian influenza H5N1 since most may assume that microbiological safety cabinet is sterile following UV- light exposure.

Further work is encouraged in the areas of laboratory and field assessment of the avian influenza H5N1 virus.

Acknowledgement

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CHARACTERIZATION OF BIOCIDES RESISTANT ISOLATES FROM DENTAL UNIT WATER LINE BIOFILMS BY CULTURE DEPENDENT APPROACH

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Abstract

The importance of biocides resistant bacterial strains in medicine, industry and the environment has gained significant attention. Microbial contamination of dental unit waterlines is thought to be the result of biofilm formation within the small-bore tubing used for these conduits. Our objectives were to characterize biocides resistant isolates from dental unit water line biofilm (DUWL) using the standard laboratory approaches. Growth curves of isolates established in biocides free and supplemented medium demonstrated less growth in the presence of biocides. O10 **|** P a g e **ptimum pH was 7 whereas; optimum temperature was 37°C. Isolates showed resistance against multiple of heavy metals while fewer antibiotics. Genetic studies were accomplished by performing conjugation and transformation experiments. In two isolates (AWT 21 and PTNPF) transconjugants were observed, while no transformant was recorded in any case. Overall, the findings of this study can be used to profile the metabolic effects of new biocides or biocide combinations upon biocides resistant biofilm isolates from clinical environment.**

Key words: Biocides, resistant isolates, dental unit water lines, characterization, heavy metals, antibiotics

Introduction

Bacterial infection remains one of the most serious complications associated with the use of indwelling medical devices. The clinical environment is particularly susceptible to contamination by bacterial pathogens that grow on surfaces in biofilms (1). The formation of surface biofilms can be regarded as a universal bacterial strategy for survival and for optimum positioning with regard to available nutrients. In addition, biofilm bacteria are substantially resistant to surfactants, biocides, and antibiotics (2). Two problems can arise from the presence of biofilms in a distributing aqueous system. First, the biofilm can clog pipes and tubings or interfere with the proper function of mechanical devices. Second, bacterial populations living in this protected mode of growth produce planktonic cells that contaminate fluids and alter their properties or, in the case of pathogens, can result in food poisoning or infections (2). As a result, microbial biofilms constitute major industrial and

medical concerns. These concerns are now being realized in the dental profession.

Dental unit waterlines were coated with a well established biofilm made up of filamentous and bacillus-like microorganisms (1). It has long been known that the water collected at the output of dental unit waterlines (DUWL) is densely populated with microorganisms. This dense, planktonic, microbial population is now known to be a reflection of the colonization of the waterlines by biofilms. Water stagnation is thought to be in part responsible for the phenomenon (3). The bacteria isolated from the water in dental units belong to the community of water bacteria (3), but the finding that some potentially pathogenic microorganisms such as *Pseudomonas aeruginosa* (1) may be present in the water used to perform intraoral and sometimes invasive treatments has led to some concern in the dental community (3). Attempts to control the DUWL contamination have included the use of filters and various biocides. However, bacteria in biofilm mode exhibit 3000 times more resistance than their planktonic counterparts (4). At the present time, commercially available options for improving dental unit water quality are limited and certainly involve additional cost. Among various methods to disinfect DUWL, one is the use of biocides (1).

Biocides are used extensively in healthcare settings for different applications: the sterilization of medical devices; the disinfection of surfaces and water; skin antisepsis; and the preservation of various formulations. In addition, there are now numerous commercialized products containing low concentrations of biocides, the use of which is controversial. Some professionals believe that the indiscriminate usage of biocides in the healthcare environment may not be justified and is detrimental in the long term, for example, by promoting the emergence of bacterial resistance to specific antimicrobials (2).

The activity of a biocide depends upon a number of factors, some inherent to the biocide, some to microorganisms. Among microorganisms most resistant to biocidal exposure are bacterial spores, followed by mycobacteria, Gram-negative, Gram-positive, and fungal microorganisms (2). Although there are exceptions within this summarized classification (e.g, some mycobacteria are relatively sensitive to disinfection), this attempt at distinguishing microorganisms according to their susceptibility to biocides gives useful information for the selection of an appropriate biocidal agent (4). However, it is not always possible to predict which microorganisms will be present on certain surfaces, although the organic load or the extent of microbial contamination, and the presence or not of a biofilm can be determined (2). An understanding of the characterization of resistant organisms is essential to ensure that a biocidal product/formulation is used properly.

The present investigation was designed to look at characterization of DUWL biocides resistant isolates from a dynamic standpoint through growth curve analysis and resistance against heavy metals as well as antibiotics, pH-temperature effect, and conjugation-transformation ability.

Materials and methods

Bacterial strains and growth conditions

Seven bacterial strains (AWT 16a, AWT 21, AWT 28, AWT 33, PT 16, PTNPF and MWPNPC) were isolated from DUWL tubing samples of a priniciple dental unit Lahore, Pakistan following the method of Liaqat and Sabri (1). All strains were resistant to 100 µg ml^{-1} of eight biocides (5.25% NaOCl, 35% H_2O_2, 4% tween 20, 1% PI, 0.2% CHX, 1% EDTA and 1% phe) in L-agar. All strains were stored in microbank tubes (Pro-lab Diagnostics, Neston, Wirral, United Kingdom) at −80°C and were routinely grown in biocides (100 µg ml^{-1}) supplemented L- broth and on LB agar (Oxoid, Basingstoke, United Kingdom) at 37°C with shaking at 150 rpm for the planktonic cultures.

Morphological and biochemical characterization

Bacterial strains were characterized morphologically and biochemically following Gerhardt *et al*. (5).

Analysis of growth kinetics

Growth characteristics of all DUWL biofilm isolates were assessed at 37 or 42°C with shaking (150 rpm). Fifty microliters ($A_{1.00}$ at 600 nm) from an overnight culture of each strain was inoculated in biocides free and biocides supplemented (100µg ml^{-1} of all biocides) medium. Bacterial growth was assessed by measuring the optical densities at 600 nm of the samples with a Bioscreen C microplate reader (Labsystems, Finland) after different time intervals i.e., 0, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42, and 48 hours.

Effect of pH and temperature on bacterial growth

The impact of pH and temperature on bacterial growth of DUWL biofilm was observed in biocides free and supplemented L-broth. Fresh inoculum from overnight cultures ($A_{1.00}$ at 600 nm) was given in all the flasks. Flasks were incubated at various pHs (6-9) and temperatures (20-42°C) with 150 rpm shaking.

Effect of Heavy metal and antibiotic

Different heavy metals and antibiotics were used to check the multiple metals and antibiotic resistance profile of the isolates. Antibiotics used included $NiSO_4$, $HgCl_2$, $ZnSO_4$, $Pb(NO_3)_2$, $MnSO_4$, K_2CrO_4, $CoCl_2$, $FeSO_4$ and $CuSO_4$, whereas heavy metals were TMP (Trimethoprim – 300 µg ml^{-1}), Cdx (Cephradoxil - 100 µg ml^{-1}), Cd (Cephradine 100µg ml^{-1}), Em (Erythromycin – 100 µg ml^{-1}), Tc (Tetracycline - 25 µg ml^{-1}), Cm (Chloramphenicol – 50 µg ml^{-1}), Dx (Doxycyclin - 25 µg ml^{-1}), Ap (Ampicillin - 2000 µg ml^{-1}), Km (Kanamycin - 100 µg ml^{-1}) and Sm (Streptomycin - 100 µg ml^{-1}).

Plasmid screening

For genetic analysis, bacteria were screened for the presence of plasmid by gel electrophoresis of total cell lysate method (6).

Conjugation and transformation

To characterize plasmid, conjugation and transformation experiments were performed.

For conjugation experiments, broth mating technique of Willets (7) was used. The recipients were *Escherichia coli* K12 strains DH5α (F- φ80dlacZΔM15 Δ(lacZYA-argF)U169 deoR, recA1 endA1 hsdR17(rk – mk + phoA supE44 λ- thi-1 gyrA96 relA1) and C600 (F^- tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1 λ^-). Transconjugants were scored at 37 °C by plating mixture on double selective plates i.e., plates containing 100 μgml^{-1} of all biocides, 300 μgml^{-1}ampicillin for C600 and 500 μgml^{-1} streptomycin for DH5α.

For transformation, plasmid DNA was extracted according to the method of Thomas (6). *E. coli* K12 strains DH5α and C600 (Birmingham university, UK) were made competent and transformed. Transformants were scored by plating 50 µl of each transformation mixture on biocide supplemented plates and incubated at 37°C for 24-48 hours.

Results

Characterization of biocides resistant DUWL isolates

Seven biocides resistant isolates (AWT 16a, AWT 21, AWT 28, AWT 33, PT 16, PTNPF and MWPNPC), isolated by Liaqat and Sabri (1), were characterized morphologically, biochemically and physiologically. All strains were gram positive and rods except for AWT 33 (gram negative) and AWT 21 (cocci) (Table-1a). Except for AWT 21, all were aerobic and able to denitrify. All were able to hydrolyse starch and reduce nitrate to nitrite. None of them could hydrolyse arginine. These isolates exhibited good growth on blood agar without any hemolysis pattern (Table-1b).

TABLE 1A: SOME MORPHOLOGICAL CHARACTERISTICS OF BIOCIDE- RESISTANT ISOLATES.

CHARACTERISTICS	BATERIAL STRAINS						
	AWT 16a	AWT 21	AWT 28	AWT 33	PT 16	PTNPF	MWPNPC
Colony							
Visual colour	Light yellow	Off white	Light yellow	Off white	Off white	Off white	Off white
Form	Irregular	Irregular	Irregular	Irregular	Circular	Arborescent	Circular
Margin	Irregular	Irregular	Irregular	Irregular	Entire	Ramose	Irregular
Elevation	Raised	Flat	Flat	Flat	Convex	Flat	Raised
Colony size (mm)	0.4-0.5	0.4-0.5	0.4-0.5	0.2-0.3	0.4-0.5	0.2-0.3	0.1-0.3
Internal Characteristics	Smooth opaque	Smooth opaque	Coarsely granular	Smooth Opaque	Smooth Opaque	Smooth Opaque	Smooth transparent
Cell							
Motility	+	+	+	+	-	-	+
Cell shape	Bacilli	Cocci	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Cell Size (µm)	1.2-0.5	1-1	2.5-0.5	1.2-0.6	1.1-0.2	1.0-0.4	1.5-0.5
Gram staining	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Capsule staining	-	-	+	+	-	+	-
Spore staining	-	-	+	-	-	-	-

TABLE 1B: BIOCHEMICAL CHARACTERISTICS OF BIOCIDES -RESISTANT ISOLATES.

CHARACTERISTICS	BACTERIAL STRAINS						
	AWT 16a	AWT 21	AWT 28	AWT 33	PT 16	PTNPF	MWPNPC
Urease test	-	+	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Cytochrome oxidase	-	-	+	-	-	-	-
Oxidation fermentation	A	F.A	A	A	A	A	A
Nitrate reduction	+	+	+	+	+	+	+
Denitrification	-	+	-	-	-	-	-
Methyl red	-	-	-	-	-	-	-
Voges Proskauer	-	-	+	+	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+
Citrate utilization	-	+	-	-	-	-	-
Arginine hydrolysis	-	-	-	-	-	-	-
Triple sugar iron (slant/butt)	K/K	K/K	K/K	K/K	K/K	K/K	K/K
Growth on EMB	-	-	-	+	-	-	-
Growth on MacConkey	-	-	-	NLF	-	-	-
Growth on Blood agar	+	+	+	+	+	+	+

-, negative., +, positive., A, aerobic., F.A, facultative anaerobe., K, alkaline reaction; NLF, non lactose fermenting.

hours

Growth Kinetic

The Gowth behaviour of all isolates in two showed better growth in biocides free medium than to biocides supplemented medium. 4-6 hours extended lag phase was observed in the presence of biocides, wheresas 2-4 hours lag phase was observed in biocides free medium. Log phase of growth started after 4, 6 and 8 hours in biocides free and supplemented It persisted for 36 (AWT 16a, AWT 21, AWT 28, AWT 33, PT 16, PTNPF, MWPNPC) in two media. Stationary phase started after 36 hours of incubation. It also commenced after 36 (AWT 21, AWT 33, MWPNPC), 42 (AWT 16a) and 48 (AWT 28, PT 16) hours in different strains. Afterwards death or decline phase was observed. Overall, it is evident from Figure-1 that after 24 hours isolates showed better growth in biocides free medium but after 48 hours growth was almost equal in biocides free and supplemented medium (Fig- 1a).

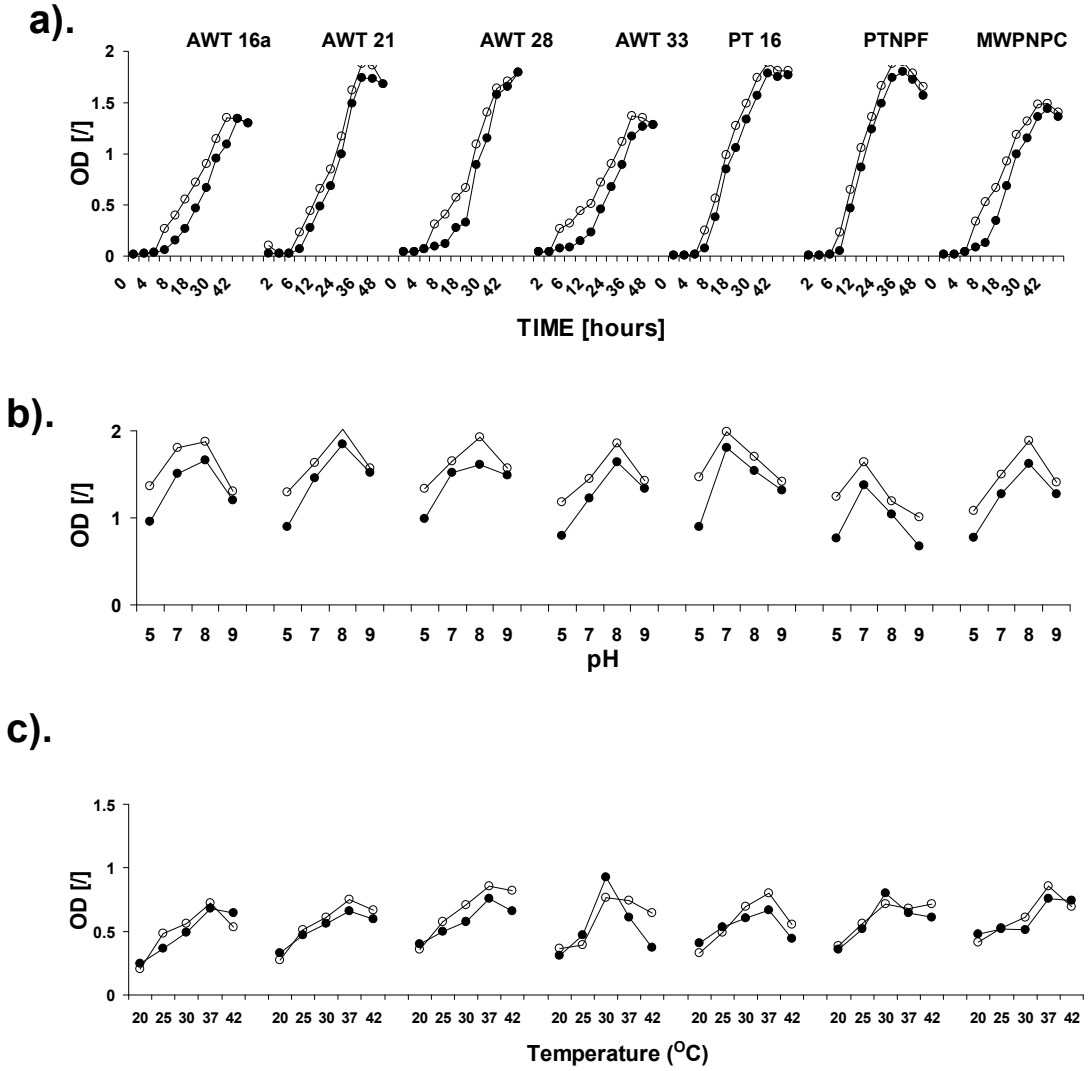

Fig. 1a. Growth curves of bioisolates in 100 µg biocides ml^{-1} supplemented (●) and biocides free (○) L- broth; b). Growth of biocides resistant isolates a) at different pHs (5, 7, 8 and 9) and c). temperatures (20°C, 25°C, 30°C, 37 °C and 42°C) in biocides free (○) and 100 µg biocides ml^{-1} supplemented (●) L- broth for 24 hours; Optical density was monitored at 600nm.

pH and temperature effect

Most of DUWL isolates preferred pH 8 for optimum growth except for PT isolates (PT 16 and PTNPF), which preferred pH 7. However, pH 5 has more detrimental effect on growth, particularly in biocides supplemented medium. Maximum growth was observed in biocides free medium compared to biocides supplemented medium. Likewise better growth was observed at 37°C and in biocides free medium in almost all isolates. However two isolates, AWT 33 and PTNPF exhibited maximum growth at

30°C. Almost equal growth was observed in AWT 16a and PTNPF in biocides free and supplemented media (Fig-1b-c).

Heavy metals and antibiotic resistance

All isolates were resistant against $NiSO_4$, $ZnSO_4$, $MnSO_4$, K_2CrO_4, $CoCl_2$, and $CuSO_4$, whereas all were sensitive to $Pb(NO_3)_2$ and $HgCl_2$ (Table-2). Antibiotic resistance profile of different isolates indicated that isolates were sensitive to majority of antibiotics including cephradine, erythromycin,tetracycline, doxycyclin, ampicillin and streptomycin. However they were resistant to trimethoprim and chloramphenicol (Table-3).

Determination of plasmid

All isolates were observed to harbour a plasmid. Plasmid residing in the biocides resistant isolates were designated as pBR6 (AWT 16a), Pbr7 (AWT 21), pBR9 (AWT 28), pBR10 (AWT 33), pBR13 (PT 16), pBR15 (PTNPF) and pBR19 (MWPNPC).

TABLE-2 HEAVY METAL RESISTANCE PROFILE OF BIOCIDES RESISTANT ISOLATES IN BIOCIDES FREE AND BIOCIDES SUPPLEMENTED (100MG ML^{-1}) L-BROTH.

	HEAVY METALS (µg ml^{-1})								
BACTERIAL ISOLATES	$NiSO_4$	$HgCl_2$	$ZnSO_4$	$Pb(NO_3)_2$	$MnSO_4$	K_2CrO_4	$CoCl_2$	$FeSO_4$	$CuSO_4$
AWT16a 0µg ml^{-1}	200	-	200	-	1000	700	5000	-	100
100µg ml^{-1}	200	-	200	-	1000	700	5000	-	100
AWT 21 0µg ml^{-1}	200	-	200	-	1000	300	5000	200	100
100µg ml^{-1}	200	-	200	-	1000	400	5000	200	100
AWT 28 0µg ml^{-1}	300	50	200	-	1000	700	5000	-	100
100µg ml^{-1}	300	-	200	-	1000	700	5000	-	100
AWT 33 0µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
100µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
PT16 0µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
100µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
PTNPF 0µg ml^{-1}	300	-	200	-	900	700	5000	200	100
100µg ml^{-1}	300	-	200	-	900	700	5000	200	100
MWPNPC 0µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
100µg ml^{-1}	300	-	200	-	1000	700	5000	200	100

- sensitive.

Conjugation and transformation

To identify the genetic determinant of biocides resistance in these strains, conjugation and transformation experiments were performed. *E. coli* strains DH5α and C600 were used as

recipients applying broth mating technique. Transconjugants in each case were selected on media supplemented with biocides (100^{-1}µg /ml)

TABLE-3 ANTIBIOTICS RESISTANCE PROFILE OF BIOCIDES RESISTANT ISOLATES IN BIOCIDES FREE AND BIOCIDES SUPPLEMENTED (100MG ML^{-1}) L-BROTH.

	ANTIBIOTICS (µg ml^{-1})									
BACTERIAL ISOLATES	TMP (300)	Cdx (100)	Cd (100)	Em (100)	Tc (25)	Cm (50)	Dx (25)	Ap (300)	Km (100)	Sm (500)
AWT16a 0µg ml^{-1}	+	-	-	-	-	+	-	-	+	-
100µg ml^{-1}	+	-	-	-	-	+	-	-	+	-
AWT 21 0µg ml^{-1}	+	+	+	+	+w	+w	-	-	-	-
100µg ml^{-1}	+	+	+	+	+w	+w	-	-	-	-
AWT 28 0µg ml^{-1}	+	-	-	-	-	+	-	-	+	-
100µg ml^{-1}	+	-	-	-	-	+	-	-	+	-
AWT 33 0µg ml^{-1}	+	-	-	-	-	+	-	-	-	+
100µg ml^{-1}	+	-	-	-	-	+	-	-	-	+
PT 16 0µg ml^{-1}	+	-	-	-	-	+	-	-	+	-
100µg ml^{-1}	+	-	-	-	-	+	-	-	+	-
PTNPF 0µg ml^{-1}	+	+	-	+	+	+	-	-	+	+
100µg ml^{-1}	+	+	-	+	+	+	-	-	+	+
MWPNPC 0µg ml^{-1}	+	-	-	-	-	+	-	-	+	-
100µg ml^{-1}	+	-	-	-	-	+	-	-	+	-

+, resistant; - sensitive; +w weak growth.

TABLE-4 RESULTS OF CONJUGATION EXPERIMENTS USING *E. COLI* STRAINS DH5A AND C600 AS RECIPIENTS.

CONJUGATION				TRANSFORMATION		
DONOR STRAINS	PLASMIDS	RECIPIENT STRAINS		DONOR PLASMIDS	RECIPIENT STRAINS	
		E. coli			*E. coli*	
		DH5α	C600		DH5α	C600
AWT 16a	pBR6	-	-	AWT 16a	-	-
AWT 21	pBR7	-	-	AWT 21	-	-
AWT28	pBR9	-	+	AWT28	-	-
AWT33	pBR10	-	-	AWT33	-	-
PT16	pBR13	-	-	PT16	-	-
PTNPF	pBR15	-	+	PTNPF	-	-
MWPNPC	pBR19	-	-	MWPNPC	-	-

+, Transconjugants/Transformants obtained; -, No Transconjugants/Transformants obtained

and antibiotics (300 µgml^{-1} ampicillin for C600 and 500 µgml^{-1} streptomycin for DH5α) for which donor isolates were sensitive and recipients were resistant. Only isolates AWT 28 and PTNPF yielded transconjugants (Ap^R +

Biocides^{R}), when *E. coli* strain C600 was used as recipient. While no transformant was recorded in this study (Table-4).

Discussion

Dental clinics have been described to be contaminated by large number of potentially harmful microorganisms. Historically, the vast majority of attempts made at enumerating and control microbes present in DUWLs (1, 8, 9).

Various biocides have been used to combat the pathogens, a potential source of infection to patients visiting dental clinics (9). Because biocides tend to act concurrently on multiple sites within the microorganism, resistance is often mediated by non-specific means. Efflux pumps have the potential to act on a range of chemically dissimilar compounds and have been implicated in both biocide- and antibiotic-resistant bacteria. Cell wall changes may also play a role in the observed cross-resistance between biocides and antibiotics, probably by reducing permeability (10). Microbial changes that result in resistance to biocides and antibiotics should therefore cause concern.

This study deals with the characterization of biocides resistant microorganisms isolated from DUWL tubing samples in Pakistan. The different bacteria isolated from the dental unit water were characterized with respect to morphological, biochemical, physiological and genetical aspects. Morphological differences were observed among various isolates. Most of the isolates were off white in color, with irregular colony and margin and have flat elevation. Majority of these were gram positive, non capsulated and non spore formers. As reported in another study (11), the morphological variants differed from the wild type in attachment, biofilm formation, and cell attachment properties. This ability of biofilm bacteria to show morphological colony variants might be a strategy to tolerate a wide variety of environmental conditions (12). Various authors reported the presence of gram positive/negative isolates in DUWL settings (1, 13).

Growth curve analysis of DUWL isolates revealed better growth in biocides free medium. Whereas, comparatively less growth was observed in the biocides supplemented medium. This might be due to the fact that biocides have inhibitory effect on the synthesis of both nucleic acids and proteins, thus affecting the endogenous respiration. In addition, biocides also interfere with the energy yielding and energy requiring processes of the isolates thus suppressing their growth (14). A long lag phase observed in strains growing in biocides supplemented medium could be explained by the fact that the strains require some acclimation period to counterfeit the harmful effect of biocides on their growth (15).

Though isolates exhibited wide pH and temperature range in both media (biocides free and supplemented), but optimum pH

and temperature were 7 and 37°C respectively. Hence, these strains can be classified as alkaliphilics. Alkaliphilic bacteria also have more amino acids and sugars in their cell walls, which are important in determining their pH tolerance in the medium (16). Less growth observed at pH 5 in biocides supplemented medium might be due to the enhanced activity of some biocides (phenolics) as reported previously by Russel, (17), hence inhibiting the growth of the isolates. On the basis of their temperature preference (30-37°C), these can be categorized as mesophilic i.e., bacteria having optimum growth temperature of 25-42°C. Poor growth observed at 24°C might be due to inhibition of enzymatic activity, resulting in reduced rates of enzymatic catalyzed reactions (18).

Biocides resistant isolates exhibited resistance against multiple of heavy metals [$NiSO_4$ (200-500 µg ml^{-1}), $MnSO_4$ (900-1000 µg ml^{-1}), $CoCl_2$ (5000 µg ml^{-1}), K_2CrO_4 (300-700 µg ml^{-1}), $ZnSO_4$ (200 µg ml^{-1}), $FeSO_4$ (200 µg ml^{-1}) and $CuSO_4$ (100 µg ml^{-1})] while few antibiotics (trimethoprim and chloramphenicol). Studies regarding tolerance as well as susceptibility of biocide resistant bacteria to different antibiotics/heavy metals have been well documented previously (19, 20, 21) It has been claimed that widespread biocides use in hospital, domiciliary, industrial, and other settings contributes to the overall rate of drug resistance (22).

Many plasmids are self-transmissible and those which can be integrated in the host chromosome can also direct the conjugal transfer of chromosomal genes. Total cell lysate method indicated the presence of single plasmid in all the isolates. Conjugation and transformation experiments were performed to characterize the plasmid in biocides resistance isolates from dental settings. Only in two isolates (AWT 28 and PTNPF), transconjugants were scored whilst in none of the case, transformant was recovered on biocides supplemented plates. In one study, Gupta and Ali (23) monitored the transferable ability of mercury resistance plasmid from the wild type mercury resistance *E. coli* strains isolated from aquatic environments of India to the mercury-sensitive, naladixic acid-resistant recipient strain of *E. coli* K12 F_ lac_ by conjugation. Recipient strains were tested for the acquisition of mercury resistance L-agar plates containing different concentrations of mercury to which the donor strains were resistant and naladixic acid (30 µg ml^{-1}) to counter select against the recipient. Transconjugants obtained were able to tolerate the same concentration (25 to 55 µg ml^{-1}) of $HgCl_2$ as the wild-type strains.

For transformation, Gupta and Ali, (23) isolated plasmids from the wild-type *E. coli* strains and transferred into *E. coli* K12 strain DH5α by transformation using standard $CaCl_2$ procedure. Transformants were selected on L-agar plates

supplemented with different concentrations of $HgCl_2$ to which the donor strains were resistant. Transformants were obtained in each case on plates supplemented with different concentrations of $HgCl_2$. For rest of bacteria negative results were obtained. The unsuccessful results reflect that there might be involved some physiochemical condition, some additional requirement of nutrients or it might be that plasmid DNA is degraded by membrane bound nucleases of the competent (recipient) strains (24). Another possible reason might be that plasmid DNA be taken up by the cell but failed to replicate there or plasmid borne markers might not be expressed in the new environment (25).

In conclusion, the scientific community must weight the risk and benefits of using biocides in DUWLs. At present insufficient scientific evidence exist to weigh these biocides and additional precautions are needed to guide biocides development and use to inhibit microbial resistance. Present study investigates the various characteristics of biocides resistant isolates from DUWL biofilm which may be helpful in determining the objective evaluation of antimicrobial and antibiofilm products against these strains.

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EFFECT OF INSTRUCTIONS ABOUT THE METHOD OF URINE COLLECTION AND STORAGE ON THE ISOLATION RATE OF URINARY BACTERIA IN CHILDREN

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ABSTRACT

A study of 65 children (29males and 36females) and aged between four weeks and 15years with significant bacteriuria was undertaking over a six month period to determine the effects of instruction received about the methods of urine collection and storage on the prevalence of urinary tract infection. The commonest clinical presentation was fever (64.6%). Only 22(35.4%) of the patients had specific symptoms suggestive of urinary tract infections. The instructions about urine collection were given to 48(73.8%) care givers. This instruction was given by the attending doctors (84%). Despite the explanation, 15(23.1%) of the patients collected the urine samples wrongly and 44(67.7%) stored the samples for longer than one hour. Significant bacteriuria was more prevalent in 74.2% of patients who submitted their urine samples more than one hour after collection. Communication skill is important and should be emphasized in the trainings of health workers in procedure on the patients.

INTRODUCTION

Urinary tract infection is a preventable cause of morbidity and mortality in the paediatric age group (1-4). The infection is often asymptomatic or the symptoms may be mild that insufficient attention is paid to the illness (3). The prevalence rate in Saudi Arabia (5) is 5.3%, in Sweden[6] is between 1 and 3% from neonatal period to school age. In Nigeria, Akinkugbe et. al. (7) reported a prevalence of 24% and 6% among rural and urban children respectively, Okafor et al (8) found the prevalence rate of 2.1% in Enugu. Morton and Lawande (9) in Zaria reported a prevalence rate of 0.4% of symptomatic bacteriuria while Adeleke et al(10) in Kano, reported a prevalence of 3.2%.

Omer and El-Haj (5) reported a rate of 26.7% in Saudi Arabia. Female sex preponderance for symptomatic and asymptomatic bacteriuria after the age of two years has been reported from within and outside Nigeria (3,5,7,10,11).

Diagnosis of UTI is based on the finding of significant bacteriuria, which is influenced by the methods of urine collection (1-5). Accurate diagnosis requires careful collection of urine samples in sterile bottles with good storage and transportation to the laboratory within

30minutes to one hour after collection(3,8,10,11). Urinary tract infections can be over diagnosed due to contamination of urine samples during urine collection and prolonged storage without refrigeration. This study was undertaken to find out the level of instruction received by patients and their caregivers and their effect on the method of collection and storage of urine specimen in children with significant bacteriuria.

PATIENTS AND METHODS

The study was carried out at Aminu Kano Teaching Hospital, Kano (AKTH) between January – June, 2008. The Patients were consecutive children aged between 1month and 14years that attended the Paediatric out-patients department of AKTH, who had significant bacteriuria and whose parents gave consent for the study. All the urine samples were analyzed in the microbiology laboratory of AKTH, Kano.

A structured questionnaire was used to collect the data. The information obtained from the care-givers included age, sex, presenting complaints, methods of urine collection, duration and places of urine storage, any instruction about cleaning external genitalia, method of collection of urine, storage of urine, source of instructions, and the organism cultured from the urine.

The data were analyzed using the chi-square test where appropriate and a probability level of $p \leq 0.05$ was taken as significant.

RESULTS

There were 65 patients; (29males and 36females) aged four weeks to 15 years who attended AKTH during the study period with significant bacteriuria of a single organism. Female predominance was observed in all age groups but this was only statistically significant after five years of age.

The symptoms on presentation included fever (64.6%) abdominal pain (43%) dysuria (23.1%), urinary incontinence (10.8%) and failure to thrive (9.2%).

The methods of urine collection are shown in Table I. No urine specimen was obtained by urethral catheterization or suprapubic tap. Twenty (30.8%) patients collected the urine specimens wrongly from early stream or chamber pot specimens. 25(38.5%) submitted the urine samples within one hour of collection. None of the urine specimen was refrigerated. Forty-eight (73.8%) patients admitted being instructed on the proper method of urine collection, storage

and transportation to the laboratory within one hour. Doctors (84%), laboratory Scientists (14.8%) and Nurses (2.2%) gave such instructions. Some of the care-givers had multiple instructions given by both the Doctors and laboratory Scientists. Seventeen (26.2%) of patients or care-givers did not receive previous instructions. Although there was no significant relationship between instructions given and the method of urine collection as well as the duration of storage, bacteriuria was higher in patients who stored urine samples longer than one hour (Table II).

TABLE I: METHODS OF URINE COLLECTION ACCORDING TO THE AGE GROUPS

Method	1 – 12months	>1 – 5 years	>5– 15years	Total
Midstream	-	14	14	28
Clean catch	15	2	-	17
Chamber pot	10	4	-	14
Early stream	2	3	1	6
Total	27	23	15	65

TABLE II: RELATIONSHIP BETWEEN INSTRUCTIONS AND METHODS OF URINE COLLECTION AND DURATION OF STORAGE.

Method of Urine collection	Received instruction		Total	X^2	p – value
	Yes	No			
	n-48	n-17			
Correct	39	11	50(76.9)	1.78	> 0.1
Wrong	9	6	15(73.1)		
Duration of Urine storage					
≤ 1 hour	14	7	21(32.3)	0.39	> 0.1
≥ 1 hour	34	10	44(67.7)		

TABLE III: RELATIONSHIP BETWEEN DURATION OF URINE STORAGE AND TYPES OF ORGANISM ON CULTURE

Duration of home storage	E. Coli	Klebsiela	Staph aureus	Pseudominas	Proteus	Total
≤ 1 hour	8	5	2	4	5	24
≥ 1 hour	19	8	9	5	-	41
Total	27	13	11	89	5	65

The commonest organism isolated was **Escherichia coli** (41.5%). There were no multiple bacterial isolates. The other organisms isolated were **Klebsiella spp** (20%), **Staphylococcus aureus** (16.9%), Pseudomonas **aeruginosa** (13.8%) and **Proteus mirabilis** (7.8%). Table III shows the relationship between instructions and methods
of urine storage and types of organism cultured.

DISCUSSION

The study has shown a female preponderance for asymptomatic bacteriuria. This was significant after the age of five years. This is similar to studies reported from elsewhere(2,4,5). The reason for this may be due to the short female urethra, which makes it
easier for organisms from the vulva to get into the bladder. Morton and Lawande[9] noted that up to the age of three years, male were more affected than females. However, Okoro and Okafor(8) in Enugu (South east Nigeria) did not observe any sex difference in patients with symptomatic urinary tract infections.

The commonest clinical presentation was fever (64.6%) of the cases. Twenty three percent of the children had dysuria while 10.8% had urinary incontinence; these two symptoms are specific for urinary infections. This finding is similar to the study by Anochie et al (12) but contrast with report by Okoro and Okafor[8] who observed dysuria and pyuria in 43% and 71% of their cases, respectively. Morton and Lawande[9] in Zaria (North-west Nigeria), reported 10% of children with fever had UTI, 22% of with dierrhoea and 43% of those with dysuria. It is therefore

recommended that UTI should be suspected and investigated accordingly, in any child that presents with a history of non-specific fever, even in the absence of symptoms referable to the renal system. Twenty-seven percent of care-givers did not receive adequate instructions in respect of proper urine collection, storage (refrigeration) and rapid transportation of samples within one hour to the laboratory. This has contributed to the wrong collection of urine from unsterile containers (Chamber-pot) or from early rather than mid-stream clean catch of urine flow. The chance of growing contaminants cultures of such collections is high. There was delay in the transportation of urine samples to the laboratory in 67% of patients; these samples were submitted later than one hour after collection. This may increase the multiplication of the organisms in the urine, resulting in false diagnosis of urinary tract infection. This over diagnosis of UTI may account for the high prevalence rates reported from Port-Harcourt, (13) Ibadan (7) and Zaria (9).

The instructions given by the doctors and laboratory scientist did not positively influence the method of urine collection and storage. Only 48(73%) agree to be instructions by both the doctors and laboratory scientists. The instructions may be too detailed in that the care-givers could not comprehend the instructions. About 67% who claimed to understand the instructions collected the urine samples wrongly and stored the samples wrongly and stored the samples for more than one hour. There were no significant difference in the storage and method of collection of urine in growing a positive culture from the study.

The commonest organism cultured in the study was **Escherichia Coli** was (41.5%). This is similar to reports from within and outside Nigeria have reported that **Escherichia Coli** was the commonest cause of UTI in symptomatic patients (3,8,10, 11,12). In Port-Harcourt[12] and Ibadan (7), **Klebsiela spp** was the commonest and also in Arabian Gulf in the paediatric age group(5). However, in rural Nigerian children, Akinkugbe et al(7) found **Staphylococcus aureus** as the commonest organism in urine specimen of asymptomatic patients.

There is uncertainty of electricity power supply in Nigeria and the low socio-economic status of most

people, refrigeration is not always possible. There is the need to communicate and educate care-givers in accurate methods of collecting urine specimens to be examined for bacteria and also how to transport it to the laboratory within one hour of collection.

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METHICILLIN RESISTANT *S. AUREUS* (MRSA) AND THEIR ANTIBIOTIC SENSITIVITY PATTERN IN KANO, NIGERIA

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Abstract

Background: Nosocomial infection caused by methicillin resistant *S. aureus* (MRSA) presents with management difficulties in infected patients due to their resistance to a number of other frontline antibiotics and constitutes significant epidemiological problems. This study was undertaken to determine the prevalence of methicillin resistant *S. aureus* and antibiotic sensitivity pattern in clinical isolates in Kano. There is dearth of information on this subject in Kano.

Method: One hundred and eighty five (185) *S. aureus* isolates from various clinical specimens obtained over a12-month period in the Microbiology Department of Aminu Kano Teaching Hospital (AKTH) were subjected to methicillin susceptibility testing, while including susceptibility testing to other antibiotics by the disc diffusion methods.

Result: Out of 185 *S. aureus* isolates tested, 53(28.6%) were found to be methicillin resistant. While 38(62%) isolates were obtained from in-patients, 15(28%) were from out-patients. Surgical wound infection had the highest prevalence of 32(60%) isolates. Antibiotics sensitivity results of methicillin susceptible staphylococcus aureus MSSA) and MRSA with the third generation cephalosporins and the quinilones were encouraging. All MRSA isolates were sensitive to vancomycin.

Conclusion: A prevalence of 28.6% MRSA in this environment calls for urgent intervention strategies due to its possible rapid spread and therapeutic problem.

Keywords: MRSA, Prevalence, Antibiotic susceptibility, AKTH.

Introduction:

S. aureus has long been established as an important pathogen in human disease. The emergence of MRSA strains found in increasing number of infections and often multi resistant in nature now pose serious therapeutic problems to clinicians.

When penicillin was introduced in 1994, over 95% of staphylococci isolates were susceptible to the drug. Today, the proportion has shrunk to 10%. The resistance of *S. aureus* to various antibiotics and particularly to methicillin has become a global nosocomial concern. The introduction and the use of gentamycin in the late 1960's and early 1970's halted the development of MRSA. However, by the late 1970's gentamycin resistant MRSA had emerged (1).

MRSA strains are usually resistant to several groups of broad spectrum antibiotics that are used on a large scale in the hospital. The mechanism of increased spreading under antibiotic pressure may have contributed to the

worldwide increase in the prevalence of MRSA in hospitals. (2)

The most remarkable feature of *S. aureus* is its ability to acquire resistance to antibiotics. Many resistance genes are acquired by plasmid mediated gene transfer and some may be transferred to the chromosome as mobile genetic elements. Methicillin resistant strains usually possess more than four genes encoding different resistant mechanism (3).

Classically, MRSA has been a nosocomial problem associated with long hospital stays, numerous or prolonged antibiotic courses, the presence of invasive devices and proximity to an already infected or colonised patient (4).

Materials and methods

Study Area: Aminu Kano Teaching Hospital is a 500 bed tertiary hospital which also serves as a referral hospital for states surrounding Kano without such health facilities. It has emergency, general and specialist units for both pediatric and adult patients.

Methods: One hundred and eighty five (185) consecutive isolates of *S. aureus* were obtained over a 12-month period from various clinical specimens in the Medical Microbiology Department of AKTH by standard procedures (5). They were subjected to methicillin susceptibility testing by using Mueller-Hinton agar supplemented with 4% NaCl. Culture plates were inoculated by dipping a sterile cotton swab into a suspension of the overnight growth of the organism prepared to the density of a McFarland no. 0.5 standard, expressed excess liquid from the swab and inoculated the surface of the agar by spread method. The 5µg methicillin discs (oxoid) were aseptically placed on the surface of the inoculated plates and incubated aerobically at 35^{o}C for 24hrs. The isolates were also similarly inoculated into the surfaces of plain Mueller-Hinton agar plates and Ofloxacin (10µg), Ceftazidim (30µg), Ceftriaxone (30µg). Ciprofloxacin (5µg), Vancomycin (30µg) discs (Abbot Laboratories) were placed and also incubate at 35^{o}C for 24hrs.

The zones of inhibition were measured and compared with National committee for clinical laboratory standards (NCCL) guidelines (6). The isolates that were resistant to the methicillin (<14mm) were termed methicillin resistant *S. aureus* (MRSA) while those with zone of inhibition as (≥ 14mm) were termed susceptible. Antibiotic susceptibility tests were carried out by modified Kirby-Bauer method (7).

Results: Out of 185 isolates of *S. aureus* testes 53(28.6%) were found to be methicillin resistant. Of the 53 MRSA isolates 38(62%) were obtained from In-patients, 15(28%) were from out-patients.

The percentage distribution of MRSA for each service unit is show in Tabe 1. Surgery department had the highest prevalence of 40% while General out-patient department (GOPD), Accident and emergency, Pediatrics, Special care baby unit and medical words had 20%, 4%, 3%, 6% and 17% respectively.

Table 2 shows the percentage distribution of isolates of MRSA from different clinical

specimens. Surgical wound infection showed the highest record with 60% while Ear swab, Urine from urinary tract infection, Skin swab and swab from Eye infections recorded 18%, 11%, 7, and 4% respectively.

Table 3 shows the antibiotic susceptibility of the MSSA and MRSA isolates to methicillin and six other antibiotics. While 71.4% of all the isolates were sensitive to methicillin, 28.6% were resistant. The antibiotic sensitivity profile of MRSA to various antibiotics was as follows, Ciprofloxacin (64%), Ofloxacin (90%), Amoxycillin/clavulanic acid (31.7%), Ceftriaxone (75%), Gentamicin (18%) and Ceftazidim (79%). The percentage sensitive for the MSSA were recorded as follows; Methicillin (100%), Ciprofloxacin (68%), Ofloxacin (93%), Amoxycillin/clavulanic acid (62%), Ceftriaxone (90%), Gentamicin (75%) while Ceftazidime was (92%).

All the isolates were sensitive to Vancomycin.

TABLE 1: DISTRIBUTION OF MRSA BY SERVICE UNIT AT AKTH, KANO

SERVICE	No. POSITIVE (%)
GOPD	11 (20)
MEDICINE	9 (17)
SURGERY	21 (40)
PAEDIATRICS	7 (13)
SPECIAL CARE BABY UNIT	3 (6)
ACCIDENT & EMERGENCY	2(4)
TOTAL	53 (100)

TABLE 2: DISTRIBUTION OF MRSA ISOLATES BY SOURCE OF SPECIMEN AT AKTH, KANO

SITE OF INFECTION	No. POSITIVE (%)
SURGICAL WOUNDS	32(60)
EAR SWAB	10 (18)
URINE	6 (11)
SKIN	3 (77)
EYE SWAB	2 (4)
TOAL	53 (100)

TABLE 3: ANTIBIOTICS SUSCEPTIBILITY PATTERN OF MSSA AND MSRA AT AKTH KANO

No. OF ISOLATES (% SENSITIVE

S. aureus	Meth	CIP	OFL	AMC	CRO	CN	CAZ
MSSA n=132	132 (100)	80 (61)	124 (93)	82 (62)	120 (90)	180 (75)	122 (92)
MRSA N= 53	53 (0)	34 (64)	48 (90)	26 (49)	45 (84)	10 (18)	42 (79)

Meth – Methicillin
CIP – Ciprofloxacin
OFL – Ofloxacin
AMC – Amoxycillin/clavulanic acid
CRO – Ceftriaxone
CN – Gentamyin
CAZ – Ceftazidim

Discussion:

Although it was not possible to carry out a polymerase chain reaction (PCR) to detect mecA gene which is regarded as the gold standard for determining methicillin resistance, sensitivity to or not with 5µg methicillin discs (oxoid) were used. This was the same method adopted by the other studies used for comparison with this present report. In this study, a prevalence rate of 28.6% MRSA was recorded. This is lower than the study in Jos (8) and Ilorin (9), Nigeria which had prevalence rates of 43% and 34.7% respectively.

In another study (10) comprising eight large hospitals in Africa and Malta, a prevalence rate of 16% was observed. MRSA was relatively high in Nigeria, Kenya and Cameroon (21-30%), and below 10% in Tunisia Malta and Algeria.

In Europe MRSA prevalence varied almost a 100 fold in a study, (11) form <1% in northern Europe to >40% in southern and Western Europe.

In the United States (12) the proportion of MRSA was rapidly increased from below 5% in the 1980's to 29% in 1991.

From the foregoing, it is clear the MRSA has become a global nosocomial pathogen with attendant therapeutic problems.

The low prevalence of 6% MRSA in the special baby care unit (SBCU) highlights the awareness interventions of the staff there to prevent nosocomial infection while the high prevalence of 40% in the surgical world would be attributed to lack of adequate precaution and very limited infection control applications.

The antibiotic susceptibility results of MRSA in this study to third generation cephalosporins and the quinolones was quite encouraging and compared favorably well with the study from Ilorin (9). However, the study (8) at Jos showed a low percentage sensitivity of MRSA to the cephalosporins but good results with the quinolones.

This result will be of local clinical relevance in the treatment of MRSA in this environment especially when vancomicin is not commonly available in this area.

There should be an effective infection control committee to coordinate implementation of its policies especially regular hand washing and strict ward antisepsis to reduce nosocomial infections.

Although vancomycin resistant MRSA is not yet common in this part of the world, the rate of spread of this pathogen and its unique ability to acquire and transfer antibiotic resistance calls for urgent and well coordinated surveillance programme to combat this situation.

There should also be strict antibiotic prescription policies enforced by the appropriate authorities to contain the abuse of antibiotics and reduce acquisition of resistance by pathogens. Educational awareness should be encouraged to update

health care workers with new intervention strategies.

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BRANDS OF AMPICLOX AGAINST CLINICAL STRAINS OF STAPHYLOCOCCUS AUREUS

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RUNNING TITLE: AMPICLOX BRANDS AGAINST STAPHYLOCOCCUS AUREUS

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Abstract

Proliferation of different brands of antibiotics including ampiclox (a notable penicillinase inactivator) was considered a relevant factor in the antibiotic resistance of *Staphylococcus aureus*. Consequently the antibiogram and susceptibility of 20 clinical strains and a control strain (NCTC 6571) of *Staphylococcus aureus* to 10 different brands of ampiclox were determined by disk diffusion and tube broth dilution methods. The control strain was found sensitive to ampicilin, augmentin^R and cloxacillin in the antibiogram, and to all the l0 brands of ampiclox, with the MIC's of either 0.125 or *0.25µg/mI* among the clinical strains. This result, compared with the MIC's obtained in the range of 0.125µg/ml to > *60µg/*mI, varying among the brands of ampiclox against the 20 clinical strains, indicates contrasting inhibitory activity among the different brands but reflective of the worrisome level of resistance to antibiotics by *Staph. aureus*. However, this resistance to most of the brands of ampic1ox could not be associated with brand variation having found the control strain sensitive to all the brands of ampiclox.

INTRODUCTION

Ampiclox is a Beta-lactam combination antibiotic comprising ampicillin (an -aminobenzylpenicillin) and cloxacillin, both of which are still obtainable as individual penicillins. Ampicillin and cloxacillin represent an improvement over the parent penicillins (penicillin G, penicillin V and benzathine penicillin) in terms of stability in the presence of the gastric juice acid and (Beta-1actam drug inactivating enzymes - penicillinase and cephalosporinase and spectrum of antimicrobial activity(l). Though ampillin is susceptible to the action of (Beta-1actamase{2), a deficiency that accounts for its combination with cloxacillin, thus producing a synergy in the antibacterial activity of ampiclox. However, there have been reports of multiple drug resistant staphylococci to antimicrobial agents(3,4,5). In this episode of multiple drug resistance (MDR) ampicillin, amoxicillin and cloxacillin (6) as well as other Beta-lactamase targeted antibiotics like ampiclox have

not been excluded: amoxicillin-clavulanate, methicillin, vancomycin, and cephalosporins (7,8,9,1O,1I,12, 13). The MDR in *Staphylococcus aureus* has' been largely attributed to resistance genes borne on R-plasmid DNA(l4). Notably, the hospital strains of this organism have been greatly implicated(l5). *Staph au*reus is an important cause of community and hospital acquired infections (l6,17). Besides plasmid, high-level resistance could also occur from a low level through a multistep process due to chromosomal gene .alteration(18).

Among the enormous number of antibiotics avmlable, pencillin antibiotics are recognizably the most commonly used. This could explain the high level of production of these antibiotics in different forms-single, combination and different brands. Antibiotic prescribing has been observed to be in favour of some particular brands of antibiotics particularly, ampiclox(19). Hence, the need for this study to evaluate the antimicrobial potency of available different brands of ampiclox.

MATERIALS AND METHODS

BACTERIOLOGY

Strains of *Staphylococcus aureus* from different clinical sources, numbering 20, were collected from the Medical Microbiology Laboratory, University College Hospital (U.C.H.), Ibadan and General Bacteriology Laboratory, Lagos University Teaching Hospital (LUTH), Lagos, Nigeria (Table 1). All the clinical strains were screened for purity by means of some coagulase test with reference to a control preserved on Nutrient agar (OXOID) slants at 4°C in a refrigerator until when they were needed.

ANTIBIOTICS

Ten brands of ampiclox (powder for injection) were purchased from local pharmacy shops, and given codes as : EL, PH, JA, CL, ML, RS, SK, RH, TR.

Antibiotic Disks (Abtek Biological Ltd.): Augmentin^{R} {30μg), Amoxicillin (25μg), Erythromycin (5μg), Tetracycline (10μg), Cotrimoxazole (25μg), Chloramphenicol (30μg), Cloxacillin (5μg), Gentamicin (10μg).

ANTIBIOGRAM

The antibiotic susceptibility pattern of each strain of *Staph. aureus* was determined by the Kirby-Bauer's Disk-Diffusion method as previously described(21). The inoculum size of each strain was 10^{7} cells/ml in O.lml taken from 10^{-2} dilution of overnight broth culture, as determined in a pour-plate culture. All the culture and sensitivity plates were incubated at 37°C for 24hrs. Zones of growth inhibition were then observed and measured in millimeters (mm) to

determine sensitivity or resistance to a particular antibiotic disc (Table 2).

MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE BRANDS OF AMPICLOX

The tube-broth dilution method was used to determine MIC. Suitable high aq. stock concentrations of each brand of ampiclox were prepared to provide 60, 50, 20, and 4μg/rnl, in four different tubes of 10ml nutrient broth (OXOD) each. Serial dilutions were made also in nutrient broth to give the following antibiotic concentrations: 30, 25, 10, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0.031μg/m1. Each dilution was then inoculated with O.lml from 10^{-2} aq. dilution (equivalent to 10^7 cells/ml) of an overnight broth culture. The mixtures were then incubated at 37°C for 24hrs, followed by observation for sensitivity or resistance revealed by the visibility of turbidity in each tube to determine the MIC.

RESULTS

In the antibiogram for the clinical strains of *Staph aureus,* amoxicillin, augmentin^R (amoxicillin-clavulanate) and cloxacillin showed no zone of growth inhibition, but did so for the control strain, NCfC 6571. Gentamicin and chloraphenicol were relatively outstanding in their anti-staphylococcal activity (Table 2). Among the 10 brands of ampiclox, 9 brands exhibited reasonable activity of MIC 0.125 - 0.5μg/ml (Table 3) against 9 of the clinical strains (i.e. 45%) of *Staph. aureus* at different levels: BH (5 strains), SK (2 strains), TR (1 strain) RH (1 strain) and CL (4 strains). Thus, one coded EL lacked activity against any one of the clinical strains. These variations are reflected as percentage resistant pattern of the clinical strains (Table 4). However, everyone of the ampiclox brands produced MIC of either 0.125$\mu g/m$l or 0.25μg/ml against the control strain of *Staph. aureus,* NCfC 6571 (Table 3).

TABLE 1: THE CLINICAL STRAINS OF STAPHYLOCOCCUS AUREUS

Strain of *S. aureus*	Clinical source/ Control	Strain of *S. aureus*	Clinical source/ Control
0263	Endorcervical swab	086	HVS
0273	Wound swab	0342	Eye swab
0301	Ear Swab	0748	HVS
1122	Sputum	1937	Urethral swab
1503	Nasal swab	1950	Wound swab
1559	Eye swab	1970	Ear swab
2464	Sputum	1975	Sinus effluent
2994	Sputum	5626	Blood
3409	Wound swab	17322	Ear swab
3413	High Vaginal swab	NCTC 6571	Type culture
078	Wound swab		

a = National Collection of Type Cultures, London.

DISCUSSION

Cloxacillin is used to treat B-Iactamase (penicillinase) - producing staphylococci(6) due to the presence of isoxazolyl as an added group(1) in the antibiotic. The resistance to this antibiotic (cloxacillin) by all the 20 clinical strains in the antibiogram and by most of them in the ampiclox brands indicates a strong possibility of the clinical strains as Blactamase bearers. The sensitivity noted for the control Oxford strain of *Staph. au reus* NCfC 6571 to cloxacillin both in the antibiogram and the ampiclox brands (at MIC of either 0.12511g/ml or 0.2511g/ml) supports this assertion.

TABLE 2: ANTIBIOGRAM OF THE CONTROL NCTC 6571 AND CLINICAL STRAINS OF *STAPHYLOCOCCUS AUREUS*

				Antibiotic disk(μ.g)				
Strain of *S.aureus*	Aug	Amx	Ery	Tet	Cxc	Gen	Cot	ChI
NCTC 6571	20.0*	18.0	22.2	25.5	21.5	23.1	6.4	14.0
078	**-	-	-	–	-	-	-	-
086	-	–	-	–	-	17	–	-
0342	-	-	-	–	–	-	-	–
0748	–	-	-	-	-	–	-	-
1937	-	-	-	-	- 'i	16	-	-
1950	-	-	-	–	–	17	-	15
1970	-	–	–	–	–	15	–	-
1975	-	-	-	–	–	23	22	25
5626	-	-	-	–	-	-	-	-
17322	–	-	-	–	–	–	-	10
0263	-	-	-	-	-	16	-	-
0273	-	-	-	-	-	15	-	-
0301	-	-	-	-	-	13	-	-
1122	-	-	-	–	-	–	-	-
1503	-	-	-	-	-	12	10	10
1559	-	-	-	-	-	17	12	11
2464	-	-	-	-	-	24	22	18
2994	-	–	-	-	-	12	10	9.0
3409	-	-	-	-	-	13	11	10
3413	-	-	-	–	-	21	15	13

KEY: Aug: augmentin (30μg) Amx = amoxicillin (25μg) .
Ery: Erythromycin (5μg) Tet = Tetracycline (10μg)
Cxc: Cloxacillin (10μg) Gen = Gentamicin (10μg)
Cot: = Cotrimoxazole (10μg) ChI = Chloramphenicol (20μg)
* = Zone of growth inhibition (Sensitivity) ** = No zone of growth inhibition (resistance).

TABLE 3: MIC's OF BRANDS OF AMPICLOX AGAINST CLINICAL AND CONTROL STRAINS OF STAPHYLOCOCCUS AUREUS

*Sa Strain		Brand of Ampiclox								
	BH	SK	ML	RH	RS	EL	PH	JA	TR	CL
0263	> 60	> 60	>60	> 60	1.0	> 60	1.0	30	50	20
5626	> 60	> 60	>60	> 60	1.0	> 60	1.0	30	50	30
0273	0.25	> 60	>60	> 60	2.0	2.0	2.0	> 60	> 60	. > 60
078	0.25	20	>60	> 60	2.0	50	0.25	0.5	5.0	0.25
0301	> 60	> 60	> 60	> 60	5.0	> 60	> 60	> 60	> 60	> 60
086	> 60	> 60	> 60	> 60	1.0	> 60	> 60	> 60	> 60	> 60
1122	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
0748	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
1503	2.0	> 60	> 60	> 60	2.0	> 60	> 60	1.0	2.0	10
1559	0.25	> 60	> 60	> 60	0.5	> 60	0.125	0.5	2.0	0.25
0342	0.125	0.5	20	5.0	0.25	20	0.125	1.0	5.0	0.5
2464	0.0	5.0	1.0	2.0	0.125	30	10	5.0	0.25	0.5
1937	10	0.125	0.25	1.0	0.5	20	0.25	0.125	1.0	0.125
1950	20	10.0	0.125	0.5	0.5	> 60	0.125	0.25	5.0	5.0
2994	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
1970	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
3409	10	> 60	> 60	> 60	4.0	> 60	> 60	10	> 60	10
1975	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60	> 60
3413	2.0	> 60	> 60	> 60	0.5	> 60	> 60	10	> 60	5.0
3413	2.0	> 60	> 60	> 60	0.5	> 60	> 60	10	> 60	5.0
17322	025	10	> 60	> 60	> 60	> 60	0.5	> 60	> 60	> 60
NCTC 6571	0.125	0.125	0.125	0.125	0.25	0.5	0.25	0.25	0.5	0.125

*Staph-aureus

TABLE 4: RESISTANCE PATTERN OF THE CLINICAL STRAINS OF *STAPH. AURUES* AGAINST 10 BRANDS OF AMPICLOX .

Brands of Ampiclox	No of resistant isolates	percentage (%)
BH	15	75
SK	18	90
ML	18	90
RH	19	95
RS	14	70
EL	20	100
PH	14	70
JA	16	80
TR	19	95
CL	15	75

The variation noted in the activity of 9 out of 10 brands of ampiclox against 9 out 20 clinical strains of *Staph aureus* and the inactivity of one brand of ampiclox (EL) against none of the 20 clinical strains, corroborates the report of higher demand of one particular brand of ampiclox than the demand for other (19). There is need therefore, for continuous monitoring of the brands of antiobiotics with respect to their activity on relevant bacteria from clinical sources. Having found the control strain, *Staph.aureus* NCfC 6571, sensitive to the 10 brands of ampiclox tested, the resistance noted in this study among the 20 clinical strains to most of the brands of ampiclox could not be associated with variation in the brands.

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PROLONGED USE OF COUGH FORMULATIONS AND THE HEALTH RISK FROM THEIR ANTIMICROBIAL ACTIVITY ON SOME NORMAL BACTERIAL FLORA

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RUNNING TITLE: COUGH FORMULATIONS AGAINST SOME NORMAL BACTERIAL FLORA.

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ABSTRACT

Cough formulations were observed to contain some chemical substances that have been associated with antimicrobial property, namely: menthol, honey, citric acid and volatile oils. A prolonged use of such formulations by patients was therefore considered a health risk on the normal bacterial flora. Nine cough formulations denoted by letter codes along with simple syrup B.P., absolute alcohol and sterile distilled water as controls, were investigated for relative antimicrobial activity on some normal flora bacteria by the agar-cup diffusion method. The respective individual single brands of cough formulation with the exception of one brand exhibited inhibitory activity against 5 - 1 2 bacterial isolates including *Escherichia coli, Klebsiella spp, Streptococcus faecalis, Strep. pneumoniae, Strep. viridians, Proteus mirabilis, Staphylococcus aureus and Pseudomonas aeruginosa.*

Simple Syrup B.P. and sterile distilled water did not exhibit any antibacterial activity while the absolute alcohol exerted activity only on *Staph aureus*. The antimicrobial activity recorded for the cough formulations could cause a depletion of the normal bacterial flora following a prolonged use of the formulations tested, hence, the attendant health risk of depressed natural immune system of the body, normally associated with such bacteria.

INTRODUCTION

Cough preparations are drugs, useful for the initiation of cough, serving either as cough suppressants in cases of non-productive cough associated with mucus secretions in the throat. Cough is recognized as an important physiological protective mechanism, particularly, while serving to clear the respiratory passage of foreign materials and excess secretions(1). Cough could however assume a pathological status when it presents as a symptom of an underlying disorder and becomes chronic. In such situations, the use of an appropriate cough formulation becomes highly imperative, though with the attendant implications in the events of a prolonged use. Cough formulations usually contain mixtures of antihistamines, decongestants, antitussives and expectorants (1), reflecting

their forms of activity but so far without established antimicrobial activity. Besides these combinations in cough mixtures, there are other chemical substances that have inhibitory activity on microorganisms, namely: menthol, citric acid, honey and volatile oils e.g. peppermint oil, aniseed and menthol. Ammonium salts and sodium citrate may also be present which along with citric acid would affect the pH of the formulations. In particular, honey and volatile oils (mixtures of esters, aldehydes, alcohols, ketones and terpenes) have been reported by various workers to have pronounced antimicrobial activity[2,3,4]. Certain herbs, also with demonstrable antimicrobial activity are known to be included in cough formulations e.g. bloodroot, eucalyptus, red clover and others. The presentation of cough formulations as oral preparations implicates their intimate contact with the normal bacterial flora against which the antimicrobial property of the relevant components of the formulations may be directed. Such bacteria are recognized for their role in the innate immune response of the body. In spite of this recognition, research effort has hitherto not been directed at a deliberate investigation of the extent of the antimicrobial activity of the cough formulations vis-a- vis its health risk on the normal bacterial flora consequent upon their prolonged administration. It was against this background that this study has been designed.

MATERIALS AND METHODS

BACTERIOLOGY

Bacterial isolates *of Staphylococcus aureus* (4 isolates), *Escherichia coli* (2 isolates), *Klebsiella* sp. 1 isolate), *Streptococcus faecalis* (1 isolate), *Strep. viridians, Strep pneumoniae* (2 isolates), *Proteus mirabilis* (3 isolates) and *Pseudomonas aeruginosa* (3 isolates) were collected from different clinical sources of throat, wound and sputum. They were confirmed by some established conventional methods: Salt tolerance on Mannitol Salt Agar, Catalase, Oxidase and Coagulase tests as well as Gram staining[10]. The bacteria were then preserved as slant cultures at 4°C in a refrigerator.

COUGH FORMULATION AND OTHER CHEMICALS

Nine cough formulations were purchased from some Pharmacy st ores in Ibadan, Nigeria. They were given letter codes as stated along with their respective chemical ingredients in Table 1. **Simple Syrup B.P:** It was prepared as described in the official monograph (B.P. 1980). Absolute alcohol: It was purchased from a local chemical supplier based in Ibadan, Nigeria.

ANTIMICROBIAL SUSCEPTIBILITY TEST

The agar-cup diffusion as described by Singleton[5] was used for assessing the susceptibility of the bacterial isolates to each of the nine cough formulations and also,

Simple Syrup B.P., absolute alcohol and sterile distilled water as controls. MS-2 that contained only ammonium chloride and ammonium bicarbonate as chemical additives was brought into mixture in ratio 1:1 with each of the other 7 cough formulations. The resulting mixtures were then similarly assessed for antimicrobial activity against the same bacterial isolates. Simply, the method involved digging holes (or wells) in solid culture media that have been seeded with each of the bacterial isolates. The wells were then filled with the respective cough formulations and the controls. After a 24-hrs period of incubation at 37°C, zones of growth inhibition produced were measured to determine bacterial sensitivity or resistance (Tables 3 & 4).

RESULTS

Amongst the 9 cough formulations tested (Table 1), MS - 1 brand in plastic container did not show antimicrobial activity against any one of the bacterial isolates tested, just like the controls except the absolute alcohol which exhibited activity only on one *Staph. aureus* isolate (Table 2). The Zones of growth inhibition produced by the cough formulations varied from 8mm to 30mm. TL brand had the widest spread of antibacterial activity against 13 isolates including at least one isolate of every bacterial sp. tested, followed by CF brand (9 isolates), CT brand (8 isolates), BN brand (6 isolates), EZ and MS - 2 brands (5 isolates each) and, ZP and DK brands (4 isolates each) (Table 2). However, decreased antimicrobial activity was noticeable for the mixtures of 7 cough formulations each with MS - 2 brand. Exceptionally, BN, CF, ZD, CF and DK brands and also absolute alcohol, each in combination with the MS - 2 brand produced higher activity against *Staph. aureus* than when each of the formulations was tested alone. Similar trend obtained with TL and CF brands against one *Proteus mirabilis* isolate (Table 3). Remarkably, Simple Syrup B. P. in combination with the same MS - 2 brand exhibited antistreptococcal activity which was absent when Sterile Syrup B. P. was tested alone.

TABLE 1: THE COUGH SYRUPS (IN LETTER CODES) AND THEIR CHEMICAL CONSTITUENTS AS STATED ON THEIR LABELS.

EZ :	**Diphenhydramine, ammonium Chloride, Sodium citrate and menthol.**
BN :	**Diphenhydramine and ammonium chloride**
CT :	**Ammonium chloride, Ipecacuanha liquid extract, Liquorice extract, pepermint oil and aniseed oil.**
ZD :	**Bromhexine, dextromethorphan, ammonium chloride, menthol, flavoured syrupy base.**
CF :	**Chlorphemiramine, ammonium chloride, Sodium citrate, menthol and ephedrine.**
DK :	**Diphenhydramine, bronhexine, ammonium chloride, Sodium Citrate, and menthol.**
TL :	**Diphenhydramine, ammonium chloride, trisodium citrate, Citric acid, menthol and flavoured Syrup base.**
MS- 1:	**Ammonium Chloride and ammonium bicarbonate (in plastic container).**
MS - 2:	**Ammonium Chloride and ammonium bicarbonate (in glass container).**

TABLE 2: THE ANTIMICROBIAL ACTIVITY OF COUGH FORMULATIONS ON SOME CLINICAL ISOLATES

Zones of growth inhibition (mm)												
ORGANISM	**EZ**	**BN**	**CT**	**ZD**	**CF**	**DK**	**TL**	**MS-1**	**MS-2**	**Syrup BP**	**Alcohol**	**Dist. water**
SA_1	**15***	**-**	**12**	**-**	**-**	**-**	**-**	**-**	**15**	**-**	**-**	**-**
SA_2	**15**	**-**	**16**	**-**	**-**	**-**	**-**	**-**	**10**	**-**	**-**	**-**
SA_3	**-**	**-**	**-**	**-**	**-**	**10**	**12**	**-**	**-**	**-**	**-**	**-**
SA_4 Typed	**17**	**16**	**16**	**-**	**18**	**16**	**17**	**-**	**-**	**-**	**-**	**-**
PA_1	**15**	**-**	**19**	**30**	**-**	**15**	**-**	**-**	**14**	**-**	**-**	**-**
PA_2	**-**	**-**	**-**	**-**	**17 (RM)**	**-**	**12**	**-**	**-**	**-**	**-**	**-**
PA_3 Typed	**-**	**-**	**-**	**-**	**14 (RM)**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
PM_1	**-**	**-**	**12**	**30**	**-**	**-**	**-**	**-**	**12**	**-**	**-**	**-**
PM_2	**-**	**-**	**11**	**30**	**-**	**-**	**-**	**-**	**12**	**-**	**-**	**-**
PM_3	**-**	**-**	**-**	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
EC_1	**-**	**-**	**-**	**-**	**29 (RM)**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
EC_2	**-**	**10**	**9 (IM)**	**8 (IM)**	**14**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
SP_I	**-**	**-**	**-**	**-**	**9 (IM)**	**-**	**12**	**-**	**-**	**-**	**-**	**-**
SP_2	**-**	**10**	**-**	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
SV	**9 (IM)**	**9 (IM)**	**10 (IM)**	**-**	**10 (IM)**	**-**	**14**	**-**	**-**	**-**	**-**	**-**
SF	**-**	**9 (IM)**	**-**	**-**	**9 (IM)**	**-**	**13**	**-**	**-**	**-**	**-**	**-**
KL Typed	**-**	**14**	**-**	**-**	**13**	**15**	**16**	**-**	**-**	**-**	**-**	**-**

KEY

SA = *Staph. aureus*
PA = *Pseud. aeruginosa*
PM = *Proteus mirabilis*
EC = *Esch. coli*
SP = *Strep. Pneumoniae*
SV= *Strep. viridians*
SF = *Strep. faecalis*
KL = *Klebsiella sp.*
Rm = Resistant mutant (few discrete colonies within the Zone)
IM = Intermediate
\- = Resistant (No Zone of growth inhibition)
* = Zone of growth inhibition in mm.

TABLE 3: ANTIMICROBIAL ACTIVITY OF COUGH FORMULATIONS EACH IN COMBINATION WITH MS - 2 BRAND ON SOME CLINICAL ISOLATES

Zones of growth inhibition (mm)										
ORGANISM	**EZ**	**BN**	**CT**	**ZD**	**CF**	**DK**	**TL**	**Syrup BP**	**Alcohol**	**Dist. Water**
SA_1	**15***	**14**	**-**	**14**	**12**	**15**	**-**	**-**	**10**	**-**
SA_2	**15**	**18**	**14**	**16**	**14**	**20**	**-**	**-**	**13**	**-**
SA_3	**-**	**-**	**-**	**-**	**-**	**-**	**10**	**-**	**-**	**-**
SA_4	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PA_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PA_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PA_3 Typed	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PM_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PM_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
PM_3	**-**	**-**	**-**	**-**	**24**	**-**	**25**	**-**	**-**	**-**
EC_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
EC_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
SP_1	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**8 (IM)**	**-**	**-**
SP_2	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
SV	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**9 (IM)**	**-**	**-**
SF	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**9 (IM)**	**-**	**-**
KL Typed	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

KEY

SA = *Staph. aureus*
PA = *Pseud. aeruginosa*
PM = *Proteus mirabilis*
EC = *Esch. coli*
SP = *Strep. pneumoniae*
SF = *Strep. faecalis*
SV = *Strep. viridians*
KL = *Klebsiella* sp.
IM = Intermediate
- = Resistant (No Zone of growth inhibition)
* = Zone of growth inhibition in mm.

DISCUSSION

The inhibitory effect of certain chemical substances - menthol, citric acid, honey, volatile oils and others, contained in cough formulations on microorganism is well recognized [6,7,8,9] yet, a deliberate effort directed at determining the extent of such antimicrobial activity offered by whole cough formulations and its health risk with respect to the human body normal bacterial flora in the event of their prolonged use by patients, has hitherto been lacking. It is interesting to note in this study the varying levels of antimicrobial activity recorded for the cough formulations tested individually but remarkably for TL, CF, CT and BN brands. Of further interest was the observation that 5 cough formulations (DK, CF, ZD, CT and BN brands) had their antistaphylococcal activity increased when combined each with MS - 2 brand. This improved combination antibacterial effect was also observed for TL and CF brands against *Streptococcus pneumoniae* as well as for Sterile Syrup B.P. Remarkably none of *Pseud.* and *Esch.* spp.

was sensitive to any one of the cough formulations in combination with MS - 2.

The bacteria used in this study are among the normal bacterial flora of man, occurring variously in mouth, throat, pharynx and gut within which they perform host body defense against microbial infections[5]. This indication suggests a depletion of these microbes by the antimicrobial activity of cough formulations, particularly, when subjected to prolonged use by patients. The health risk involved becomes worrisome when cough formulations have to be taken concomitantly with some antibiotics usually in the conditions of microbial infections that may present cough as a symptom. The awareness generated in this study on the health risk in the prolonged use of cough formulations should form part of health education to patients.

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ANTIBIOTIC RESISTANT *SALMONELLA* AND *ESCHERICHIA COLI* ISOLATED FROM DAY-OLD CHICKS, VOM, NIGERIA.

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Abstract

Reports of large scale mortality of day-old-chicks were received at the National Veterinary Research Institute, Vom, Nigeria in 2007 to 2008. We investigated the cause of death using several virological and bacteriological techniques, isolated the pathogenic agents and carried out sensitivity tests. Our investigation revealed that *Escherichia coli* and *Salmonella* organisms were isolated in the outbreaks. A pattern of antibiotic resistance that seems to be increasing was also found. Considering the role of chickens and its products in the human food chain in Nigeria; and the close interaction between poultry and man, these resistant organisms may pose dangers to humans through the food chain or zoonotic infection and precipitate a similar pattern of resistance in man. We advocated for informed use of antibiotics in the food animals, especially poultry.

Keywords: *Escherichia coli, Salmonella paratyphi*, poultry, Nigeria

INTRODUCTION

Antibiotics have been used successfully in human and veterinary medicine in the past sixty years to turn many of the life threatening bacterial infections into treatable conditions. However, in recent times, antibiotic resistance has become an important health and food safety issue with the emergence of many drug-resistant species of microbial pathogens in human (1). The use of several veterinary antibiotics/conbiotics for therapeutic or prophylactic administration, especially in the poultry operations is particularly worrisome in view of the potential to extend such drug into the human food chain or the possibility of reduce efficacy of such drugs sometimes administered by non qualified personnel (2, 3, 4, 5, 6).

Poultry production is an important income activity; its success is largely hinged on good management practices and sourcing of the stock from reputable hatchery. In recent times, there has been a gradual and documented increase (30-50%) in day-old-chicks mortality in many farms in parts of north central Nigeria (Plateau, Kaduna and Bauchi). This is linked most often to hatchery originated infections and efforts to treat using many of the commonly available veterinary antibiotics have met with little or no success.

It should be noted that the inability of many major hatchery to meet the national requirements for day-old-chicks has led to the establishment of many small scale hatchery facilities without the recourse to very strict and stringent but necessary conditions of hatchery operations.

A recent survey in Ekiti state, Nigeria indicated that there has been a gradual increase in the use of various antibiotics including quinolones, gentamycin, neomycin, tylosin and chloramphenicol between the year 2002 and 2004 (7). Undocumented field surveys in other parts of the country had presented with comparable statistics.

This work therefore aims to investigate and isolate the causative agent (s) of the recent increasing cause of death in day-old-chicks; and report on its management and controls.

Materials and Methods

Reports of increasing mortality in day-old-chicks were made by poultry farms around Vom ($8^{o}45'E$, $9^{o}43'N$). Similar confirmed and unconfirmed reports were received from other parts of Plateau, Bauchi, and Kaduna states. Data from Ekiti state indicated similar findings (7). Though the chicks were sourced from different hatcheries and distributors; histories of huddling, somnolence, weakness, droopy wings, sudden death without premonitory signs and off-feed were reported by most farmers. Increasing mortality usually starts at about day three to five of chicks' lives. One of the case farms confirmed the usage of combined Gentamycin and Diaziprim based on manufacturers' instruction. Based on the relative ineffectiveness of the recommended dosages, the dosage of the Gentamycin in the combination was doubled to achieve the desired results (initial reduction in mortality followed by complete cessation of mortality).

Visits were made to farms and carcasses were collected. All carcass samples collected were transported in sealed containers to the laboratory on wet ice ($+4^{0}C$).

Carcasses were opened aseptically inside a microbiological safety cabinet class II (SterilGARD® III Advance, The Baker Company, Sanford ME, www.bakerco.com). At post mortem, carcasses were investigated for post mortem lesions indicative of aspergillosis, management errors (dehydration, asphyxiation, etc) and other diagnostic pathological lesions of sudden death in day-old chicks. The congested tissue samples (lung, liver, spleen, heart and intestine); and the brain were harvested for further laboratory analyses.

Virologic analyses

Pooled tissue samples were homogenized to make a 20% suspension using PBS enriched with penicillin, streptomycin, gentamycin and amphotericin B (PSGA), inoculated into 9-day-old specific antibodies negative chicken embryonating

eggs (SAN-CEE) and monitored for pathogenicity for seven days using standard protocols (OIE, 2004a; OIE, 2005). The non-haemagglutinating allantoic fluid was passaged into another set of 9-day-old SAN-CEE and monitored as previously described (8, 9). Agar-gel-immuno-diffusion test was performed on the 20% pooled tissue homogenate using standard protocol to test for antigen to infectious bursal disease virus (IBDV) (10).

Bacteriologic analyses

Whole samples of lung, liver, spleen and heart were sectioned aseptically; the parenchyma portion of each organ was plated directly on 5% sheep blood agar (Oxoid, Basingstoke, England) using sterile wire loop and incubated for 24 hours in a humidified incubator (temperature: 37^{o}C approx., Humidity: 44.50 approx.). The resultant colonies were sub-cultured onto blood, MacConkey (Oxoid) and Salmonella-Shigella (Fluka, UK) agars and incubated at 37^{o}C for 24 hours (11, 12).

These colonies were further sub-cultured and Gram-stained. Catalase and oxidase tests were performed according to previously described procedures (13). The isolates from the plates were pre-enriched in peptone water and incubated for 4-6 hours. Motility test was performed using the hanging drop method (14). Indole test was conducted by using Kovac's reagents according to previously described methods (15). Triple sugar iron agar test was performed by streaking three colonies of the pure culture on the sloped surface and the stabbing of the butt centrally, followed by incubation for 24-48 hours at 37^{o}C.

Sugar fermentation test was carried out on Mannitol, Dulcitol, Lactose, Sucrose, Inositol, Glucose, Arabinose and Xylose. Citrate and urease tests were also performed.

Antibiotic Sensitivity

Antibiotic sensitivity test was conducted using antibiotic disc (Oxoid, UK) according to Kirby-Bauer antibiotic disc diffusion techniques. Briefly described, Mueller-Hinton agar was prepared in petri-dishes (Bibby Sterilin, UK). Pure colonies of the isolated organisms were emulsified in normal saline and the turbidity matched against McFarland No. 0.5 turbidity standard. The bacteria were plated on the Mueller-Hinton agar and antibiotic disc was placed centrally using the antibiotic disc dispenser (Oxoid, UK). The Petri-dish and its content were incubated for 24 hours in a humidified incubator at 37^{o}C. The organisms were observed for antibiotic sensitivity by measuring the zone of inhibition on the plate.

Results

Virology

The allantoic and chorio-allantoic fluids were declared negative for Newcastle disease (ND) and avian influenza (AI)

viral infection respectively following negative haemagglutination tests, and the performance of haemagglutination-inhibition on allantoic fluid (for ND) and agar-gel-immuno-diffusion on chorioallantoic membrane (for AI) tests using monospecific antisera of ND and AI. The 20% homogenate was negative for IBDV antigen since no line of identity was recognized between the test antigen and the IBDV monospecific antibodies used.

Bacteriology

On blood agar, uniform growth of smooth, circular and convex colonies without haemolysis was observed from the different organ plated. The cultures in MacConkey revealed both pale non lactose fermenting translucent colonies suggestive of Salmonella, and reddish lactose fermenting colonies surrounded by a turbid zone suggestive of *E. coli*; while yellowish/pale colonies with black dots indicative of hydrogen sulphide (H_2S) production was seen on Salmonella-Shigella agar 24-48 hours post incubation. Gram Staining revealed numerous Gram negative short rods. All isolates were catalase positive, oxidase negative and motile confirming *E.coli* and *S. paratyphi*. Indole test revealed both indole negative and positive organisms. On Triple Iron Sugar, some organisms produce acid and gas, a characteristic of *E. coli* while others produce acid, gas and H_2S, an indication of *S. paratyphi*.

Sugar fermentation test revealed some lactose fermenting organisms and some non-lactose fermenters; the organisms ferment mannitol with gas production (*E. coli* and *S. paratyphi*), on dulcitol, the organism ferment it with gas production (*S. paratyphi*), there was no fermentation on sucrose (Salmonella organisms). The reaction in inositol test was delayed but there was fermentation (*S. paratyphi*), fermentation on glucose with acid and gas production was observed (*E. coli* and *S. paratyphi*). The fermentation reaction on arabinose was also delayed but acid and gas production was observed (*S. paratyphi* and *E. coli*) and fermentation reaction was also observed on xylose. Based on the bacteriological and biochemical tests performed above, a confirmation of *Salmonella paratyphi* and *Escherichia coli* was reached.

Antibiotic Sensitivity

The pure isolate of *S. paratyphi* was sensitive to Ciprofloxacin, Kanamycin, Chloramphenicol, Gentamycin and Norbafloxacin but resistant to Penicillin. However, the isolate of *E. coli* was sensitive to Chloramphenicol, Kanamycin, Gentamycin but resistant to Ciprofloxacin, Norbafloxacin and Penicillin. In view of the virologic and bacteriologic analyses of the samples, paratyphoid diseases complicated by colibacillosis was diagnosed.

Discussion

Our investigation revealed a pattern of infection that appears to be spreading in the country in the form of epizootics. The causative organisms-in this case *S. paratyphi* and *E. coli* are resistant to a range of antibiotics commonly used in the veterinary and human practices in Nigeria. This presents a serious cause for concern considering that the uninformed farmers/quacks may continue to use increasing level of ineffective antibiotics in the management of infection in farms with possible residues in poultry meat, eggs and other products meant for human consumption. Previous workers have confirmed similarity in antibiotic resistance pattern in poultry and humans within the same locality (3, 16, 17). The cause of the resistance to these antibiotics needs to be investigated more thoroughly, to prevent possible continued resistance to currently sensitive and newer range of antibiotics.

The transfer of potential residues of these antibiotics through the food chain to human presents an opportunity for the development of resistance by human pathogens to this range of antibiotic products.

In conclusion, this work has demonstrated that there appear to be resistant strains of Salmonella organism and Escherichia coli circulating in the field in Nigeria, and calls for concerted effort from the field veterinarians in informed drug administration; and for the policy makers to make and implement standardized legal classification of veterinary drugs to prevent continued abuse of these various products. More work need to be done to comprehensively assess the national prevalence of these organisms and carry out full characterization of the various serotypes that exist.

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ORIGINAL ARTICLE

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SOCIO-DEMOGRAPHIC CHARACTERISTICS OF ADULTS SCREENED FOR HIV/AIDS IN A RURAL COMMUNITY IN BENUE STATE, NIGERIA

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Running title: HIV/AIDS socio-demographic characteristics

ABSTRACT

Background: Benue state has been considered among the 'hot zones' for HIV/AIDS in Nigeria with a prevalence rate of 9.3 among adults aged 15 to 45 years. Yet, there is paucity of information on the socio-demographic factors associated with this level of prevalence. We hereby report the prevalence and socio-demographic factors associated with HIV among adults in Abwa-Mbagene, Benue state, Nigeria.

Methods: Adults in Abwa-Mbagene community who accepted to participate between 9th and 24th of August 2007 were recruited. After counselling, screening was done using 'Determine HIV' kit.

Results: 153 subjects (74 males and 79 females) were recruited. Their age ranges from 15 to 60 years (Mean: 26.2years). Fourty-two (27.5%) consisting of 12 (16.2%) males and 30 (38%) females were positive for HIV (male to female ratio, 2:5). The prevalence of HIV was higher among the divorcees (50%); those whose level of education was primary school (41.3%) and below and; farmers (60%). Majority (96%) knows AIDS exists, 85% are informed on mode and prevention of HIV transmission. Cases of AIDS deaths, unprotected casual sex, blood transfusion with unscreened blood and polygamy are common in the community. The single most important cultural factors in HIV transmission identified is indulgence in night parties, which increased the rate of unprotected casual sex.

Conclusion: This community represents a high HIV/AIDS prevalence spot in Benue State, Nigeria. There is therefore the need for the establishment of HIV Counselling and Testing (HCT), Prevention of Mother-To-Child Transmission (PMTCT), save blood and antiretroviral treatment services in the community. Vocational centers to engage single women and improve their financial state will boost preventive strategies. Finally, partnership with community leaders towards discouraging night parties will reduce the prevalence of HIV in the community.

Index words: Factors, prevalence, prevention, transmission.

Introduction

The AIDS epidemic continues to spread at an alarming rate all over the world. In 2006 alone, 4.3 million people were infected with HIV. Despite new advancement in the availability of HIV treatment in developing countries, 2.9 million people died from AIDS in 2006[1,2]. This is the highest number reported for a single year since the beginning of the pandemic. Sub-Sahara Africa is the worst affected regions and 59% of those infected were women (1-3). In this region, where resources and aids are poor and access to health care and new treatment is limited, the vast majority (72%) of death from

HIV/AIDS is seen and AIDS is now a leading cause of death between the ages 15-59 years (1). In Nigeria, AIDS cases have been reported ranging from 1.4% to 42.7% in different states of the federation with majority of cases in their prime of life (4-10). The current national average prevalence of HIV/AIDS has been found to be 3.9% (3). Considering a national population figure of 160 million and at an average prevalence of 3.9%, over 6 million Nigerians may be under varied burden of HIV infection. However, the prevalence varies across states and different subgroups within Nigeria (4-12). Benue state has been found to be among the highest zones of HIV/AIDS in Nigeria, with prevalence rate of 9.3 (13). Yet, there is paucity of information on the socio-demographic factors responsible for this level of prevalence from this environment. Jegede[14] found that the context of the prevalence of cultural heterosexual relationship such as early marriage, virginity, son preference, patriarchy, widowhood and inheritance practice, extramarital and premarital sexual behaviours has negative influence on sexual behaviours and impact negatively on HIV/AIDS control. Akanni & Erhabor

(6) found that absence of formal education, history of alcoholism, drug use, multiple sexual partners and age of first debut, are independent risk factors in adolescents HIV infection in Port Harcourt. Jegede related inadequate knowledge of the cultural context of transmission of HIV/AIDS to high level of HIV infection despite various preventive measures (14). Hence the need for more specificity with regards to different cultural and social groups rather than the current generalized 'ABC' mode of prevention.

A major factor in the control of AIDS is individual and community knowledge of HIV status through voluntary counseling and testing and the number of people willing to undergo HIV counseling and testing is higher when the test is free and treatment programme is available (15-17). This is because the poor socioeconomic factors which reduce individual willingness to pay for the test on one hand and the understanding that in the absence of life saving medicines, an HIV/AIDS diagnosis for most people may mean a notification of early death (15,16). Regularly available and affordable voluntary counseling and testing usually help people to know their HIV status and affected patients can benefit from the various antiretroviral treatment programs in the country. Community Involvement in counseling and testing helps them to participate in HIV awareness and prevention activities thereby protecting their community against HIV/AIDS.

The aim of this study is to support individuals and the community of Abwa in Buruku Local Government, Benue state, Nigeria in the determination of their HIV status, determine the socio-demographic factors related to HIV/AIDS transmission

in the community, with the intention of applying our findings in the control of HIV/AIDS transmission specifically in Abwa community and Nigeria in general.

We hereby report the prevalence of HIV/AIDS and the sociodemographic Characteristics related to HIV/AIDS transmission among adults screened for HIV/AIDS in Abwa community, Benue state, Nigeria.

Methods

This study was conducted between 9th and 24th of August 2007 in Abwa, Buruku local government, located about 110 km east of Markurdi, the capital city of Benue state. The community is located along a major road leading to Taraba state on the left, Cross river state on the right and straight to the Cameroon border. The community consists of four quarters, 622 compounds and about 10,000 people.

Ethical approval was given by the Ethical Review Committee of the college of Health Sciences, Benue State University, Makurdi. On arrival at the local government, the chairman of the local Government and other members of the council were informed of the presence of the group and the various projects to be conducted during the period. The traditional leader and members of his council were met and the mission of the group was discussed with them in detail. We were accepted by the community and the people promised full support and cooperation.

Randomly selected individual within reproductive age group who accepted to participate was recruited into the study. Participants were sent for counseling and HIV testing. The counseling was done following a format in the questionnaire (designed by the authors), which included bio data, marital status, previous risk factors, knowledge of the respondent on the various preventive measures, cultural factors in the locality perceived by respondent to be responsible for HIV transmission and their perception and reactions to individual with HIV/AIDS among other questions.

Sample size was determined using the formula: $n = Z^2pq/d^2$

Where n $q = 1.0-p$ i.e $1.0 - 0.093 = 0.907$

d = degree of accuracy desired, which will be set at 0.05.

Therefore the minimum sample size is: $n = Z^2pq/d^2$

$n = (1.96)^2 (0.093) (0.91)/ (0.05)^2$

$= 0.3388291/0.0025 = 130$

= the desired sample size (when population is greater than 10,000)

Z = the standard normal deviate, usually set at 1.96 (or more simply at 2.0) which corresponds to the 95% confidence level.

P = the proportion in the target population estimated to have a particular characteristic, in this study, the previous finding in this environment that shows HIV/AIDS prevalence in Benue state as 9.3% (13) was used.

Testing was done at no cost to the volunteers after pre test counseling using third generation ELISA kit called 'Determine HIV' produced by Abbott diagnostics. Manufacturer's manual was followed, briefly as follows: after identification of the patient, patient's finger was cleaned with 70% alcohol, using sterile lancet, a prick was made on the pulp of the thumb. The strip was opened, a drop of blood was collected on the specimen site and 2 drops of diluents were then added and the mixture was allowed to migrate to the viewing site. Interpretation was done in which red or pink band at the test and control sites was considered positive. Similar band only at the control site alone was interpreted as negative, while band occurring only at the test site was considered an invalid result.

Post test counseling was then conducted: HIV positive patients were referred to treatment centers and negative patients were advised on preventive measures against HIV/AIDS. Issues related to stigmatization and cultural practices perpetuating the disease prevalence were noted and thoroughly discussed. Towards the end of the programme, health talks were organized emphasizing the prevailing health problems in the community and the various modes of prevention. The attendance at the health talk was impressive cutting across age, occupational and sociopolitical boundaries in the community, comments and questions were properly addressed.

Results are presented in tables and figures as applicable. Comparisons were made using standard statistical methods in which categorical data were compared by Chi-Square. Conclusions and recommendations were based on scientific evidence from the results.

Results

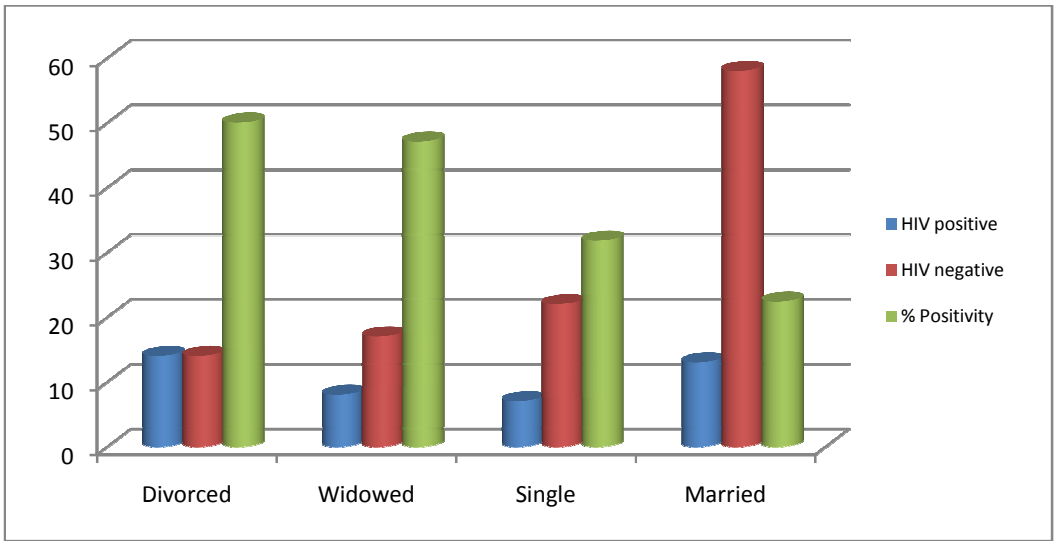
One hundred and fifty-three subjects were recruited into the study consisting of 74 males and 79 females, age ranging from 15 to 60 years. Fourty-two (27.5%) subjects consisting of 30 (38%) females and 12 (16.2%) males were positive giving a male to female ratio of 1:2.5. The mean age of positivity among males was 23.9 years while that among females was 28.5 years. The highest incidence was found among the 20-29 year age groups in both sexes. There was none positive above the age of 55years. (Table 1)

The highest number of subjects recruited into the study were the married (71), followed closely by the single, divorced and the widowed. The highest prevalence of HIV was found among the divorcees or with the history of divorce (50%) while the lowest was among the married (22.4%) and the difference is statistically significant. (Figure 1)

Sixty percent of the subjects recruited into the study were farmers. 22 (14.4%) were students while civil servants were the least common group. Others, which include community workers, house wives and medical laboratory technicians represent 11.1% of the study group. The highest

HIV prevalence was seen among farmers (46%), while the lowest prevalence was seen among traders (16.7%). The prevalence of the infection was significantly higher among farmers when compared with other occupational groups. (Table 2)

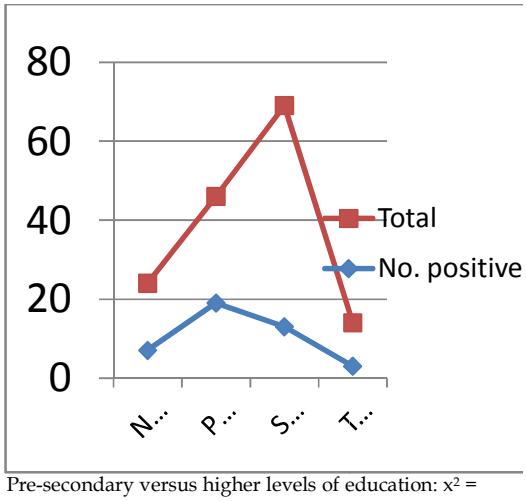
FIGURE 1: MARITAL STATUS OF SUBJECTS



Married versus divorced $x^2 = 10.27$; p-value < 0.05

Sixty-nine (45.1%) of the subjects had secondary school as their highest level of education while 14 (9.1%) had post-secondary school education, which were National certificate in Education (NCE), School of health, or other diploma courses. The highest number of HIV prevalence was found among subjects with the lowest level of education, that is; primary school (41.3%), followed by those with no formal education (29.2%). HIV was least prevalent among those with post secondary education (18.8%). (Figure 2). Over 96% of the subjects believed HIV/AIDS exists. 85.6% were well informed about the mode of transmission and preventive measures. Majority (65.4%) had seen one or more HIV/AIDS patients the neighbourhood and 60.8% had seen one or more people die of AIDS in the last 6 months. Only 9.8% of the subjects would discriminate against HIV/AIDS patients (Figure 3).

FIGURE 2: LEVEL OF EDUCATION AMONG SUBJECTS



Pre-secondary versus higher levels of education: x^2 = 12.17; p-value < 0.5

FIGURE 3: KNOWLEDGE AND PERCEPTION OF SUBJECTS ON HIV/AIDS

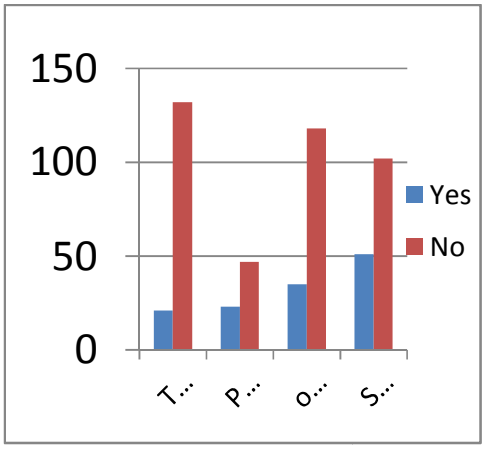
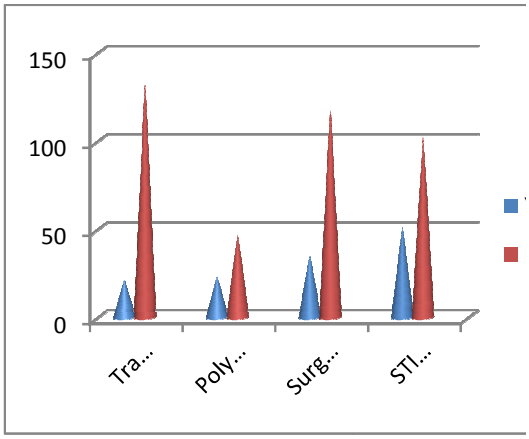


FIGURE 4: PREVIOUS EXPOSURE TO HIV/AIDS RISK FACTORS



Fourteen percent of subjects had blood transfusion at least once in their life time. 33% had previous sexually transmitted infection or are practicing polygamy, and 23% had previous surgical operation, which ranged from bilateral tubal ligation to total abdominal hysterectomy (Figure 4). Over 90% of the subjects believed that a major cultural factor in HIV transmission in the environment is unprotected sex following night parties usually during burial wake keeping.

Discussion

The 27.5% of HIV positivity among subjects seen in this study is higher than the average reported earlier in Benue state[13] (9.3%), from Kano (1.44%; 3.8%) in 2006[5], 2003[7] respectively, from Calabar (2.7%) in 2003 (11) and from Abeokuta (14.7%) in 2007 (8). It is however lower than that from Port Harcourt 42.7% in 2005[6] and comparable with the finding of Bakare et al (12) where 34.3% HIV prevalence was seen among commercial sex workers (2003). The rate found in this study is significantly higher than the national (3.9%) and state (9.3%) average prevalence (3, 13). This calls for concern as this type of pockets of high prevalence can adversely militate against the various national and international control efforts.

TABLE 1: AGE AND SEX DISTRIBUTION VERSUS HIV STATUS OF SUBJECTS

Age group (years)	HIV positive		HIV negative		Total	
	M	F	M	F	M	F
<15	None	None	None	None	None	None
15-19	2	3	9	6	11	9
20-24	4	10	9	7	13	17
25-29	2	7	10	8	12	15
30-34	1	3	8	10	9	13
35-39	1	3	8	4	9	7
40-44	1	2	8	5	9	7
45-49	-	1	4	3	5	4
50-55	1	1	4	3	4	4
>55	Nil	nil	2	3	2	3
Total	**12**	**30**	**62**	**49**	**74**	**79**

Average Prevalence rate = 27.5%; mean age; male =23.9years; female = 28.5years; male to female positivity ratio = 1:2.5

TABLE 2: OCCUPATIONAL ENGAGEMENT AMONG SUBJECTS

Occupation	No positive	No. negative	Total (%)
Farmers	**29**	**63**	**92 (60)**
Students	**4**	**18**	**22 (14.4)**
Traders	**2**	**12**	**14 (9.2)**
Civil servants	**2**	**6**	**8 (5.2)**
Others	**5**	**12**	**17 (11.1)**
Total	**42**	**111**	**153 (100)**

Farmers versus other groups X^2 = 3.840; p-value = >0.05

The male to female positivity ratio of 2:5 seen in this study is similar to other findings[6,18] in which women have been found to be two to four times more vulnerable to HIV infection than men during unprotected sexual intercourse because of the larger surface area exposed to contact. Besides, the female is the recipient of semen and is prone to micro trauma during sexual intercourse.

HIV was significantly higher among subjects who were previously or currently divorced in this study. This prevalence is followed closely by the widowed, showing that single women are more vulnerable to HIV infection than married ones. This may be accounted for by absence of

family cover, which could shield or restrain them from multiplicity of sexual partners. Financial demand, efforts to re-marry, sexual assault and unusual freedom, among others, may be responsible for the high prevalence seen among single women in this study. Further study is needed to determine the relative importance of these factors in the higher prevalence of HIV/AIDS among unmarried females in this environment.

The importance of financial factors in HIV transmission is further buttressed by highest prevalence of the infection among farmers in this study. Efforts at stress reduction resulting from unattained goals may facilitate trading of sex for money and may be a major factor among this group of subjects. Ability to assert self and relative financial independence may be important factors in lowering the rate of HIV prevalence seen among traders and civil servants in this study.

Majority of the HIV positive subjects in this study were individuals whose highest level of education was primary school and below. There was significant difference between subjects with no secondary schools education and the more educated ones, factors responsible may be related to their low understanding of the knowledge acquired by subjects on HIV/AIDS and their willingness and ability to utilize such information in taking informed decision related to HIV/AIDS preventions. This is because the majority of the subjects know that AIDS exists. Though only a few have the consciousness that transfusion with HIV infected blood may be a major factor in HIV transmission, most are well informed about other modes of transmission and prevention of HIV/AIDS. Poverty and emotional instability may explain why the people were still involved in the various risk factors after seeing many AIDS patients and even deaths. Issue of self denial and the feeling of 'it can only affect them, not me', 'we are many', 'man must die of something' are among some personal feelings noted from interaction with infected patients. Worthy of note among the group of HIV positive individual is that females who are less exposed to the various risk factors associated with HIV are emotionally more disturbed about their HIV status as reflected by the usual emotional outbursts associated with the realization of their HIV positivity.

Majority of the subjects in this study will not discriminate against AIDS patients, showing that stigmatization is not a major issue in this environment. The care for the sick among the family members in terms of show of love and concern demonstrated by the people is obviously impressive. Varied percentage of the study population has been exposed to risk factors associated with HIV transmission such as blood transfusion, previous operation, previous history of sexually transmitted infections (especially purulent urethral discharge among men and lower back pain among women) and polygamy. Of major concern and worthy of note is the interest in surgical operation by the people. Yet, there are only a few numbers of qualified staff for such procedures in the

environment. From our personal relationship with some of the health workers, it was found that community members prefer unscreened blood for transfusion basically for financial reasons. Little wonder that only a few of our subjects in this study will consider blood transfusion with HIV infected blood as a major risk factor in HIV transmission. Thus, involvement of the community in partnership for HIV/AIDS control is needed[19].

We therefore conclude that HIV infection is a major health issue in the community, especially among the single women. The level of awareness of the disease is high among the people but their understanding of unscreened blood in HIV transmission is low. Majority engage in the various risk factors such as unprotected casual sex, which predisposes them to sexually transmitted infections, blood transfusion with unscreened blood and polygamy despite their awareness of the associated risks for HIV infection. The single most important cultural factor identified is night parties. This has increased the rate of unprotected casual sex.

We hereby recommend that Voluntary Counseling and Testing (VCT), Prevention of Mother to Child Transmission (PMTCT), provision of save blood and antiretroviral treatment centers are urgently needed in Abwa community to help control the tide of the HIV/AIDS rampage. Vocational centres are paramount to engage the single women and improve the financial state of the populace. Finally, there is urgent need to engage the community leaders in cultural practices that will lead to the discontinuation of night parties.

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