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Antibiogram of Bacterial Isolates from the Anterior Nares and Hands of Health Care Workers in University of Uyo Teaching Hospital (UUTH) Uyo, Akwalbom State, Nigeria

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Abstract

Hands and anterior nares of Health Care Workers may serve as sources for transmission of pathogens causing nosocomial infection. The importance of hands in the transmission of nosocomial infection is known worldwide. However, it is difficult to induce hand washing behavior in health-care workers.

This work is designed to ascertain the frequency of bacterial colonization and the antibiotic sensitivity pattern of the isolates from the anterior nares and hands of healthcare workers in University of Uyo Teaching Hospital (UUTH).

Sterile cotton wool swabs, which were pre-moistened in sterile normal saline, were used to swab the anterior nares, and another used to swab the interdigital spaces of both hands of the participants. The samples were cultured on Mannitol salt agar, Blood agar and MacConkey agar. The plates were incubated at 35°C for 24-48 hrs. The bacterial isolates were identified and antibiotic susceptibility testing carried out on them using CLSI standard.

Out of the 60 samples analysed (30 nasal and hand swabs), 48 (80%) yielded bacterial growth and 12 (20%) showed no bacterial growth. Of the 48 isolates, 46 (95.8%) were identified as *Staphylococcus* spp and 2 (4.2%) were identified as Gram negative bacteria (*Escherichia coli* and *Proteus mirabilis).* Out of the 46 Staphylococcal isolates, 30 (65.2%) were coagulase positive and 16 (34.8%) were coagulase negative. Out of the 30 coagulase positive *Staphylococcus* spp, 12 (40%) were found to be Methicillin resistant, and one of the Gram negative bacteria isolated (*Proteus mirabilis*) was extended spectrum beta lactamase producing. Also, of the 48 Staphylococcal isolates, 5 (10.4%) were inducible Clindamycin resistant. *Staphylococcus aureus* was found to be sensitive to Clindamycin (80%), followed by Ciprofloxacin (77%), Amoxicillin clavulanic acid (73.3%), Oxacillin (60%), Erythromycin (43%), Ceftriaxone (40%) and Trimethoprim Sulphamethoxazole (23.3%). On the other hand, *Staphylococcus epidermidis* was found to be sensitive to Ciprofloxacin (81%), Clindamycin (70%), Amoxicillin Clavulanic Acid (68.8%), Erythromycin (56.2%), Ceftriaxone (19%) and Trimethoprim Sulphamethoxazole (2.5%). *E. coli* was 100% sensitive to Ceftriaxone, Ciprofloxacin, Gentamycin, Ceftazidime and Cefotaxime, and *Proteus mirabilis* showed 100% sensitivity to Ceftriaxone, Amoxicillin clavulanic acid, Gentamycin and Cefotaxime.

Since hands and anterior nares of health care worker attending to patients could be a source of transmission of nosocomial infection with its attendant consequences in patients care, it will be helpful to screen them regularly as a measure towards the prevention and control of hospital acquired infection.

Keywords: Hands; Anterior nares; Hospital acquired infection; ESBLs; MRSA

Introduction

Hospital acquired infections are increasingly becoming a major concern in both developed and developing economies. In hospital admitted patients, surgical site infections, catheter associated urinary tract infections, intravenous devices infection and respiratory infections are frequent causes of prolonged hospital stay, morbidity and mortality.

Frequently, most of the nosocomial pathogens are multidrug resistant bacteria which pose serious therapeutic challenges. Hospital outbreaks of methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), extended-spectrum beta lactamases (ESBLs) producing Gram negative bacteria which are resistant to cephalosporins and monobactams, and multidrug resistant *Pseudomonas* have severally been reported [1-3].

The role of health care workers in the transmission of infections has been extensively described [2]. Most often, spread is from patient to patient on the hands of health care workers, person to person through direct contact and on medical devices. The importance of hands in the transmission of hospital infections is worldwide accepted [4,5]. However, it may not be the regular practice to include hand washing (HW) as routine behavior in health-care workers, since microorganisms are invisible. or there are no adequate elements to carry out this practice [6].

Health-workers hands by themselves, or after contact with patients, increase the risk of virus and bacteria transmission that are sometimes resistant to antimicrobial agents (AMA) [4]. This is a two-way hazard that could be noxious to both patients and health-care workers, and which depends on the nature and frequency of contact with infectious materials, inoculum and prevalence of susceptible patients [4]. Despite

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the knowledge of the importance of this fact by health-care workers (HCW), they do not practice hand washing [6-8], which is more neglected by physicians nurses [9,5].

The need for intervention is imperative with several reports still documenting poor hand washing among health-care workers [10,11]. A targeted surveillance culture to identify asymptomatic carriers of multidrug resistant bacteria and subsequent isolation and treatment had been recommended for minimizing their spread within hospitals [1]. The knowledge of sensitivity patterns of isolates will also be useful in predicting possible risk of infection with multi-drug resistant bacteria in health-care settings.

This study is therefore undertaken as part of a wide scale hospital infection control surveillance programme of the University of Uyo Teaching Hospital, (UUTH), Uyo, Nigeria. It seeks to ascertain the frequency of bacterial colonization of the anterior nares and hands of healthcare workers who are in regular contact with patients. It also seeks to determine multidrug resistant isolates for possible intervention.

Materials and Methods

This study was carried out at the University of Uyo Teaching Hospital (UUTH) located in Uyo, AkwaIbom State. The hospital is a tertiary center that receives referrals from primary and secondary health institutions in the State and beyond.

A total of 60 samples consisting of 30 nasal swabs and 30 hand swabs were collected from 30 healthcare workers in University of Uyo Teaching Hospital. Of all the healthcare workers, 15 (50%) were doctors and 15 (50%) were nurses, which consisted of 11 (36.7%) males and 19 (63.3%) females. The media used in this study were Mannitol Salt Agar, Blood Agar, MacConkey Agar and Mueller Hinton, which were provided by the tertiary hospital. Sterile cotton wool swabs, which were pre-moistened in sterile normal saline, were used to swab the anterior nares and another used to swab the interdigital spaces of both hands of the participants. All media were prepared according to the manufacturer's instructions. The nasal swabs were aseptically swabbed and cultured on Mannitol salt agar plates. Yellowish colonies from the Mannitol salt agar plates were sub-cultured on blood agar base supplemented with 5% sheep blood. Plates were incubated aerobically at 35°C for 24-48 hrs. Isolates were presumptively identified as *S. aureus* using coagulase test. The swabs from interdigital spaces of both hands were cultured on Mannitol salt agar, Blood agar, MacConkey agar and Mueller Hinton agar plates. The bacterial isolates were identified using bacteriological procedures, involving morphology, microscopy and biochemical tests [12,13]. Antimicrobial susceptibility testing was carried out using the modified Kirby-Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2008).

Methicilin resistance was tested using 1 µg oxacillin disc (Oxoid Ltd, Cambridge, UK), according to CLSI guidelines. Mueller Hinton Agar was inoculated with 0.5 McFarland preparation of the inoculum using the spread plate technique. 1 µg Oxacillin disc was placed on the agar and incubated aerobically at 35°C for 24 hrs. Organisms with zones of inhibition ≤ 12 mm were interpreted as methicillin resistant, while those with zones of inhibition ≥ 13 mm were interpreted as methicillin susceptible.

Inducible Macrolide Lincomycin Streptogramin B (iMLS$_B$) was done for all *Staphylococcus* isolates which were resistant to Erythromycin, but susceptible to Clindamycin, as described by the CLSI (CLSI, 2008). Mueller Hinton Agar plates were inoculated with 0.5 McFarland preparation of the inoculum using the spread plate technique. Then a 15 µg Erythromycin (Oxoid ltd, Cambridge, UK) and 2 µg Clindamycin discs were placed at 15-26 mm apart. The plates were incubated for 18-24 hrs at 35°C aerobically. Isolates that showed flattening of the Clindamycin zone of inhibition adjacent to the erythromycin disc (referred to as a D-zone) was regarded as exhibiting inducible Clindamycin resistance.

Enterobacteriaceae isolates with cefotaxime, ceftazidime and cefpodoxime zones of inhibition less than 27 mm, 22 mm, 17 mm, respectively, were suspected to be ESBL producing. A confirmatory test using the Double Disc Synergy technique was carried out according to CLSI (2008) guidelines. Mueller Hinton Agar plates were inoculated with 0.5 McFarland preparation of the inoculum using the spread plate technique. Then amoxicillin-clavulanate (20 µg-10 µg) disc was sandwiched by 30 µg ceftazidime, 30 µg cefpodoxime and 30 µg cefotaxime discs placed 15 to 20 mm, edge to edge from the amoxicillin-clavulanate disc. The plates were incubated aerobically for 16-18 hrs at 35°C. Isolates which showed increase in the inhibition zone of the cephalosporin adjacent to the amoxicillin-clavulanate disc was considered to be ESBL-producing.

Staphylococcus aureus ATTC 25923 and *Escherichia coli* ATTC 25922, and locally isolated MRSA and ESBL producing *E. coli* designated ASU11 and AFU11, respectively, were used as controls during the study.

Results

A total 60 samples, consisting of 30 nasal swabs and 30 hand swabs, were collected from 30 healthcare workers in University of Uyo Teaching Hospital. Of all the healthcare workers, 15 (50%) were doctors and 15 (50%) were nurses, which consisted of 11 (36.7%) males and 19 (63.3%) females.

Of the 60 samples analysed, 48 (80%) yielded bacterial growth, while 12 (20%) showed no bacterial growth. Out of the 48 isolates, 15 (31.3%) and 13 (27.1%) which were all Gram positive, were obtained from the nostrils of doctors and nurses, respectively. Also 13 (27.1%), which included 11 (84.6%) Gram positive and 2 (15.4%) Gram negative bacteria were isolated from the hands of doctors, while 7 (14.5%), which were all Gram positive were isolated from the hands of nurses (Table 1).

Of the 60 samples analyzed, 48 organisms were isolated. Fourty-six isolates were identified as *Staphylococcus* spp, of which 30 (65.2%) were *Staphylococcus aureus* and 16 (34.8%) were *Staphylococcus epidermidis*. The other two isolates were identified as *E. coli* and *Proteus mirabilis,* and were isolated from 2 hand swabs of doctors. *Staphylococcus* spp were isolated from 26 (56.5%) specimens obtained from doctors and 20 (43.5%) from nurses. Of the 26 isolates from doctors, 20 (76.9%) were *Staphylococcus aureus* and from the total 20 isolates from nurses, 10 (50%) were *Staphylococcus aureus*. Generally, 28 (60.9%) Staphylococcal isolates were obtained from the noses of the healthcare workers, while 18 (39.1%) Staphylococcal isolates were obtained from their hands (Table 2a and 2b). A total 12 (40%) Methicillin resistant *Staphylococcus aureus*

Source		Total number of isolates	Gram positive	Gram negative
Nasal	Doctors	15	15 (100%)	0 (0.0%)
	Nurses	13	13 (100%)	0 (0.0%)
	Total	**28**		
Hands	Doctor	13	11(84.6%)	2 (15.4%)
	Nurses	7	7 (100%)	0 (0.0%)
	Total	**20**		

Table 1: Distribution of isolates by Gram reaction.

Organism type	Healthcare workers	
	Doctors (n=28)	Nurses (n=20)
Staphylococcus aureus (%)	20 (76.9%)	10 (50%)
Staphylococcus epidermidis (%) *E. coli* *Proteus mirabilis*	6 (23.1%) 1(5%) 1(5%)	10 (50%) 0 (0%) 0 (0%)

Doctors (n=nasal+hands isolates)=(15+13=28)
Nurses (n=nasal+hands isolates)=(13+7=20)

Table 2a: Prevalence of isolates from nose and hands of healthcare workers.

Organism type	Source	
	Nose (n=28)	Hands (n=20)
Staphylococcus aureus (%)	17 (60.7%)	13 (72.2%)
Staphylococcus epidermidis (%)	11 (39.3%)	5 (27.8%)
E. coli	0 (0%)	1 (5%)
Proteus mirabilis	0 (0%)	1 (5%)

Table 2b: General sources of organism and organism type at different sources for both doctors and nurses.

(MRSA) isolates were obtained from 30 *Staphylococcus aureus* isolated from healthcare workers. Out of the 30 healthcare workers included in this study, 6 (20%) showed MRSA colonization in their noses, and 6 (20%) also showed MRSA colonization in their hands (Table 3).

Also, 5 (10.9%) of the Staphylococccal isolates were inducible Clindamycin resistant, one of the Gram negative bacteria isolated (*Proteus mirabilis*) was Extended Specrum Beta Lactamase (ESBL) producing (Table 4).

The frequency of bacterial colonization was seen to be higher among doctors than the nurses, and a higher frequency of colonization was seen in the noses than the hands of healthcare workers (Figure 1).

The antibiotic sensitivity of the *Staphylococcus aureus* showed 60% to Oxacillin, 43.3% to Erythromycin, 80% to Clindamycin, 40% to Ceftriaxone, 77% to Ciprofloxacin, 23.3% to Trimethoprim Sulphamethoxazole and 73.3% to Amoxicillin clavulanic acid. Thirteen percent of the *Staphylococcus aureus* were found to inducible clindamycin resistant. The antibiotic sensitivity of *Staphylococcus epidermidis* showed 56.2%, 75%, 19%, 81.3%, 12.5% and 68.8% to Erythromycin, Clindamycin, Ceftriaxone, Ciprofloxacin, Trimethoprim Sulphamethoxazole and Amoxicillin clavulanic acid, respectively. Inducible Clindamycin resistant *Staphylococcus epidermidis* were 6.25%. *Escherichia coli* showed 100% sensitivity to Ceftriaxone, Ciprofloxacin, Gentamycin, Ceftazidime and Cefotaxime, but were resistant to Amoxicillin clavulanic acid and Cefpodoxime. It was non-ESBL producing. *Proteus mirabilis* showed 100% sensitivity to Ceftriaxone, Amoxicillin clavulanic acid, Gentamycin and Cefotaxime. It was ESBL producing. Also, the MRSA isolates showed 0%, 50%, 91%, 33.3% and 75% sensitivity to Oxacillin, Erythromycin, Clindamycin, Ceftriaxone, Ciprofloxacin, Trimethoprim Sulphamethoxazole and Amoxicillin clavulanic acid, respectively, and 16.6% of the MRSA isolates were inducible Clindamycin resistant.

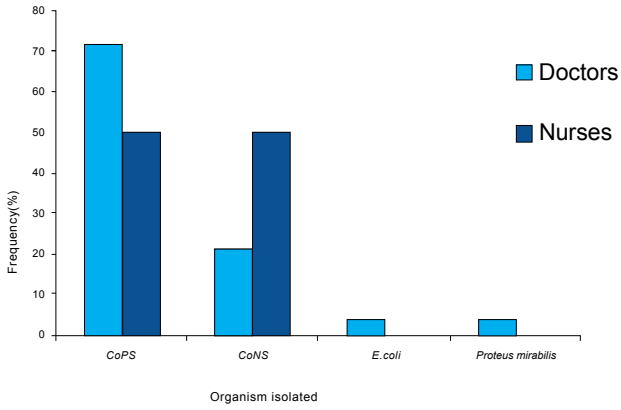
Discussion

A total of 30 healthcare workers, which included 15 doctors and 15 nurses, were included in this study. Out of the 60 (30 nasal swabs and 30 hand swabs) obtained from these healthcare workers, 46 (76.7%) yielded *staphylococci,* of which 30 (65.2%) were coagulase positive and 16 (34.8%) were coagulase negative, 2 (3.3%) yielded Gram negative bacteria (*E. coli* and *Proteus mirablis),* while 12 (20%) showed no bacterial growth. This can be compared to a research carried out by Kumar et al. [14], which included a total of 84 healthcare workers, consisting 60 doctors and 24 laboratory technicians. Of the 84 samples collected from the healthcare workers, 66 (78.6%) yielded *Staphyloccus* spp, which included 40 (60.6%) *Staphylococcus aureus* and 26 *Staphylococcus epidermidis,* while 18 (21.4%) specimens showed no growth of *Staphylococci* colonies, but showed growth of Gram negative bacteria from the nasal swab collected from the anterior nares. The difference in the number of isolates could be attributed to the difference in sample size.

In this study, the prevalence of *Staphylococcus aureus* was 50%; out of these, 40% were MRSA, and the rate of *Staphylococcus epidermidis* was 26.7%. Farzana et al. [15] conducted a similar study and reported the carriage of *S. aureus,* 48%, out of these, 29% were MRSA. Another study by Akoua et al. [16] showed the carriage rate of *S. aureus* 45.4%, and out of these, 38.7% strains were methicillin resistant.

This study revealed that 93% of healthcare workers carry *Staphylococcus* in their nostrils and 66.6% on their hands; this goes to prove that one of the ecological niches for colonization of *Staphylococcus* is the anterior nares, as most of the nasal specimens yield *Staphylococcal* growth on culture.

The greater number of bacteremia cases recorded has been due to *Staphylococcus aureus* of endogenous origin, since they originated from colonies of nasal mucosa. Although nasal carriage of *S. aureus* is harmless in healthy individuals, they can pose the risk of spreading infections to the community at large, and since the section of individuals under this study were healthcare workers, their interaction and exposure to hospital environment could cause major risks in transmitting to hospital patients and spreading nosocomial infections. Kumar et al. [14] also carried out similar research and showed that almost 25% of healthcare workers are stable nasal carriers and 30%-50% of them possess the bacteria in their hands. Tammelin et al. [17] also showed that 50.7% of healthcare workers carry bacteria in their nose and 26.3% in their hands.



Keys: *COPS=Staphylococcus aureus,; CONS=Staphylococcus epidemidis*

Figure 1: Frequency of bacterial colonization in health care workers.

Organisms type	No isolated	Percentage
MRSA MSSA	12 18 **30**	40% 60%

Staphylococcus aureus (n=30)

Keys: MRSA=Methicillin resistant *Staphylococcus aureus*; MSSA=Methicillin sensitive *Staphylococcus aureus.*

Table 3: Colonization with mrsa by healthcare workers.

Antibiotic organism	OX 2 µg	E 15 µg	DA 2 µg	CRO 30 µg	CIP 5 µg	SXT 5 µg	AMC 20-10 µg	CN 10 µg	CPD 30 µg	CAZ 30 µg	CTX 30 µg	ESBL	$iMLS_B$
Staphylococcsaureus (n=30)	18 (60%)	13 (43.3%)	24 (80%)	12 (40%)	23 (77%)	7 (23.3%)	22 (73.3%)	NT	NT	NT	NT	NT	4(13%)
Staphylococcus epidermidis (n=10)	NT	9 (56.2%)	12 (75%)	3 (19%)	13 (81.3%)	2 (12.5%)	11 (68.8%)	NT	NT	NT	NT	NT	1(6.25%)
E.coli (n=1)	NT	NT	NT	1 (100%)	1 (100%)	NT	0 (0%)	1 (100%)	0 (0%)	1 (100%)	1 (100%)	0(0%)	NT
Proteus Mirabilis (n=1)	NT	NT	NT	1 (100%)	0 (0%)	NT	1 (100%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	1(100%)	NT
MRSA (n=12)	0 (0%)	6 (50%)	11 (91%)	4 (33.3%)	9 (75%)	4 (33.3%)	9 (75%)	NT	NT	NT	NT	NT	2 (16.6%)

Keys
NT-Not tested; ESBL-Extended Spectrum Beta Lactamases; $iMLS_B$-Inducible Macrolide Lincomycin Streptogramin B-OX-Oxacillin, 0020.
E-Erythromycin, DA-Clindamycin; CRO-Ceftriaxone; CIP-Ciprofloxacin; SXT-Trimethoprim Sulphamethoxazole.
AMC-Amoxicillin clavulanic acid; CN-Gentamycin; CPD-Cefpodoxime; CAZ-Ceftazidime; CTX-Cefotaxime.

Table 4: Antibiotic sensitivity pattern.

Isolated *Staphylococcus aureus* were most sensitive to Clindamycin (80%) and least sensitive to Trimethoprim sulphamethoxazole (12.5%). The *Staphylococcus aureus* were 60% sensitive to Oxacillin, 43.3% to Erythromycin, 40% to Ceftnaxone, 77% to Ciprofloxacin and 73.3% sensitive to Amoxicillin clavulanic acid and *Staphylococcus epidermidis* were 56.29%, 75%, 19%, 81.3%, 12.5% and 68.8% sensitive to Erythromycin Clindanycin, Ceftnaxone, Ciprofloxacin, Trimethoprum Sulphamethoxazole and Amoxicillin clavulanic acid, respectively. A similar antibiogram was seen in the study carried out by Farzana et al. [15], which revealed 100%, 92%, 90%, 81%, 74%, 70%, 70%, 55%, 11% and 3% sensitivity of *Staphylococcus aureus* to Vancomycin, Cephalothin, Ciprofloxacin, Doxycycline, Amikacin, OxacillinFusidic acid, Erythromycin, Ampicillin and Penicillin, respectively. Also *Staphylococcus epidermidis* showed 100%, 100%, 100%, 93%, 93%, 90%, 92% 64 % and 59% sensitivity to Vancomycin, Ciprofloxacin, Cephalothin, Doxycyclin, Amikacin, Fusidic acid, Eryothromycin, Ampicillin and Penicillin.

Majority of the *Staphylococci* were multidrug resistant. Clindanycin was found to be very effective, but the rate of resistance against Trimethoprim Sulphamethoxazole was highest in the study.

The carriage of Gram negative bacteria among healthcare workers was found in 6.6% of the healthcare workers. Fifty percent of the Gram negative bacteria isolated were ESBL producing. A research by Metri et al. [3] shows that out of 218 *Enterobacteriaceae* isolates, 200 (91.7%) were ESBL producing. Previous studies from India have reported the prevalence of ESBL producers to be 6.6 to 91% [3].

Several researchers have revealed that most of the bacteria that cause nosocomial infections are those that have developed resistance to antibiotics used in treating those [15].

This study indicated high incidence of nasal carriage of *Staphylococci* and a low incidence of Gram-negative bacteria carriage among healthcare workers. There was also the presence of MRSA, ESBL producing bacteria and also inducible Clindamycin resistance *Staphylococcus.* There was also the presence of Methicillin Resistant *Staphylococcus aureus* (MRSA), extended spectrum Beta lactamases (ESBLs) producing bacteria, and also inducible clindamycin resistant *Staphylococcus.* The antibiotic sensitivity of these isolates was seen to be higher for Clindamycin in *Staphylococcus aureus* and ciprofloxacin in *Staphylococcus epidermidis*. *E.coli* was 100% sensitive to five of the antibiotics, while *Proteus mirabilis* was 100% sensitive to four of the antibiotics used, and completely resistant to the rest. The least sensitivity was seen for Trimethoprim Sulphamethoxazole.

There is need for Healthcare workers to wash their hands regularly with antiseptic soap, or to disinfect the hand by rubbing with alcohol solution. Proper infection control measures should be adopted and further research on multidrug resistant organisms and surveillance of nosocomial infection should be carried out. Public enlightenment against the abuse of antibiotics should be carried out.

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