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Research

Incidence of Cephalosporin Resistant Bacterial Flora in Palms of Some University Students and Staff in Ilishan-Remo, Ogun State

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Abstract

Handshake is a common mode of greeting among humans, including both adults and youths. But potentially pathogenic microbial agents have been reported to be transferable through hands. There is, however, a paucity of information on the nature and resistant status of bacterial flora in palms of University students and staff in Ilishan community. Hence, this study was carried out to determine the nature and antibiotic resistant status of bacterial flora in palms of some University students and staff in Ilishan community. Fifty subjects namely, students and staff of tertiary institutions, residing in Ilishan-Remo of Ogun State, Nigeria, were recruited for this study. The surfaces of their palms were aseptically swabbed and inoculated into bijou bottles containing 0.1% peptone water, which were serially diluted ten-fold by Miles and Misra viable plate count technique. Bacterial counts were determined on nutrient agar (for total count) and MacConkey agar (for Enterobacteriaceae count) and the stock suspensions from the 0.1% were aseptically streaked on blood agar and MacConkey agar plates for isolation of bacteria while incubation was carried out aerobically at 37 °C for 24 hours. Identities of the isolated bacteria were confirmed by biochemical test and antimicrobial susceptibility was done on Mueller Hinton agar. Out of the 50 subjects whose palms were screened for microflora, 46 (92.0%) of them were culture positive while 4 (8.0%) were culture negative. Bacillus subtilis recorded the highest frequency of 38.0% while Klebsiella pneumoniae recorded the lowest frequency of 2.8%. Mixed culture of Bacillus subtilis and Staphylococcus epidermidis was highest with a frequency of 16.9%, followed by mixed growth of Staphylococcus epidermidis and Pseudomonas species (15.5%). Highest frequency of antimicrobial resistance was observed against cefuroxime (100.0%), followed by augumentin (77.5%). Antibiotic resistance profile of the isolated bacterial flora from palms of the University students and staff revealed a relatively high frequency of 38.0% multiple drug resistance with augumentin (AMC) and cefuroxime (CXM). In conclusion, this study has established that human palms serve as medium for the carriage and transfer of pathogens, including cephalosporin resistant strains.

KEY WORDS:

INTRODUCTION

The skin flora, more properly referred to as the skin microbiome or skin microbiota, are the microorganisms which resides on the skin (Grice *et al.*, 2009). The total number of bacteria on an average human has been estimated at 1 trillion (Todar, 2006). Most are found in the superficial layers of the epidermis and the upper parts of hair follicles (Todar, 2006). Skin flora are usually non-pathogenic, and are either commensals or symbionts (Cogen *et al*., 2008).

The benefits these bacteria can offer include preventing transient pathogenic organisms from colonizing the skin surface, either by competing with the pathogens for nutrients, secreting chemicals against them, or stimulating the skin's immune system (Cogen *et al*., 2008).

Before now, controversies have lingered over the role of human palms as a medium for the carriage and dissemination of antibiotic resistant pathogens in the human community. However, recent reports from some parts of the world have implicated resident microbes as potential causes of skin diseases and can also enter the blood system to create life-threatening diseases particularly, in immuno-suppressed individuals (Cogen *et al*., 2008). Hygienic control of such flora is therefore, important in preventing the transmission of antibiotic resistant, hospital-acquired infections (Cogen *et al*., 2008).

Examples of skin microbes include; *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Streptococcus mitis, Staphylococcus warneri, Corynebacterium* species, *Acinetobacter johnsonii, Propionibacterium acnes* e.t.c. (Cogen *et al.,* 2008). Transmission of pathogen can occur in various ways. Such means of transmission include physical contact with contaminated food, body fluids, objects, airborne inhalation, or through arthropods.

In developing countries like Nigeria where high mortality have been associated with sepsis, there is a paucity of report on the spread of pathogens via human hands (WHO, 2006) as well as their antibiotic resistant status.

Babcock University (BU) is a residential community that habouring human population of both students and staff up to a capacity of over 12,000 people. Since greetings by hand shake are common practice among BU residents and there is also a paucity of knowledge on the nature as well as the antibiotic resistant status of microbial flora in the palm of BU residents.

The aim of this study is therefore, to ascertain the nature and the antibiotic resistant status of bacterial flora in palm of BU students and staff while the objectives are to:

1. Isolate the bacterial flora on the palms of University students and staff
2. Determine the antibiotic resistance patterns and profile of the isolated bacterial flora from the palms of University staff and students.
3. Come up with suggestions on ways to curtail the incidences and the possible spread of antibiotic resistance strains of microorganisms in Babcock University community.

MATERIALS AND METHODS

This study was carried out in Babcock University, Ilishan-Remo, Ogun State of Nigeria. Babcock University is a private institution, constituted by a community of staff and students from various backgrounds. This study was restricted to the students and staff in the school of Public and allied health (SPAH), School of Nursing Science (SNS), School of Basic and Applied Sciences (BAS) and Babcock University Teaching Hospital (BUTH). The study was conducted between May-July.

Sample collection

With the aid of moistened sterile swab sticks, the entire surfaces of both left and right palms were thoroughly swabbed and the swab was inoculated into bijou bottle containig 0.1% peptone water and was assigned code. Questionnaires were administered to retrieve demographic data from the subjects after which the samples were immediately taken to the laboratory for analysis.

Culture and viable count

The viable count was carried out with Miles and Misra technique. The inoculated 0.1% peptone water (i.e. specimen) was serially diluted ten-fold (i.e. from 10^{-1}-10^{-5}). With the aid of grease pencil, well dried nutrient agar (for total bacterial count) and MacConkey agar (for Enterobacteriaceae count) were partitioned into segments (10^{-1}-10^{-5}). With the aid of sterile 50 dropper Pasteur pipettes, a drop of each dilution was inoculated into each corresponding segment. The plates were covered with lids, left on the bench for a period of 30minutes for adequate absorption of the inoculated sample. The inoculated 0.1% peptone water was streaked unto blood agar and MacConkey agar plates after 1hour incubation period. The cultured plates were aerobically incubated at 37°C for 24 hours. **Note**; the plate count were done in duplicates. After incubation, the colonies of the isolated bacteria in each segment (especially segments containing 20-200 colonies) were counted. The colonies of the two plates were counted and the average taken as the bacterial count. Colony count (CFU/ml) = 50 x no. of colonies x dilution factor. Identity of the isolated organisms was done by Gram staining, spore staining and biochemical tests.

Antimicrobial susceptibility test

With the aid of graduated pipette 0.5ml of 1% (weight/volume) barium chloride was added to 99.5ml of 1% sulphuric acid (volume/volume) to prepare a turbid suspension of barium sulphate (0.5 Mc Farland standard) equivalent to $1x10^8$organisms/mL. With the aid of a pre-sterilized wire loop, loopful inoculums of bacterial colonies were introduced into 0.1% of peptone water. These were incubated overnight at 37℃. About 8mL of the overnight culture was introduced into a test tube and the turbidity of the overnight culture was matched with the turbidity of the 0.5 Mac Farland standard against a transparent light. If the bacterial culture is too turbid, the culture is diluted with fresh sterile peptone water until the bacterial turbidity matches the Mac Farland standard which has an equivalent density of 1×10^{-8} organism/mL. The sensitivity agar plates were allowed to dry properly in an incubator at 40℃ for 2 hours. Using a sterile 50 dropper pipette a drop of the overnight broth culture of organism (containing an equivalent density of 2×10^{-6} organism/mL) was placed at the centre of the plate and spread evenly on the entire surface of the plate with the aid of a pre-sterilized wire loop. A pre-sterilized pair of forceps was used to pick and place antibiotic discs on the surface of the agar. Maximum of 6 discs (of some selected quinolones and cephalosporins) were used per plate as recommended by National Committee of Clinical Laboratory Standard (NCCLS). The sensitivity agar plates were incubated aerobically at 37℃ overnight. The zone of inhibition was measured using a transparent ruler to the nearest millimeter. The measured zone of inhibition was interpreted with the aid of standard reference chat from NCCLS as cited by Ochei and Kolhatkar (2007).

Results

Figure 1 displayed a frequency distribution of microflora in palms of Babcock University (BU) staff and students. Out of the 50 participants whose palms were screened for microflora, 46 (92.0%) of them were culture positive while 4 (8.0%) of them were culture negative.

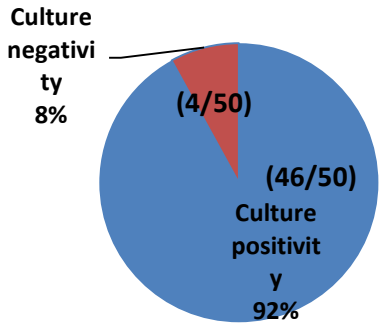

Figure 1: Frequency distribution of microflora culture positivity from palms of BU staff and students.

Table 1 showed a frequency distribution of bacterial isolates from palms of Babcock staff and students. *Bacillus subtilis* recorded the highest frequency of 38.0% while *Klebsiella pneumoniae* recorded the lowest frequency of 2.8%. Mixed growth culture of *Bacillus subtilis* and *Staphylococcus epidermidis* was highest with a frequency of 16.9%, followed by mixed growth culture of *Staphylococcus epidermidis* and *Pseudomonas* species (15.5%).

Table 1: Frequency distribution of bacterial isolates from palms of Babcock staff and students.

Bacterial isolates	Frequency n (%)
Bacillus subtilis	27 (38.0)
Kleb. pneumoniae	2 (2.8)
Staph. epidermidis	11 (15.5)
Bacillus subtilis & Staph. epidermidis	12 (16.9)
Staph. epidermidis & Pseudo. spp	11 (15.5)
Kleb. pneumoniae & Staph. Epidermidis	2 (2.8)
Bacillus subtilis & Pseudo. spp	2 (2.8)
Staph. epidermidis & Providencia spp	2 (2.8)
Kleb. pneumoniae & Pseudo. spp	2 (2.8)
TOTAL	71 (100.0)

Figure 2 showed a frequency distribution of resistance from the isolated bacteria to some selected cephalosporins and quinolones. The highest frequency of antimicrobial resistance was observed against cefuroxime (100.0%) and augumentin (77.5%).

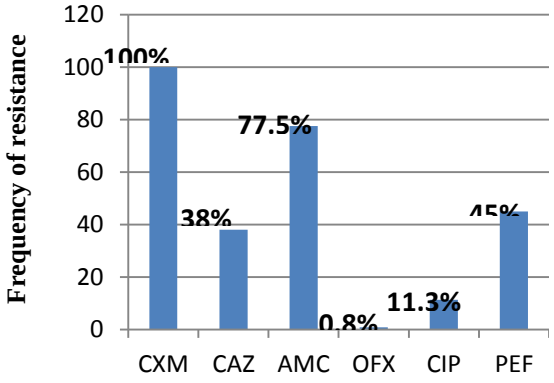

Figure 2: Frequency distribution of antibacterial resisistance from the bacterial isolates observed

KEY:
CXM-Cefuroxime
CAZ-Ceftazidime
PEF-Pefloxacin
OFX-Ofloxacin
CIP-Ciprofloxacin
AMC-Augumentin

Table 2 displayed an antibiotic resistant profile of isolated bacteria from the palms of Babcock staff and students. Highest frequency of multiple drug resistance (38.0%) was registered by the isolated bacteria against augumentin and cefuroxime (AMC/CXM), followed by multiple drug resistance against augmentin, ceftazidime and cefuroxime (AMC/CAZ/CXM).

Table 2: Antibiotic resistance profile of bacterial isolates from palms of babcock staff and students.

Profile of antibacterial agents	n	(%)
CXM	10	(14.0)
AMC/CXM	27	(38.0)
AMC/CXM/PEF	6	(8.5)
AMC/CAZ/CXM/PEF	6	(8.5)
AMC/CAZ/CXM/PEF/OFX/CIP	6	(8.5)
AMC/CAZ/CXM	10	(14.0)
CAZ/CXM/PEF	6	(8.5)
Total	71	(100.0)

Microflora culture positivity was compared in palms of health and non-health workers in Table 3. No significant association was observed in the microflora culture positivity of both health and non-health workers (χ^2 = 2.22, P>0.05).

Table 3: Comparison of frequency of microflora in palms of health and non health workers.

Microflora culture from palms of subjects	Health workers n	(%)	Non-health workers n	(%)	Total
POSITIVE	29	(96.7)	17	(85.0)	46
NEGATIVE	1	(3.3)	3	(15.0)	4
TOTAL	30	(100.0)	20	(100.0)	50

χ^2 =2.22, P > 0.05

Microflora culture positivity was compared in palms of students and staff in Table 4. A significant association was observed in which higher microflora culture positivity was obtained in palms of students (100.0%) than workers (82.6%). (χ^2 = 5.10 , P<0.05).

Table 4: Comparison of frequency of microflora in palms of BU students and staff.

Microflora culture From palms of Subjects	Students n	(%)	Workers n	(%)	Total
POSITIVE	27	(100.0)	19	(82.6)	46
NEGATIVE	0	(0)	4	(17.4)	4
TOTAL	27	(100.0)	23	(100.0)	50

χ^2 =5.10, P < 0.05

In Table 5, the relationship between the occurrence of microflora and the wearing of protective gloves was determined. Microflora culture positivity was found to be significantly lower among subjects that frequently use hand gloves (73.3%) than those who do not (100.0%) when handling dirt. (χ^2 = 10.15 , P < 0.05).

Table 5: Relationship between the occurrence of microflora and wearing of protective hand gloves.

Microflora culture From palms of Subjects	Wearing of protective gloves when handling dirt Yes n	(%)	No n	(%)	Total
POSITIVE	11	(73.3)	35	(100.0)	46
NEGATIVE	4	(26.7)	0	(0)	4
TOTAL	15	(100.0)	35	(100.0)	50

χ^2 =10.15, P < 0.05

Discussion, Conclusion and Recommendation

Both life-threatening diseases and nosocomial infections have been reportedly linked with skin microflora and skin contaminating pathogens (Cogen *et al.*, 2008). In this study, the rate of microflora carriage in palms of Babcock staff and students was about 92%. This high figure is in conformity with the hypothesis that microorganisms are ubiquitous and could found on surfaces including human skin and palms. Different types of bacterial species were isolated from the palms of Babcock staff and students. They are *Bacillus subtilis*, *Staphylococcus epidermidis*, *Pseudomonas* species, *Klebsiella pneumonia* and *Providencia* species. The isolated bacterial flora from the palms of Babcock staff and students were somewhat similar to that observed by Tamberka and Shirsat (2009), in a study conducted in india and Cogen *et al.*, (2008), but differ in the sense that no *Salmonella* species was isolated.

It is important to state that neither yeast nor yeast like organism was isolated from the palms of all the subjects in this study. The isolated bacteria demonstrated a high degree of resistance against cephalosporins, especially cefuroxime (CXM) and Augumentin (AMC). Although, double-disc synergy test (DDST)

could not be conducted in order to ascertain the Extended Spectrum Beta Lactamase (ESBLS) - producing status of the isolated organisms, Rupp and Fey (2003), reported that ESBLS production is one of the commonest mode of bacterial resistance to the cephalosporins. Majority of the isolates demonstrated multiple-drug resistance of which the highest frequency was recorded against augumentin and cefuroxime (AMC/CXM), followed by multiple drug resistance against augmentin, ceftazidime and cefuroxime (AMC/CAZ/CXM).

Comparison of microflora culture positivity in palms of health and non-health workers showed no significant difference (P>0.05). This implies, that contamination of human palms by microorganisms is not occupation-dependent as both health and non-health workers share equal risk of contamination from these category of organisms. Isolation of fecal bacteria (enterobacteriaceae) such as; *Klebsiella pneumoniae* and *Providencia* species from palms is an indication that human palms can serve as a medium for the carriage and spread of fecal pathogens.

In conclusion, this study has established that human palms can serve as a medium for the carriage and transfer of pathogens, including cephalosporin resistant strains. However, microflora contamination of palms can be effectively reduced by wearing of protective gloves when handling dirt and frequent washing of hands.

Based on the outcome of this study, it is therefore recommended that the BU Authority should commence a periodic and regular enlightenment programmes on "how both staff and students can effectively maintain healthy palm and handshake".

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