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A Survey of Bacterial Isolates Cultured from Apparently Healthy Individuals in South-Western Nigeria

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Abstract: The study was undertaken to isolate and identify bacterial species colonising six different body sites of apparently healthy students. Three hundred and one students participated in the study. The mean age of male participants was 22.1 years and that of the females' was 22.2 years. About 886 samples were taken from the participants. Altogether, 1394 bacterial isolates were cultured from the samples averaging 1.57 bacteria per sample. Samples from the foot, hand and face were collected into sterile saline and a loop-ful of each sample was applied on blood agar, selective and differential media and other conventional media and incubated at 37°C for 24-48 h. Samples from the ear, nose and throat were collected with sterile cotton-tipped applicators after which each applicator had been dipped into sterile saline and processed. Characterisation of bacterial isolates was initially based on gram-reaction. Cocci that appeared in clusters and fermented mannitol on mannitol salt agar, coagulated human pooled plasma were confirmed as *Staphylococcus aureus* isolates. Those that neither fermented mannitol on (MSA) nor coagulated human pooled plasma were deemed *Staphylococcus* sp. Cocci in small chains on blood agar were deemed Streptococci and their pattern of hemolysis or lack of it on blood agar plates was used to classify them. Gram negative rods were categorised as lactose fermenters or non-lactose fermenters based on their reaction on Triple Sugar Iron agar (TSI). Others gram-negative enteric rods were characterised based on their reactions on Eosin Methylene Blue agar (EMB) and other conventional media. Antibiotic susceptibility tests were done on some isolates using the disc dilution method. Out of the 1394 bacterial isolates cultured, gram-positive isolates constituted 57.8% and gram-negative enteric rods 42.2%. Staphylococci accounted for 32% of gram-positive bacterial isolates with *Staphylococcus aureus* being 72.8% and *Staphylococcus* sp. was 27.1%. *Staphylococcus aureus* (23.3%) was the single most predominant gram-positive bacterial isolate cultured followed by *E. coli* (14.0%) which was the single most predominant gram-negative bacteria seen. Other gram-positive isolates seen include *Streptococcus* sp. (11.3%), *Bacillus* sp. (9.9%) and *Corynebacterium* sp. (2.4%). Gram negative rods seen were: *Klebsiella* sp. (10.6%), *Pseudomonas* sp. (5.5%) *Proteus* sp. (5.0%), *Pseudomonas aeruginosa* (3.4%), *Salmonella* sp. (1.9%) *Citrobacter* sp. (1.1%) and *Shigella* sp. (0.6%). The antibiotic resistant profile showed 69.9% *S. aureus* isolates were resistant to amoxicillin 60.3% to augmentin and 60.8% to cloxacillin however, some *S. aureus* isolates were more amenable to ofloxacin (12%) while other *S. aureus* isolates demonstrated multi-resistance.

Key words: Skin bacteria-flora, apparently healthy, students, antibiotic resistance, Nigeria

INTRODUCTION

The human body harbours bacteria that reveal a vast diversity in several human-associated bacterial communities (Dethlefsen *et al*., 2007). Among one of the largest human-associated microbial habitats is the skin, a body habitat with complex variations in cellular orientation and environmental exposures where bacterial population may sometime be as high as 10^7 cells per square centimetre (Turnbaugh *et al*., 2007; Fredricks, 2001). Many of the bacteria thriving on or within the skin are not passive or transient colonisers but rather appear to be adapted to specific challenges associated with living in different regions of the skin like antimicrobial host defences, exposure to harsh conditions like soap and detergent and other milieu which test the habitat's

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resilience continuously (Roth and James, 1988; Cogen *et al*., 2008). It has been shown that both culture-based and molecular studies reveal significant amount of intra and inter-individual variability in the composition of skin-associated bacterial communities (Roth and James, 1988; Gao *et al*., 2007). The dynamic nature of bacterial population replenishment in the skin is impressive as many factors driving this variability in skin community composition are yet to be fully comprehended (Grice *et al*., 2008). The indigenous micro flora often described as normal flora plays a vital role in the host in preventing colonization with pathogens. Normal flora plays a role both in health and in causation of disease (Chiller *et al*., 2001; Dechen *et al*., 2011). Although, microorganisms constituting normal flora are non-pathogenic in their usual anatomic location, they can be pathogenic in other parts of the body (Dechen *et al*., 2011). The study was carried out to determine the bacteria colonising six different body surfaces of apparently healthy students in a tertiary institution setting. Because these students are physically active and live in overcrowded hostels or dormitories an often in close proximity to one another, sharing common physical facilities such as water and sanitation facilities, they are vulnerable to infections caused by bacterial agents hence, the study. The antibiotic sensitivity pattern of some of these isolates was also determined in order to assist clinicians in treating infections that may be caused by these agents in the event of an epidemic in this environment.

MATERIALS AND METHODS

Study centre: The study was undertaken between June to December 2011 at the Obafemi Awolowo University, Ile-Ife, in South-Western Nigeria. The institution is one of the premiere universities in the country with a population of over twenty eight thousand students. Participants were students of the institution randomly recruited on voluntary basis. Information relating to each participant was obtained from a structured questionnaire response.

Inclusion criteria: Subjects were admitted into the study if they were apparently healthy, showed no signs of illness or suffered from any skin disease. No subject on antibiotics or/and on illicit drugs use such as narcotics was admitted to the study.

Collection of sample from hands and feet and determination of total bacterial load: For determination of total bacterial count per sample, 20 mL of distilled water was dispensed into clean 50 mL glass bottles and autoclaved at 121°C for 15 min. Several of such bottles were prepared. Similarly, 4.5 mL of distilled water was dispensed into clean Pyrex test-tubes and autoclaved at 121°C for 15 min such test-tubes served as diluent for quantitative serial dilution of samples collected from each subject. Each subject was instructed to place the foot upon one another and 20 mL of the distilled sterile water was poured on the top foot and subject was instructed to rub the top foot over the second foot into a clean plastic bowl collector (The foot refers to the top skin and sole of the foot).

The hands were also washed, each hand washing the other with 20 mL of sterile distilled water (The hand here refers to the palm, the fingers web and back skin of the palm). Thereafter, a tenfold serial dilution was carried out using 0.5 mL of aliquot obtained from each sample and introducing into 4.5 mL of distilled sterile saline diluent and further serial dilutions were made as appropriate depending on the turbidity of initial sample. The 0.1 mL of each diluent was subsequently introduced into plating agar usually plate count agar and spread over the plating medium with a glass spreader then incubated at 37°C for 24-48 h to determine total bacterial load per sample.

Collection and processing of sample from the face: Each subject was instructed to wear sterile disposable gloves on the hand and then 20 mL of sterilised distilled water was poured to gently wash the face (The face here refers to the margin of the fore head not encroaching the hair, the surface of face skin not including the earlobes and not below the subject's chin). Determination of total bacterial count per sample and qualitative analysis of bacterial isolates were done as described above for the foot and hand.

Collection, processing of sample from ear, nose and throat: Samples were collected from ear, nose and throat of each subject with the aid of cotton-tipped applicators (one applicator for each sample).

Each applicator was initially dipped into sterile saline and introduced into the inner ear, rolled gently and retrieved. Same procedure was done for the anterior nare and the throat. Each of such applicator samples was applied onto selective and differential media, streaked with aid of heat flamed loop on each medium and the plates were thereafter incubated at 37°C for 24-48 h for growth. Each colony that grew on these media was picked and characterized.

Isolation and identification of bacterial isolates in samples: A loopful each of aliquot obtained from the initial sample collected from each subject was applied onto conventional media such as Mannitol Salt Agar (MSA), Eosin Methylene Blue agar (EMB), Blood Agar (BA), Nutrient Agar (NA) and Chocolate Agar (CA).

Each sample was streaked onto the plates by heat flamed inoculating loop. Such samples were initially incubated at 37°C for 24-48 h. Colonies appearing on these media were picked and studied individually. Each colony was initially gram stained and on basis of the gram reaction was inoculated onto selective and differential media (Cowan and Steel, 1985; Murray *et al*., 1999). Cocci in clusters that fermented mannitol on MSA were deemed as Staphylococci and confirmed as *Staphylococcus aureus* isolates by slide and tube coagulase tests using pooled human plasma (Cowan and Steel, 1985; Murray *et al*., 1999). Those cocci in clusters that did not ferment mannitol on MSA were considered Coagulase Negative Staphylococci (CNS). Cocci in chains were applied onto blood agar plates and based on haemolysis on blood further studied.

Gram-negative rods that grew on EMB agar were also studied. Those that produced haemolysis on blood agar and also developed greenish metallic sheen were deemed as *Escherichia coli* and further confirmed as lactose fermenters on Triple Sugar Iron agar (TSI). Mucoid colonies on EMB agar that produced acid on slant and butt in TSI agar without gas production were deemed as *Klebsiella* species. Non-lactose fermenters were also confirmed by colour and production of greenish yellow colonies on nutrient agar as *Pseudomonas* sp. other gram negative rods were confirmed using conventional methods (Cowan and Steel, 1985; Murray *et al*., 1999).

Antibiotic sensitivity test: The antibiotic sensitivity of some of the bacteria isolated was determined by the method of Ericsson and Sherris (1971). The plating medium employed was Mueller-Hinton agar (provided by Merck, Darmstadt, Germany). The antibiotics used included chloramphenicol (30 μg), erythromycin (5 μg), gentamicin (10 μg), augmentin (30 μg), ofloxacin (30 μg), cloxacillin (5 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), amoxicillin (25 μg), cotrimoxazole (25 μg) and tetracycline (30 μg), obtained from Abtek Biological Ltd. Liverpool, UK. *S. aureus* 25923 was used as the control organism. The diameter of the zones of inhibition was measured to the nearest millimetre and results were interpreted based on the National Committee for Clinical Laboratory Guidelines (NCCLS in 1997) (CLSI, 2007).

RESULTS AND DISCUSSION

Six different body sites were cultured for bacteria Table 1 shows the profile of the distribution of the bacterial isolates recovered from these sources. One thousand three hundred and ninety four bacterial isolates were cultured from eight hundred and seven samples obtained from three hundred and one subjects. Figure 1 reflects this pattern. The bacterial isolates cultured from

Table 1: Profile of distribution of bacterial isolates from six body sites of subjects

			Number (%)					
Bacteria	Bacterial isolate	Total No. cultured	From feet	From hands	From face	From ear	From nose	From throat
Gram positive bacteria	Gram positive cocci Staphylococci							
	Staphylococus aureus	325 (23.3)	63 (18.6)	64 (18.7)	22 (12.4)	76 (30.8)	84 (35.6)	16 (31.4)
	Staphylococcus sp.	121 (8.7)	48 (14.2)	48 (14.0)	5 (2.8)	15 (6.1)	5 (2.1)	-
	Streptococci							
	Streptococcus sp.	158 (11.3)	35 (10.3)	23 (6.7)	13 (7.3)	21 (8.5)	34 (14.4)	32 (62.7)
	Micrococci							
	Micrococcus sp.	31(2.2)	9 (2.6)	6 (1.7)	5 (2.8)	8 (3.2)	3 (1.3)	-
	Gram positive rods							
	Bacillus sp.	138 (9.9)	27 (8.0)	42 (12.2)	13 (7.3)	26 (10.5)	29 (12.3)	1 (2.0)
	Corynebacterium sp.	34 (2.4)	1 (0.3)	11 (3.2)	12 (6.8)	6 (2.4)	4 (1.7)	-
Gram negative bacteria	Gram negative enteric rods							
	Escherichia coli	194 (14.0)	51 (15.0)	75 (21.9)	38 (21.3)	12 (4.9)	16 (6.8)	2 (3.9)
	Klebsiella sp.	148 (10.6)	42 (12.4)	16 (4.7)	31 (17.4)	30 (12.1)	29 (12.3)	-
	Genus *Pseudomonas*	124.0	23.0	32.0	6.0	41.0	22.0	-
	Pseudomonas sp.	76 (5.5)	7 (2.1)	18 (5.2)	4 (2.2)	35 (14.2)	12 (5.0)	-
	Pseudomonas aeruginosa	48 (3.4)	16 (4.7)	14 (4.1)	2 (1.1)	6 (2.4)	10 (4.2)	-
	Proteus sp.	70 (5.0)	20 (5.9)	17 (4.9)	25 (14.0)	5 (2.0)	3 (1.3)	-
	Salmonella sp.	27 (1.9)	15 (4.4)	4 (1.2)	2 (1.1)	3 (1.2)	3 (1.3)	-
	Citrobacter sp.	15 (1.1)	5 (1.5)	3 (0.9)	1 (0.6)	2 (0.8)	4 (1.7)	-
	Shigella sp.	09 (0.6)	-	2 (0.6)	5 (2.8)	2 (0.8)	-	-
	Total (%)	1394.0	339 (24.3)	343 (24.6)	178 (12.8)	247 (17.7)	236 (16.9)	51 (3.7)

Table 2: Pattern of distribution of bacterial isolates from feet, hands and face of subjects

Bacteria	Bacterial isolate	Total No. cultured	Number (%) From feet	From hands	From face
Gram positive bacteria	Gram positive cocci Staphylococci				
	Staphylococus aureus	149 (17.3)	63 (18.6)	64 (18.7)	22 (12.4)
	Staphylococcus sp.	101 (11.7)	48 (14.2)	48 (14.0)	5 (2.8)
	Streptococci				
	Streptococcus sp.	71 (8.3)	35 (10.3)	23 (6.7)	13 (7.3)
	Micrococci				
	Micrococcus sp.	20 (2.3)	9 (2.6)	6 (1.7)	5 (2.8)
	Gram positive rods				
	Bacillus sp.	82 (9.5)	27 (8.0)	42 (12.2)	13 (7.3)
	Corynebacterium sp.	24 (2.8)	1 (0.3)	11 (3.2)	12 (6.8)
Gram negative bacteria	Gram negative enteric rods				
	Escherichia coli	164 (19.1)	51 (15.0)	75 (21.9)	38 (21.3)
	Klebsiella sp.	89 (10.3)	42 (12.4)	16 (4.7)	31 (17.4)
	Proteus sp.	62 (7.2)	20 (5.9)	17 (4.9)	25 (14.0)
	Genus *Pseudomonas*	61.0	23.0	32.0	6.0
	Pseudomonas sp.	29 (3.4)	7 (2.1)	18 (5.2)	4 (2.2)
	Pseudomonas aeruginosa	32 (3.7)	16 (4.7)	14 (4.1)	2 (1.1)
	Salmonella sp.	21 (2.4)	15 (4.4)	4 (1.2)	2 (1.1)
	Citrobacter sp.	9 (1.0)	5 (1.5)	3 (0.9)	1 (0.6)
	Shigella sp.	7 (0.8)	-	2 (0.6)	5 (2.8)
	Total (%)	860.0	339 (39.4)	343 (39.9)	178 (20.7)

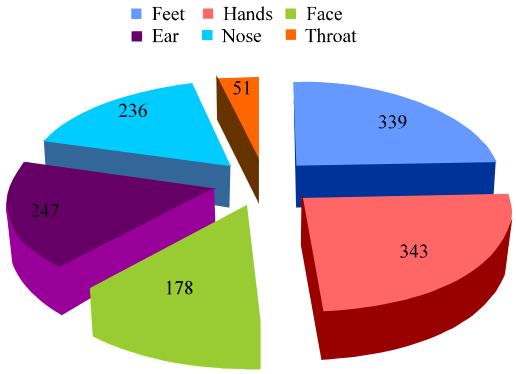

Fig. 1: Distribution of bacterial isolates from different six body sites

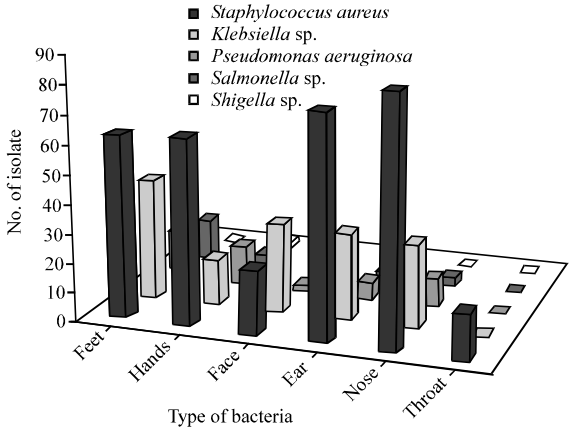

Fig. 2: Distribution of pathogenic organisms isolated from subjects

limbs that is feet and hands (fore-arm) and the faces of subjects are shown in Table 2 and 3 while the antibiotic resistance profile of some of the bacterial isolates, 273 in all of total isolates cultured from the six sites is shown in Table 4.

Table 5 shows the multiple antibiotic resistance profile of some of the isolates while Fig. 2 is the distribution of the opportunists and pathogenic enteric rods cultured from the subjects.

The study was carried out to determine the bacterial isolates colonising the six different body sites of apparently healthy students in order to provide information regarding the types and form to assist clinicians to better treat infections that may be caused by these agents. Three hundred and one students participated in the study. The mean age of the female participants was 22.2 and 22.1 years for the male participants. Altogether, 886 samples were taken from the six sites and cultured for bacteria. One thousand, three hundred and ninety four bacterial isolates were recovered from the six different body sites averaging 1.57 bacteria per sample (Fig. 1).

The distribution of bacterial isolates recovered from each body site is shown in Table 1. About 48.9% of the total bacterial isolates were cultured from feet (24.3%) and hands (24.6%) followed by the ear (17.7%), nose (16.9%) and throat (3.7%), respectively. About a half (48.9%) of the total bacterial isolates recovered from the study were from the limbs (feet/hands). The reason for the high frequency of bacteria from the limbs particularly the fore arm may be due the multifaceted functions of the hand (shaking of hands, processing many activities

Table 3: Pattern of distribution of bacterial isolates from ear, nose and throat of subjects

Bacteria	Bacterial isolate	Total No. cultured	Number (%) From ear	From nose	From throat
Gram positive bacteria	Gram positive cocci Staphylococci				
	Staphylococus aureus	176 (33.0)	76 (30.8)	84 (35.6)	16 (31.4)
	Staphylococcus sp.	20 (3.7)	15 (6.1)	5 (2.1)	-
	Streptococci				
	Streptococcus sp.	87 (16.3)	21 (8.5)	34 (14.4)	32 (62.7)
	Micrococci				
	Micrococcus sp.	11 (2.1)	8 (3.2)	3 (1.3)	-
	Gram positive rods				
	Bacillus sp.	56 (10.5)	26 (10.5)	29 (12.3)	1 (2.0)
	Corynebacterium sp.	10 (1.9)	6 (2.4)	4 (1.7)	-
Gram negative bacteria	Gram negative enteric rods				
	Genus *Pseudomonas*	63	41.0	22	-
	Pseudomonas sp.	47 (8.8)	35 (14.2)	12 (5.0)	-
	Pseudomonas aeruginosa	16 (3.0)	6 (2.4)	10 (4.2)	-
	Klebsiella sp.	59 (11.1)	30 (12.1)	29 (12.3)	-
	Escherichia coli	30 (5.6)	12 (4.9)	16 (6.8)	2 (3.9)
	Proteus sp.	8 (1.5)	5 (2.0)	3 (1.3)	-
	Citrobacter sp.	6 (1.1)	2 (0.8)	4 (1.7)	-
	Salmonella sp.	6 (1.1)	3 (1.2)	3 (1.3)	-
	Shigella sp.	2 (0.4)	2 (0.8)	--	-
	Total (%)	534.0	247 (46.2)	236 (44.2)	51 (9.6)

Table 4: Pattern of antibiotic resistance of some bacterial isolates cultured from subjects

Bacteria	Bacteria species	NO	AUG	AMX	ERY	TET	CXC	COT	GEN	CHL	OFL	NIT	NAL
Gram+	Gram positive cocci Staphylococci												
	Staphylococus aureus	156	94 (60.3)	109 (69.9)	80 (51.3)	81 (51.9)	95 (60.8)	87 (55.7)	53 (33.9)	62 (39.7)	19 (12.2)	32 (20.5)	27 (17.3)
	Staphylococcus sp.	28	27 (96.4)	26 (92.9)	24 (85.7)	19 (67.9)	23 (82.1)	23 (82.1)	1 (3.6)	23 (82.1)	17 (60.7)	17 (60.7)	17 (60.7)
	Streptococci												
	Streptococcus sp.	79	51 (64.5)	59 (74.7)	56 (70.9)	45 (56.9)	50 (63.3)	51 (64.5)	23 (29.1)	52 (65.8)	10 (12.7)	21 (26.6)	11 (13.9)
	Micrococci												
	Micrococcus sp.	12	11 (91.7)	10 (83.3)	12 (100.0)	12 (100.0)	11 (91.7)	12 (100.0)	10 (83.3)	12 (100.0)	9 (75.0)	11 (91.7)	10 (83.3)
	Gram positive rods												
	Bacillus sp.	82	59 (71.9)	67 (81.7)	58 (70.7)	57 (69.5)	53 (64.6)	53 (64.6)	33 (40.2)	47 (57.3)	42 (51.2)	50 (60.9)	35 (42.7)
	Corynebacterium sp.	9	9 (100.0)	9 (100.0)	9 (100.0)	9 (100.0)	9 (100.0)	9 (100.0)	5 (55.6)	9 (100.0)	4 (44.4)	9 (100.0)	9 (100.0)
Gram -	Gram negative enteric rods												
	Escherichia coli	35	30 (85.7)	30 (85.7)	15 (42.9)	26 (74.3)	9 (25.7)	18 (51.4)	19 (54.3)	10 (28.6)	11 (31.4)	33 (94.3)	12 (34.3)
	Klebsiella sp.	60	38 (63.3)	42 (70.0)	12 (20.0)	51 (85.0)	34 (56.7)	33 (55.0)	23 (38.3)	4 (6.7)	6 (10.0)	28 (46.7)	27 (45.0)
	Genus *Pseudomonas*												
	Pseudomonas sp.	30	30 (100.0)	26 (86.7)	24 (80.0)	27 (90.0)	-	4 (13.3)	21 (70.0)	13 (43.3)	15 (50.0)	28 (93.3)	3 (10.0)
	Pseudomonas aeruginosa	30	12 (40.0)	9 (30.0)	1 (3.3)	4 (13.3)	1 (3.3)	8 (26.7)	22 (73.3)	1 (3.3)	4 (13.3)	30 (100.0)	9 (30.0)
	Proteus sp.	29	26(89.7)	22 (75.7)	16 (55.2)	18 (62.1)	20 (69.0)	23 (79.3)	15 (51.7)	20 (69.0)	10 (34.5)	23 (79.3)	18 (62.1)
	Salmonella sp.	4	4 (100.0)	4 (100.0)	-	2 (50.0)	-	4 (100.0)	1 (25.0)	-	1 (25.0)	4 (100.0)	3 (75.0)
	Citrobacter sp.	4	4 (100.0)	3 (75.0)	-	2 (50.0)	-	3 (75.0)	-	-	1 (25.0)	3 (72.0)	1 (25.0)
	Shigella sp.	2	2 (100.0)	2 (100.0)	-	1 (50.0)	-	2 (100.0)	-	-	-	2 (100.0)	-
	Total	560	397 (70.9)	418 (74.6)	307 (54.8)	354 (63.2)	305 (54.5)	330 (58.9)	226 (40.4)	253 (45.1)	149 (26.6)	291 (51.9)	182 (32.5)

including eating in this environment) and for the feet (their proximity to the soil) where they are constantly in contact with the saprophytic microorganisms may account for their relative high number. Overall, gram-positive bacteria constituted (57.9%) of the total bacterial isolates cultured from the six sites with gram-negative bacteria accounting for (42.1%) (Table 1). Gram-positive cocci accounted for 78.7% of the gram-positive bacterial isolates organisms of which staphylococci were 70.2% and *S. aureus* led the group with 72.8% and CONS 27.2%. *Bacillus* sp. and *Corynebacterium* sp. constituted 21.3% of gram-positive bacilli. Non-haemolytic streptococci also accounted for 24.9% of cocci and *Micrococcus* sp. 3.9%.

The predominance of *S. aureus* on the skin surface of apparently healthy individuals suggests the frequency of colonization of these active individuals in this community. The results of the study showed *Staphylococcus aureus* was the single most predominant isolate cultured from subjects. Of the total 325 *S. aureus* isolates recovered, 18.6% were recovered from feet, 18.7% from hands and 12.4% from face. The frequency of distribution of *S. aureus* isolates in the orifices was much higher. Of the 325 isolates cultured, 30.8% were recovered from ear, 35.6% from the nose and 31.4% from the throat. It also suggests the possibility of these individuals being carriers that can easily disseminate these organisms in the community.

Table 5: Pattern of multiple antibiotic resistance of some bacterial isolates cultured from subjects

		No. of antibiotics to which isolares are resistant					
Bacteria specie	No. tested	8	7	6	5	4	3
Gram positive bacteria							
Staphylococus aureus	78	2	3	8	16	36	6
Staphylococcus sp.	11	-	-	1	5	2	2
Streptococcus	47	2	3	9	15	13	6
Bacillus sp.	24	-	-	1	7	9	5
Gram negative bacteria							
Escherichia coli	11	-	1	3	4	3	-
Klebsiella sp.	34	-	1	5	8	12	5
Genus *Pseudomonas*							
Pseudomonas sp.	4	-	1	1	1	1	-
Pseudomonas aeruginosa	20	-	-	2	1	2	1
Salmonella sp.	2	-	-	-	1	1	-
Citrobacter sp.	4	-	-	1	1	1	-
Shigella sp.	2	-	-	-	1	1	-
Total	229	4	9	31	60	81	25

Many investigators have reported different values for *S. aureus* isolates nasal carriage in this environment, Paul *et al.* (1982) recorded a 50% *S. aureus* carrier rate among hospital patients with the incidence in patients and staff similar. Lamikanra *et al.* (1985) reported 55.7% overall carriage rate with 46.5% rate occurring in males and 65.0% among females students whose age ranged from 21-23 years. In a long term study of nasal carriage of *S. aureus* among 323 healthy Nigerian students, the overall carriage rate was 38% (Lamikanra and Olusanya, 1988). However, the current study showed a 35.6% carriage rate among males whose mean age was 22.1 years and females mean age 22.2 years which is lower than other values reported in this environment. However, this carriage rate is within the 35.6-56% nasal carrier rate reported in this environment in the last 30 years.

Recent reports of *S. aureus* protein profiling in the laboratory suggest identical community *S. aureus* biotypes have been found to also exist in hospital environment (Nkem, 2001). The implication of these findings is that community isolates may easily spread to hospital environment if the frequency of nasal colonization is not checked. Nasal carriers of *S. aureus* isolates have been reported to transfer these isolates to siblings and co-workers. Studies by Ako-Nai *et al.* (1991) showed the transfer of identical *S. aureus* phage-types between mothers and neonates and hospital personnel and neonates underscoring the epidemiological significance of curtailing the spread of these isolates in the community.

The frequency of isolation of the predominant bacterial isolates was compared. The frequency of *S. aureus* isolates from the three orifices ear 30.8%, throat 31.4% and nose 35.6% was significantly higher than the values obtained from samples from the feet 18.6% hands 18.7% and face 12.4% which underscores the fact that the frequency of the single most predominant isolate *S. aureus* was more prominent in orifices than in the limbs and the face. On the contrary, the frequency of single most predominant enteric gram-negative rod cultured from the limbs (feet and hands) *E. coli* on the feet (15.0%), hand (21.9%) and face (21.3%) was significantly higher than the frequency from the ear (4.9%) nose (6.8%) and throat (3.9%), suggesting that this opportunistic pathogen is more likely to be encountered at these sites than in the three orifice surfaces (Table 2 and 3).

The results obtained from the orifices namely: ear, nose and throat also showed that gram-negative enteric rods constituted 42.1% of which gram-negative lactose fermenters *E. coli* and *Klebsiella* sp. accounted for 58.3%. *E. coli* was the single most predominant enteric rod (33.0%) followed by *Klebsiella* sp. (25.2%).

It is interesting to note that opportunistic enteric gram-negative rods, *Pseudomonas* sp., *Pseudomonas aeruginosa* and pathogens like *Salmonella* sp. and *Shigella* sp. altogether accounted for 36.0% of the total enteric rods. The isolation of potentially pathogenic bacteria among apparently healthy students is worrisome and indicates these individuals are carrying bacteria that could be deleterious to their health. The sources of these pathogenic bacteria could be associated with subjects' lifestyles. Clean hygienic living is vital in reducing colonization with bacteria including enteric bacteria. In a situation where participants live in overcrowded environment with poor sanitation and polluted water supply, culturing of these organisms is certain.

Antibiotic susceptibility testing of 273 of the total bacteria isolates recovered from subjects was carried out. The results showed 69.9% of the *S. aureus* isolates were resistant to amoxicillin, 60.8% to cloxacillin and 60.3% to augmentin. However, only 17.3% of *S. aureus* isolates were resistant to nalidixic acid, 20.5% to nitrofurantoin and 12.2% to ofloxacin. The results suggest penicillins were ineffective among *S. aureus* isolates tested.

However, *S. aureus* strains susceptibility to quinolones was better as only 12.2% of the *S. aureus* isolates tested were resistant to ofloxacin (Table 4).

Resistance profile among enteric lactose fermenters was even higher than for *S. aureus* isolates (Table 4). While *E. coli* may not be considered a major pathogen, 85.7% of strains tested were resistant to both augmentin and amoxicillin and 25.7% to cloxacillin.

The opportunistic pathogen *Pseudomonas* sp. resistance pattern was strikingly high. All the 30 (100%) isolates tested were resistant to augmentin and 86.7% to amoxicillin. Resistance to ofloxacin was 31.4% (Table 4). The multiple antibiotic resistance profile among the bacterial isolates was also evaluated. Out of the 78 *S. aureus* isolates tested, 2 were resistant to 8 antibiotics, 3-7 antibiotics; 8-6, 16-5, 36-4 and 6 isolates to 3 antibiotics (Table 5). The results showed the total bacterial load recorded for different body sites differed. Samples cultured from the fore arms and the feet had relatively high microbial counts (2.00×10^5-3.10×10^6 CFU/sample) compared to those samples from the ear, nose and the throat (2.50×10^2-3.00×10^3 CFU/sample). It has been reported that most of these organisms inhabit the stratum corneum and the upper parts of the hair follicles. A small number however are present deeper within follicles and serve as a reservoir for replenishing flora after washing (Larson, 2001; Langley, 2002). Other studies showed while washing may decrease skin counts by 90% however, normal numbers are found by 8 h. Abstinence from washing does not lead to an increase in numbers of bacteria on the skin. Normally 1000-10000 organisms are found per square centimetre. However, counts may increase to one million/cm in humid areas such as groin and other areas where the skin overlaps.

CONCLUSION

The study showed gram-positive bacterial isolates predominated among microflora recovered from apparently healthy subjects screened with *S. aureus* isolates predominating. The results also showed the prevalence rate of (35.5%) nasal carriage of *S. aureus* isolates seen this study is remarkably within the 30-56% rates obtained in different studies in the last 30 years. The results also revealed the *S. aureus* isolates were more likely to be cultured from the three orifices than the limbs and face of apparently healthy students. The results also showed high prevalence of multi-resistant *S. aureus* isolates among these individuals which is of epidemiologic importance in controlling infections that may be caused by these isolates. The high frequency of enteric rods (42.1%) in skin flora of the subjects suggests contamination of these individuals probably due to personal hygiene. The study suggests improvement of individuals' personal hygiene and living conditions in the hostels and dormitories should be advocated to reduce the frequency of contaminations with enteric gram-negative rods.

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