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Qualitative and quantitative analysis on colonizing facial bacterial strains among tertiary students of Iligan City

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Abstract

The most prominent skin-covered anatomical region is the human face which is a privileged site for growth of microorganisms. This study was conducted to provide information in establishing healthy baseline of facial microflora. This study involved a convenience sample of 135 healthy tertiary students with no evident skin infections. The skin surface samples were obtained by the swab method and all samples were allowed to grow on nutrient agar to determine colonization rates. All clinical samples exhibited bacterial growth on nutrient agar while 87% of the MSA plates had growth of bacterial colonies. Two hundred sixty-seven isolated bacterial colonies were randomly picked from NA and MSA plates and were purified and subjected to different identification techniques - colonial characterization, staining for cellular morphologies and biochemical tests. Through these conventional methods of identification, four bacterial species were identified up the genus level: *Staphylococcus* remained the predominant genus of the superficial skin microbiota at 39% (*Staphylococcus aureus*) and 19% (coagulase negative staphylococci); followed by *Micrococcus* (2%), and *Bacillus* sp.(1%).

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Introduction

The skin is the human body's largest organ, colonized by a diverse milieu of microorganisms (Grice and Segre, 2011). Microorganisms inhabiting superficial skin layers are known as skin microbiota which include bacteria, viruses, archaea and fungi (Murilla and Raoult, 2013) and are classified as pathogens, potential pathogens or innocuous symbiotic organisms (Cogen *et al.*, 2008). Collectively, the normal microflora are beneficial to the skin because they prevent colonization of pathogens from excreting chemicals such as fatty acids, gases, alcohols, and antibiotics and they control overgrowth of other microoganisms present on the skin through antagonism and competition for nutrients (Todar, 2012).

However, the epidermis is not a favorable environment for colonization of diverse bacterial species due to several factors: it is subjected to periodic drying that drives many resident microbiota into dormant state, it has a slightly acidic pH- owed to the organic acids produced by staphylococci and secretions from skin oil and sweat glands that discourages colonization by many microorganisms, presence of high concentration of sodium chloride from sweat that makes the skin hyperosmotic causing osmotic stresses to microorganisms and the existence of certain inhibitory substances that help control colonization, overgrowth and infection from resident microorganisms (Prescott, 1990). An enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel promicrobial and antimicrobial therapeutic approaches for their treatment (Grice and Segre, 2011). This study aimed to determine the qualitative and quantitative composition of the facial microbiota of volunteer tertiary students of Iligan City, Philippines

Materials and methods

Collection of Facial Swabs

Representative tertiary students of Iligan City, Philippines formed the study population. Each subject was ask to fill-up a self-administered questionnaire pertaining to their social and health conditions which included age, sex, status, inclination to sports, use of any cosmeceuticals and medical history. Facial specimens from cheeks of each respondent were collected by a premoistened sterile swab. The specimens were immediately processed on Nutrient Agar (NA) and Mannitol Salt Agar (MSA) plates for the detection and identification of aerobic bacteria and salt-tolerant bacterial, respectively.

Determination of Facial Microbial Colonization

Nutrient Agar was used to allow the growth of all heterotrophic bacteria obtained from the facial swabs while MSA was used to confirm carriage and define topography of *Staphylococcus* colonization. NA and MSA plates were incubated at ambient room temperature for 48 hours at inverted position. After 24 and 48 hours of incubation, colony characterization was done.

Isolation, Characterization and Presumptive Identification of Facial Bacterial Isolates

All randomly picked bacterial isolates were streaked three times to ensure purity of the bacterial cultures. These were further subjected to various traditional methods of characterization of presumptive identification purposes.

Cultural Characterization: Pure cultures of bacterial isolates were restreaked on NA and MSA plates. Colonial morphology of isolates were observed after 24 and 48 hours of incubation at ambient room temperature. Another batch of pure cultures were inoculated onto NA slants and nutrient broth and growth pattern characteristics were observed after 24 and 48 hours of incubation.

Morphological Characterization: Before the staining procedures, preparation of hear-fixed bacterial smears were done. Gram, spore and acid-fast staining were done. All stained slides were then examined in a microscope under immersion oil objective.

Physiological Characterization: An inoculum from a 24-hour old culture to be tested was transferred to a

clean glass slide was added with one to two drops of 3% hydrogen peroxide. Catalase test detects the presence of the enzyme superoxide dismutase. Bubble formation was indicative of a positive result (Mac Faddin, 2000).

Differentiation from pathogenic and non-pathogenic strains of *Staphylococcus* done by performing the coagulase test. The 24-hour old bacterial isolate was inoculated onto 0.5 ml of human plasma which was incubated at room temperature for two hours. A positive test was denoted was by a clot formation in the test tube after the alloted time (Coagulase Test, 2018).

Determination of oxidation and fermentation of lactose was done by inoculating a 24-hour old culture onto Triple Sugar Iron (TSI) slant. A color change from brown to yellow indicated acid fermentation and the formation of bubble showed gas production (Triple Sugar Iron Agar, 2018).

A 24-hour old bacterial isolate was subcultured into blood agar plate and was then incubated at ambient room temperature for 24-and-48 hours. Hemolysis was visible as an area of clearing around the colony (zone of hemolysis). If the organism produces enzymes that completely lyse the red blood cells (RBC), this is termed beta hemolysis, Partial destruction of the RBC produced a greenish color to the zone of hemolysis and is termed alpha hemolysis. Organisms lacking hemolysins cause no change in the color or opacity of the media and are termed gamma hemolytic.

Results and discussion

Demographical Data

One hundred thirty-five tertiary students of Iligan City formed the study population of this cross-sectional study. Each respondent was given a self-administered questionnaire pertaining to demographic and clinical information which includes age, sex, inclination to sports and cosmeceuticals products used and medical history (antibiotic use, hospitalization and dermatological tests undertaken). The age of the study population ranged from 17-20 years old, wherein females dominate over the males (85 vs 50).

Table 1. Demographic profiles of the study population and the characteristics of students with positive growth cultures on NA and MSA.

Characteristic	Number of respondents	Bacterial colonies	
		With growth on MSA (n=118)	With growth on NA (n=135)
History of Antibiotic Use			
Yes	11	10/11 (91%)	11/11 (100%)
No	124	108/124 (87%)	124/124 (100%)
History of Hospitalization			
Yes	0	0	0
No	135	135/135 (100%	135/135 (100%)
Use of Facial Regimens			
Antibacterial soap user	109	106/109 (97%)	109/109 (100%)
Astringent user	13	12/13 (92%)	13/13 (100%)
Facial mask user	2	1/2 (50%)	2/2 (100%)
Facial scrub user	46	42/46 (91%)	46/46 (100%)
Whitening cream user	14	14/14 (100%)	14/14 (100%)
Undertaken Dermatological Test	5	5/5 (100%)	5/5 (100%)
Inclined to Sports	41	35/41 (88%)	41/41 (100%)
Make-up User	25	23/25 (92%)	25/25 (100%)

Table 1 shows that from a total number of respondents, 41 (30%) were inclined to sports, 26 (19%) were make-up users, 11 (8%) claimed to have taken antibiotics two weeks prior to the sampling period and only 4 (4%) had sought dermatological help.

However, majority of the students (130/135) reported to self-medicate using different facial regimens bought without formal presciptions: bacterial soaps were claimed to have used by 113 individuals (84%) followed by facial scrub (46, 35%) and whitening cream (14, 11%). These different products are readily available and are believed by most of the respondents to help prevent pimples. Use of astringents and facial masks were not prevalent among the respondents (13 and 2) due to the notion that these products might lead to irritation and redness.

Table 2. Cellular characteristics and biochemical profiles of the facial bacterial isolates.

Bacterium	Cellular morphology			Biochemical reaction					# of isolates
	Gram rxn	Shape	Spore	CAT	MANN	COAG	BLD HEM	GLU FERM	
S. aureus	+	cocci	-	+	+	+	α	+	105 (39%)
CoNS	+	cocci	-	+	+	-	α	+	52 (19%)
Micrococcus	+	cocci	-	+	-	-	-	-	4 (2%)
Bacillus sp.	+	rod	+	+	-	-	-	+	2 (1%)
Unknown 1	+	rod	-	-	-*	-	-	-	43 (16%)
Unknown 2	+	rod	-	+	-	-	-	+	61 (23%)

Detection of Facial Bacterial Colonization

All facial swab samples exhibited bacterial growth on NA plates: 130 plates (97%) had a mixture of colonies of different colonial morphologies while only 5 (3%) had monoculture growth. Due to high sat content of the MSA medium, only 118 plates (87%) exhibited bacterial growth and 4 (3%) were monocultures (Table 1). From the 118 positive growth cultures on MSA plates, 106 came from antibacterial soap users.

Presumptive Identification of Bacterial Isolates

A total of 267 bacterial isolates were randomly picked from positive growth cultures on both NA and MSA plates and were subjected to traditional microbiological identification methods (as shown in Table 2).

The 267 bacterial colonies subculture onto NA rendered 15 types of colonial morphologies wherein six colony types dominate. Gram staining procedure revealed that 100% were gram positive in which 64% (161/267) were cocci: 157 were arranged in clusters while only four were in pockets of four. The remaining 40% (106/267) of the isolates were bacillus in shape, three percent (3/106) of which appeared to be chain and the rest are in singles. There were two bacillus isolates which were spore-formers but none were positive in acid-fast staining.

Bubble formation in catalase test was exhibited by 224 bacterial isolates and growth in mannitol was positive for 157 cocci isolates, 105 of which were mannitol fermenters while 52 were non-fermenters. Forty-three bacilli isolates also had growth on MSA but were not able to show fermentation.

The 157 cocci isolates with growth on MSA were subjected to catalase test. One hundred five were coagulase positive thus are presumed to be *Staphylococcus aureus* and 52 were coagulase negative staphylococci (CoNS).

Blood hemolysis was conducted to identify *Streptococcus sp*. and the test performed showed absence of the strain from the isolated facial bacteria. Four *Micrococcus sp*. was identified through glucose fermentation test which was tested to all cocci isolates.

Table 3. The prevalence and distribution of the identified bacterial isolates from facial swab samples of the tertiary students.

Bacterial species recovered	Number of respondents	
Staphylococcus aureus	66	(49%)
CoNS	26	(19%)
Bacillus	2	(1%)
Micrococcus	3	(2%)
Unknonwn 1	20	(15%)
Unknown 2	43	(32%)

Prevalence of Bacterial Strain Colonization and Distribution of the Bacterial Species among the Study Population

Table 2 shows that *Staphylococcus aureus* was the predominant bacterial species of the total bacterial strains isolated from facial swab samples of the student respondents with 39% (105/267) proportion, followed by CoNS (52), *Micrococcus sp*. (4) and *Bacillus sp*. (2). Typically, the major population of the normal skin microbiota consists of coagulase negative *Staphylococcus* species, *Propionibacterium acnes*, and *Malassezia* species which all live on the surface of the skin and in the hair follicles (Dekio, 2012). The presence of *S. aureus* on facial skin has been associated with patients with atopic dermatitis (Gloor *et al*., 1982). Coagulase-negative *Staphylococcus* provides a protective function to the skin by producing antimicrobial peptides against *S. aureus* (Cogen *et al*., 2010). Other major bacterial species such as *Micrococcus*, *Streptococcus*, *Aerobacter*, *Proteus* and *Bacillus* have also been cultured from the facial skin (Dekio, 2012). Thus, all presumptively identified bacterial species isolated from the volunteers are all commonly found in human facial skin.

Table 4. Distribution of the pure and mixed bacterial isolates from facial swabs taken from 135 students.

Facial bacterial isolates	Number of isolates
S. aureus	47
Unknown 2	14
CoNS	12
Unknown 1	9
S. aureus/Unknown2	15
Unknown2/CoNS	14
Unknown2/Unknown1	11
Unknown2/*Micrococcus* sp	3
S. aureus/Bacillus sp	2
S. aureus/CoNS	2
S.aureus/Unknown1/Unknown2	3
S. aureus/CoNS/Unknown2	2

However, 39% (104/267) gram positive, rod-shaped and non-spore former bacteria were unidentified but their characteristics have been noted. Of which, 43 isolates (41%) are negative in catalase and glucose fermentation test but survived on MSA. The remaining 61 isolates (58%) do not grow in MSA but are catalase and glucose fermentation positive.

The prevalence and distribution of characterized and presumptively identified bacterial isolates is shown in

Table 3. Sixty-six of the subjects were found to harbor *S. aureus* followed by unknown species 2 (43), CoNS (26), unknown species 1 (20), *Micrococcus* (3) and *Bacillus* (2).

Most of these isolated bacterial strains were normal inhabitants of the skin. Staphylococcal isolates were considered to be the major colonizers while *Micrococcus* and *Bacillus* are seldom isolated. Unknown species 1 and 2 were presumed to be bacterial species that require an environment same as the skin.

The antagonistic behavior *of S. aureus* to other bacterial strains usually leads to competitive interaction which favors the high occurrence of *S. aureus* and the lesser number of other bacterial strains. Moreoever, some of the strains might have been also affected by the skin conditions such as pH, dryness, and frequent exposure to the environmental conditions making it less habitable to sensitive bacteria.

Eighty-two subjects (61%) yielded monoculture plates. Forty-seven of which had only *S. aureus*, 14 had unknown species 2, 12 had CoNS, and 9 had unknown species 1 (Table 4).

Mixed cultures could be attributed to the nutrients present in the media which favors growth of the bacterial isolates and the lesser competitive interaction that took place which is a common cause for monoculture growth.

Forty seven (35%) of the respondents had mixed cultures consisting of two facial bacterial strains. The combination which was detected most frequently was that of S. aureus/Unknown 2, found among 15 students, followed by Unknown 2/CoNS with 14 respondents, Unknown2/Unknown1 with 11, *S. aureus*/CoNS (2) and *S. aureus/Bacillus* with two isolates. Only 5 students (4%) had three different bacterial strains present in each facial swab sample: *S. aureus*/CoNS/Unknown2 (2) and S aureus/ Unknown2/Unknown1 (3).

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