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Nasal microflora of marginalized adult populace in residential proximity to an abandoned dumpsite in Iligan City, Philippines

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Abstract

Nasal swab cultures of seventy adults who are proximal residents of the abandoned city dumpsite were collected in order to determine the colonizing microflora of their anterior nares. Nasal bacterial colonization rates of microorganisms were greater during wet season, which might be due to proliferation of microorganisms from fecal matter that were left behind when there is a hard rain wash. Conventional methods were employed for the morphological (growth patterns in nutrient agar plate, slant and nutrient broth) and cellular (Gram stain, simple sporulation stain and acid-fast stain) characterization of the 214 bacterial strains that were isolated for their preliminary classification. Physiological characterization was done through catalase and coagulase tests, blood agar hemolysis, mannitol salt and glucose fermentation reactions. The presumptively identified bacterial strains were *Staphylococcus aureus* (79), coagulase negative staphylococci (17), *Streptococci* (17), *Corynebacterium* (32), *Bacillus* (24), *Micrococcus luteus* (14), *Mycobacterium* (4) and *Lactobacillus* (27).

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Introduction

As nations today become more aggressive in their workload of developing activities, one inevitable outcome is the production of solid wastes which are indiscriminately thrown and disposed of without due planning and consideration for the environment and consequent possible hazards that may have on human health. It is unfortunate though that improperly managed open dumps are still in existence all over the country. Many studies have been carried out to investigate the possible adverse health effects and assessed the respiratory and general health of waste pickers and populace living near open dumps (Ray *et al.*, 2004; Nguyen *et al.*, 2001; Pukkala and Ronka, 2001).

The nose is the most accessible site of bacterial infiltration where most probable contaminations occur and with its moist condition and frequent direct contact with the physical environment, it is a hospitable environment for microbial colonization and growth. The frequency of contact to improperly managed abandoned garbage, especially hospital wastes, among slum-dwellers poses a significant risk factor in increasing rates of potentially pathogenic bacteria (PPB), which in turn might facilitate infection and subsequent diseases.

An abandoned improperly managed open dump of Iligan City is now an area with temporary dwellings of marginalized urban population. Due to the high risk of exposure to potential health hazards, this study was conducted to ascertain the nasal colonization of potential pathogenic bacteria amongst the proximal residents of this improperly abandoned uncontrolled dumpsite.

Methodology

Study Population and Design

Residents of the general area of the abandoned open dump were recruited to be part of the study. The individuals were interviewed and data collected included age, sex, civil status, medical history, common illnesses, medication taken, antibiotics used, smoking habits, pregnancy status and appearance of skin lesions.

Collection of Nasal Swabs

Nasal swab collection was done following a modified method of the study by Heikkinen *et al.*, (2002). Nasal specimens were collected using sterile premoistened cotton swabs. This was inserted into the first nostril going downwards in a rotating motion from a depth of two to three centimeters. The same method was applied to the second nostril using the same cotton swab. This procedure was done quickly and efficiently to avoid contamination. After which, the swab was placed into a properly labeled sterile screw-cap tube containing seven millilitres of Amies Transport Medium, which was used for inhibiting the decrease of bacteria growth by providing suitable environment rich in nourishment, and was placed in an icebox. The clinical specimens were brought immediately to the laboratory within an hour of collection.

Determination of Nasal Microbial and Colonization Rates

After the collection of nasal swab samples. Stabilization was done by decreasing to room temperature. The swabs were then directly inoculated in a zigzag pattern to properly labelled nutrient agar plates. Inoculated plates were screened after 24 and 48 hours of incubation at ambient room temperature for bacterial colonies.

Isolation, Purification and Maintenance of Suspected Bacterial Strains

Bacterial colonies were randomly picked and purified three times in order to obtain a pure, unmixed colony type (Kaiser, 2002). Each pure bacterial culture stocked in a viable medium that will enable the bacterial strain to propagate and then be stored in a sufficient amount of time. These stock culture were periodically checked for signs of drying or fungal contamination. The stock cultures were kept at 4°C.

Three control strains from the culture collection of UPLB Biotech were used as reference strains in this

study, namely *Staphylococcus aureus* ATCC 1823, *Staphylococcus aureus* ATCC 1800 and *Micrococcus luteus* ATCC 1793.

Characterization and Identification of Bacterial Isolates using Traditional Methods

All bacterial isolates and control strains were subjected to various identification methods: cultural, cellular, morphological and biochemical characterization.

Cultural characterization

Pure cultures were subcultured on nutrient agar (NA) and mannitol salt agar (MSA), nutrient agar slants and nutrient broth for 48 hours at ambient room temperature to ensure optimal growth conditions. With the expected colony morphology on nutrient agar and mannitol salt agar (Reynolds, 2012), the suspected bacterial isolates and control strains were observed only for specific cultural characteristics.

Cell morphology of bacterial isolates

Bacterial isolates that were previously grown on NA and incubated at ambient room temperature for 24 hours were characterized by Gram, simple spore staining and acid fast staining procedures (Moreth-Kebernik and Schmidt 1991). All stained smears made were examined under low power, high power and oil immersion objectives in a compound microscope.

Physiological characterization

Bacteria can be identified through their enzymatic reactions to different metabolic components:

Catalase Test (Benson, 2001). Bacterial slant culture was allowed to grow at room temperature for 24 hours. Few drops of 3% hydrogen peroxide was added slowly allowing it to flow down the agar slant. The appearance of bubbles was indicative of positive reaction of the catalase test.

Coagulase Test (Benson, 2001). In a plasma tube, a 24-hour old bacterial culture was aseptically inoculated to the human plasma. Several loopfuls were considered, for success was more rapid with a heavy inoculation. Solidification may be complete in the lower tube or show up as a semi-solid ball in the middle of the tube. Coagulase activity was examined after four hours and those which were negative were further incubated overnight.

Blood Agar Hemolysis (Johnson, 2001). An inoculum was streaked on a blood agar plate and incubated for 18-24 hours to check if blood hemolysis occurs. Appearance of hemolysis shows clearing of the medium around the colonies (beta-hemolysis) or a brownish or greenish zone around the colonies (aplha-hemolysis).

Glucose Fermentation (Carbohydrate Fermentation Test, 2016). Using aseptic technique, a 24-hour old bacterial culture was streaked in a triple sugar iron slant and then stabbed in the butt of the slant. It was then incubated for 18-24 hours to allow bacterial growth. Indicative of positive glucose fermentation is a change of color in the butt of the slant to yellow from red. If there was no color change after 24 hours, incubation was continued and the slant was observed until the slant has changed color.

Mannitol Salt Fermentation (Kaiser, 2002). A bacterial culture was streaked into a mannitol salt agar medium and was allowed to grow after 24 and 48 hours. A change of color of the medium from red to yellow was indicative of the bacterial culture have fermented mannitol.

Statistical Analyses

For the analysis on the relationship of the detection of the PBBs and associated factors, Mann-Whitney Independent Sample Test as employed. The Chi-square test was also used to determine the significant relationship ($P<0.05$) susceptibility profiles of the bacterial isolates to its source.

Results and discussion

Initial Interview of the Study Population

Before the nasal swab collection was done, an initial interview, a house-to-house call, was made to the adult residents of the selected area pertaining to their

age, medical history, and occupational status and were then recruited to be part of the six sampling periods that ran for three months. The study population consisted of 70 adult individuals (45 males and 25 females), within the age range of 18-50 years old. Factors such as gender, smoking habits, common and serious illnesses experienced, history of antibiotic use, occupational status and waste picking activities were considered as categorical variables of the study population.

Table 1. Descriptive profile of the adult population included in the study.

Variable	Number of Individuals (Percentage)		
	Male	Female	Total
Smoker	5 (11)	13 (52)	18 (26)
Experienced common illnesses	44 (98)	24 (96)	68 (97)
Experienced serious illnesses	5 (11)	3 (12)	8 (11)
Antibiotic user	26 (58)	11 (48)	37 (53)
Waste picker	20 (44)	13 (52)	33 (47)

The respondents have mean age of 31-35 years in both the female and male populations. There were more females who reported to engage in smoking (13/52%) than males (5/11%). Information on the prevalence and extent of common and serious illnesses among the study population were restricted on the information given by the subjects, who were not properly diagnosed by medical specialists. Most of the subjects have experienced coughs, colds and fever which they attributed to the changing weather conditions that varied from harsh, sunny days to rainy evenings. Some also had reported to have experienced health conditions like kidney infections, ulcers and migraines.

The same situation was observed in the study of Abul (2010) where similar high-risk groups (those living close to waste dumps) have resulted to gastrointestinal, dermatological, respiratory, genetic, and several other kind of infectious diseases.

Table 2. Distribution of subjects throughout the six nasal swab collection periods.

Sampling Period	Number of Individuals	
1	42	(60%)
2	32	(46%)
3	30	(42%)
4	32	(46%)
5	27	(39%)
6	35	(50%)

There were more males than females with history of antibiotic use (58 % vs. 48%) with amoxicillin as the most common antibiotic taken. These individuals with reported illnesses who were not able to follow the antibiotic therapy, did not actually choose to suppress intake of antibiotic medications, but financial problems hindered them in taking prescribed medicines and instead use herbal alternatives.

Collection of Nasal Swabs from the Adult Population

There were 70 individuals that were initially recruited for the study whose nose swabs were taken at 2-week interval for three months. All throughout the sampling course, weather monitoring generally showed a sunny, dry weather for six weeks; wet and

rainy weather during the last six weeks. There were differences in the number of subjects per sampling period (Table 2). Most often than not, a subject was present only in two or three sampling schedules.

The first sampling had the highest number of individuals (42: 29 females and 13 males) which constituted 60% of the total population followed by the sixth sampling period with 50% (35 subjects: 23 females and 12 males) of the subjects. The least number of subjects was during the fifth sampling period which had 39% of the study population (27: 19 females and 8 males).

Table 3. Average bacterial colonization rates during dry and wet seasons.

Demographic Factor	Colonization Rate (CFU/swab)	
	Dry Season	Wet Season
General	159	>300
Smoker	10	>300
Experienced common illnesses	101	>300
Experienced serious illnesses	39	>300
With history of antibiotic use	223	>300

Detection and Determination of Nasal Bacterial Colonization Rates

All respondents yielded positive nasal bacterial cultures on nutrient agar of which colonization rate of each plate was determined by counting the different isolated colonies. Differences of colonization rates were noted during the wet and dry seasons and was one of the study factors which might have affected the number of microorganisms per swab. Table 3 showed that during the wet season, the average cfu/swab (159 in dry season and >300 in wet season) has significantly increased ($p<0.0001$). The increase in colonization rates in wet season may be attributed to the washing off of decaying matter, which is a hospitable environment for bacterial proliferation (Nguyen *et al.*, 2002).

The nasal colonization rates among individuals belonging to different categorical subgroups during wet and dry seasons were determined (Table 3). Nasal cfu/swab among the smokers had the highest increase from dry to wet season, than any categorical variable. Although generally attributed to interference with oral and airway innate and adaptive host defences, recent research has also revealed significant pathogen-directed effects of cigarette smoking. Not only do these enhance microbial virulence, but may also lead to the emergence of antibiotic resistance, as well as to an altered composition of the airway microbiota (Feldman and Anderson, 2013).

Subjects with history of antibiotic use have a greater average cfu/swab during the wet season (223 vs >300). Antimicrobial agents are the most valuable means for treating bacterial infections, however, the administration of therapeutic doses of these agents is a leading cause of disturbance of normal microflora.

This disturbance may then result in diminishing the natural defense mechanisms provided by the microbial ecosystem, making the host vulnerable to infection by commensal microorganisms or nosocomial pathogens (Rafii *et al.,* 2008).

Isolation, Characterization, and Identification of the Suspected Nasal Bacterial Isolates

A total of 214 bacterial strains were isolated from the nasal swab cultures of the subjects and these were purified and maintained using the nutrient agar medium. These were then observed for cultural, cellular and physiological characteristics.

The distribution of isolated bacterial species per subgroup of the study population is shown in Table 4. The identified bacterial strains were not only the members of the nasal microflora (*S. aureus*, coagulase-negative staphylococci, *Streptococcus*, and *Corynebacterium*) but also *Bacillus*, *Micrococcus*, *Lactobacillus*, and *Mycobacterium*. The presence of *Staphylococcus* and *Corynebacterium* is similar to the findings of Rasmussen *et al.* (2008).

Table 4. Colonization rates of nasal microorganisms in different subgroups.

Microorganism		Number of Isolates					
		A	B	C	D	E	F
S. *aureus*	(79)	37	42	20	59	47	32
CoNS	(17)	7	10	5	12	6	11
Bacillus sp.	(24)	11	13	3	21	11	13
Streptococcus sp.	(17)	7	10	5	12	8	9
Micrococcus sp.	(14)	5	9	5	9	10	4
Lactobacillus sp.	(27)	14	13	7	20	15	12
Corynebacterium sp.	(32)	15	17	6	26	19	13
Mycobacterium sp.	(4)	3	3	0	4	2	2
TOTAL	(124)	99	117	51	163	118	96

Note: Values in parentheses are the total number of each bacterial type.

Legend: A- Waste Pickers, B - Non-waste pickers, C- Smokers, D-Non-smokers, E-With history of antibiotic use, F- without history of antibiotic use.

The detection of these non-microfloral strains in the anterior nares might be due to the constant exposure of the subjects to the piled up mismanaged wastes and the mucus membranes of the human nasal cavities serve as a very hospitable environment for these microorganisms.

The predominant presumptively identified bacterial strain was the *Staphylococcus aureus* (79), a normal microflora of the nose that is potentially pathogenic. Other detected and identified microorganisms were coagulase negative staphylococci (17), *Streptococcus sp.* (17), and *Corynebacterium sp.* (32). However there were four bacterial groups detected that are not usually found on the anterior nares of humans namely *Bacillus sp.* (24), *Lactobacillus sp.* (27), *Mycobacterium sp.* (4) and *Micrococcus luteus* (14).

Most bacterial strains were isolated from non-smokers (163) followed by the subjects with history of antibiotic use (118), non-waste pickers (117), waste pickers (99) and the subjects without any history of antibiotic use (96).

The least amount of bacterial strains isolated were from the smokers (56). However, the frequency of isolation of bacterial strains could not be attributed to any factor for random colony isolation was used.

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