INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 2, p. 36-41, 2018

RESEARCH PAPER OPEN ACCESS

Nasal carriage of common bacterial pathogens among healthy children of proximal residence to dumpsites

Leonell Albert L. Quitos, Lucilyn L. Maratas*

Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines

Key words: Pathogen, nasal carriage, microbiota.

http://dx.doi.org/10.12692/ijb/13.2.36-41 Article published on August 18, 2018

Abstract

The human nasal microbiota plays an important role in the health of the hosts including the proper functioning of the immune system, nutrition and resistance to infections. This study was conducted to gain knowledge on the nasal microbiota of healthy children who are constantly exposed to open dumps. Nasal swab cultures were taken from 124 healthy asymptomatic children with mean age of 7.7 years, who were living near open dumpsites. There was an average of 142 colony forming units per swab (CFU/swab) from all nasal swab culture plates. The isolated bacterial strains were characterized by colonial, cellular and morphological properties and were subjected to series of biochemical tests. These are the presumptively identified isolates from the nasal swab cultures, in which some are potentially pathogenic: *Staphylococcus aureus* (69), *Mycobacterium sp.* (37), *Corynebacterium sp.* (26), *Lactobacillus sp.* (22), *Bacillus sp.* (21), coagulase negative staphylococci (7), *E. coli* (1) and G- non *E. coli* (3).

* **Corresponding Author:** Lucilyn L. Maratas ✉ lucilyn.lahoylahoy@g.msuiit.edu.ph

Introduction

The human nasal passages host major human pathogens and recent research suggests that the microbial communities inhabiting the epithelial surfaces of the nasal passages are a key factor in maintaining a healthy microenvironment by affecting both resistance to pathogens and immunological responses (Bomar *et al*., 2018). The nasal microflora generally consists of *Staphylococcus epidermidis*, *Corynebacterium diphtheriae, Proteus* and several bacterial strains that possess pathogenic potential such as *Staphylococcus aureus*, *Steptococcus pneumoniae*, *Nesseria meningitidis* and *Haemophilus influenza (*Uehara, 2000; Todar 2002; Gluck and Gabbers, 2003).

The composition of the normal microbiota depends upon various factors including age, gender, genetics, stress, nutrition and diet of individual, oxygen availability, pH and appropriate receptor sites for bacterial attachment (Todar, 2002). Environmental factors also influence the carriage of microorganisms and disease occurrence.

Open and uncontrolled dumpsites still remain as a major problem for most cities in the Philippines. These are the main terminals for trash where massive amounts of assorted wastes are situated. Piled among these large mounds of garbage are household, hospital and medical wastes. Residents within the vicinity of these poorly maintained dumpsites are documented to have higher prevalence of infection and diseases. Exposure to higher concentration of potentially pathogenic bacterial agents piled up in mismanaged wastes might lead to higher nasal colonization rates of bacteria and possible alterations in the composition of the nasal microbiota.

Children are at a higher risk of contracting diseases due to underdeveloped immune system and frequent close contact to infected adults. However, there is a definite lack of information regarding the nasal bacterial microbiota among asymptomatic children in these areas. Thus, this study was undertaken to generally isolate and identify nasal bacterial strains from the paediatric population living within the vicinity of open dumpsites in Iligan City.

Materials and methods

Data collection and design

Individuals with ages ranging from two to twelve who were residents of the areas surrounding open dumpsites of Iligan City were recruited to be part of this study. Parents and designated guardians were interviewed to determine socio-demographic and clinical information.

The study was planned for the recovery, isolated and identification of possible bacterial species colonizing the anterior nares of these children using agar-based traditional methods.

Collection of nasal swabs

The collection of nasal swabs were done twice a month for three consecutive months encompassing dry and wet seasons. Daily weather conditions were constantly monitored all throughout the sampling periods. Nasal swab specimens were obtained by swabbing pre moistened sterile cotton swab to both the left and right anterior nares four times around the inside of each nostril. The swabs were immediately placed in screw-cap tubes with Amies transport medium and were kept cool until inoculation.

Determination of bacterial colonization rates from nasal swab cultures

Nasal swabs were aseptically streaked unto the surface of nutrient agar which were later incubated for 24-to-48 hours at ambient room temperature. Isolated colonies were then counted to determine the bacterial nasal colonization rates.

Characterization and presumptive identification of microorganisms obtained from the nasal swab cultures

Pure cultures of the collected bacterial isolates were subjected to cultural, morphological (gram staining, spore staining and acid fast staining) and biochemical characterization (catalase test, mannitol fermentation test, coagulase test, growth on eosin

methylene blue agar) in determination of its presumptive identification.

Results and discussion

Nasal Swabs from Pediatric Population

One hundred twenty-four children, with the mean age of 7.7 year, participated in the study. The number of subjects, however, were not consistent throughout the six sampling periods (Table 1) and the fluctuations in the number of subjects was due to the absence of some individuals and the addition of new recruits in certain sampling periods. The first, second and third sampling periods had relatively high numbers of participants (66, 55 and 88 respectively) for these periods coincided with the summer vacation. Several factors were taken into consideration in the study: gender, waste-picking activities and history of antibiotic use. Table 1 summarizes the socio-demographic profile of the study population.

Table 1. Socio-demographic factors of the paediatric subject and population distribution throughout the six sampling periods.

Gender		Sampling period	Number of subjects
Male	52 (42%)	1	65 (52%)
Female	72 (58%)	2	55 (44%)
		3	68 (55%)
Waster pickers (n=37)		4	48 (39%)
Male	16 (13%)	5	51 (41%)
Females	21 (17%)	6	31 (25%)
With history of antibiotic use	47 (38%)		

From the study population, 30% (37 individuals) reported to be waste pickers or scavengers. Several studies have reported that these children are at more risk of injury and contracting diseases from possible pathogens obtained from the wastes. A study in India (Hunt, 1996) indicated that children scavengers are more at risk than adult waste-pickers for they lack judgment and knowledge about what to pick and what wastes to avoid. With their developing young body, they have an underdeveloped immune system and at the same time, are unable to excrete toxins in the same manner as adults can (Agarwal, 2003). Children also have a faster rate of breathing which may make them more vulnerable to airborne hazards.

Thinner skin layers of paediatric individuals make them more vulnerable to chemical absorption, cuts and burns (Hunt, 1996).

Table 2. Nasal bacterial colonization rates among the paediatric population.

Category	Average CFU/swab	
	Dry season	Wet season
Waste-picker	102	>300
Non waste-picker	162	>300
With history of antibiotic use	144	>300

Despite close proximity to the dumpsite, majority of the subjects (87 individuals or 70%) did not engage in scavenging which may be due to parental censure regarding to the associated health hazards of such activity. However, the paediatric subjects, whether waste-picker or not all reported to frequently exhibit signs of colds, low-grade fever and some other common illnesses such as cough and stomach pains. Despite of the reported prevalence of medical complaints, only 38% (47) of the study population have a history of antibiotic use. Some have reported to the use of herbal concoctions as treatments and

others even just ignored the claimed ailments.

These may be due to financial difficulty and the lack of awareness about public health care.

Detection and determination of the nasal bacterial colonization rates among the paediatric subjects

Three hundred eighteen nasal swabs were collected throughout the six sampling periods and all 318 nasal swab specimens yielded positive nasal bacterial cultures on nutrient agar (NA) plates after 24 and 48 hours of incubation at ambient room temperature.

An average of 142 colony forming units per swab (CFU/swab) was recorded from all nasal swab culture plates. Majority of the participants were females (n=72) and some studies have indicated that gender is a major risk factor for higher bacterial colonization rates and acquisition of pathogenic strains in the nasal cavity other than the normal nasal microbiota (Kuehnert *et al.*, 2006). However, in this study, the number of males and female individuals were not normally distributed and for this reason, it is not logical to compare the occurrence of potentially pathogenic bacteria and the rates of bacterial colonization between the two population.

Table 3. Distribution of identified bacterial species from the nasal swab cultures of paediatric subjects.

Bacterial species	Number of isolates
Staphylococcus aureus	69 (37%)
Coagulase-negative staphylococci	7 (4%)
Bacillus sp.	21 (11%)
Lactobacillus sp.	22 (12%)
Corynebacterium sp.	26 (14%)
Mycobacterium sp.	37 (20%)
Escherichia coli	1 (1%)
G- Non *E. coli*	3 (2%)

Non waste-pickers had the highest average CFU of 162 (Table 2) while waste-pickers yielded an average of 102 CFU/swab. Waste pickers should logically have had the higher colonization rates compared to non-waste-pickers considering that these individuals are at a higher risk of bacterial colonization for they are in direct contact with hazardous and potentially infectious wastes. However, there is also a possibility that scavengers have contracted some potentially pathogenic bacteria in their anterior nasal cavity and which may have caused bacterial antagonism, thus, consequently decreasing the colonization diversity in the nasal swab culture of the waste-pickers.

Children with history of antibiotic use yielded an average CFU/swab of 144. The use of antibiotics may not just eliminate certain pathogens in the body systems but also may create resistant strains. Some common mistakes regarding antibiotic use are then insufficient or over-use of antibiotics and the of erroneous antibiotic prescription for nonbacterial infections (Kardar, 2005) which may lead to proliferation of antibiotic strains (Lewis, 2003). Considering that about one-third of the study population were taking or have taken antibiotics, there may be a high possibility of culturing resistant strains from the collected nasal swabs.

Season has been identified to have a significant effect on the colonization rates of bacterial species. Thus, weather monitoring was done all throughout the sampling period. The first weeks (April to second week of May) were considered to be part of the dry season and the remaining six weeks were categorized under the wet season (third week of May until June). Mann Whitney statistical test yielded a significant difference on the nasal colonization rates ($P<0.0001$) between the dry and wet seasons in all the three

subgroups of the asymptomatic paediatric subjects (waste-pickers, non-waste-pickers, and with history of antibiotic use) for CFU/swabs significantly increased in the wet season.

Presumptive identification of suspected bacterial strains from the
Nasal swab cultures of the paediatric subjects

One hundred eighty-six isolated colonies randomly picked from the positive culture plates and were subcultured thrice on NA plates in order to ensure purity and viability of the isolates. Purified bacterial strains were kept in NA slants and were stored at four degrees Celsius which then later served as stock cultures.

These isolates were then presumptively identified using traditional methods of identification. The cultural morphologies and cellular characteristics were noted and used in the first step of identification. Gram staining yielded 182 (98%) gram positive (G+) and only 4 (2%) gram negative (G-) isolates.

All 76 G+ cocci were then subjected to catalse test, mannitol fermentation and coagulase test which then led to the differentiation of *Staphylococcus aureus* (69) and coagulase-negative staphylococci (7). All the other 106 G+ bacilli were tested for spore formation, acid fast staining and presence of catalase enzyme which led to the differentiation and presumptive identification of *Bacillus sp.* (21), *Mycobacterium sp.* (37), *Corynebacterium sp.* (26) and *Lactobacillus sp.* (22).

The other G- isolates were run on EMB agar which singled out *E. coli* (1) from three other G- bacilli. All bacterial strains that were isolated and identified (summarized in Table 3) from the nasal swab cultures of the study population were the usual members of the normal nasal microbiota.

The high nasal carriage of these potentially pathogenic bacteria might not immediately lead to infections, however, it is a potential risk factor for subsequent infection. No assumptions could be made regarding the type and frequency of bacterial strains that were isolated due to the study design of random picking of colonies.

References

Agarwal R. 2004. Children: Hidden Population Under Threat. Regional Health Forum **7,** 1.

Bomar L, Brugger SD, Lemon KP. 2018. Bacterial microbiota of the nasal passages across the span of human life. Current Opinion in Microbiology **41,** 8–14. https://doi.org/10.1016/j.mib.2017.10.023.

Gluck U, Gebbers JO. 2003. Ingested probiotics reduce nasal colonization with pathogenic bacteria (Staphylococcus aureus, Streptococcus pneumoniae, and β-hemolytic streptococci). American Journal of Clinical Nutrition **7(2),** 517-520.

Hunt C. 1996. Child waste pickers of India: the occupation and its health risks. Environment and Urbanization **8(2),** 111-118.

Kardar SS. 2005. Antibiotic Resistance: New Approaches to a Historical Problem. American Institute of Biological Sciences. http://www.actionbioscience.org/biotechnology/kardar.html

Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister SK, Fosheim G, McDougal LK, Chaitram J, Jensen B, Fridkin SK, Killgore G, Tenover FC. 2006. Prevalence of Staphylococcus aureus Nasal Colonization in United States, 2001-2002. Journal of Infectious Diseases **193(2),** 172-179.

Lewis R. 2003. The Rise of Antibiotic-Resistant Infections. United States of America Food and Drug Administration. https://pdfs.semanticscholar.org/1332/a1f21271ebfdc97543b16881f183f338afb7.pdf

Todar K. 2002. The Bacterial Flora of Humans. Todar's Online Textbook of Bacteriology. http://textbookofbacteriology.net/normalflora.html

Uehara Y, Nakama H, Agematsu K, Uchida M, Kawakami Y, Abdul Fattah AS, Maruchi N. 2000. Bacterial interference among nasal inhabitants: eradication of Staphylococcus aureus from nasal cavities by artificial implantation of Corynebacterium sp. **44(2),** 127-133.