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Nasal Carriage of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* Among Healthy Children of Iligan City, Philippines

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Abstract

Surveillance and monitoring of nasal carriage of *Staphyloccocus aureus* and MRSA among the vulnerable pediatric population is a necessity as it is associated with increased risk for development of various infections. This study aimed to determine the prevalence of *S. aureus* and MRSA nasal carriage and its probable risk factors among healthy children in Iligan City, Philippines. Seventy-three out of the 114 children included in the study were found to be nasal carriers of *S. aureus,* where two factors were found to have significant association (p value < 0.001): the use of herbal medicine in the past six months and passive exposure to cigarette smoke. Kirby-Bauer disk diffusion method was used to determine the antibiotic susceptibility of the 73 isolates, of which 21 isolates (29%) were MRSA. The rate of MRSA colonization in this study is significantly higher than previous published researches and may be attributed to the overcrowded situation in daycare centers. The only identified potential risk factor for MRSA colonization in this study was the manifestation of clinical symptoms of common childhood illness (p=0.000948).

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Introduction

Staphylococci are ubiquitous colonizers of human epithelia and mucous membranes (Hamdan-Partida *et al.,* 2014) Frequent carriage of *Staphylococcus aureus* in the anterior nares of children has been consistently been documented (Wertheim *et al.*, 2005; Esposito *et al.*, 2013; Vieira *et al.*, 2014).

The duration of *S. aureus* colonization and burden of organisms varies among individuals; however, nasal colonization is a potent risk factor for *S. aureus* infection (Bessesen *et al.*, 2015). Furthermore, *S. aureus* is characterized by its high capacity to adapt to antimicrobials by the acquisition of resistance mechanisms particularly against methicillin, further complicating the treatment of infections (Aires de Sousa & de Lencastre, 2004).

Numerous studies confirm that there is an increasing incidence of asymptomatic carriage of methicillin resistant *S. aureus* (MRSA) encompassing various types of populations which are non-institutionalized. A study in 2007 reported that healthy children are more likely to be infected with MRSA (Elston, 2007).

Despite the substantial documentation of the prevalence of nasal carriage of *S. aureus* and MRSA in children, there is a definite lack of information about investigations carried out in the Philippines. This study aims to provide information *S. aureus* and MRSA nasal carriage in otherwise healthy children and identify probable risk factors for colonization.

Methodology

Nasal swab collection

An observational cross-sectional study was conducted amongst children of ages 3-6 years attending day care centers. Prior to sample collection, written informed consent from the guardians of the day care attendees was obtained and a brief interview with each guardian was conducted about the demographic and clinical history the pediatric subject.

Information collected included demographic aspects, medical history, antimicrobial usage, history of previous hospitalization, smokers in the household and other possible factors linked to colonization.

Nasal swabs were collected from 114 children attending various daycare facilities in Iligan City and the swabbing was carried out in a specific area of each room at the end of the school session.

The general procedure of nasal swabbing followed the published protocol of Susanna Esposito *et al.* (2014) where it involved inserting the sterile swab tip into one nostril and rotating it for three seconds and swabs were immediately placed in a sterile screw cap tubes filled with 1 ml Ames transport medium.

All clinical specimens were transported to the laboratory and were processed within two hours after collection.

Traditional identification of S. Aureus

The specimens were plated onto Mannitol Salt Agar and after 24–48h of incubation at 37˚C, colonies having mannitol-salt fermentation and morphology suggestive of *S. aureus* were confirmed by Gram staining and catalase and coagulase activities.

MRSA determination

Methicillin resistance of *S. aureus* isolates was determined by disk diffusion for isolates showing inhibition zones of ≤10mm around 1mg oxacillin were characterized as MRSA in accordance to the guidelines set by the Clinical Laboratory Standards Institute (CLSI).

Results and discussion

Nasal Staphylococcal Carriage of the Study Population

One hundred fourteen children within the age range of three to six years underwent the nasal specimen acquisition. Table 1 shows general characteristics of the study population wherein more than half of the population (76%) had a history of antibiotic use in the last six months as agents of treatment of childhood illnesses. Eighty subjects reported being with general symptoms of infection with coughs, colds, fever and

respiratory ailments as the common complaints.

Seventy-three subjects (64%) were found to be nasal carriers of *S. aureus*. A study has documented that the carriage of respiratory bacteria is strictly age dependent as it is significantly higher in younger children than in adolescents (Esposito *et al.*, 2013).

Table 1. Characteristics of children screened for *S. aureus* and MRSA nasal carriage.

Variables	N/Total	%
With history of antibiotic use (in the last 6 months)	77/114	76
With history of herbal medicine use (in the last 6 months)	13/114	11
Hospitalization (in the last 6 months)	26/114	23
Contact with Healthcare worker	43/114	38
Contact with Previously Hospitalized Individuals (in the last 6 months)	18/114	16
Exposure to Cigarette Smoke	45/114	39
Symptomatic of an Illness during time of Screening	80/114	70
Kinds of illnesses experienced		
Cough	52/114	46
Colds	48/114	42
Fever	21/114	18
Asthma	14/114	12
Flu	13/114	11
Wounds	5/114	4
Allergic Rhinitis	4/114	4
Stomachache	2/114	2
Acute Bronchitis	2/114	2
Unknown Ailment	1/114	1
Cyst in Feet	1/114	1

However, the findings in this study are significantly higher than other studies of similar study population like studies cited by Rodriguez *et al.,* (2014) wherein colonization rates ranged from 7.6 - 53.8%. The substantial proportion of staphylococcal isolates in a nonhospital setting as identified in this surveillance is of great clinical concern.

Risk factors associated with nasal *S. aureus* colonization are shown in Table 2. Multivariate analysis indicated that only two epidemiological variables were of significant association with staphylococcal colonization.

The use of herbal medicine in the past six months and passive exposure to cigarette smoke within the residences were both found to have significant association (p value < 0.001). Nasal staphylococcal colonization of those children exposed to cigarette smoke is in agreement with two studies inferring that there is an increased risk of colonization due to passive smoking (Bogaert *et al.*, 2004; Rodriguez *et al.*, 2014).

The calculated p values in association between all other considered probable risk factors to staphylococcal colonization were observed to have no significant difference (p>0.05).

Children who were experiencing common childhood illnesses at the time of sampling were noted to have visible secretions (66%) was found to have higher staphylococcal colonization. Based on several studies conducted, symptomatic individuals are designated to have higher bacterial inhabitation rates as well as of greater risk for acquisition of potentially pathogenic microorganisms because of its suitable moist environment for bacterial proliferation as well as

possibly compromised immune system (Butwin, 2004).

Most staphylococcal strains were reported to be of nosocomial derivation with healthcare or hospital workers being commented as potential carriers and culprits of staphylococcal diseases (Florentino, 2000). However, high staphylococcal rates (75%) were still detected from those who do not have any history of hospital admittance. Regardless of the lack of contact among recently hospitalized individuals (which were designated as those who are closely related to the subjects i.e. siblings, elderly relatives), 51 children were found to be colonized with *S. aureus*. Colonization might be mediated by some factors that were not tackled in the study such as fomites and other indirect modes of transmission. This is in contrast with adult studies of nasal staphylococcal carriage, colonization is significantly more frequent in subjects in close contact with other *S. aureus* carriers, such as frequently hospitalized patients (Mertz *et al.*, 2009).

Table 2. Epidemiological factors of children colonized with *S. Aureus*.

Variables	n	%	p < 0.05
History of Antibiotic Use	42	58	0.843473
History of Herbal Medicine Use	10	14	< 0.001
Hospitalized in the past 6 months	18	25	0.644069
Contact with Healthcare worker	26	36	0.552212
Contact with Previously Hospitalized Individuals	14	19	0.284385
Exposure to Cigarette Smoke	10	14	< 0.001
Symptomatic of an Illness during time of Screening	48	66	0.203765

The symptomatic state of the children revealed insignificant relationship in the colonization of *S. aureus*. This means that colonization is independent of the illness experienced by the subjects.

This is similar to the results obtained in the study of Susanna Esposito *et al.* (2014). Health complaints by the children were considered to be in mild form since these are considered typical childhood illnesses which are unlike reported cases of chronic diseases and infections among the pediatric populace at different regions were designated to have a strong correlation in terms of staphylococcal colonization.

Methicillin-resistant S. aureus Among the Healthy Children

The clinical significance of staphylococcal colonization has been discussed detail in previous studies but all in nosocomial environments; it importance in the community is still controversial (Rodriguez *et al.*, 2014) However, an increase in nasal colonization has been implicated as the principal risk factor in the emergence of MRSA infections, especially in healthy children (Creech *et al.,* 2005; Huang *et al.*, 2007).

The clinical significance of staphylococcal colonization has been discussed detail in previous studies but all in nosocomial environments; its importance in the community is still controversial (Rodriguez *et al.*, 2014). However, an increase in nasal colonization has been implicated as the principal risk factor in the emergence of MRSA infections, especially in healthy children (Creech *et al.*, 2005; Huang *et al.*, 2007).

Previous studies suggest that nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) occurs infrequently in healthy pediatric population (0.2-9.2%) (Creech *et al.*, 2005; Chen *et al.*, 2011; Tavares *et al.,* 2010). It is important to take note that the result of this study is significantly higher than the previous studies at 29%.

There were no significant associations between the identified potential risk factors and MRSA

colonization except for exhibiting some clinical symptoms of common childhood illness (p=000948). However, it is postulated that crowding in daycare centers may be a probable independent environmental risk factor attributing to the high MRSA colonization rate.

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