Genital Staphylococcal Flora in Women of Reproductive Age

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Abstract

Self-collected vaginal swab specimens of 156 reproductive aged women of Iligan City were taken during the period of December 2005 to December 2006 to isolate staphylococcal strains. Only one hundred fifty-four clinical specimens exhibited positive bacterial growth on mannitol salt agar where 237 isolates were randomly picked. The isolates were subjected to conventional microbiological tests: cultural, morphological, and physiological (mannitol salt fermentation, catalase and coagulase tests) characterizations where 121 *Staphylococcus aureus* and 116 coagulase-negative staphylococci (CoNS) were presumptively identified. Different categorical variables were identified as probable risk factors, however, staphylococcal colonization was found to be not associated with any of these identified categorical factors.

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All presumptively identified *S. aureus* were tested for their phenotypic profiles against oxacillin (1 μg), cefoxitin (30 μg), vancomycin (30 μg), erythromycin (15 μg), and clindamycin (2 μg). Vancomycin was found to be the most effective among the five antibiotics being able to have only nine percent (11/121) resistant isolates while erythromycin have displayed the least efficiency by having almost half of the isolates resistant to it (48%). In addition, majority of the isolates (56%) were susceptible dose dependent to clindamycin implying that clindamyin works in borderline efficiency. Eighteen isolates were detected as true methicillin-resistant *S. aureus* (MRSA) strains being resistant to both oxacillin and cefoxitin. Erythromycin-induced clindamycin resistance, on the other hand, was exhibited by five of the isolates. Half of the confirmed MRSA isolates (9/18) were multidrug-resistant *S. aureus* (MDRSA) and these isolates could probably be of nosocomial origins while the other nine nonmultidrug-resistant MRSA (NMD-MRSA) were assumed to be community-associated. Detection of *S. aureus* and MRSA has shown that these strains can be isolated from the vagina of asymptomatic women. Statistically, there is no significant correlation between the subgroups and the identified risk factors and the susceptibility patterns of the *S. aureus* strains.

The human vagina provides physical and chemical environment that allows microorganisms to use its underlying tissue as a normal habitat (Larsen, 1993) making it a good host to a variety of microorganisms.

During the female reproductive life, from puberty to menopause, women acquire an adult vaginal microflora with *Staphylococcus epidermidis* and *Lactobacillus* as its constant members, occurring at nearly a hundred percent of the women population. There are, however, other bacteria that may thrive in the vagina, which may become possible pathogens. Among this array of potentially-pathogenic bacteria that colonize the vagina is *S. aureus*, which occur at about 25% of the female populace (Davis, 2006).

S. aureus, a potential pathogen, lives in mucous membranes that line the nose, mouth, intestinal tract, and vagina (Treney, 2005) and is naturally occurring in vagina in small numbers (Lindley, 2005). This potential pathogen has long been recognized as one of the most important bacteria that cause diseases in humans (Minnesota Department of Health, 2006).

Today, antibiotic-resistant strains of *S. aureus* are common and pose a global health problem in hospitals. *S. aureus* is one of the major resistant pathogens being extremely adaptable to antibiotic pressure (Wikipedia, 2007). It was the first bacterium in which penicillin resistance was found. In the United States, almost every strain of *S. aureus* is now resistant to penicillin. After occurrence of penicillin-resistant *S. aureus*, methicillin was then the antibiotic of

choice. Moreover, strains have begun to develop resistance to newer drugs like methicillin and vancomycin. The threat of prolonged illness or even death from *S. aureus* infection increases with this rise in resistance, as fewer and fewer drugs are able to effectively control or eliminate this microorganism. While 25% to 35% of the population carries *S. aureus*, approximately 1% carries methicillin-resistant *S. aureus* (MRSA) (Chambers, 2001).

Objectives of the Study. The study generally aims to determine the vaginal staphylococcal population cultured from self-collected vaginal swab samples of randomly chosen women of reproductive age in Iligan City.

Specifically, it intends:

1. To detect, culture, isolate, purify, and presumptively identify halophilic staphylococci on MSA culture medium from the self-collected vaginal swab samples using conventional methods and tests;
2. To evaluate factors associated with the colonization of potentially pathogenic bacteria in the vagina;
3. To determine phenotypic susceptibility of *Staphylococcus aureus* against oxacillin, cefoxitin, vancomycin, erythromycin, and clindamycin;
4. To conduct preliminary assessment on the methicillin-resistant *S. aureus* (MRSA) colonization rates among the women respondents; and
5. To ascertain frequency of community-associated MRSA antibiograms.

Materials and Methods

Study area, study population, and data collection. The study was conducted in different areas of eight barangays of Iligan City. Sampling of clinical specimens was done from December 2005 to December 2006. Women from 18-35 years old were asked to participate in the study. Prior to the collection of the vaginal swab samples, the subjects were interviewed and made to answer brief structured questionnaires designed to identify potential risk factors for vaginal staphylococcal colonization.

Collection of vaginal swab specimens. Standard cotton swab technique was used to obtain vaginal swab samples from the women. Each subject was asked to provide a swab sample, which they collected themselves, according to instructions given. Samples were placed inside a properly labeled screw-capped tubes containing Amies transport medium. Tubes were placed in an icebox, maintained cool until processing, and were brought to the laboratory within an hour of collection for inoculation onto the primary culture media.

Detection, isolation, purification, and maintenance of suspected bacterial strains from vaginal swab cultures. Swab samples were then aseptically swab-streaked into properly labeled Mannitol Salt Agar (MSA) plates and incubated at room temperature and were observed. Colony types and isolated colonies were counted after 24 and 48 hours of incubation. Bacterial colonies were purified thrice in the same culture medium to obtain pure colony isolates. Pure isolates were stock cultured in microcentrifuge tubes with 1-mL nutrient broth, stored, and kept cool inside the refrigerator.

Characterization and identification of the bacterial isolates using traditional methods. All bacterial isolates were subjected to various conventional identification methods: cultural characterization (colonial morphology on MSA plates); cellular morphology (Gram staining and microscopy); and physiological characterization (mannitol salt fermentation, catalase and coagulase tests).

Antibiotic susceptibility testing: Kirby-Bauer disc diffusion method. Antibiotic susceptibility of the presumptively identified *S. aureus* isolates were tested by screening their phenotypic profiles to oxacillin (10 μg), cefoxitin (30 μg), vancomycin (30 μg), erythromycin (15 μg), and clindamycin (2 μg) by employing the standard Kirby-Bauer disc diffusion technique. Bacterial suspension was made based on 0.5 MacFarland turbidity standard. Suspension was then swab-streaked and allowed to grow on Mueller Hinton Agar (MHA) plates. Standard antibiotic discs were aseptically placed at a standard distance from each other on the culture plates. Diameter of the zone of inhibition produced by each antibiotic disc was measured and isolates were categorized as resistant, intermediate resistant, or susceptible based on the standard Zone Diameter Interpretation Chart. The double disc diffusion test was performed to detect erythromycin-inducible clindamycin resistant *S. aureus* strains.

Statistical Analyses. To determine differences between groups, data were analyzed using the chi-square and Fisher exact tests. These tests were used to determine the significant relationship ($P<0.05$) between colonization rate of bacterial isolates and the subgroups of the study population they were isolated from. These were also used to determine the significant correlation ($P<0.05$) considering each of the antibiotic and the antibiotic combinations.

Results and Discussion

Distribution of different bacterial species among the respondents. One hundred fifty-four bacterial cultures yielded positive bacterial growth with 327 isolates and one hundred twenty-one of these Gram-positive catalytic staphylococci isolates were found to ferment mannitol and were positive for the enzyme coagulase. These isolates were then presumptively identified as *Staphylococcus aureus* (121), while those non-fermenting coagulase negative isolates were referred to as coagulase-negative staphylococci (CoNS) (116).

The distribution of isolated and presumptively identified bacterial strains per subgroup of the study population is shown in Table 1. Detection staphylococci is expected since these bacteria are frequent members of the vaginal microflora (~100), however, *S. aureus* is found only among 25% of the women populace (Todar, 2005).

Table 1. Colonization rates of vaginal staphylococcal microorganisms among the different subgroups.

MICROORGANISMS	NUMBER OF ISOLATES															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
S. aureus (121)	13	9	11	33	67	17	4	30	29	59	70	111	26	35	61	72
CoNS (116)	11	6	11	29	68	16	3	33	25	53	54	105	23	30	62	56
TOTAL (237)	24	15	22	62	135	33	7	63	54	112	124	216	49	65	123	128

Legend:

A-pregnant
B- menstruating
C- smokers
D- feminine wash user
E- soap user
F- water user
G- other products
H- with current illness/es
I- with medical history of disease
J- antibiotic user
K- contraceptive user
L- cotton underwear user
M- fabric conditioner user
N- panty-liner user
O- tight-fit clothes user
P- prefer sweets

Between the two different staphylococcal isolate types, the presumptively identified *S. aureus* was slightly predominant bacteria (121) with 5 bacterial isolates more than the coagulase-negative staphylococci (116). Seventy-eight percent of the subjects were colonized with *S aureus*. The frequency of *S. aureus* isolation higher compared to the 9.2 % *S. aureus* isolated from women subjects in a study conducted by Aldritch (1982). It is also considerably higher than the expected 25% occurrence (Todar, 2006).

Most of the *S. aureus* strains isolated were from the cotton underwear users (111/121), which also have the highest number of subjects, followed by the women who prefer to have sweets in their diet (72/121) and contraceptive users (70/121). The least number of *S. aureus* strains were isolated from the women who used products like shampoo and toothpaste in washing their genital areas (4/121).

The same is true with the colonization distribution of CoNS isolates with regard to the subgroups that have obtained the most and the least number of CoNS: cotton underwear users (109/116) shampoo/toothpaste users (3/116), respectively.

The disparity in number of the bacterial isolates per subgroup is due to the difference in number of the subjects per subpopulation. It was found that there is no significant relationship between *S. aureus* and CoNS colonization and all of the identified subgroups.

Antimicrobial susceptibility patterns of *Staphylococcus aureus*. *Staphylococcus aureus* remains one of the most frequently isolated pathogens in both community and hospital practices (Land and Orrett, 2006). Changes in the pattern of antimicrobial susceptibility of *S. aureus* have been reported worldwide, especially in developing countries making antibiotic agents increasingly less effective in treating bacterial infection.

Over the past years, there have been dramatic changes in the susceptibility of *S. aureus* in both hospital and community settings. The older ß-lactam drugs, penicillin and ampicillin, are ineffective to more than 80% of isolated strains, and resistance to many of the non- ß-lactam agent such as tetracycline, gentamicin, chloramphenicol, erythromycin, and clindamycin has gradually increased and reached alarming levels by the year 1990 in many parts of the world (Land and Orrett, 2006).

One hundred twenty-one presumptively identified *Staphylococcus aureus* from the female subjects were subjected to Kirby-Bauer Disc Diffusion test, following the guidelines recommended by CLSI, to determine the phenotypic susceptibility of the *S. aureus* isolates to oxacillin (1 µg), cefoxitin, (30 µg), vancomycin (30 µg), erythromycin (15 µg), and clindamycin (2 µg). Only one *S. aureus* isolate per subject was taken into consideration in the test. Susceptibility or resistance of each isolate was determined by measuring zones of inhibition around each antibiotic disc after 18 hours of incubation.

The graph (Figure 1) shows the different phenotypic profiles of the isolates against the five antibiotics: oxacillin, cefoxitin, vancomycin, erythromycin, and clindamycin. It depicts that vancomycin remains the most effective antibiotic since it has the least number of resistant isolates (11/121) and therefore, the most number of susceptible strains. It also had the second least number of susceptible dose-dependent isolates. On the other hand, erythromycin, a commonly used antibiotic which can be bought as an over-the-counter drug, had

the most number of resistant strains (58/121). Susceptible dose dependent isolates are numerous among the clindamycin-tested strains (68/121), which inferred that continued clindamycin use among these individuals may lead to conversion of intermediate resistance into high clindamycin-resistance.

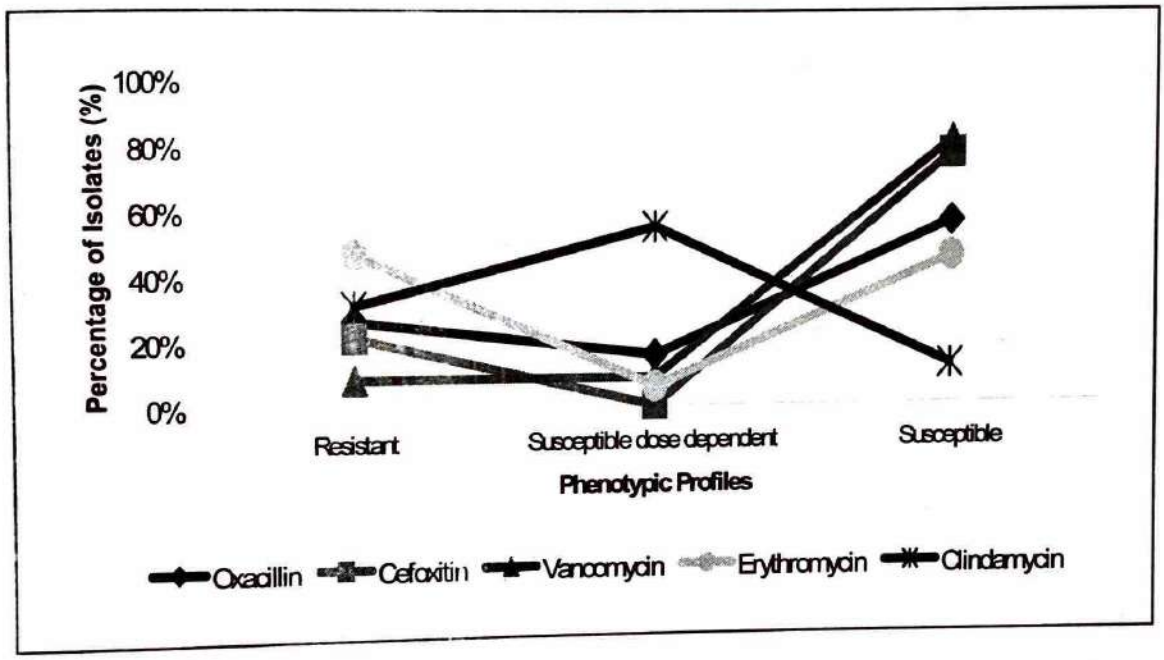


Figure 1. Phenotypic categorization of *S. aureus* towards the five antibiotics.

Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Susceptible *Staphylococcus aureus* (MSSA). Methicillin-resistant *S. aureus* (MRSA), a serious threat to hospitalized patients globally, now represents a challenge for public health, as community acquired infection appear to be on the increase. Recently, a shift in the epidemiology of MRSA infection has been documented, whereby community-associated MRSA infections have become more common (Hanselman, 2003).

MRSA identification for this study is based on resistance of isolates towards the antibiotics oxacillin and cefoxitin, otherwise it is referred to as methicillin susceptible *S. aureus* (MSSA).

The table shows the number of isolates per subgroup exhibiting the different oxacillin-cefoxitin combinations where eighteen isolates out of 121 were confirmed to be MRSA isolates (Ox_R+Fox_R). Pregnant subjects had the highest

rate of MRSA colonization (31%), which could be attributed to the fact that pregnant women are more susceptible to changes in their normal flora due to hormonal factors associated with pregnancy; this is followed by the smokers (27%), and feminine wash users (24%) while the subgroups that used water and shampoo/toothpaste in cleaning their genital parts harbored no MRSA (Table 2).

Table 2. Distribution of oxacillin-cefoxitin resistant isolate pattern per categorical variable.

CATEGORICAL VARIABLES	ANTIBIOTIC COMBINATIONS				
	Ox_S+Fox_S (63)	Ox_I+Fox_S (16)	Ox_I+Fox_R (3)	Ox_S+Fox_R (6)	Ox_R+Fox_R (18)
A (13)	4	1	0	1	4
B (9)	1	1	0	0	0
C (11)	6	1	0	0	3
D (33)	14	6	0	0	8
E (67)	34	9	2	4	10
F (17)	11	1	1	1	3
G (4)	4	0	0	0	0
H (30)	14	5	2	1	4
I (29)	14	4	2	0	5
J (59)	31	8	2	2	7
K (70)	39	8	1	2	12
L (111)	59	16	2	6	16
M (26)	10	6	0	0	5
N (35)	17	8	2	0	5
O (61)	27	7	2	5	9
P (72)	34	11	3	3	9

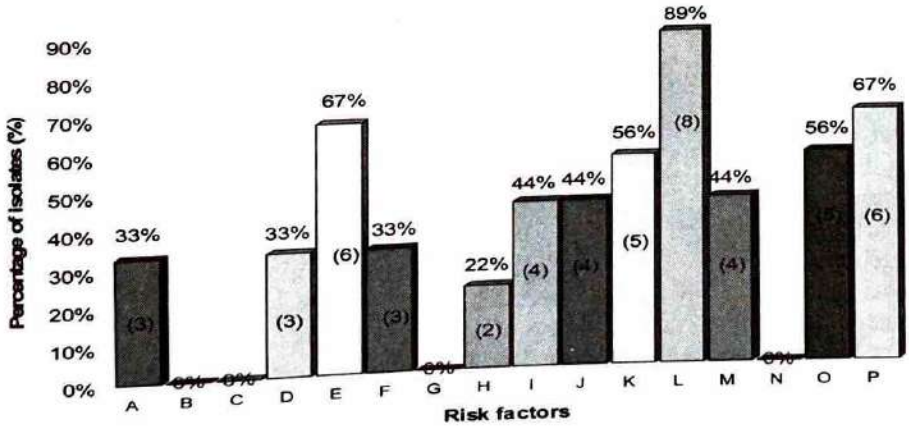
Legend

A-pregnant
B- menstruating
C- smokers
D- feminine wash user
E- soap user
F- water user
G- other products
H- with current illness/es
I- with medical history of disease
J- antibiotic user
K- contraceptive user
L- cotton underwear user
M- fabric conditioner user
N- panty-liner user
O- tight-fit clothes user
P- prefer sweets

Detection of these MRSA isolates proved that these antibiotic resistant strains can also be isolated from the vagina. These would pose a great concern because these strains could become systemic and cause infection in organs near the vagina or in any other parts of the body. It could also be passed on to a partner during sexual contact and can be transmitted to a newly-born baby during delivery.

Detection of Multidrug-Resistant *Staphylococcus aureus* (MDRSA) and non Multidrug-Resistant *Staphylococcus aureus* (NMDRSA). Of the 18 MRSA isolates, nine were found to be resistant to multiple antibiotics.

Multidrug resistance is defined to be nonsusceptibility to at least two of the other three antibiotics, otherwise it is referred to as nonmultidrug-resistant *S. aureus* (NMDRSA). Five of these MDRSA isolates showed resistance to both erythromycin and clindamycin. Five of the nine MDRSA carriers were contraceptive users. Demographic profiles of these individuals are shown in Figure 2. Cotton underwear users had the highest percentage of MD-MRSA strains (8/9), followed by the soap users and women who prefer sweets in their diet (6/9), while there were no MD-MRSA strains detected from those who have their menstruation and used shampoo or toothpaste in cleaning their genital areas.



Legend:

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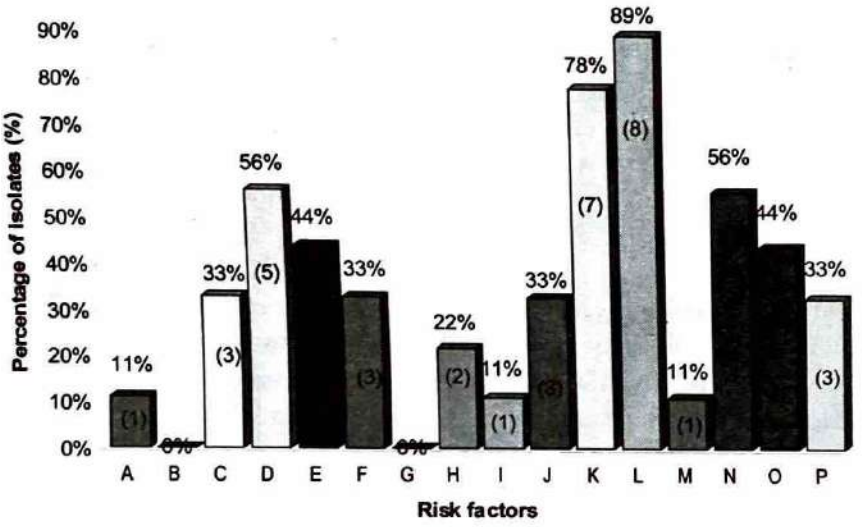
K- contraceptive user
L- cotton underwear user
M- fabric conditioner user
N- panty-liner user
O- tight-fit clothes user
P- prefer sweets

Note: Values in parentheses are actual number of subjects.

Figure 2. Demographic profiles of individuals with MD-MRSA strains.

These MDRSA strains could be nosocomial in origin and are associated with the hospital settings while the nine nonmultidrug-resistant strains were probably community acquired. The demographic profiles of the CA-MRSA carriers were shown in Figure 3.



Legend:

A-pregnant	I- with medical history of disease
B- menstruating	J- antibiotic user
C- smokers	K- contraceptive user
D- feminine wash user	L- cotton underwear user
E- soap user	M- fabric conditioner user
F- water user	N- panty-liner user
G- other products	O- tight-fit clothes user
H- with current illness/es	P- prefer sweets

Note: Values in parentheses are actual number of subjects.

Figure 3. Demographic profiles of individuals with CA-MRSA strains.

Cotton underwear users had the highest percentage of CA-MRSA strains (8/9), followed by the contraceptive users (7/9) and women who prefer to use soap as part of their hygiene practices and panty-liner frequent users (5/9), while there were no MD-MRSA strains detected from those who have their menstruation and used shampoo or toothpaste in cleaning their genital areas.

None of the identified risk factors, however, is associated with colonization of any of the two types of MRSA strains. This idea has conformed to the fact that there are no reliable risk factors found for community-acquired MRSA.

Five out of these nine MR-MDRSA and seven of MR-NMDRSA carriers were contraceptive users. It was statistically tested that there is no significant association between colonization of MRSA and MDRSA strains and the use of contraception methods, the use of any form of contraception could not influence the colonization of MRSA strains in the vagina.

Conclusion and Recommendation

Staphylococcus aureus, bacteria commonly found in the anterior nares of humans is found to be present at a high level in the vagina of the female subjects. Colonization was observed to be statistically-associated with any of the risk factors taken into consideration. Though, only a small proportion of the population was found out to be MRSA carriers, it should still be noted that these strains were still able to colonize the vagina and that they may cause serious systemic and gynecological infections in what is considered a woman's most private part.

It is recommended to study a larger population with well-defined risk factors. Establishing the risk factors would help in identifying the source of these resistant bacteria. It should also be taken into account the use of other medium to detect other vaginal bacteria and correlate its prevalence with *Staphylococcus*. In addition, study on the molecular level of the bacteria should also be given consideration to identify genetic factors that are responsible source of bacterial resistance or infection. A wider range of antibiotics should also be used to determine the phenotypic profiles of these bacteria to other antimicrobial agents.

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