RESEARCH PAPER

Phenotypic profiles of nosocomial *Staphylococcus aureus* isolates of Iligan City, Philippines

Lucilyn L. Maratas*, Muriel Faith A. Goles

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

Abstract

The increase of resistance of *Staphylococcus aureus* against commonly used antibiotics is perceived as a threat in therapeutic fields. The present study investigates the susceptibility of nosocomial strains of *S. aureus* against four major antibiotics: erythromycin, clindamycin, oxacillin and vancomycin. Ninety-four previously identified *S. aureus* nosomonial isolates were reconfirmed following the standard microbiological procedures for the isolation and identification of *S. aureus:* cultural, morphological and biochemical characterizations. Antibiograms of all isolates were determined employing the current guidelines of the Clinical and Laboratory Standards Institute (CLSI) on Müeller Hinton Agar. The resistance phenotypes were highest against clindamycin at 51% (48 isolates), making it the least effective antibiotic. Resistance against oxacillin and erythromycin were 46% (43 isolates) and 38% (36 isolates), respectively. The results indicated that the isolates are still susceptible to vancomycin which has the lowest resistance at 4% (4 isolates), making it the most effective antibiotic.

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

Introduction

*Staphylococcus aureus*is an important opportunistic pathogen commonly present in the natural environment. While it is harbored in the nares of 20–30%of healthy people (Rong *et al.*, 2017), it is considered as one of the most important bacterial pathogens in clinical practice and is a major diagnostic focus for the routine microbiology laboratory (Aryee and Edgeworth, 2016). *S. aureus*is known not only for its virulence factors but also for its multidrug resistance (Rong *et al.*, 2017). Multidrug-resistant strains of *S. aureus* have become increasingly prevalent worldwide and represent a leading cause of morbidity and mortality (Kashani *et al.*, 2018).

Surveillanceon the nosocomial prevalence of antibiotic resistant strains is essential in providing information on the magnitude of, and trends in, antimicrobial resistance (Kang and Song, 2013). Despite extensive research in other parts of the world, published data on antibiotic resistant, nosocomial strains of *Staphylococcus* in the Philippines is very limited. Therefore, in this study, the prevalence of nosocomial strains of antibiotic resistant *Staphylococcus* in Iligan CIty was determined. Data from this research will serve as the baseline information in the development of comprehensive strategies for the control and prevention of antimicrobial resistance.

Materials and methods

Study population

Nasal swab specimens of 112 patients from the different wards of a tertiary hospital in Illigan City, Philippines were collected. Demographic characteristics, clinical information and treatments were determined: age, gender, length of recent hospitalization, smoking habits, presence of skin lesions, use of nasal sprays, antibiotic treatment, history of hospital exposure prior to recent hospitalization, herbal medicine use and pregnancy.

Reconfirmation of previous identification of Staphylococcus aureus

Ninety-four previously presumptively identified *Staphylococcus aureus* from the combined inpatient nasal cultures were subjected to the standard traditional microbiological identification: growth on Mannitol Salt Agar for cultural characterization and detection of mannitol fermentation, microscopy and gram staining for cellular observation, and lastly, catalase and coagulase tests.

Antibiotic susceptibility testing

Susceptibility testing was done by agar screening method on Mueller Hinton agar (MHA). Plates were swab-streaked with bacterial suspension matched to 0.5 McFarland Turbidity Standard. Commercial discs impregnated with known amount of antibiotics were placed manually on MHA plate at approximately 28 millimeters (mm) from each disc except between clindamycin and erythromycin discs, which were 15mm apart from each other (measured edge to edge). Antimicrobial sensitivity was performed for oxacillin (1ug), vancomycin (30ug), erythromycin (15ug) and clindamycin (2ug) using the guidelines of the Clinical Laboratory for Standards Institute for disc-diffusion susceptibility. The plates were incubated for 18 hours at ambient room temperature.

Statistical analyses

Distribution of relationships between variables and subpopulations were evaluated. Comparison of categorical variables between groups was done by means of the Fisher's Exact and Chi-square (Pearson's) tests.

Results and discussion

Demographic and clinical profiles of the study population

The sampling done was through opportunistic method where 70 females and 42 male patients recruited to be part of the study (Table 1). Eighty-two patients have been in the hospital for more than three days and the remaining 30 were considered as newly-admitted patients. All 112 subjects reported to have been used antibiotics in the past six months, 67 patients claimed they have been to a hospital in the past six months (whether to visit a patient or they themselves have been hospitalized) also. The rest of the variables were relatively low with less than one-third of the population who were nasal spray users

(26%), with evident skin lesions (11%), confirmed smokers (9%) and those who had taken herbal medicines (10%). The identified variables for the study have been documented to be of significant

association for facilitating possible contraction of diseases from nosocomial pathogens (Perera and Hay, 2005).

Table 1. Sociodemographic data of the study population.

Variables	n (%)	Variables	n (%)
Sex		Age group (yo-years old)	
Males	42 (63%)	0-1 yo	8 (7%)
Females	70 (37%)	2-4 yo	17 (15%)
		5-7 yo	12 (11%)
Risk Factors		8-19 yo	19 (17%)
Presence of skin lesions	12 (11%)	20-65 yo	54 (49%)
History of antibiotic use	112 (100%)	66 and above	1 (1%)
Previous hospitalization	67 (60%)		
Smoker	10 (9%)	Length of hospital stay	
Use of nasal sprays	29 (26%)	Newly admitted	30 (27%)
Use of herbal medicines	11 (10%)	More than 3 days	82 (73%)

Phenotypic susceptibilities of S. aureus to different antimicrobials

The 94 confirmed *S. aureus* were from the previously collected 112 nasal swab specimens from both pediatric and adult ward patients of a tertiary hospital in Iligan City, Philippines. All these isolates were then subjected to the Kirby Bauer Disc Diffusion Technique for determination of phenotypic profiles against four antibiotics. These antibacterial agents are the most commonly used antibiotics against staphylococcal infections and preliminary surveillance was to assess its efficacy on the local picture. Table 1 shows the summary of the results obtained in the determination of the isolates' antibiograms.

Susceptibility profiles against oxacillin

Oxacillin resistance may be used interchangeably with methicillin resistance for they are in the same class of drugs and thus the same mode of action. Methicillin was used in the past but now oxacillin is used because it is more resistant to degradation in storage, is readily available and is more likely to detect most heteroresistant strains (Laboratory Detection of: Oxacillin/Methicillin-resistant *Staphylococcus aureus,* 2010).

Of the 94 isolates recovered, 46% (43 isolates) were oxacillin resistant which is considerably higher than the latest documented incidence in the Philippines which was 16.67% (San Juan *et al.*, 2012). The nosocomial rates of MRSA in USA from 2003-2008 was one MRSA infection per 1,000 hospital admissions (David *et al.*, 2012). The Center for Disease Control have reported in 2017 that the current rates of both nosocomial and community-associated MRSA in USA is at 2%. It is important to note though that while the isolates were limited in number and were based only from one local hospital, the results suggests the emergence of a significant oxacillin resistant *S. aureus* (ORSA) problem in Iligan City.

Seventy-two percent of the subjects who were able to stay in the hospital for at least three days (59/82) harbored ORSA while all the newly admitted patients were colonized with *S. aureus* still susceptible to oxacillin. There is a significant statistical correlation between the hospital stay and detection of ORSA of the study population (p <0.0001) thus implying that longer hospital stay presdisposes patients to acquisition of ORSA.

Hospital exposure prior to recent hospitalization was also found to be of significant association with ORSA detection at p <0.0001 in this study. The overall assessment on the capacity of the drug oxacillin to eliminate the collected *S. aureus*was that it was not a competitive drug (p=0.0013).

Susceptibility profiles against vancomycin
Vancomycin is considered as the most effective antimicrobial agent against staphylococcal strains and is the first-line and most inexpensive therapy to treat methicillin-resistant *S. aureus* (Panesso *et al.*, 2015).

However there has been an alarming emergence of vancomycin-resistant *S. aureus* (VRSA) (Hasan *et al.*, 2016). In the study population, a low resistance rate of four percent was observed from the total *S. aureus* isolates, all of which from patients with longer hospital stay. All the newly admitted patients had vancomycin-susceptible *S. aureus*. The length of hospital stay was not significant in the isolation of VRSA from the patients (p=0.5629). The results obtained in this study is significantly low compared to the 38% resistance of nosocomial *S. aureus* isolates from patients in a burn unit in Bangladesh (Hasan *et al.*, 2016).

Table 2. Summary of the phenotypic profiles of the nosocomial *S. aureus* isolates.

Antibiotic	Phenotypic profile	
	Susceptible	Resistant
Oxacillin	51	43
Vancomycin	90	4
Erythromycin	58	36
Clindamycin	46	48

Susceptibility profiles against erythromycin
Erythromycin has been used for many years in the treatment of bacterial infections and the value of testing this antibiotic as part of the preliminary evaluation could be especially meaningful in the view of increasing resistance of *S. aureus* against erythromycin. Asian countries had significant high *S. aureus* resistance rates against this drug: Vietnam (92.1%), Taiwan (86%) and HongKong (76%) (Song *et al.,* 2004).

The rate of non-susceptibiltiy of *S. aureus* is relatively low at 38% compared to the previously cited countries. Forty-one percent of the patients with longer hospital stay (34/82) harbored resistant strains and 30% of the newly admitted patients (9/30) were colonized with *S. aureus* that were non-susceptible to erythromycin (statistically not significant at p=0.3804). Nasal spray users, subjects with history of hospital exposure prior to recent hospitalization, , patients with evident skin lesions, smokers and herbal users also had high resistant

strains against erythromycin at 60%, 50%, 50%, 40% and 35% respectively, thereby implying the common use of this antibiotic may have caused the emergence of resistant forms of microorganisms.

Susceptibility profiles against clindamycin
Clindamycin is widely used in the treatment of Gram-positive infections and the expanded use of this antibiotic has been accompanied by increased numbers of resistant strains among staphylococci (Lim *et al.*, 2002). Fifty-one percent of the study population were colonized with *S. aureus* that were resistant against clindamycin. High resistance to clindamycin was observed among subjects undergoing antibiotic therapy (78/112), nasal spray users (35/52) and pregnant woment (2/4). Less than half of the population of the patients with hospital exposure prior to recent hospitalization, subjects with skin lesions, smoker and herbal medicine users at 45% (30/67), 33% (4/12), 20% (2/10) and 13% (2/16) respectively were found to have resistant strains against clindamycin.

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