



Phenotypic profiles of nasal methicillin resistant *Staphylococcus aureus* from asymptomatic children of Iligan City

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Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) has become a global healthcare threat, even colonizing healthy asymptomatic individuals with no traditional risk factors for MRSA. The objectives of this study was to determine sensitivity patterns of MRSA, isolated from healthy asymptomatic children, against three antibiotics commonly used to treating outpatients; and to establish the prevalence of multidrug resistant MRSA strains. Modified Kirby Bauer disc diffusion technique was employed to determine the susceptibilities, with zones of inhibition interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. Tetracycline is the best choice for empiric treatment of suspected MRSA infections with the lowest resistance at 3%. Clindamycin and ampicillin had resistance rates of 28% and 45% respectively. Fifteen percent of the MRSA isolates were multidrug resistant. Routine screening of clinical *S. aureus* isolates for methicillin resistance, regular surveillance studies as well as institution of infection control measures and antibiotic stewardship programme are recommended.

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Introduction

Since the 1960s, methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged, disseminated globally and become a leading cause of bacterial infections in both health-care and community settings. However, there is marked geographical variation in MRSA burden owing to several factors, including differences in local infection control practices and pathogen-specific characteristics of the circulating clones (Lee *et al.*, 2018).

While MRSA has been extensively studied in the United States and Europe, data from developing countries like the Philippines is severely lacking. The information gap is In addition to dire consequences of infections, MRSA strains are important for their resistance to many other commonly used antibiotics. Thus, it is immensely important to study the antibiogram of MRSA in order to minimize the irrational use of vancomycin when other antibiotics could cure the infection (Tiwari, Sapkota, Sen 2008).

This study was carried to determine the antibiotic susceptibility profiles of methicillin-resistant *S. aureus* from asymptomatic children of Iligan City with aim of providing information that will guide rational choice of antibiotics in the therapy of associated infections.

Materials and methods

Presumptive Identification of Methicillin Resistant Staphylococcus aureus

Staphylococcal isolates were isolated and presumptively identified using standard laboratory methods. Antibiotic susceptibility test using the Kirby Bauer disc diffusion technique was employed using cefoxitin (30 µg) in the determination of methicillin resistance.

Susceptibility Testing (Coyle, 2005) : The Modified Kirby Bauer Disc Diffusion Method

The Mac Farland turbidity standard was prepared by adding 0.5 ml of a 1% solution of barium chloride

hydrate into a 100-ml cylinder, and filling to 100ml with 1% sulfuric acid.

The inoculum of the methicillin resistant staphylococci was prepared from a primary culture plate onto a tube with 10ml distilled water and was compared with a turbidity standard. Proper adjustment to the turbidity of the inoculum was essential to ensure that the resulting lawn of growth is confluent or almost confluent. A sterile cotton swab was dipped into the inoculum, and excess inoculum was removed by pressing and rotating the swabs firmly against the side of the tube above the level of the liquid. The swab was streaked all over the surface of the Mueller-Hinton plate three times, rotating the plate through an angle of 45° after each application. The plates with inoculum were left to dry for a few minutes at ambient room temperature with the lid closed. The antibiotic discs (tetracycline 30µg, clindamycin 2µg and ampicillin 10µg) were then placed on the inoculated plates, approximately 15 millimeters (mm) from the edge of the plate, using a pair of sterile forceps. Each disc was pressed down gently to ensure even contact with the medium and the plates were then incubated at room temperature. After an overnight of incubation, the diameter of each zone (including the diameter of the disc) was measured and recorded in mm. The results were then interpreted according to the current critical diameters as published by the Clinical Laboratory Standards Institute. The measurements were made with the a caliper under the surface of the plate without opening the lid. The end-point of inhibition was judged by the naked eye at the edge where the growth has started.

Results and discussion

Asymptomatic carriage of methicillin-resistant *S. aureus* can predispose the host to a wide range of infections (Aqel *et al.*, 2015) making it a threat to healthcare worldwide. The high level of resistance to beta-lactam antibiotics in MRSA is due to the presence of mec A which encodes for penicillin – binding protein (PBP2a). PBP2a is able to prevent the action of methicillin and enables the bacteria to

synthesize peptidoglycan and grow (Fishovitz *et al.*, 2014).

Phenotypic profiles of the 69 methicillin resistant *S. aureus* which were isolated from nasal swab cultures of healthy, asymptomatic children living near open

dumpsites were tested for activities against tetracycline, clindamycin, and ampicillin.

Table 1 shows the number of susceptible isolates to the three antibiotics.

Table 1. Antibiotic susceptibility profiles of MRSA from nasal swab cultures of pediatric population living near open dumpsites of Iligan City.

Antibiotic	Susceptible isolates
	MRSA (n=67)
Tetracycline	65 (97%)
Clindamycin	48 (72%)
Ampicillin	37 (55%)

An understanding of various phenotypic profiles of methicillin resistant staphylococci is a significant contribution on the optimization of strategies to effectively control MRSA. Among the three antibiotics, tetracycline was the most effective wherein nearly all MRSA isolates were still susceptible (97%) and beta-lactam ampicillin was the least effective wherein only 55% of MRSA isolates showed susceptibility. The result conforms to the reports to the reports (Hizel *et al.*, 1997; Eckart, Hospenthal, Fishbain, 2000) on the emergence of beta-lactam-specific resistant mutants of staphylococci, which may eventually result to resistance of these bacterial stains to the antibiotic.

MRSA is frequently associated with having acquired resistance to a variety of drugs including tetracyclines. In fact, tetracycline resistance is the second most common resistance phenotype in MRSA strains isolated in Poland Tetracycline resistance is also prevalent in MRSA in Bulgaria, Turkey and England (Trzcinski *et al.*, 2000). However, in this study, tetracycline was the most effective antibiotic with only 3% of the MRSA isolates being resistant to it. An investigation on the phenotypic profiles of MRSA isolates in the United States (Diekema *et al.*, 2001) showed similar pattern where tetracycline has the lowest resistance at 16% while the antibiotics ciprofloxacin, erythromycin, and clindamycin in 89%, 93%, and 79% of all isolates.

The lowered susceptibility of the isolates to the beta-lactam ampicillin conforms to the reports (Hizel *et al.*, 1997; Eckart *et al.*, 2000) on the emergence of the beta-lactam-specific resistant mutants of staphylococci, which may eventually to the total resistance of these bacterial strains to the antibiotic.

The enzymes beta-lactamases produced by *S. aureus* act in the hydrolysis of the ring beta-lactamic of the penicillin, which is transformed in acid neutralizing its bactericidal effect (Bernardo *et al.*, 2006).

Given the fact that staphylococci spread by direct or indirect person-to-person contact, the emergence of multidrug-resistant stains of MRSA is worrisome in the present therapeutic scenario (Tiwari, Sapkota, Sen, 2008). Multidrug resistance was defined as resistance of a MRSA strain towards three or more antibiotics at a given point of time.

Table 2 shows the susceptibility profiles of the isolates to different antibiotic collaborations. Multidrug resistance rates varies and most of the data available are of nosocomial origins, reaching as high as 80%. In this study, multidrug resistance was exhibited by 10 (15%) MRSA isolates. Despite the relatively low rates of MDR-MRSA, it is but imperative that there should be regular MRSA surveillance and strict drug policies should be implemented. Otherwise, the threat of MDR-MRSA will exponentially increase.

Table 2. The response of methicillin-resistant *S. aureus* isolates to the different antibiotics.

Antibiotic Patterns	Number of MRSA isolates (n=67)	
Tr-Cr	1	(1%)
Tr-Cs	1	(1%)
Ts-Cr	19	(28%)
Ts-Cs	46	(67%)
Tr-Ar	0	(0%)
Tr-As	2	(3%)
Ts-Ar	29	(43%)
Ts-As	36	(54%)
Cr-Ar	9	(13%)
Cr-As	10	(15%)
Cs-Ar	20	(30%)
Cs-As	28	(42%)
Tr-Cr-Ar	0	(0%)
Tr-Cr-As	1	(1%)
Tr-Cs-Ar	0	(0%)
Tr-Cs-As	1	(1%)
Ts-Cr-Ar	9	(13%)
Ts-Cr-As	9	(13%)
Ts-Cs-Ar	20	(30%)
Ts-Cs-As	27	(40%)

Tetracycline-clindamycin collaboration was the most effective wherein 67% (46) of MRSA isolates were still susceptible. The cell growth inhibition by tetracycline and the bacteriostatic effect of clindamycin was found to be very useful and effective in killing MRSA isolates. It can therefore be inferred that antibiotics other than vancomycin can be used as anti-MRSA agent.

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