

Presence of methicillin resistance and heteroresistance among Coagulase Negative Staphylococci (CoNS) isolates obtained from Health Sciences students at a Public University

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Received 21 March 2016; received in revised form 3 March 2016; accepted 5 March 2016

Abstract. This study was carried out to investigate the Coagulase Negative Staphylococci (CoNS) nasal carriage and the presence of methicillin resistant Coagulase Negative Staphylococci (MR-CoNS) among health sciences students at Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. A total of 120 isolates of CoNS (62.5%) was isolated from 192 student volunteers. The *mecA* gene was detected in 15 isolates of CoNS (12.5%). Eight out of the 15 isolates of *mecA* positive CoNS were resistant to cefoxitin in disc diffusion test whereas the remaining seven isolates of *mecA* positive CoNS were susceptible to cefoxitin. Analysis of questionnaires showed no significant association between CoNS nasal carriage and the socio-demographic and risk factors except for the genders and history of cold ($P \leq 0.050$). Generally, this finding showed a relatively low level of methicillin resistance among CoNS nasal carriage from student volunteers.

INTRODUCTION

Staphylococci are opportunistic pathogens that commonly colonize skin and mucous membrane of human. Apart from *Staphylococcus aureus*, coagulase negative staphylococci (CoNS) such as *S. epidermidis* and *S. haemolyticus* are also frequently isolated from these sites. *S. epidermidis* is the most widespread CoNS species and is frequently isolated from human epithelia, axillae (armpits), head and nostril (Otto, 2009). Generally, CoNS species are distributed throughout the human body, indicating the importance of CoNS in maintaining healthy skin flora. In addition to mutual relationship with human hosts, CoNS,

particularly methicillin resistant strain can also cause infections in high risk individuals such as newborns, elder individuals and immunocompromised patients. Based on previous studies, 60% to 85% of clinical strains are methicillin resistant CoNS (MR-CoNS) whereas the prevalence rate of MR-CoNS in the community settings is between 11% to 31% (Silva *et al.*, 2001; Jamaluddin *et al.*, 2008; Otto, 2009; Ruppé *et al.*, 2009; Barbier *et al.*, 2010).

Resistance to methicillin in staphylococci is due to *mecA* gene which expresses penicillin binding protein 2a (PBP2a), a transpeptidase with a low affinity for β -lactams (Zong *et al.*, 2011). The *mecA* gene is carried in mobile genetic elements (MGE)

known as the staphylococcal cassette chromosome *mec* (SCC*mec*) (Zong *et al.*, 2011). As most of studies are concentrating on *S. aureus* and MRSA, not much research has been carried out on the prevalence of community-acquired CoNS or MR-CoNS in Malaysia. The purpose of this study was to investigate the prevalence of CoNS nasal carriage among student volunteers who lived in vicinity and its association with socio-demographic and various risk factors. In addition, *mecA* gene screening was carried out to detect the presence of MR-CoNS among the student volunteers.

MATERIALS AND METHODS

This cross-sectional study was carried out at Faculty of Medicine and Health Sciences, Universiti Putra Malaysia involving 192 health sciences students in November 2013. Before the collection of nasal samples, each student who consented in participating in this study was given a set of guided self-administered questionnaire which included questions on socio-demographic data, health status and hygienic practices (Amin Nordin *et al.*, 2012). The socio-demographic and various risk factors collected from questionnaires were analyzed in relation to distribution of CoNS using Chi-square and Fisher's exact tests (Motulsky, 2015). The association with P-value of ≤ 0.050 was considered significant.

The nasal samples were obtained from each participant by rubbing and rotating a sterile cotton swab against the inner surface of each nostril. The specimen was directly inoculated on mannitol salt agar and incubated at 37°C for 24 hours. The identity of CoNS was further confirmed by gram staining and coagulase test. *S. epidermidis* ATCC 12228 and *S. aureus* ATCC 700699 were included as reference strains in all subsequent analyses.

Antibiotic susceptibility test was carried out by using Kirby Bauer disc diffusion method based on guidelines by Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2012). Cefoxitin (30 µg) were used to check

the phenotypic methicillin resistance. The diameter of zone of inhibition was interpreted according to the standard range in CLSI (CLSI, 2012). As for PCR, all the CoNS isolates were subjected to *mecA* gene screening. The primers used were *mecA*-F (5'-AAA ACT AGG TGT TGG AGA TAT ACC-3') and *mecA*-R (5'-GAA AGG ATC TGT ACT GGG TTA ATC AG-3') (Suhaili *et al.*, 2013). The PCR conditions included an initial denaturation at 94°C at 4 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C and extension at 72°C for 1 min, and a final extension at 72°C for 4 min. The bacterial isolates that were detected for 147 bp amplicon were characterized as *mecA* positive CoNS.

RESULTS

In this study, 120 isolates (62.5%) of CoNS were collected from 192 student volunteers. These isolates matched the characteristics of CoNS, including lack of mannitol fermentation, gram positive cocci in clusters and coagulase negative. Among all the isolates that were subjected to antibiotic susceptibility test, ten were resistant against cefoxitin. Nevertheless, for the latter, only eight of them were positive for *mecA* gene whereas the other two isolates were *mecA* negative. PCR also detected *mecA* gene in seven isolates of CoNS which were susceptible to cefoxitin. These isolates were considered as methicillin-heteroresistant CoNS.

The association between the health sciences student volunteers and the risk factors are indicated in Table 1. Based on Chi-square and Fisher's exact test analyses, two risk factors, gender and history of cold or fever in the past two weeks had significant association in relation to CoNS nasal carriage. The other socio-demographic and risk factors were deemed as insignificant. Fourteen out of the 15 isolates of *mecA* positive CoNS were carried by female students. Two of them were with chronic illness (asthma).

Table 1. CoNS nasal carriage in relation to risk factors

Risk factor	Health Sciences Students		P-value
	Positive for CoNS n = 120 (62.5%)	Negative for CoNS n = 72 (37.5%)	
Gender			
Male	15 (12.5)	17 (23.6)	0.046 ^a
Female	105 (87.5)	55 (76.4)	
Race			
Malay	88 (73.3)	49 (68.1)	0.43 ^a
Others	32 (26.7)	23 (31.9)	
History of chronic illness	4 (3.3)	3 (4.2)	1.0 ^b
History of cold or fever in the past two weeks	15 (12.5)	19 (26.4)	0.015 ^a
Consumption of antibiotics in the past two weeks	3 (2.5)	2 (2.8)	1.0 ^b
History of hospitalization	3 (2.5)	0 (0)	0.29 ^b
Presence of unhealed wound	1 (0.8)	4 (5.6)	0.067 ^b
Use of intravascular devices	2 (1.7)	5 (6.9)	0.11 ^b
Habit of touching nose	74 (61.7)	46 (63.9)	0.76 ^a
Habit of washing hand after touching nose	81 (67.5)	50 (69.4)	0.78 ^a

a – Chi-square test

b – Fisher's exact test

DISCUSSION

There are limited studies on prevalence of CoNS at local setting for comparison. Nevertheless, the relatively high prevalence of CoNS (62.5%) was in accordance to a previous study which showed a high CoNS nasal carriage (73.3%) among health care workers in a Pakistani hospital (Akhtar, 2010). The significant factors related to nasal carriage of CoNS were gender and history of cold or fever in the past two weeks. The results showed a higher rate of CoNS nasal carriage in female than male students. Previously, Chen *et al.* (2013) reported gender as the significant factor in *S. aureus* colonization in Taiwanese adolescent population. However, they found a lower incidence of *S. aureus* colonization in female. The rate of CoNS nasal carriage was slightly lower in individuals with history of cold or fever in the past two weeks before sample collection. This indicated that having cold or fever might have effects in microbial colonization in nares.

In this study, MR-CoNS is defined as CoNS isolates carrying *mecA* gene regardless the susceptibility against cefoxitin. The prevalence of MR-CoNS among the student volunteers (12.5%) was in harmony to several studies on community acquired MR-CoNS which had reported the prevalence rate within 11 to 31% (Silva *et al.*, 2001; Jamaluddin *et al.*, 2008; Ruppé *et al.*, 2009; Barbier *et al.*, 2010). Several factors, for example the status of hygiene and sanitation, use of antibiotic and climate were reported to explain the variation in the incidence of MR-CoNS colonization in community (Jamaluddin *et al.*, 2008; Widerström *et al.*, 2011; Lebeaux *et al.*, 2012). Lebeaux *et al.* (2012) reported that poor hygienic condition may contribute to abnormally high prevalence of MR-CoNS (51% in 2006 and 47% in 2008) in the remote population in French Guiana. Furthermore, the method used in isolation may also affect the prevalence rate of MR-CoNS (Silva *et al.*, 2001; Widerström *et al.*, 2011). In our study, the nasal swab samples were directly grown on MSA without

any antibiotic supplement. Therefore, with the possibility of MR-CoNS being outnumbered by commensal bacteria, the chances for isolating MR-CoNS was significantly decreased. In the case of methicillin-heteroresistant CoNS isolates, this was most likely due to insufficient regulation of *mecA* expression and the absence of methicillin or other β -lactam antibiotic as inducers in the host environment (Martineau *et al.*, 2000). Lastly, regarding the two *mecA* negative isolates showing phenotypic resistance against cefoxitin, a similar scenario had been encountered in previous studies (Martineau *et al.*, 2000; Swenson *et al.*, 2005; Zhang *et al.*, 2009). The methicillin resistance in *mecA* negative staphylococci is possibly mediated by remnants of SCC*mec* elements in the bacterial genomes (Martineau *et al.*, 2000; Zhang *et al.*, 2009).

In conclusion, this study showed a high prevalence rate of CoNS nasal carriage among the student volunteers but a relatively low prevalence of *mecA* positive CoNS isolates. The CoNS nasal carriage was associated with two factors (genders and history of cold and fever) and the other risks factors showed no significant association. A well-designed study which involves a larger sample size is warranted to provide further information on the prevalence of MR-CoNS and its association with various risk factors, as well as the issues on the heteroresistance.

Ethical considerations. This study was approved by the Ethics Committee for Research involving Human Subjects of Universiti Putra Malaysia.

Acknowledgement. This study was partly funded by grant no. 04-02-12-1764RU and ERGS/12013/5527167. We are grateful to the assistance of technical staff at the Department of Biomedical Science and Department of Medical Microbiology and Parasitology, Faculty Medicine and Health Sciences, Universiti Putra Malaysia.

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