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Prevalence, Influencing Factors, Antibiotic Resistance, Toxin and Molecular Characteristics of *Staphylococcus aureus* and MRSA Nasal Carriage among Diabetic Population in the United States, 2001–2004

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Abstract

Diabetic population were reported more likely to suffer carriage and infection with *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *Staphylococcus aureus* (MRSA) than non-diabetic population. We aim to elucidate the prevalence and characteristics of *S. aureus* and MRSA nasal carriage among diabetic population in the United States National Health and Nutrition Examination Survey, 2001–2004. Univariate analyses were conducted using Chi-square test, Fisher's exact probability test or student *t* test, as appropriate. Multivariate analysis using logistic regression was conducted to assess the association between influencing factors and *S. aureus* and MRSA nasal carriage. 1010 diabetic participants were included in the study. The prevalence of *S. aureus* and MRSA nasal carriage were 28.32% and 1.09%, respectively. After the logistic regression, ever had a painful sensation or tingling in hands or feet past three months (Odds Ratio [OR] = 0.359, 95% Confidence Interval [CI], 0.146–0.882) was significant among *S. aureus* nasal carriage and gender (OR = 3.410, 95% CI, 1.091–10.653) was significant among MRSA nasal carriage. The proportions of staphylococcal enterotoxin (SE) A, SEB, SEC, SED, Toxic-shock syndrome toxin-1, and Panton Valentine Leukocidin toxin among *S. aureus* strains were 18.75%, 3.13%, 12.50%, 15.63%, 28.13%, and 9.38%, respectively. 63.63% of MRSA strains were community-acquired, 27.27% were hospital-acquired, and 9.09% were non-typeable. Diabetic patients might be more likely to carry *S. aureus* and MRSA in the United States. Improving hand hygiene compliance, reducing antibiotic overuse, screening for carriers, and decolonization are recommended to reduce the spread of *S. aureus* and MRSA, especially in community.

Key words: *Staphylococcus aureus*, diabetics, MRSA, nasal carriage, NHANES

Introduction

Staphylococcus aureus (*S. aureus*) is a common pathogen and can cause different kinds of infection in both hospitals and communities (Knox *et al.*, 2015; Samanta *et al.*, 2015). Several parts of human body can carry *S. aureus* and the nasal cavity is the main part. It was reported that *S. aureus* nasal carriage can increase the possibility of infection (Wertheim *et al.*, 2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) was first discovered in 1961 and then widely spread around the world (Chen *et al.*, 2012; Hernandez-Porto *et al.*, 2015). In the United States, the proportion of methicillin resistance in *S. aureus* strains approached almost 60% in 2003, with an average resistance rate of approximately 50% over the period from 1998 to 2002 (NNIS, 2004). In Europe, the proportion of methicillin resistance in *S. aureus* strains isolated from infected patients increased from 21% in 2002 to 23% in 2005 and then decreased to 19% in 2008. Between 2002 and 2005, the annual increase was 7.6%, followed by an average annual decrease of 4.8% (de Kraker *et al.*, 2013).

MRSA can increase the mortality rate, prolong hospitalization and aggravate the economic burden (Gastmeier *et al.*, 2012; Lodise and McKinnon, 2005). MRSA infection, hepatitis B and Acquired Immune Deficiency Syndrome has currently become three global infectious diseases.

Diabetes is a common chronic disease. With the development of social economy, the improvement of living standard and the aging of population, the number of diabetic population is increasing worldwide. The

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International Diabetes Federation Annual Report in 2014 showed that 387 million people were estimated to be living with diabetes, an alarming number that is set to rise to 592 million within the next twenty years. A further 316 million with impaired glucose tolerance are at high risk from the disease, with projections indicating that over one billion people will be living with or at high risk of diabetes in 2035 (IDF, 2014). Due to poor long-term blood glucose control or various complications, diabetic population is prone to suffer *S. aureus* or MRSA infection, which can cause death to a certain extent. Several studies reported that diabetes can increase *S. aureus* and MRSA carriage and infection (Chen and Pass, 2013; Daeschlein *et al.*, 2015). In other words, diabetic population was more likely to suffer carriage and even infection with MRSA than non-diabetic population.

There were a few studies focusing on *S. aureus* and MRSA nasal carriage among diabetic population and the conclusions were different. A Turkey study reported that the rates of *S. aureus* and MRSA nasal carriage among diabetic outpatient population were 41.9% (127/304) and 0.87% (30/304), respectively (Kutlu *et al.*, 2012). An Indian study reported that the *S. aureus* nasal carriage rate among hospitalized diabetic patients was 56.6% (34/60) (Ahluwalia *et al.*, 2000). A Chinese study reported that the rates of *S. aureus* and MRSA nasal carriage among hospitalized diabetic patients were 20.5% (41/200) and 0.50% (1/200), respectively (Junhua *et al.*, 2005). These studies, however, mainly focused on the prevalence and risk factors of *S. aureus* and MRSA nasal carriage among diabetic population, which were not comprehensive. Therefore, the objective of this current study was to elucidate the prevalence, influencing factors, antibiotic resistance, toxin, and molecular characteristics of *S. aureus* and MRSA nasal carriage among diabetic population who participated in the United States National Health and Nutrition Examination Survey (NHANES), 2001–2004.

Experimental

Materials and Methods

Study design and population. The continuous NHANES is a large, annual, cross-sectional survey that is designed to evaluate the nutrition and health status of the US population. The surveys were approved by the National Center for Health Statistics Research Ethics Review Board. Data releases are available in two-year increments. The *S. aureus* and MRSA nasal carriage data were only collected from this NHANES 2001–2004 dataset, so we used these data for analyzing. All participants provided written informed consent and the research ethics boards of the National Center for Health Statistics approved all protocols. More details regarding the informed consent of subjects, survey design, data collection, data procedures and laboratory assessment can be found elsewhere (CDC). Those who were tested the assessment of *S. aureus* and were self-reported diabetes were included in the current study.

Assessment of prevalence of *S. aureus* and MRSA. Assessment of *S. aureus* was conducted for the entire sample, thus, all participants aged one year or older were tested. Specimens collected from the nares were plated on mannitol salt agar, a selective medium for the isolation of *S. aureus*. After overnight cultures, specimens were used to perform Staphaurex and tube coagulase test. Staphaurex-positive and tube coagulase-positive isolates were identified as *S. aureus* and saved for further testing. *S. aureus* isolates were screened for methicillin resistance by the disk diffusion method of the National Clinical and Laboratory Standards Institute.

Diagnosed diabetes status. Diagnosed diabetes was identified in participants who answered "yes" to the self-reported question, "Have you ever been told by a doctor or health professional that you had diabetes?" and/or who used diabetes medication *(i.e.* oral hypoglycemic agents and/or insulin).

Potential influencing factors. Several variables were investigated as potential characteristics indicative of nasal carriage of *S. aureus* and MRSA among diabetic population. These factors included the self-reported diabetic characteristics variables, self-reported demographic variables, body measures variables, self-reported current health variables, self-reported medical conditions variables, self-reported alcohol use variables, self-reported drug use variables, self-reported physical activity variables, self-reported prescription medications variables, and self-reported exposure to cigarette smoke (operationalized as either being a smoker or living in a house with a current smoker). Given the limited population carriage of MRSA, we investigated a limited number of characteristics based on whether the sample size was large enough to make a reliable population estimate.

Assessment of antibiotic resistance of *S. aureus* and MRSA. Antibiotic resistance of *S. aureus* and MRSA was through antibiotic susceptibility testing by broth microdilution using Clinical and Laboratory Standards Institute reference methods. We calculated the resistance of 18 common antibiotics including tetracycline, clindamycin, erythromycin, penicillin, imipenem, vancomycin, cefazolin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, rifampin, amoxicillin, chloramphenicol, doxycycline, daptomycin, quinupristin-dalfopristin, and linezolid. Moreover, we calculated multidrug resistance (MDR), which was defined as resistant to three or more antibiotics with different mechanisms of action

(note that these strains are already resistant to all beta-lactam antibiotics).

Assessment of toxin characteristics and molecular characteristics of *S. aureus* and MRSA. Strain typing by singleplex polymerase chain reaction (PCR) for detection of staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1 (TSST-1), Panton-Valentine Leukocidin (PVL) toxin, and staphylococcal cassette chromosome *mec* (SCC*mec*) type. SCC*mec* type is an important classification of MRSA that can be used to trace the source of the bacteria. According to some international reports, SCC*mec* I–III are designated as hospital-acquired (HA), SCC*mec* IV and V are designated as community-acquired (CA), and others are designated as non-typeable (NT) strains (Ito *et al.*, 2003; Okuma *et al.*, 2002). Although there have been studies (Freitas *et al.*, 2010) that reported possible misclassification errors for this typing, we still referenced it because there are no other accurate classifications.

Statistical analysis. Means and standard errors were calculated for continuous variables, and frequencies (percentages) were calculated for categorical variables. Univariate analyses were conducted using Chi-square test, Fisher's exact probability test or student t test, as appropriate. Multivariate analysis using logistic regression was conducted to assess the association between influencing factors and *S. aureus* and MRSA nasal carriage. We also conducted a logistic regression analysis of all variables with a P value of < 0.10 and then removed variables with a P value of ≥ 0.10. All statistical analyses were two-sided. All the statistical analyses were performed using Stata 13.1 (College Station, Texas, USA).

Results

Prevalence of *S. aureus* and MRSA nasal carriage. According to the inclusion criteria of study population there were 1010 diabetic population included in the current study. The prevalence of *S. aureus*, MRSA, and methicillin-sensitive *Staphylococcus aureus* (MSSA) nasal carriage were 28.32% (286/1010), 1.09% (11/1010), and 27.23% (275/1010) respectively.

Influencing factors of *S. aureus* and MRSA nasal carriage. We found that military status ($P = 0.027$), marital status ($P = 0.001$), ever still had a liver condition ($P = 0.033$), ever had cancer or malignancy ($P = 0.038$), ever blood relatives had diabetes ($P = 0.030$), ever taken antibiotics past month ($P = 0.017$), ever taken diabetic pills to lower blood sugar ($P = 0.040$), ever had numbness ($P = 0.071$), ever had a painful sensation or tingling in hands or feet past three months ($P = 0.026$), number of days used street drugs over past year ($P < 0.001$), and ever had five or more drinks every day ($P = 0.043$) were associated with *S. aureus* nasal carriage among diabetic population in this current study.

Gender ($P = 0.068$), number of alcoholic drinks per day over past 12 months ($P = 0.048$), number of days had five or more drinks past 12 months ($P = 0.036$), ever had vigorous activity past 30 days ($P = 0.070$), ever had moderate activity past 30 days ($P = 0.081$), frequency of playing or exercising hard every week ($P = 0.050$), and number of hours using TV, video or computer every day ($P = 0.002$) were associated with MRSA nasal carriage among diabetic population in this current study. More details can be found in Table I and Supplementary materials.

To account for potential confounding among the influencing factors, we further analyzed the relationship between the potential predictors with a logistic regression model. This model showed that when controlling for the effect of the other influencing factors, the relationships found in the univariate analyses changed. Ever had a painful sensation or tingling in hands or feet past three months was still associated with *S. aureus* nasal carriage among diabetic population in this logistic regression model. Having a painful sensation or tingling in hands or feet past three months was a protective factor. Having a painful sensation or tingling in hands or feet past three months among diabetic population were 0.359 times (95% Confidence Interval [CI], 0.146–0.882) less likely than those not having a painful sensation or tingling in hands or feet past three months to carry *S. aureus*. Gender was still associated with MRSA nasal carriage among diabetic population in this logistic regression model. Female was a risk factor. Female diabetic population was 3.410 times (95% CI, 1.091–10.653) more likely than male diabetic population to carry MRSA. More details can be found in Table II and Table III.

Antibiotic resistance of *S. aureus* nasal carriage. Among the proportions of antibiotic resistance in MRSA strains, penicillin (100.00%), imipenem (100.00%), cefazolin (100.00%), oxacillin (100.00%), and amoxicillin (100.00%) were the highest, followed by erythromycin (72.73%), tetracycline (27.27%), levofloxacin (27.27%), clindamycin (18.18%), chloramphenicol (11.11%), vancomycin (0.00%), gentamicin (0.00%), ciprofloxacin (0.00%), rifampin (0.00%), doxycycline (0.00%), daptomycin (0.00%), quinupristin-dalfopristin (0.00%), and linezolid (0.00%). Specially, there was no MRSA strain resistant to vancomycin, gentamicin, ciprofloxacin, rifampin, doxycycline, daptomycin, quinupristin-dalfopristin, and linezolid. Among the proportions of antibiotic resistance in MSSA strains, erythromycin (90.48%) and penicillin (90.48%) were the highest, followed by tetracycline (0.00%), vancomycin (0.00%), clindamycin (0.00%), imipenem (0.00%), cefazolin (0.00%), oxacillin (0.00%), gentamicin (0.00%), ciprofloxacin

Table I
Significant influencing factors of *Staphylococcus aureus* and MRSA nasal carriage

Influencing factors	Number of population	*Staphylococcus aureus*			MRSA		
		Number (%)	χ^2	P	Number (%)	χ^2	P
Gender			–	–		3.340	0.068
Male	553	155 (28.03)			3 (1.94)		
Female	534	131 (24.53)			8 (6.11)		
Military status			4.868	0.027		–	–
Yes	77	16 (20.78)			1 (6.25)		
No	507	169 (33.33)			6 (3.55)		
Marital status			20.062	0.001		–	–
Married	277	105 (37.91)			3 (2.86)		
Widowed	55	12 (21.82)			0 (0.00)		
Divorced	46	11 (23.91)			2 (18.18)		
Separated	13	2 (15.38)			0 (0.00)		
Never married	252	58 (23.02)			2 (3.45)		
Living with partner	34	14 (41.18)			0 (0.00)		
Do you still have a liver condition			4.530	0.033		–	–
Yes	27	3 (11.11)			0 (0.00)		
No	25	9 (36.00)			0 (0.00)		
Ever told you had cancer or malignancy			4.310	0.038		–	–
Yes	147	32 (21.77)			1 (3.12)		
No	828	250 (30.19)			10 (4.00)		
Blood relatives have diabetes			4.723	0.030		–	–
Yes	717	222 (30.96)			9 (4.05)		
No	230	54 (23.48)			2 (3.70)		
Which biological [blood] family member have diabetes?			–	–		–	0.093
Mother	114	31 (27.19)			1 (3.23)		
Father	63	19 (30.16)			0 (0.00)		
Mother's mother	31	6 (19.35)			2 (33.33)		
Mother's father	17	6 (35.29)			0 (0.00)		
Father's mother	20	8 (40.00)			1 (12.50)		
Father's father	9	3 (33.33)			0 (0.00)		
Brother	90	29 (32.22)			2 (6.90)		
Sister	204	68 (33.33)			2 (2.94)		
Other	167	51 (30.54)			1 (1,96)		
Taken antibiotics past month			5.695	0.017		–	–
Yes	54	8 (28.89)			1 (12.50)		
No	576	174 (30.21)			5 (2.87)		
Take diabetic pills to lower blood sugar			4.229	0.040		–	–
Yes	684	208 (30.41)			6 (2.88)		
No	323	78 (24.15)			5 (6.41)		
Has the numbness been in hands, feet, or both			5.287	0.071		–	–
Hands	92	20 (21.74)			0 (0.00)		
Feet	116	41 (35.34)			1 (2.44)		
Both	144	37 (25.69)			2 (5.41)		
Had a painful sensation or tingling in hands or feet past three months			4.954	0.026		–	–
Yes	353	87 (24.65)			5 (5.75)		
No	562	177 (31.49)			5 (2.82)		

Table I continued

Influencing factors	Number of population	*Staphylococcus aureus*			MRSA		
		Number (%)	χ^2	P	Number (%)	χ^2	P
Number of days used street drugs over past year			43.144	< 0.001		–	–
< 30	67	20 (29.85)			0 (0.00)		
30 –	0	0 (0.00)			0 (0.00)		
90 –	0	0 (0.00)			0 (0.00)		
180 –	0	0 (0.00)			0 (0.00)		
270 –	943	266 (28.21)			11 (4.14)		
Ever have five or more drinks every day			4.090	0.043		–	–
Yes	66	12 (18.18)			0 (0.00)		
No	328	100 (30.49)			2 (2.00)		
30 –	5	1 (20.00)			0 (0.00)		
90 –	6	2 (33.33)			0 (0.00)		
180 –	2	0 (0.00)			0 (0.00)		
270 –	718	203 (28.27)			11 (5.42)		
Vigorous activity past 30 days			–	–		3.279	0.070
Yes	298	78 (26.17)			0 (0.00)		
No	421	122 (28.98)			5 (4.10)		
Moderate activity past 30 days			–	–		3.039	0.081
Yes	367	106 (28.88)			1 (0.94)		
No	362	99 (27.35)			5 (5.05)		
Frequency of playing or exercising hard every week			–	–		3.841	0.050
< 7	92	27 (29.35)			2 (7.41)		
7 –	112	39 (34.82)			3 (7.69)		
15 –	4	0 (0.00)			0 (0.00)		
21 –	2	1 (50.00)			0 (0.00)		
30 –	800	219 (27.38)			6 (2.74)		
Number of hours using TV, video or computer everyday			–	–		9.332	0.002
None	5	0 (0.00)			0 (0.00)		
< 1	42	14 (33.33)			0 (0.00)		
1	47	9 (19.15)			1 (11.11)		
2	77	27 (35.06)			0 (0.00)		
3	63	15 (23.81)			0 (0.00)		
4	32	11 (34.38)			0 (0.00)		
> 5	67	24 (35.82)			0 (0.00)		

(0.00%), levofloxacin (0.00%), rifampin (0.00%), amoxicillin (0.00%), chloramphenicol (0.00%), doxycycline (0.00%), daptomycin (0.00%), quinupristin-dalfopristin (0.00%), and linezolid (0.00%). Specially, there was no MSSA strain resistant to tetracycline, vancomycin, clindamycin, imipenem, cefazolin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, rifampin, amoxicillin, chloramphenicol, doxycycline, daptomycin, quinupristin-dalfopristin, and linezolid. Moreover, there was no MDR *S. aureus* strain. There were significantly differences between MRSA and MSSA strains in tetracycline (P = 0.012), imipenem (P = 0.007), cefazolin (P = 0.007), oxacillin (P < 0.001), levofloxacin (P = 0.007), and amoxicillin (P = 0.070). More details can be found in Table IV.

Toxin characteristics of *S. aureus* nasal carriage. No MRSA strain was positive to SEA while 28.57% of MSSA strains were positive to it. No MRSA strain was positive to SEB while 4.76% of MSSA strains were positive to it. 18.18% of MRSA strains were positive to SEC while 9.52% of MSSA strains were positive to it. 36.36% of MRSA strains were positive to SED while 4.76% of MSSA strains were positive to it. Both MRSA and MSSA strains were not positive to SEE or SEH. 9.09% of MRSA strains were positive to STTS-1 while 38.10%

Table II
Logistic regression analysis of *Staphylococcus aureus* nasal carriage

Influencing factors	OR	95% CI
Military status		
Yes	0.371	0.091–1.514
No	1.00	
Ever told you had cancer or malignancy		
Yes	0.255	0.051– 1.286
No	1.00	
Blood relatives have diabetes		
Yes	1.680	0.567–4.982
No	1.00	
Taken antibiotics past month		
Yes	0.552	0.100–3.045
No	1.00	
Take diabetic pills to lower blood sugar		
Yes	1.797	0.691–4.677
No	1.00	
Had a painful sensation or tingling in hands or feet past three months		
Yes	0.359	0.146–0.882
No	1.00	
Number of days used street drugs over past year		
< 30	1.00	
30 –	–	–
90 –	–	–
180 –	–	–
270 –	1.951	0.526–7.236
Ever have five or more drinks every day		
Yes	0.317	0.079–1.269
No	1.00	

OR, Odds Ratio; CI, confidence interval.

Table III
Logistic regression analysis of MRSA nasal carriage

Influencing factors	OR	95% CI
Gender		
Male	1.00	
Female	3.410	1.091–10.653
Frequency of play or exercise hard every week		
< 7	1.00	
7 –	0.976	0.148–6.427
15 –	–	–
21 –	–	–
30 –	0.323	0.061–1.722

OR, Odds Ratio; CI, confidence interval.

of MSSA strains were positive to it. With regard to PVL, 18.18% of MRSA strains were positive to it while no MSSA strain was positive to it. There were significantly differences between MRSA and MSSA strains in SEA (P = 0.049), SED (P = 0.019), TSST-1 (P = 0.083), and PVL (P = 0.044). More details can be found in Table V.

Molecular characteristics of MRSA nasal carriage. With regard to outcomes of SCC*mec* type among 11 MRSA strains, IV (63.64%) was the highest, followed by II (27.27), NT (9.09), I (0.00), III (0.00), and V (0.00). 63.64% of MRSA strains were CA, 27.27% were HA, and 9.09% were NT.

Discussion

This current study comprehensively elucidate the prevalence, influencing factors, antibiotic resistance, toxin and molecular characteristics of *S. aureus* and MRSA nasal carriage among diabetic population who participated in the NHANES, 2001–2004. Among 1010 diabetic population in this current study, there were 286 *S. aureus* strains, 11 MRSA strains, and 275 MSSA strains. The prevalence of *S. aureus*, MRSA, and MSSA nasal carriage were 28.32% (286/1010), 1.09% (11/1010), and 27.23% (275/1010) respectively. The prevalence of *S. aureus* (28.32%, 286/1010) nasal carriage among diabetic population in this current study was lower than that of diabetic outpatient population in Turkey (41.78%, 127/304) (Kutlu *et al.*, 2012), that of long-term hemodialysis type 2 diabetes patients in Saudi Arabia (72.41%, 42/58) (Saxena *et al.*, 2002), that of hospitalized diabetic patients in India (56.67%, 34/60) (Ahluwalia *et al.*, 2000), and that of community-based diabetes patients in Australia (39.09%, 258/660) (Hart *et al.*, 2015), but was higher than that of hospitalized diabetic patients (20.50%, 41/200) (Junhua *et al.*, 2005) and community-based type 2 diabetes patients in China (10.31%, 43/417) (Yan *et al.*, 2015). The prevalence of MRSA (1.09%, 11/1010) nasal carriage among *S. aureus* diabetic population in this current study was lower than community-based type 2 diabetes patients in China (5.28%, 22/417) (Yan *et al.*, 2015), that of diabetic outpatient population in Turkey (9.87%, 30/304) (Kutlu *et al.*, 2012), and that of long-term hemodialysis type 2 diabetes patients in Saudi Arabia (18.97%, 11/58) (Saxena *et al.*, 2002), but was higher than hospitalized diabetic patients in China (0.50%, 1/200) (Junhua *et al.*, 2005), that of community-based diabetes patients in Australia (1.21%, 8/660) (Hart *et al.*, 2015), and that of type 1 diabetes pediatric outpatients in Turkey (in 2005, 0.99%, 1/101; in 2013, 0.75%, 1/134) (Karadag-Oncel *et al.*, 2015). From the above statistics, we can know that the prevalence of *S. aureus* and MRSA nasal carriage among diabetic population were different in different countries and regions, and diabetic population might be more likely to carry *S. aureus* and MRSA in United States.

Table IV
Antibiotic resistance of *Staphylococcus aureus* nasal carriage

Antibiotic	MRSA		MSSA		χ^2	P
	Number of population	Resistant (%)	Number of population	Resistant (%)		
Tetracycline	11	3 (27.27)	21	0 (0.00)	6.320	0.012
Clindamycin	11	2 (18.18)	21	0 (0.00)	–	0.111
Erythromycin	11	8 (72.73)	21	19 (90.48)	1.725	0.189
Penicillin	11	11 (100.00)	21	19 (90.48)	–	0.534
Imipenem	2	2 (100.00)	15	0 (0.00)	–	0.007
Vancomycin	11	0 (0.00)	21	0 (0.00)	–	–
Cefazolin	2	2 (100.00)	15	0 (0.00)	–	0.007
Oxacillin	11	11 (100.00)	21	0 (0.00)	32.000	< 0.001
Gentamicin	11	0 (0.00)	21	0 (0.00)	–	–
Ciprofloxacin	2	0 (0.00)	15	0 (0.00)	–	–
Levofloxacin	11	3 (27.27)	21	0 (0.00)	6.320	0.012
Rifampin	11	0 (0.00)	21	0 (0.00)	–	–
Amoxicillin	2	2 (100.00)	15	0 (0.00)	–	0.007
Chloramphenicol	9	1 (11.11)	6	0 (0.00)	–	1.000
Doxycycline	2	0 (0.00)	1	0 (0.00)	–	–
Daptomycin	5	0 (0.00)	3	0 (0.00)	–	–
Quinupristin-dalfopristin	6	0 (0.00)	3	0 (0.00)	–	–
Linezolid	9	0 (0.00)	6	0 (0.00)	–	–

Table V
Toxin characteristics of *Staphylococcus aureus* nasal carriage [n (%)]

Toxin	Total (N = 32)	MRSA (N = 11)	MSSA (N = 21)	χ^2	P
SEA	6 (18.75)	0 (0.00)	6 (28.57)	3.868	0.049
SEB	1 (3.13)	0 (0.00)	1 (4.76)	–	1.000
SEC	4 (12.50)	2 (18.18)	2 (9.52)	0.495	0.482
SED	5 (15.63)	4 (36.36)	1 (4.76)	5.468	0.019
SEE	0 (0.00)	0 (0.00)	0 (0.00)	–	–
SEH	0 (0.00)	0 (0.00)	0 (0.00)	–	–
TSST-1	9 (28.13)	1 (9.09)	8 (38.10)	3.004	0.083
PVL	2 (6.25)	2 (18.18)	0 (0.00)	4.073	0.044

SE, staphylococcal enterotoxin; TSST-1, toxic-shock syndrome toxin-1; PVL, panton valentine leukocidin; N, number of population; n, number of positive strains.

There were studies reported that the main influencing factors of *S. aureus* and MRSA nasal carriage were gender (Geofrey *et al.*, 2015; Soltani *et al.*, 2014; Yan *et al.*, 2015), age (Geofrey *et al.*, 2015; Huifen *et al.*, 2015; Soltani *et al.*, 2014), weight (Campbell *et al.*, 2015), history of hospitalization (Huifen *et al.*, 2015; Karanika *et al.*, 2015), history of invasive operation (Karanika *et al.*, 2015; Qiongxiang, 2014), conditions of antibiotic using (Qiongxiang, 2014; Soltani *et al.*, 2014), chronic diseases (Campbell *et al.*, 2015; Chen and Pass, 2013; Daeschlein *et al.*, 2015; Geofrey *et al.*, 2015; Huifen *et al.*, 2015; Karanika *et al.*, 2015), history of infection (Chen and Pass, 2013; Daeschlein *et al.*, 2015) and so on. After performing a logistic regression, ever had a painful sensation or tingling in hands or feet past three months was still associated with *S. aureus* nasal carriage among diabetic population in this current study. Having a painful sensation or tingling in hands or feet past three months was a protective factor, which might be resulted from more likely of this population to notice the hygiene of their hands or feet. We found that having a painful sensation or tingling in hands or feet past three months among diabetic population was 0.359 times less likely than those not having a painful sensation or tingling in hands or feet past three months to carry *S. aureus*. With regard to

MRSA nasal carriage among diabetic population in this current study, gender was still associated with it in this logistic regression model. Female was a risk factor, which might be resulted from their weaker immune system. Female diabetic population was 3.410 times more likely than male diabetic population to carry MRSA, which was consistent to a Chinese study (Yan *et al.*, 2015) and a Tanzanian study (Soltani *et al.*, 2014). Therefore, female diabetic population should be paid more attention to perform surveillance of *S. aureus* and MRSA so as to avoid carriage and/or infection.

We found that both MRSA and MSSA strains in this current study were highly resistant to erythromycin and penicillin, which was similar to several studies (Geofrey *et al.*, 2015; Junhua *et al.*, 2005; Kejela and Bacha, 2013; Morgenstern *et al.*, 2016) and this might be resulted from the extensive use of these antibiotics in medical institutions. But both MRSA and MSSA strains were 100% sensitive to vancomycin, gentamicin, ciprofloxacin, rifampin, doxycycline, daptomycin, quinupristin-dalfopristin and linezolid, which was similar to several studies (Junhua *et al.*, 2005; Morgenstern *et al.*, 2016), and it could indicate that these antibiotics can be used to treat *S. aureus* infection in clinical medication. The propotions of resistance to tetracycline ($P = 0.012$), imipenem ($P = 0.007$), cefazolin ($P = 0.007$), oxacillin ($P < 0.001$), levofloxacin ($P = 0.007$), and amoxicillin ($P = 0.070$) among MRSA strains were significantly higher than MSSA strains. This could indicate that the antibiotic resistance of MRSA strains was more severe than MSSA strains and should be emphasized by healthcare workers. It suggested that healthcare workers should rationally utilize antibiotics, prevent antibiotic abuse and long-term use, and can use antibiotic combination or rotation to avoid reducing sensitivity of *S. aureus* and MRSA to antibiotics.

The pathogenesis of *S. aureus* infections depends on the production of surface proteins that mediate bacterial adherence to host tissues, secretion of a series of extracellular toxins, and enzymes that destruct host cells and tissues, avoidance of, or incapacitating, the host immune defense, and growth and spread of bacteria in host cells (Lowy, 1998). Toxins are proteins secreted by *S. aureus* into the extracellular matrix during the post-exponential and early stationary phases. These proteins are usually involved in tissue penetration and enable the bacteria to invade its host. They are also cytolytic and help bacterial growth by acquiring essential nutrients such as iron from lysed-cells (Kong *et al.*, 2016). The SEs genes are super antigens which trigger T-cell activation and proliferation, and their mode of action probably includes activation of cytokine release and cell death *via* apoptosis and potentially lethal toxic shock syndrome (Lin *et al.*, 2010). In this current study, 18.18% and 36.36% of MRSA strains were positive to SEC and SED, respectively. 28.57%, 4.76%, 9.52%, and 4.76% of MSSA strains were positive to SEA, SEB, SEC, and SED, respectively. Because SEs can cause vomiting and diarrhea and the toxins are one of the most common causes of food-borne diseases (Kong *et al.*, 2016), we should pay more attention to those who were positive to them. It has been reported that TSST-1 will stimulate the release of chemokines, such as IL-8 and MIP-3α, IL-2, and TNFα (Otto, 2014). Activation of immune cells will enhance inflammation and cause mucosal cell barrier disruption, allowing further interaction of the toxin with T-cells and macrophages, leading towards toxic shock syndrome (Larkin *et al.*, 1982). MSSA (38.10%) strains were more likely than MRSA (9.09%) strains to be positive to TSST-1 ($\chi^2 = 3.004$, $P = 0.083$), which meant that those who carried MSSA should be paid more attention. PVL has been reported to be associated with CA-MRSA infections (Otto, 2010), which was consistent with this current study. 28.57% (2/7) of CA-MRSA strains in this current study were positive to it. 18.18% of MRSA strains were positive to PVL, which was higher than health care personnel in Egypt (2.24%, 5/223) (Hefzy *et al.*, 2016) and patients in Indonesia (6.56%, 17/259) (Santosaningsih *et al.*, 2016), which might be resulted from poor hand hygiene compliance and non-judicious use of antibiotics.

Among 11 MRSA strains, seven (63.63%) were CA, three (27.27%) were HA, and one (9.09%) was NT, which could indicate that the majority of MRSA strains in this current study were from community and relevant departments should pay attention to strengthen the surveillance and disinfection in community.

Overall, there were several limitations in this current study. Firstly, we did not take the environmental characteristics into consideration due to the complex data due to limited financial support. Secondly, we did not follow up the outcome of the included population due to the limited data, which can be studied in the future. Thirdly, there are limited data on the antibiotic resistance, toxin, and molecular characteristics of *S. aureus* and MRSA, which might lead to some kind of errors.

Conclusions

Diabetic population might be more likely to carry *S. aureus* and MRSA in United States. Having a painful sensation or tingling in hands or feet past three months might be a protective factor. Female might be a risk factor and female diabetic population should be paid more attention to perform surveillance of *S. aureus* and MRSA so as to avoid carriage and/or infection. The antibiotic resistance of MRSA strains was more severe than that of MSSA strains and should be emphasized by health-

care workers. The proportions of toxins in *S. aureus* were high and those who detected positive should be paid more attention. Therefore, improving compliance, reducing antibiotic overuse, screening for carriers, and decolonization are recommended strategies for reducing the spread of *S. aureus* and MRSA. The majority of MRSA strains in this current study were from community and relevant departments should pay greater attention to strengthen the surveillance of *S. aureus* and MRSA and disinfection measures in community.

Supplementary Materials

Supplementary materials were tables used in the study for the univariate results of influencing factors among *S. aureus* and MRSA nasal carriage. Supplementary materials accompanies the paper on Polish Journal of Microbiology website.

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Conflict of interest

The authors declare that they have no conflict of interest.

Literature

Ahluwalia A., A. Sood, A. Sood, R. Lakshmy, A. Kapil and R.M. Pandey. 2000. Nasal colonization with *Staphylococcus aureus* in patients with diabetes mellitus. *Diabet. Med*. 17: 487–488.

Campbell K.A., C. Cunningham, S. Hasan, L. Hutzler and J.R. Bosco. 2015. Risk Factors for Developing *Staphylococcus aureus* Nasal Colonization in Spine and Arthroplasty Surgery. *Bull. Hosp. Jt. Dis. (2013)*. 73: 276–281.

Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey. http://www.cdc.gov/nchs/nhanes/about_nhanes.htm.

Chen C.C. and S.E. Pass. 2013. Risk factors for and impact of methicillin-resistant *Staphylococcus aureus* nasal colonization in patients in a medical intensive care unit. *Am. J. Infect. Control*. 41: 1100–1101.

Chen C.S., C.Y. Chen and Y.C. Huang. 2012. Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students at a Taiwanese university. *Int. J. Infect. Dis.* 16: e799–e803.

Daeschlein G., S. von Podewils, T. Bloom, O. Assadian, M. Napp, H. Haase and M. Junger. 2015. Risk factors for MRSA colonization in dermatologic patients in Germany. *J. Dtsch. Dermatol. Ges.* 13: 1015–1022.

de Kraker M.E., V. Jarlier, J.C. Monen, O. E. Heuer, van de N. Sande and H. Grundmann. 2013. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clin. Microbiol. Infect*. 19: 860–868.

Freitas E.A., R.M. Harris, R.K. Blake and C.D. Salgado. 2010. Prevalence of USA300 strain type of methicillin-resistant *Staphylococcus aureus* among patients with nasal colonization identified with active surveillance. *Infect. Control Hosp. Epidemiol*. 31: 469–475.

Gastmeier P., F. Schwab, E. Meyer and C. Geffers. 2012. [Excess mortality and prolongation of stay due to bloodstream infections caused by multiresistant pathogens in Germany]. *Dtsch. Med. Wochenschr*. 137: 1689–1692.

Geofrey A., A. Abade and S. Aboud. 2015. Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization among Intensive Care Unit (ICU) patients and health care workers at Muhimbili national hospital, Dar Es Salaam, Tanzania, 2012. *Pan. Afr. Med. J*. 21: 211.

Hart J., E.J. Hamilton, A. Makepeace, W.A. Davis, E. Latkovic, E.M. Lim, J.R. Dyer and T.M. Davis. 2015. Prevalence, risk factors and sequelae of *Staphylococcus aureus* carriage in diabetes: the Fremantle Diabetes Study Phase II. *J. Diabetes Complications*. 29: 1092–1097.

Hefzy E.M., G.M. Hassan and E.R.F. Abd. 2016. Detection of panton-valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* nasal carriage among Egyptian Health Care workers. *Surg. Infect* (*Larchmt*) 17: 369–375.

Hernandez-Porto M., M. Lecuona, A. Aguirre-Jaime, B. Castro, T. Delgado, M. Cuervo, Y. Pedroso and A. Arias. 2015. Antimicrobial resistance and molecular analysis of methicillin-resistant *Staphylococcus aureus* collected in a Spanish hospital. *Microb. Drug Resist.* 21: 201–208.

Huifen Y., Z. Junshao, Q. Wenzhou, F. Yi, Y. Ling and G. Defan. 2015. Study on colonization status and risk factors of methicillin resistant *Staphylococcus aureus* in patients of intense care units. *Chin. J. Disinf.* 32: 24–26.

IDF. 2014. International Diabetes Federation Annual Report 2014. *International Diabetes Federation*. 2014: 6.

Ito T., K., Okuma X.X. Ma, H. Yuzawa and K. Hiramatsu. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist. Updat*. 6: 41–52.

Junhua M., C. Junchang, W. Rui and Z. Chunrong. 2005. Nasal carriage of *Staphylococcus aureus* and drug resistance in patients with diabetes Mellitus. *Chin J. Nosocomiol*. 15: 830–831.

Karadag-Oncel E., N. Gonc, Altay O., A.B. Cengiz, A. Ozon, A. Pinar, M. Ceyhan, A. Alikasifoglu, Y. Akyon, N. Kandemir and others. 2015. Prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* in children with diabetes mellitus: Trends between 2005 and 2013. *Am. J. Infect. Control*. 43: 1015–1107.

Karanika S., F.N. Zervou, I.M. Zacharioudakis, S. Paudel and E. Mylonakis. 2015. Risk factors for meticillin-resistant *Staphylococcus aureus* colonization in dialysis patients: a meta-analysis. *J. Hosp. Infect.* 91: 257–263.

Kejela T. and K. Bacha. 2013. Prevalence and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia. *Ann. Clin. Microbiol Antimicrob*. 12: 11.

Knox J., A.C. Uhlemann and F.D. Lowy. 2015. *Staphylococcus aureus* infections: transmission within households and the community. *Trends Microbiol.* 23: 437–444.

Kong C., H.M. Neoh and S. Nathan. 2016. Targeting *Staphylococcus aureus* toxins: A potential form of anti-virulence therapy. *Toxins* (*Basel*) 8: 72.

Kutlu S.S., N. Cevahir, S. Akalin, F. Akin, C.S. Dirgen, M. Bastemir and K. Tekin. 2012. Prevalence and risk factors for methicillin-resistant *Staphylococcus aureus* colonization in a diabetic outpatient population: a prospective cohort study. *Am. J. Infect. Control.* 40: 365–368.

Larkin S.M., D.N. Williams, M.T. Osterholm, R.W. Tofte and Z. Posalaky. 1982. Toxic shock syndrome: clinical, laboratory, and pathologic findings in nine fatal cases. *Ann. Intern. Med.* 96: 858–864.

Lin C.F., C.L. Chen, W.C. Huang, Y.L. Cheng, C.Y. Hsieh, C.Y. Wang and M.Y. Hong. 2010. Different types of cell death induced by enterotoxins. *Toxins* (*Basel*) 2: 2158–2176.

Lodise T.P. and P.S. McKinnon. 2005. Clinical and economic impact of methicillin resistance in patients with *Staphylococcus aureus* bacteremia. *Diagn. Microbiol. Infect. Dis*. 52: 113–122.

Lowy F.D. 1998. *Staphylococcus aureus* infections. *New Engl. J. Med*. 339: 520–532.

Morgenstern M., C. Erichsen, S. Hackl, J. Mily, M. Militz, J. Friederichs, S. Hungerer, V. Buhren, T.F. Moriarty, V. Post and others. 2016. Antibiotic resistance of commensal *Staphylococcus aureus* and coagulase-negative staphylococci in an International Cohort of Surgeons: A prospective point-prevalence study. *PLoS One*. 11: e148437.

National Nosocomial Infections Surveillance (NNIS). 2004. System Report, data summary from January 1992 through June 2004, issued October 2004. *Am. J. Infect. Control*. 32: 470–485.

Okuma K., K. Iwakawa, J.D. Turnidge, W.B. Grubb, J.M., Bell F.G. O'Brien, G.W. Coombs, J. W. Pearman, F.C. Tenover, M. Kapi and others. 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J. Clin. Microbiol.* 40: 4289–4294.

Otto M. 2010. Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. *Annu. Rev. Microbiol.* 64: 143–162.

Otto M. 2014. *Staphylococcus aureus* toxins. *Curr. Opin. Microbiol.* 17: 32–37.

Qiongxiang Z. 2014. Multivariate logostic regression analysis of risk among community-acquired methicillin resistant *Staphylococcus aureus* infection and colonization. *Hebei Medical Journal*. 36: 1876–1878.

Samanta D., J.L. Batte, S.N. Brown, A.G. Crosby, L.A. Marcos and M.O. Elasri 2015. Molecular and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* isolates causing bacteremia at a major hospital in southern Mississippi. *Am. J. Infect. Control*. 43: 540–542.

Santosaningsih D., S. Santoso, N.S. Budayanti, Suata K., E.S. Lestari, H. Wahjono, A. Djamal, K. Kuntaman, A. van Belkum, M. Laurens and others. 2016. Characterization of clinical *Staphylococcus aureus* isolates harboring *mec*A or panton-valentine leukocidin genes from four tertiary care hospitals in Indonesia. *Trop. Med. Int. Health*. 21: 610–618.

Saxena A.K., B.R. Panhotra, C.K. Venkateshappa, D.S. Sundaram, M. Naguib, W. Uzzaman and M.K. Al. 2002. The impact of nasal carriage of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* (MRSA & MSSA) on vascular access-related septicemia among patients with type-II diabetes on dialysis. *Ren. Fail*. 24: 763–777.

Soltani B., A.A. Taghavi, A. Moravveji, M. Erami, R.M. Haji, R. Moniri and M. Namazi. 2014. Risk factors for methicillin resistant *Staphylococcus aureus* nasal colonization of healthy children. *Jundishapur. J. Microbiol*. 7: e20025.

Wertheim H.F., D.C. Melles, Vos M.C., W. van Leeuwen, A. van Belkum, H.A. Verbrugh and J.L. Nouwen. 2005. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.* 5: 751–762.

Yan L., X. Ping, D. Lin, Q. Ou and Z. Yao. 2015. Nasal colonization prevalence and risk factors of methicillin-resistant *Staphylococcus aureus* in type 2 diabetic patients from communities. *J. Pract. Med.* 31: 4133–4135.