Meticillin-resistant *Staphylococcus aureus* isolated from foot ulcers in diabetic patients in a Chinese care hospital: risk factors for infection and prevalence

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A retrospective case–control study of 118 (male : female, 68 : 50) Chinese type 2 diabetic patients with foot ulcers (Wagner's grade 3–5) was conducted to determine the prevalence and risk factors for meticillin-resistant *Staphylococcus aureus* (MRSA) infection in relation to the original community or hospital parameters. Ulcer specimens were processed for Gram staining, aerobic culture and antimicrobial susceptibility testing. *Staphylococcus* species were tested for meticillin resistance using oxacillin. *S. aureus* was the most frequent pathogen (25.6 %) in diabetic patient specimens (160 isolates), and a high proportion of *S. aureus* isolates were MRSA (63.4 %). A high percentage of *S. aureus* isolates (65.4 %) satisfied the definition for hospital-associated MRSA (HA-MRSA) infection. The size of ulcers [adjusted odds ratio (OR) 1.61; 95 % confidence interval (CI) 1.22–2.12] and osteomyelitis (adjusted OR 18.51, 95 % CI 2.50–137.21) were independent predictors of MRSA infection. The HA-MRSA group had a significantly different distribution from the community-associated MRSA group with respect to age, history of diabetes and length of hospital stay (all $P < 0.001$). Neuropathy, vascular disease (all $P = 0.049$) and osteomyelitis ($P = 0.026$) were the most common underlying conditions observed in the HA-MRSA group. This study contributes to the establishment of precautions against the emergence of MRSA including MRSA acquired from different sources among the Chinese population with diabetic foot ulcers based on their original or clinical parameters.

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INTRODUCTION

Foot lesions in diabetic patients are a major medical, social and economic problem. Infectious agents are associated with amputation of the infected foot if not treated promptly. Infection with multidrug-resistant organisms may increase the duration of hospital stay and cost of management, as well as morbidity and mortality (Hartemann-Heurtier *et al.*, 2004).

Staphylococcus aureus is the most common pathogen among Gram-positive bacteria isolated from ulcers. Almost 50 % of *S. aureus* isolates are meticillin-resistant *S. aureus* (MRSA). MRSA has been increasingly isolated from diabetic foot ulcers, and several studies have found its emergence in as many as 15–30 % of diabetic wounds (Goldstein *et al.*, 1996;

Shankar *et al.*, 2005; Tentolouris *et al.*, 1999). MRSA emergence may be associated with adverse prognosis. Patients with community-acquired MRSA (CA-MRSA) infections often do not exhibit the risk factors known in patients with hospital-associated MRSA (HA-MRSA) infections. These risk factors include recent hospitalization, dialysis, nursing-home residence and the presence of other co-morbid conditions such as diabetes, chronic renal failure and chronic pulmonary diseases, which bring patients into contact with healthcare settings.

However, the clinical characteristics and factors that predispose diabetic patients with foot ulcers towards infection with different associated MRSA remain unclear. This study aimed to compare the prevalence and antimicrobial susceptibility profile of CA-MRSA with HA-MRSA isolated from Chinese diabetic patients with foot ulcers. The risk factors for infection of ulcers with MRSA and the outcome of these infections were also studied.

Abbreviations: CA, community-acquired; CI, confidence interval; HA, hospital-associated; HbA_{1c}, glycosylated haemoglobin; MRSA, meticillin-resistant *Staphylococcus aureus*; OR, odds ratio.

METHODS

Patients and infection definition. Samples for this retrospective study were collected from 118 type 2 diabetic patients with infected foot ulcers (Wagner's grade 3–5). The patients were admitted to the Diabetic Foot Care Center at the Zhongda Hospital of Southeast University, Nanjing, PR China, from 1 January 2004 to 31 December 2006. Infection was diagnosed according to the criteria proposed by the International Consensus on the Diabetic Foot (Lipsky *et al.*, 2004). Ulcers were graded using the Wagner classification (Wagner, 1981). The control group comprised 97 type 2 diabetic patients with foot ulcers (Wagner's grade 3–5) without CA-MRSA or HA-MRSA infection.

HA-MRSA infection is defined as that occurring in a patient whose MRSA isolate was cultured more than 48 h after admission, who has a history of hospitalization of more than 24 h, who has had dialysis, surgery or residence in a long-term healthcare facility within 6 months prior to the culture date, or who has an indwelling intravenous line, catheter or any other percutaneous medical device present at the time the culture was taken. CA-MRSA is defined as an MRSA isolate obtained within 48 h of hospitalization from a patient without the above-mentioned risk factors (Fridkin *et al.*, 2005; Naimi *et al.*, 2003). Patients who had infections in other body sites or an MRSA-positive isolate prior to the study period were excluded from the study.

Data collection. Data were collected via a case record form. The data included demographic data, duration of diabetes, glycaemic control and presence of retinopathy, nephropathy (creatinine ≥ 150 mol l⁻¹ or the presence of micro- or macroalbuminuria), neuropathy (absence of perception of the Semmes–Weinstein monofilament at two of ten standardized plantar sites on either foot) and peripheral vascular disease (ankle brachial pressure index <0.9 , or a history of intermittent claudication or rest pain, with or without pedal pulses).

The duration and size of the ulcer, clinical outcome, duration of hospital stay and glycaemic control during the hospital stay were noted for each patient. Clinical assessment for signs of infection (swelling, exudate, surrounding cellulitis, odour, tissue necrosis, crepitation and pyrexia) was performed. Hospitalizations within the previous 6 months and residence in nursing homes were systematically recorded. Laboratory values were determined at the laboratory centre of Zhongda Hospital.

Ulcer size, expressed in cm², was determined by multiplying the longest and widest diameters. Osteomyelitis was diagnosed from suggestive changes in radiographs and bone scans. All patients were monitored until discharge from the hospital.

Microbiological methods. Each ulcer specimen for culture was obtained at least twice, once within 48 h and the other more than 48 h after admission, both taken after the surface of the wound had been washed vigorously with saline, followed by debridement of superficial exudates. Specimens were obtained by scraping the ulcer base or the deep portion of the wound edge with a sterile curette, and were promptly sent to the laboratory to be processed for Gram staining, aerobic culture and antimicrobial susceptibility testing.

Characterization of isolates. All MRSA cultures were confirmed in the microbiology laboratory of Zhongda Hospital. Antimicrobial susceptibility testing of aerobic isolates was performed using a standard disc diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002). Susceptibility to gentamicin, tetracycline, erythromycin, clindamycin, nitrofurantoin, fusidate, sulfamethoxazole, rifampicin, vancomycin, teicoplanin, levofloxacin, quinupristin/dafopristin, penicillin, minocycline and norfloxacin was determined. *Staphylococcus* species were tested for meticillin resistance using a 1 µg oxacillin disc and oxacillin screen agar (6 µg oxacillin ml⁻¹).

Statistical analysis. Quantitative data were expressed as means $\pm$ SD, and qualitative variables were expressed as frequencies. For statistical analysis, Fisher's exact test, Pearson's χ^2 test and Student's *t*-test were used as appropriate. Continuous variables were analysed using a two-tailed *t*-test, and $P < 0.05$ was considered statistically significant. For the case–control study, conditional univariate and multivariate logistic regression models were used to estimate the odds ratio (OR) and the associated 95% confidence interval (CI). All statistical analyses were performed using statistical software (SPSS for Windows, version 11.5).

RESULTS

Characteristics of patients and wounds

Over half of the patients in the study were male (57.6%). The mean age of the subjects was 60.8 ± 10.4 years. The mean duration of diabetes was 8.8 ± 7.0 years, and 46 (39.0%) had had the condition for ≥ 10 years. The mean level of glycosylated haemoglobin (HbA_{1c}) was $7.4 \pm 1.9\%$; 87 patients (73.7%) had neuropathy, 88 (74.6%) had peripheral vascular disease, 59 (50.0%) had nephropathy, 48 (40.7%) had retinopathy and 80 (67.8%) were hypertensive. Osteomyelitis was present in 20 patients (16.9%).

Total number of bacteria and specific strains

A total of 160 isolates were detected from the 118 ulcer specimens, giving a mean of 1.4 species per patient. Nearly 40% of patients had an infection from two or three species, whilst infection from three species was found in 6.0% of patients.

A profile of the organisms isolated is given in Table 1. Of the 160 isolates, 81 (50.6%) were Gram-positive aerobic bacteria and eight were fungal isolates (5.0%). Of the 81 Gram-positive bacterial isolates, the majority were *Staphylococcus* species ($n=51$; 63.0%). *S. aureus* was the

Table 1. Profile of bacteria isolated from infected foot ulcers in specimens from diabetic patients ($n=118$ patients)

The total number of isolates was 160.

Category	Frequency (%)
Gram-negative bacteria	
<i>Proteus</i> species	17 (10.6)
<i>Escherichia coli</i>	32 (20.0)
<i>Pseudomonas aeruginosa</i>	11 (6.9)
<i>Acinetobacter</i> species	7 (4.4)
<i>Klebsiella</i> species	4 (2.5)
Gram-positive bacteria	
Staphylococci species	51
<i>S. aureus</i>	41 (25.6)
<i>S. haemolyticus</i>	4 (2.5)
<i>S. epidermidis</i>	6 (3.8)
Enterococcus species	17 (10.6)
Streptococci	13 (8.1)
Fungi	8 (5.0)

most frequent pathogen, found in about one-quarter of the total infections (41; 25.6 %). In our data, a high proportion ($n=26$; 63.4 %) of *S. aureus* isolates were MRSA; 17 (65.4 %) satisfied the definition of HA-MRSA, whilst the rest ($n=9$; 34.6 %) were classified as CA-MRSA.

Characteristics of patients infected with MRSA and their wounds

Twenty-one type 2 diabetic patients (Wagner's grade 3–5) with foot ulcers were infected with MRSA. The clinical features of the cases and controls are given in Table 2. They did not differ significantly in gender, duration of ulcer or duration of hospital stay. Chronic diabetic complications, Wagner grade and discharge status were not associated with MRSA infection. Age, duration of diabetes, level of HbA_{1c}, size of ulcer and osteomyelitis were significantly associated with CA-MRSA infection. The number of MRSA-infected patients that had an increased requirement for surgical treatment was also significantly higher than controls.

Six variables were eligible for inclusion in the multivariable model. In this model (Table 3), the size of ulcer (adjusted OR 1.61; 95 % CI 1.22–2.12) and osteomyelitis (adjusted

OR 18.51; 95 % CI 2.50–137.21) were independent predictors of MRSA infection. Age, duration of diabetes, HbA_{1c} and requirement for surgical treatment were not statistically significant predictors of MRSA infection in the multivariable model.

The HA-MRSA group had significantly different distributions from the CA-MRSA group with respect to age and length of hospital stay (both $P<0.001$; Table 2). Patients with HA-MRSA ulcers had a long history of diabetes ($P=0.001$); neuropathy, vascular disease (both $P=0.049$) and osteomyelitis ($P=0.026$) were the most common underlying conditions observed in the HA-MRSA group. There was no significant difference between CA-MRSA and HA-MRSA in either duration or size of ulcer, although HA-MRSA colonization of ulcers was characterized by longer histories and larger ulcers compared with those of CA-MRSA.

Antimicrobial susceptibility in patients infected with MRSA and their prognosis

The prevalence of MRSA resistance to each antimicrobial tested is presented in Table 4. CA-MRSA isolates were more likely than HA-MRSA isolates to be susceptible to

Table 2. Association of study characteristics in two groups of diabetic patients according to infection of foot ulcers with HA-MRSA and CA-MRSA

Twenty-one type 2 diabetic patients (Wagner's grade 3–5) with foot ulcers were infected with MRSA. The control group included 97 type 2 diabetic patients with foot ulcers (Wagner's grade 3–5) without CA-MRSA or HA-MRSA infection.

Characteristic	MRSA	Control	OR (95 % CI)	P value	HA-MRSA	CA-MRSA	P value
<i>n</i>	21	97	–	–	14	7	–
Age (years)	66.8 ± 8.1	59.4 ± 10.5	1.10 (1.03–1.17)	0.004	71.2 ± 5.3	58.0 ± 4.7	<0.001
Gender							
Male	15	53	2.08 (0.74–5.80)	0.164	10	5	1.00
Female	6	44			4	2	1.00
Duration of diabetes (years)	14.1 ± 8.3	7.6 ± 6.2	1.13 (1.05–1.21)	0.001	17.9 ± 7.4	6.4 ± 2.9	0.001
HbA _{1c} (%)	8.5 ± 2.1	7.1 ± 1.7	1.46 (1.13–1.88)	0.003	9.5 ± 1.8	6.6 ± 0.9	0.001
Duration of ulcer (months)	1.5 ± 1.6	1.0 ± 1.4	1.22 (0.92–1.62)	0.168	1.8 ± 1.9	1.1 ± 0.5	0.361
Duration of hospital stay (days)	26.4 ± 13.7	24.9 ± 8.7	1.02 (0.97–1.06)	0.527	33.1 ± 11.9	13.0 ± 2.7	<0.001
Wagner's grade	–	–	1.77 (0.75–4.20)	0.196	–	–	–
Size of ulcer (cm ²)	6.5 ± 3.8	2.5 ± 1.9	1.69 (1.34–2.12)	<0.001	7.1 ± 4.3	5.3 ± 2.3	0.334
Case with number of ulcers ≥ 2	7	10	2.83 (0.92–8.71)	0.069	6	1	0.19
Complications							
Hypertension	14	66	0.94 (0.35–2.56)	0.903	11	3	0.102
Retinopathy	12	36	2.26 (0.87–5.89)	0.095	9	3	0.35
Nephropathy	14	45	2.31 (0.86–6.23)	0.098	11	3	0.102
Neuropathy	17	70	1.56 (0.48–5.06)	0.462	13	4	0.049
Peripheral vascular disease	17	71	1.56 (0.48–5.06)	0.462	13	4	0.049
Osteomyelitis	13	7	20.89 (6.49–67.27)	<0.001	11	2	0.026
Treatment							
Interventional	3	4	3.88 (0.80–18.81)	0.093	1	2	0.186
Surgical	6	7	5.14 (1.52–17.42)	0.009	6	0	0.04
Discharge status							
Alive	20	96	0.21 (0.01–3.47)	0.274	13	7	0.469
Dead	1	1	0.21 (0.01–3.47)	0.274	1	0	0.469

Table 3. Multivariate conditional regression analysis of risk factors for MRSA infection: matched case-control study

Twenty-one type 2 diabetic patients (Wagner's grade 3–5) with foot ulcers were infected with MRSA. The control group included 97 type 2 diabetic patients with foot ulcers (Wagner's grade 3–5) without CA-MRSA or HA-MRSA infection.

Characteristic	OR (95 % CI)	P value
Age (years)	1.02 (0.94–1.12)	0.606
Duration of diabetes (years)	1.02 (0.92–1.13)	0.735
HbA _{1c} (%)	1.15 (0.78–1.71)	0.488
Size of ulcer (cm ²)	1.61 (1.22–2.12)	0.001
Osteomyelitis	18.51 (2.50–137.21)	0.004
Surgical treatment	0.53 (0.06–4.97)	0.527

clindamycin ($P=0.012$), sulfamethoxazole ($P=0.019$), rifampicin ($P=0.042$), levofloxacin ($P=0.031$) and norfloxacin ($P=0.001$). Surgical debridement was performed in six MRSA patients, all of whom were HA-MRSA patients.

DISCUSSION

MRSA has emerged as a serious and commonly occurring problem in diabetic patients with foot ulcers (Game & Jeffcoate, 2004; Tentolouris *et al.*, 1999). This study presents a comprehensive clinical and microbiological survey of Chinese hospitalized diabetic patients with foot ulcers infected with MRSA. We focused on the prevalence of and factors that predispose patients to colonization or infection with MRSA, as well as differences in the clinical

or demographic characteristics and antimicrobial susceptibility observed for community- or hospital-origin infection. Soft tissue samples were used for bacteriological culture in all cases, even though a bone biopsy may be a better sample in patients with osteomyelitis (Senneville *et al.*, 2006). However, surgical excision can be potentially traumatic for the patient, and samples taken from the base of the wound after debridement are adequate for identifying the infecting organism (Kessler *et al.*, 2006; Pellizzer *et al.*, 2001; Perry *et al.*, 1991). Accordingly, we adopted the current procedure.

Over the last 3 years, the number of isolates in our foot clinic has agreed with that found in a previous report (Dang *et al.*, 2003), averaging 1.4 isolates per case, which is still lower than that found in other studies (Goldstein *et al.*, 1996; Lipsky *et al.*, 1990). The present study confirms the findings of previous studies in which Gram-positive aerobes were predominant in diabetic foot infections (Dang *et al.*, 2003; Mantey *et al.*, 2000). *S. aureus* was the most common pathogen among the Gram-positive bacteria isolated from ulcers (Tentolouris *et al.*, 2006), and almost two-thirds (63.4 %) of *S. aureus* isolates were MRSA. However, the ratios of Gram-positive aerobes to Gram-negative aerobes and MRSA to *S. aureus*, and the prevalence of *S. aureus*, differed from the above-mentioned reports. These dissimilarities could be attributed to the differences in age/gender composition, ulcer grades and study settings between our study patients and those of previous studies.

This study demonstrated that a high proportion of HA-MRSA patients (66.7 %) were identified among the diagnosed MRSA patients in our institution. Although

Table 4. Antimicrobial susceptibility patterns of MRSA isolates from infected foot ulcers in diabetic patients ($n=26$)

All strains of MRSA were uniformly susceptible to the study drugs.

Antimicrobial agent	No. (%) of susceptible MRSA isolates		P value
	HA-MRSA ($n=17$)	CA-MRSA ($n=9$)	
Gentamicin	4 (23.5)	0 (0)	0.114
Tetracycline	5 (29.4)	0 (0)	0.070
Erythromycin	2 (11.8)	4 (44.4)	0.060
Clindamycin	3 (17.6)	6 (66.7)	0.012
Nitrofurantoin	14 (82.4)	9 (100)	0.180
Fusidate	15 (88.2)	9 (100)	0.284
Sulfamethoxazole	7 (41.2)	8 (88.9)	0.019
Rifampicin	11 (64.7)	9 (100)	0.042
Vancomycin	17 (100)	9 (100)	
Teicoplanin	17 (100)	9 (100)	
Levofloxacin	4 (23.5)	6 (66.7)	0.031
Quinupristin/dafopristin	15 (88.2)	5 (55.6)	0.060
Penicillin	0 (0)	0 (0)	
Minocycline	7 (41.2)	7 (77.8)	0.075
Norfloxacin	2 (11.8)	7 (77.8)	0.001
Oxacillin	0 (0)	0 (0)	

previous studies have indicated that there were no differences in ulcer size between ulcers positive for methicillin-sensitive *S. aureus* and MRSA (Tentolouris *et al.*, 1999), in the current study, ulcer size was an independent predictor of MRSA infection. Furthermore, osteomyelitis was another predictive factor of MRSA infection. This finding is consistent with previous studies in which *S. aureus* (Embil *et al.*, 2006; Senneville *et al.*, 2006, 2008), including MRSA (Aragón-Sánchez *et al.*, 2009), was the most commonly found bacterium causing foot osteomyelitis in diabetic patients. Furthermore, 65.4 % of all MRSA isolates were identified as HA-MRSA in our institution. CA-MRSA was also recognized in diabetic foot ulcers, accounting for about 6 % of the patients. CA-MRSA was reported to account for some 12 % of the cases identified in a study from the USA (Naimi *et al.*, 2003). There is a suggestion that CA-MRSA may be more virulent (Baba *et al.*, 2002). However, there is currently little evidence to support the view that the involvement of CA-MRSA affects the outcome of diabetic foot ulcers. Patients with CA-MRSA infections often do not exhibit risk factors known in patients with HA-MRSA infections. These risk factors include recent hospitalization, dialysis, nursing-home residence and other co-morbid conditions, such as diabetes (Fridkin *et al.*, 2005; Naimi *et al.*, 2003). In the current study, however, none of the CA-MRSA or HA-MRSA patients were on renal replacement therapy, and none had resided in a chronic-care facility within the previous 3 months. Our data showed that no significant effect could be found for these factors (including diabetes), which have been shown previously to predispose patients towards colonization with HA-MRSA in the diabetic foot. The factors predisposing patients toward HA-MRSA infection included age, duration of diabetes and glucose control.

Our data also indicated that infection with HA-MRSA resulted in a prolonged hospital stay. An increased hospital stay may be due to the higher prevalence of diabetic complications, including neuropathy, peripheral vascular disease and osteomyelitis, in patients infected with HA-MRSA; surgery was always required in these patients. This is reinforced by the fact that HA-MRSA isolates were more resistant to multiple antibiotics, such as clindamycin, sulfamethoxazole, rifampicin, levofloxacin and norfloxacin, compared with CA-MRSA isolates. We noticed a slight difference between CA-MRSA and HA-MRSA in susceptibility to multiple antibiotics such as erythromycin, fusidate, nitrofurantoin and minocycline, which were more active against CA-MRSA. Moreover, although HA-MRSA displayed more sensitivity to quinupristin/dafopristin, the difference did not reach statistical significance. Consistent with other investigations, we found vancomycin and teicoplanin to be highly active, whilst penicillin was inactive against MRSA, with no differences in susceptibility observed for isolates of community or hospital origin. These findings will be helpful in assessing the appropriate empirical antibiotic regimen for diabetic foot ulcers infected with MRSA in order to shorten hospital stays and reduce costs.

There are several limitations to our study. First, this was a healthcare-based retrospective case series study. Thus, we were unable to estimate the true prevalence and antimicrobial susceptibility profiles of CA-MRSA and HA-MRSA infections in general diabetic foot ulcers of the Chinese population. Secondly, although medical charts were carefully reviewed, the absence of personal interviews made it possible to misclassify MRSA acquisition due to lack of a detailed history of hospital-related exposures and medical treatment, especially for those patients whose charts were incomplete.

In conclusion, we believe that this is the first Chinese study to highlight CA- and HA-MRSA infections in diabetic foot ulcers. The prevalence of MRSA was alarmingly high, especially for HA-MRSA. The size of the ulcer and osteomyelitis were independent predictors of MRSA infection, and HA-MRSA infection reflected increased diabetic complications. The age of patients, duration of diabetes and glucose control appeared to be factors that predisposed patients toward HA-MRSA. HA-MRSA was associated with an increased requirement for surgical treatment. These findings suggest that prospective multi-centre studies are required to assess the appropriate empirical antibiotic regimen for diabetic foot ulcers, taking into consideration the results found here for HA- and CA-MRSA infections in diabetic foot ulcers in a Chinese care hospital. In addition, the results alert us to the need for proper management of antibiotics to decrease the incidence of MRSA infection in this population, to reduce the risk of running out of effective antimicrobials for patients.

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