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Methicillin-resistant *Staphylococcus aureus* nasal carriage among patients on haemodialysis with newly inserted central venous catheters

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Abstract

Background Although methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization is common among end-stage kidney disease patients undergoing haemodialysis, few studies were focused on MRSA nasal carriers among haemodialysis patients with central venous catheters (CVCs). The aim of this study is to evaluate the risk factors, various clinical outcomes and effect of decolonization for MRSA nasal colonization among patients on haemodialysis via CVCs.

Methods This was a single-centre non-concurrent cohort study of 676 patients who had new haemodialysis CVCs inserted. They were all screened for MRSA colonization via nasal swabs and were categorized into two groups: MRSA carriers and MRSA noncarriers. Potential risk factors and clinical outcomes were analysed in both groups. All MRSA carriers were given decolonization therapy and the effect of decolonization on subsequent MRSA infection was also performed.

Results Eighty-two patients (12.1%) were MRSA carriers. Multivariate analysis showed that MRSA carrier (OR 5.44; 95% CI 3.02–9.79), long-term care facility resident (OR 4.08; 95% CI 2.07–8.05), history of *Staphylococcus aureus* infection (OR 3.20; 95% CI 1.42–7.20) and CVC in situ > 21 days (OR 2.12; 95% CI 1.15–3.93) were independent risk factors for MRSA infection. There was no significant difference in all-cause mortality between MRSA carriers and noncarriers. The MRSA infection rates were similar between MRSA carriers with successful decolonization and those who had failed/incomplete decolonization in our subgroup analysis.

Conclusion MRSA nasal colonization is an important cause of MRSA infection among haemodialysis patients with CVCs. However, decolonization therapy may not be effective in reducing MRSA infection.

Keywords Haemodialysis · MRSA infection · Vascular access

Introduction

Among patients with end-stage kidney disease (ESKD), *Staphylococcus aureus (S. aureus)* infections often lead to multiple complications associated with high costs and prolonged hospitalization [1]. *S. aureus* has become increasingly resistant to antibiotics, such as methicillin-resistant *S. aureus* (MRSA), which is a prevalent nosocomial pathogen nowadays. There is an increased risk of MRSA infection and/or colonization in patients with ESKD due to frequent attendance in healthcare settings, immunocompromised status, and exposure to prolonged antibiotics [2, 3]. Among dialysis patients in the USA, the incidence of invasive MRSA infection was found to be 100 times higher than in the general population [4].

MRSA nasal carriage is a predominant risk factor for subsequent MRSA infection [5]. In chronic haemodialysis patients, nasal carriage of MRSA has been associated with a 2.46-fold increase in all-cause mortality [6]. In view of the high prevalence of MRSA and the heavy burden on the healthcare system, there have been recommendations for active surveillance for MRSA colonization, though the effectiveness is still controversial [7].

Patients who have a central venous catheter (CVC) are at higher risk for bloodstream infections than those with other forms of access. In the North American cohort, the bloodstream infection rate for patients with permanent CVCs was 4.2 per 100 patient months, compared with 0.9 and 0.5 per

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100 patient months, respectively, for arteriovenous grafts and fistulas [8]. Although there were studies of MRSA nasal colonization in patients with ESKD [9–11], the majority of these patients used arteriovenous grafts or fistulas as their vascular access for haemodialysis. There have been no similar studies that solely focus on CVCs. A better understanding of MRSA epidemiology and the associated risk factors is important for planning strategies to improve patients' outcomes in this vulnerable population.

We hypothesized that a higher MRSA-related infection risk will be found in MRSA nasal carriers compared with MRSA noncarriers among haemodialysis patients with CVCs. The aim of our study is to examine whether active surveillance of MRSA nasal carriage in haemodialysis patients with CVCs could predict higher MRSA-related infection risks. Moreover, the MRSA nasal carriage rates among haemodialysis patients with CVCs, risk factors of MRSA infection and the effectiveness of decolonization therapy are also studied.

Materials and methods

This non-concurrent cohort study was conducted in Queen Elizabeth Hospital (QEH), one of the largest acute hospitals in Hong Kong SAR with approximately 2000 beds, serving approximately 15% of our 7.5 million population. All patients, aged 18 or above, who had a new haemodialysis CVC inserted in QEH between 1st January 2016 and 31st December 2018 were recruited. Those receiving haemodialysis via arteriovenous fistulas or grafts were excluded. All patients were identified by the Clinical Data Analysis and Reporting System (CDARS) of the Hospital Authority, Hong Kong. The baseline demographic and clinical data were collected from patient's medical records. Potential risk factors for MRSA infection [3, 12–14] were analysed in all patients.

Care of the vascular access and catheter site was based on our local guideline [15]. Patients were followed-up for any MRSA-related infection within one year from the date of CVC insertion or until death, whichever was earlier. For purpose of studying mortality, patients were censored on 31st December 2020. The primary outcome was MRSA-related infection in 1 year. The secondary outcome included all-cause mortality and the effect of decolonization therapy. The event rate of MRSA-related infection and all-cause mortality were compared between MRSA carriers and noncarriers. The rate of MRSA-related infection was also analysed between MRSA carriers who had successful decolonization versus incomplete or failed decolonization.

MRSA screening

All patients receiving haemodialysis for any reason had nasal swab for MRSA screening performed on the day of CVC insertion. Nasal swab was obtained from both anterior nares using sterile saline moistened swab and sent to the microbiology laboratory for MRSA culture. The results would be available approximately 5 days later. For those who had more than one CVC insertion during the period, only the data on the first CVC were analysed. Patients were categorized into MRSA carriers or MRSA noncarriers. MRSA carriers were defined as any patients with nasal swab done at first screening growing MRSA. All the others were defined as MRSA noncarriers.

Decolonization therapy

After confirmation of MRSA nasal colonization, patients were given a routine course of decolonization therapy. Our decolonization regime included 2% mupirocin to nasal cavities three times a day for 5 days, and body and hair wash with 2% chlorhexidine for 5 days [16]. Instruction sheets with illustrative pictures were given. After decolonization, nasal swab culture was repeated twice on different dates and at least 48 h apart to confirm effectiveness. Successful decolonization was defined as two consecutive MRSA-negative nasal swabs. If initial decolonization failed, a second course of decolonization was performed with a nasal swab to be repeated as above afterwards. No further decolonization was necessary if the nasal swab still remained positive for MRSA after the second course of decolonization therapy.

Statistical analysis

The statistical analyses were performed by SPSS (SPSS 27, Inc., Chicago, IL USA). Categorical data were expressed as percentages and continuous data were expressed as mean ± standard deviation (SD) or median (25th, 75th percentile). Categorical data were compared with chi-square or Fisher's exact tests while continuous data were compared with *t* test or Mann–Whitney *U* test. Kaplan–Meier estimates and log-rank test were used to demonstrate event rate during follow-up and all-cause mortality. Cox proportional hazard model was used to assess the effect of MRSA carriage on the study end points. All tests were 2-tailed, and differences for p values of less than 0.05 were considered significant. Potential risk factors were first assessed in univariate analysis. The multivariable model was developed in a forward, stepwise fashion. Candidate variables were those with $p < 0.1$ in univariate analysis.

Results

Total of 676 patients were recruited into this study. The mean age was 63.4 ± 14.4 (range 18–94) years. There were 402 men (59.5%) and 347 diabetic patients (51.3%). Fifty-five patients (8.1%) were long-term care facilities (LTCF) residents, and 100 patients (14.8%) had ICU admission in the previous three months. Five hundred and seventy-two patients (84.6%) were dialyzed through a temporary uncuffed CVC, while the remaining 104 patients (15.4%) were dialyzed through a tunnelled cuffed CVC. Five hundred and twenty-four (77.5%) of the CVCs were at the jugular region, while the rest of 152 (22.5%) CVCs were at the femoral site. There were 431 (63.8%) incident haemodialysis patients, with indications including acute kidney injury and uraemia or fluid overload in ESKD; 170 (25.1%) patients were previously on peritoneal dialysis and then started on haemodialysis due to failed peritoneal dialysis, such as refractory peritonitis requiring removal of peritoneal dialysis catheters, leakage of peritoneal fluid through pleuro-peritoneal fistula or hernias, or intra-abdominal surgeries; 67 (9.9%) patients received a new CVC insertion as their previous haemodialysis access failed, such as thrombosed or stenotic arteriovenous fistulas or grafts requiring intervention; the remaining 8 (1.2%) patients had a new CVC inserted for both plasmapheresis and haemodialysis.

MRSA carriers and decolonization rates

Among the 676 patients, 82 (12.1%) were MRSA carriers. Baseline characteristics of the MRSA carriers and MRSA noncarriers were shown in Table 1. There were significantly more male patients in MRSA carriers, more MRSA carriers being LTCF residents compared with noncarriers, and more MRSA carriers having recent ICU admission in the last three months than noncarriers. Seventy-five MRSA carriers (91.5%) received their first course of decolonization therapy; while 7 carriers did not receive any decolonization

Table 1 Demographics and characteristics of patients by MRSA nasal carriage

Characteristics	MRSA noncarriers ($N=594$)	MRSA carriers ($N=82$)	p value
Number	594 (87.9%)	82 (12.1%)	
Age in years (mean)	63.6 ± 14.5	63.8 ± 14.1	0.78
Gender, men	344 (57.9%)	58 (70.7%)	0.02*
Site of catheter			0.49
Jugular	461 (77.6%)	63 (76.8%)	
Femoral	133 (22.4%)	19 (23.2%)	
Type of catheter			0.17
Uncuffed	506 (85.2%)	66 (80.5%)	
Tunnelled cuffed	88 (14.8%)	16 (19.5%)	
Indication of haemodialysis			0.66
Incident haemodialysis	375 (63.1%)	56 (68.3%)	
Failed peritoneal dialysis	154 (25.9%)	16 (19.5%)	
Failed haemodialysis access	58 (9.8%)	9 (11%)	
Plasmapharesis and haemodialysis	7 (1.2%)	1 (1.2%)	
Chronic haemodialysis > 3 years	23 (3.9%)	1 (1.2%)	0.19
Diabetes mellitus	305 (51.3%)	42 (41.2%)	0.54
Long-term care facilities resident	39 (6.6%)	16 (19.5%)	< 0.01*
ICU admission in the previous 3 months	82 (13.8%)	18 (22%)	0.04*
On immunosuppressants	72 (12.1%)	8 (9.8%)	0.34
History of *S. aureus* infection in the previous 1 year	40 (6.7%)	4 (4.9%)	0.36
Follow-up duration in days (median)	365 (182, 365)	365 (73, 365)	0.06
CVC in situ > 21 days	355 (59.8%)	56 (68.3%)	0.09
Days of catheter survival (median)	31 (12, 74)	35 (19, 75)	0.30
Uncuffed	25 (10, 40)	30 (17, 68)	0.30
Tunnelled cuffed	177 (80, 365)	88 (51, 190)	0.30

CVC central venous catheter, *MRSA* methicillin-resistant *Staphylococcus aureus*, *ICU* intensive care unit

*Data present as mean ± standard deviation or median (25th, 75th percentile) for continuous data and total number (percentage) for categorical data

therapy, as they were either transferred to another hospital where the protocol for MRSA decolonization was not implemented, or they died before screening results or decolonization therapy was available. These 7 patients were classified as having incomplete decolonization. Out of the 75 carriers who received the first course of decolonization therapy, 60 of them had MRSA cleared afterwards, while 15 patients still had a positive MRSA nasal swab. These 15 patients then underwent a second course of decolonization therapy—12 had MRSA cleared, while 3 still had persistent positive MRSA nasal carriage and were defined as failed decolonization. Thus the successful decolonization rate in our cohort is 96% (72/75) (Fig. 1).

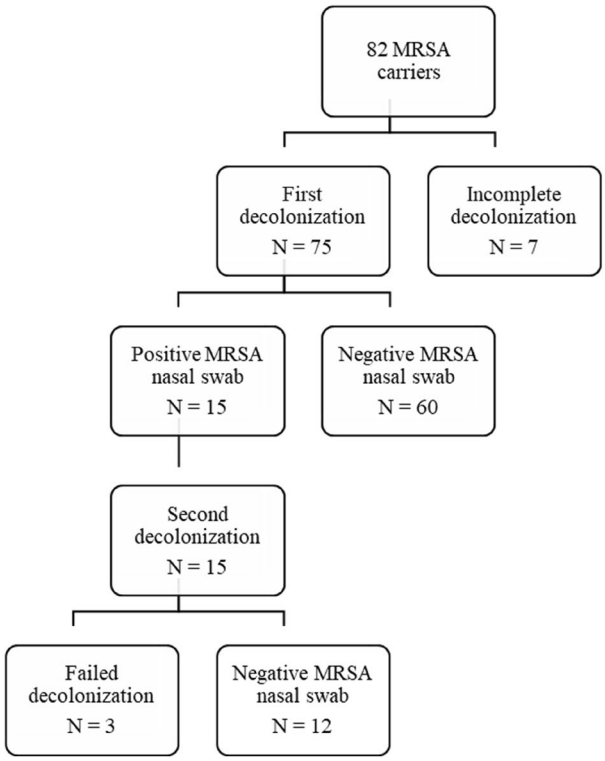

Fig. 1 Decolonization results

MRSA infection

MRSA infection occurred in 69 patients (Table 2). MRSA carriers had a significantly higher rate of MRSA infection when compared with noncarriers (31.7% vs 7.2%, $p < 0.01$). There was a significantly higher rate of MRSA bloodstream infections among MRSA carriers than noncarriers (11% vs 3.2%, $p < 0.01$). The median number of days to first MRSA infection was shorter in MRSA carriers (64 days; 25th percentile 18 days, 75th percentile 115 days) than in noncarriers (95 days; 25th percentile 29 days, 75th percentile 169 days, $p = 0.15$). Other types of MRSA infection including haemodialysis CVC exit site, soft tissue, peritoneal dialysis catheter exit site, lung, peritonitis or urinary tract occurred more frequently in MRSA carriers but were not statistically significant.

The significant risk factors for MRSA infection include MRSA carrier (odds ratio [OR] 5.95; 95% confidence interval [CI] 3.40–10.41), residing in LTCF (OR 5.44; 95% CI 2.89–10.23), history of *S. aureus* infection (either methicillin-sensitive or resistant) within the previous year (OR 2.86; 95% CI 1.34–6.07), CVC in situ > 21 days (OR 2.30; 95% CI 1.29–4.12) and cuffed haemodialysis catheters (OR 2.14; 95% CI 1.19–3.83) (Table 3). Multivariate logistic regression analysis showed that MRSA carrier (OR 5.44; 95% CI 3.02–9.97, $p < 0.01$), LTCF resident (OR 4.08; 95% CI 2.07–8.05, $p < 0.01$), history of *S. aureus* infection in the previous year (OR 3.20; 95% CI 1.42–7.20, $p < 0.01$) and CVC in situ > 21 days (OR 2.12; 95% CI 1.15–3.93, $p = 0.02$) were the four independent risk factors for MRSA infection (Table 4).

Since there was a significantly higher portion of LTCF in the MRSA carrier group, this group might already have

Table 2 MRSA infection among MRSA noncarriers and carriers

	MRSA noncarriers ($N = 594$)	MRSA carriers ($N = 82$)	p value
MRSA infection (any site)	43 (7.2%)	26 (31.7%)	< 0.01
Days to 1st MRSA infection (median)	95 (29, 169)	64 (18, 115)	0.15
MRSA exit site infection at haemodialysis CVC	12 (2%)	2 (2.4%)	0.52
MRSA bacteraemia	19 (3.2%)	9 (11%)	< 0.01
Other sites of MRSA infection			
Soft tissue	8 (1.3%)	3 (3.6%)	0.30
Peritoneal dialysis catheter exit site	7 (1.2%)	5 (6.1%)	
Lung	7 (1.2%)	2 (2.4%)	
Peritonitis	1 (0.2%)	2 (2.4%)	
Urinary tract	1 (0.2%)	3 (3.6%)	

MRSA methicillin-resistant *Staphylococcus aureus, CVC* central venous catheter

Table 3 Univariate analysis of potential risk factors for MRSA infection

Potential risk factors	Odds ratio	95% confidence interval	*p* value
Age	1.00	0.99–1.02	0.76
Sex (female)	1.52	0.89–2.58	0.13
Site of catheter: jugular	2.06	1.00–4.24	0.05
Cuffed catheter	2.14	1.19–3.83	0.01
MRSA carrier	5.95	3.40–10.41	< 0.01
Diabetes mellitus	1.35	0.81–2.23	0.25
Long-term care facilities resident	5.44	2.89–10.23	< 0.01
Chronic haemodialysis more than 3 years	0.79	0.18–3.45	0.76
ICU admission in previous 3 months	1.10	0.56–2.19	0.78
On immunosuppressants	0.83	0.36–1.87	0.65
CVC in situ > 21 days	2.30	1.29–4.12	0.01
History of *S. aureus* infection in the previous 1 year	2.86	1.34–6.07	0.01

CVC central venous catheters, *MRSA* methicillin-resistant *Staphylococcus aureus, ICU* intensive care unit

Table 4 Multivariate analysis of potential risk factors for MRSA infection

Potential risk factors	Odds ratio	95% confidence interval	*p* value
MRSA carrier	5.44	3.02–9.97	< 0.01
Long-term care facilities resident	4.08	2.07–8.05	< 0.01
History of *S. aureus* infection in the previous 1 year	3.20	1.42–7.20	< 0.01
CVC in situ > 21 days	2.12	1.15–3.93	0.02

CVC central venous catheters, *MRSA* methicillin-resistant *Staphylococcus aureus*

more MRSA infection by its nature and the results would be skewed. If LTCF residents were excluded and the results were re-analyzed, MRSA carrier was still a significant risk factor for MRSA infection (OR 8.17, 95% CI 4.33–15.42, $p < 0.01$).

All-cause mortality

Total of 346 (51.2%) patients died during the study period. Among them, 46 were MRSA carriers, whereas 300 were MRSA noncarriers. There was no statistically significant difference in all-cause mortality between MRSA carriers and noncarriers ($p = 0.13$).

Subgroup analysis between successfully decolonized MRSA carriers and carriers who had failed or incomplete decolonization

Further subgroup analysis was performed to compare the outcome of MRSA infection between MRSA carriers who were successfully decolonized and those who had failed or incomplete decolonization (Table 5). Twenty-three out of 72 (31.9%) successfully decolonized MRSA carriers had MRSA infection within one year, which is similar to 3 out of 10 (30%) MRSA carriers who had failed or incomplete decolonization. Regarding haemodialysis CVC exit site MRSA infections, there were 2 (2.7%) among the successfully decolonized group but there were none among the other group. There were 6 (8.3%) successfully decolonized MRSA carriers who had MRSA bloodstream infection, compared with 3 (30%) of the failed or incompletely decolonized group. All these findings were not statistically significant.

Table 5 Subgroup analysis between MRSA carriers who had successful decolonization and failed or incomplete decolonization

	Successful decolonization ($N = 72$)	Failed or incomplete decolonization ($N = 10$)	*p* value
MRSA infection (any site)	23 (31.9%)	3 (30%)	0.59
MRSA exit site infection at haemodialysis CVC	2 (2.7%)	0 (0%)	0.79
MRSA blood stream infection	6 (8.3%)	3 (30%)	0.06

MRSA methicillin-resistant *Staphylococcus aureus, CVC* central venous catheter

Discussion

Patients with ESKD have an increased risk for MRSA colonization and infection, especially those undergoing dialysis [3, 13, 17]. A study in Singapore reported the prevalence of MRSA colonization in hospitalized chronic haemodialysis patients at 15.1%, which is threefold higher than the prevalence of MRSA colonization among all inpatients (5.8%) [18]. Johnson et al. found an MRSA colonization rate of 21.7% among dialysis patients, compared with 8.3% among the general population [19]. The MRSA nasal colonization rate in our cohort was 12.1%, which was similar to the reported rates of 10% in the United Kingdom and 15.1% in Singapore; but lower than 27.3% among an American Indian population [18, 20, 21]. The variation in rates of colonization could be due to geographical and ethnic differences which was described by Ray et al. [22]. A possible reason behind the lower rate of MRSA nasal colonization in our cohort, when compared to other countries, could be that more than half of our patients were new to dialysis, whereas the majority of the population in other studies were on chronic haemodialysis, who might have more frequent hospital attendances with increased risk of MRSA exposure.

In our study, MRSA nasal colonization was an independent risk factor for subsequent MRSA infection for patients on haemodialysis with CVC. Neilsen J et al. reported *S. aureus* nasal carriage was a sensitive method to predict *S. aureus* septicaemia among haemodialysis patients on CVC, with a negative predictive value (NPV) of 90%, which was just second to positive S. aureus culture from the insertion site (NPV 98%) [23]. MRSA nasal carriers were 5.4 times more likely to develop MRSA infection than noncarriers within one year of screening. Our finding is in concordance with the literature, which reported a higher *S. aureus* infection rate in nasal carriers on haemodialysis than noncarriers, with relative risks ranging from 1.8 to 4.7 [9–11]. It has been proposed that the nose acts as a reservoir for *S. aureus*, which then propagates to the skin and causes subsequent infection in patients with impaired skin sites, such as patients on haemodialysis with CVCs.

Another independent risk factor for MRSA infection from our study was LTCF residents. They were 4 times more likely to have MRSA infection within one year in our study. In fact, other studies in Hong Kong reported that LTCF residents contributed to half of the MRSA carriers among hospitalized patients older than 65 [24]. A study in Germany observed that staying in LTCF for more than 6 months was a risk factor for MRSA colonization [25]. Reasons behind the high MRSA colonization rates in LTCF residents were host-related: advanced age, poor functional status, multiple co-morbidities, indwelling invasive devices, decubitus ulcers, prior hospitalizations and antibiotic therapy; and environment-related: size and density of LTCF and location of LTCF in a deprived area.

Similar to other studies [14, 26], patients with a history of *S. aureus* infection were also an independent risk factor for MRSA infections in our cohort. This could be due to patient's previous exposure to *S. aureus* which led to increased risks of colonization and thus subsequent infection; also patients might have been hospitalized for previous *S. aureus* infection which increased the chances of colonization. Our study observed a higher risk of MRSA infection with the use of cuffed tunnelled haemodialysis catheters compared with temporary uncuffed haemodialysis catheters. Possible reasons could be that our patients who received temporary uncuffed haemodialysis catheters might have switched to peritoneal dialysis afterwards, or that they have renal recovery, thus there was a reduced need to return to the hospital regularly and this minimised the risk of MRSA exposure.

Among dialysis patients, mupirocin is highly effective in the eradication of *S. aureus* nasal carriage [27–29]. A systematic review and meta-analysis found an MRSA decolonization success rate of 0.88 (95% CI 0.75–0.95) among haemodialysis patients [30]. Boelaert et al. reported a nasal *S. aureus* eradication rate of 96.3% by nasal mupirocin among surveillance cultures done on haemodialysis patients [31]. This was similar to our successful decolonization rate of 96%. On the contrary, evidence on the effect of eradication of MRSA nasal carriage by intranasal mupirocin on the reduction of infection rate among haemodialysis patients has been controversial. Despite a high successful decolonization rate, there were still significantly higher rates of MRSA infection and bloodstream infections among our MRSA carriers, who, in fact, had been decolonized after being screened positive. Possible reasons behind this include that MRSA screening and decolonization were only performed once during the insertion of CVC with no interval follow-up screening after successful decolonization, so intermittent MRSA carriers could not be identified in our study, as patients might have been colonized again afterward which was not detected by our protocol; the culprit MRSA strain causing the infection might be of a different strain from that found in the nose, so nasal decolonization could not have prevented such an MRSA infection; our overall MRSA carrier cohort had included those who had failed or incomplete decolonization albeit a small population only. Further in our subgroup analysis between MRSA carriers who had been decolonized successfully and those who had failed or incomplete decolonization, there were no significant differences in MRSA infection of any sites, CVC exit site infections or bloodstream infections. This could be attributed to the inadequate power because there were only 10 MRSA

carriers who had failed or incomplete decolonization. In fact, among patients other than those on dialysis, evidence of the effect of MRSA nasal eradication with mupirocin has also been inconclusive. A double-blind, randomized, placebo-controlled study recruited 614 patients receiving an orthopaedic surgical intervention who were then randomized to receive mupirocin or placebo. The mupirocin group had a significantly more effective eradication of nasal carriage (83.5%) compared with the placebo group (27.8%), but *S. aureus* surgical site infection rates were not reduced significantly (3.8% in the mupirocin group versus 4.7% in the placebo group) [32].

To our knowledge, this is the first study analysing the risk factors and clinical outcomes of MRSA nasal colonization solely among patients on haemodialysis via CVCs. However, there are several limitations to our study. First, the sample size of MRSA carriers was relatively small, particularly those that had failed or incomplete decolonization. This may not be able to generate adequate power to study the effectiveness of decolonization. This together with the lack of interval MRSA screening, molecular studies and genotyping of MRSA isolates may affect our study of decolonization outcome. In addition, due to the retrospective nature of the study, not all of the potential confounders could be fully adjusted, and the association identified might not imply causality. Furthermore, due to our study design where MRSA screening was performed during the insertion of a new CVC, more than half of our cohort was new to dialysis, which most of them would later be converted to peritoneal dialysis; and less than 10% of our cohort was on long term haemodialysis. Our findings could not be directly translated to the general population of chronic haemodialysis patients. Since only nasal swabs were taken for MRSA screening, the carriage rate might be underestimated. Finally, nearly all of our MRSA carriers received decolonization therapy. Our findings of clinical outcomes and mortality thus might not have reflected the direct impact of MRSA nasal colonization.

Conclusion

MRSA nasal carriage, LTCF residents, history of *S. aureus* infection in the previous one year and CVC in situ > 21 days were independent risk factors for MRSA infection in haemodialysis patients using CVCs. In view of the doubtful efficacy of intranasal mupirocin decolonization for preventing MRSA infection, the individual renal unit has to develop tailored strategies for active surveillance and mupirocin use. Further studies with larger sample size and the use of control groups with a placebo are necessary to confirm these outcomes and the effectiveness of decolonization.

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Declarations

Conflict of interest The authors have declared that no conflict of interest exists.

Ethical approval This study was conducted in accordance with the Declaration of Helsinki and was approved by the local Research Ethics Committee [REC (KC/KE)-20-0158/ER-3].

Informed consent The requirement to obtain informed consent was waived based on the observational design.

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