The Role of Healthcare Workers with Methicillin-Resistant *Staphylococcus aureus* Carriage and their Association with Clinical Isolates from Post-neurosurgical Wound Infections

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading pathogen of postoperative wound infections. A series of cases of post-neurosurgical wound infection with MRSA were detected in 1 and half months. A prospective survey of carriage in health-care workers (HCWs) and environmental contamination was conducted. The rate of MRSA nasal carriage in HCWs was higher than the rate of environmental contamination (11.3% versus 0.6%, $p < 0.05$). HCWs who were directly in contact with the infected patients had higher carriage rates than others (31.0% versus 3.8%, $p < 0.05$), while doctors had the highest carriage rates among all professionals (21.7% versus 8.3%, $p < 0.05$). Clinical isolates from post-neurosurgical wounds belonged to 3 different clusters; however, all had genotypes identical to those obtained from HCWs with staphylococcal carriage, including neurosurgeons, resident doctors, nurses, anesthesiologists, and anesthesia assistants. HCWs have an important role in the intra-hospital transmission of MRSA. This study highlighted the importance of hand hygiene in preventing contact transmission. Importantly, decolonization of MRSA carriage can be considered as a method of adjuvant infection control for interrupting an ongoing MRSA spread. (J Intern Med Taiwan 2013; 24: 123-130)

Key Words: Methicillin-resistant *Staphylococcus aureus*; Nasal carriage; Postoperative surgical wound infection; Mupirocin decolonization

Introduction

Staphylococcus aureus is a natural commensal that is capable of causing a wide range of human infections. In Taiwan, methicillin-resistant *Staphylococcus aureus* (MRSA) contributed to more than 60% of the in-hospital associated infections in recent years[1]. Surgery disrupts the integrity of the skin and makes wounds susceptible to invasion by microorganisms. Unwanted postoperative wound infection is always accompanied by prolonged duration of admission and an increased rate of post-operative

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morbidity and mortality. Among all surgery types, neurosurgery has had the highest rate of surgical site infections in the past[2].

Horizontal transmission of MRSA occurs mainly through direct contact[3]. Therefore, the importance of hand hygiene has been emphasized since recent years. In spite of the continuing efforts to promote hand hygiene, episodic postoperative wound infections still occur. In previous reports, environmental contamination caused nosocomial MRSA outbreaks, and MRSA has been isolated from uniforms, gowns, gloves, medical equipment, computer keyboards, and even faucet handles[4,5]. Humans may also carry MRSA temporally or chronically. Active screening for nasal MRSA carriage and decolonization of patients during admission has been proven to reduce postoperative wound infections[6]. However, the role of health-care workers (HCWs) involved in hospital spread of MRSA is under-recognized[7].

From November 2008 to January 2009, a series of cases including 5 patients with post-neurosurgical MRSA wound infection was detected in the neurosurgery ward. This had no post-neurosurgical MRSA wound infection in the previous 6 months; therefore, we conducted a prospective survey to investigate possible transmission routes from HCWs or the medical environment.

Materials and Methods

People and environment investigation

Five patients were noted to have post-neurosurgical MRSA wound infection in series from November 2008 to January 2009. The neurosurgery ward was free from MRSA wound infections since the previous 6 months until the first index case. Fearing an outbreak, we conducted a prospective survey 3 days after the fifth index case. In total, 107 HCWs, including all medical staff, from the neurosurgical ward or operating rooms, were screened for MRSA carriage by using nasal cultures, which were collected by rotating a sterile swab 4 times in the anterior nares (Transwab; Medical Wire and Equipment Co. Ltd., Corsham, England). The staff included 48 nurses in the neurosurgery ward, 8 interns, 4 resident doctors (as operation assistants), 5 attending physicians (as operators), 22 nurses in the operation room, 6 anesthesiologists, and 14 anesthesia assistants. The environment was also investigated using superficial wet swab culture. Those samplings focused on patients and HCWs who might frequently come into contact with surfaces such as door knobs, computer keyboards, medical charts, sphygmomanometers, stethoscopes, ward beds, operating tables, and dressing carts used for wound dressing at least 1 to 2 times daily. Surgical equipment was not involved in this investigation because routine disinfection and sterilization monitoring showed no abnormality in the study period.

Identification of *Staphylococcus aureus*

Swabs were streaked on to sheep blood agar (BAP, Creative Microbiologicals Ltd, Taiwan) and incubated overnight at 37°C. All swabs were processed on the same day. The colonies resembling S. aureus were investigated by the coagulase test (BBL Microbiology Systems, Cockeysville, MD, USA). The isolates that showed positive agglutination as compared with the no agglutination of the negative control were identified as *Staphylococcus aureus.*

Antimicrobial Susceptibility Testing

The methicillin susceptibility of S. aureus was determined using a cefoxitin disk (BBL Microbiology Systems) using the disk diffusion test following manufacturer's instructions and the revised 2008 Clinical and Laboratory Standards Institute guidelines.

Genotyping

Pulsed-field gel electrophoresis (PFGE) was

used for genotyping and was performed according to the procedure described previously with some modifications[8]. Bacterial colonies grown overnight on blood agar plates were suspended in 10 mM Tris–0.1 mM EDTA and cast into gel plugs. The plugs were treated with lysis solution (6 mM Tris-HCl [pH 7.6], 1 M NaCl, 100 mM EDTA [pH 7.5], 0.5% Brij, 0.2% deoxycholate, 0.5% sodium lauroyl sarcosine, 30 μg of RNase [DNase free] per mL, 1 mg of lysozyme per mL) containing 1 mg of lysostaphin/mL at 37°C for 24 h and were further incubated in ESP buffer (0.5 M EDTA [pH 9 to 9.5], 1% sodium lauroyl sarcosine, 500 μg of proteinase K per mL) at 50°C for 24 h. Plugs were thoroughly washed; thin slices of the DNA plugs were then cut and incubated overnight with 50 U of SmaI (New England Biolabs, Beverly, Mass.) at 25°C. Subsequently, the plugs were loaded onto a 1% agarose gel, and PFGE was carried out with a CHEF Mapper XA system (Bio-Rad Laboratories) at 14°C. An auto algorithm mode was chosen, with the running molecular size standards ranging from 30 to 500 kb. The gel was stained with ethidium bromide and photographed with UV illumination. The criteria proposed by Tenover et al. were employed to analyze the DNA fingerprints generated by PFGE[9]. Briefly, strains with banding patterns identical in the size and number of bands were considered genetically indistinguishable and assigned to the same type; strains with banding patterns that differed by only 3 or fewer bands were considered closely related and described as subtypes of a given clonal type. Strains with banding patterns that differed by 4 or more bands were considered different and assigned to separate types. Similarity of more than 80% in the phylogenic analysis was defined as the same cluster.

Decolonization of Nasal MRSA Carriage and Follow-up

All HCWs with positive staphylococcal carriage were treated with mupirocin 2% nasal ointment (SmithKline Beecham, Rijswijk, Netherlands) twice daily for 5 days, according to the manufacturer's guidelines. Therapy failure was defined as having persistent positive nasal culture with S. aureus at the repeat culture tests performed 1 week after the treatment.

Statistical Analyses

The chi squire test was used to analyze the positive rate of MRSA between HCWs and the environment, doctors, and other HCWs. Tests were carried out using the SPSS statistical software (version 17, SPSS Inc., Chicago, Illinois), and p values <0.05 were considered to be statistically significant.

Results

A total 5 patients with post-neurosurgical wound infection were involved in this study. Three of them underwent cranioplasty, 1 underwent removal of a subdural hematoma, and 1 patient underwent a ventriculoperitoneal shunt. They were cared for by overlapping HCWs and developed postoperative wound infections in an average of 10.6 days (range, 4–17 days) after their surgery (Table 1).

Of the 107 HCWs who were screened, 12 staff were noted to carry MRSA, including 3 nurses in the neurosurgery ward, 1 intern, 1 attending physician, 1 resident doctor (belonging to the same surgical team), 2 nurses in the operating room, 2 anesthesiologists, and 2 anesthesia assistants. When cross-referenced to the staff schedules, 10 HCWs with MRSA carriage were involved either in the surgical procedure or in the postoperative care in the neurosurgery ward (marked in Table 1). Among 107 HCWs, 29 had direct contact with 5 index patients during clinical care. The nasal carriage of HCWs providing direct care for the 5 patients was higher than that in those were not directly involved (10/29, 34.5% versus 3/78, 3.8%, $p < 0.05$). Notably, doctors had a higher rate of MRSA nasal colonization than other HCWs (5/23, 21.7% versus 7/84,

Table 1. Demographic data of the 5 patients with post-neurosurgical wound infections with methicillin-resistant *Staphylococcus aureus* and the distribution of health-care workers

Age(years)/ sex	Patient	Type of surgery	Operation date	Infection date	Time to infection after operation (Days)	Neurosurgeon (O)	Resident doctor (assistant) (R)	Nurse (ward) (Nwd)	Nurse (operating room) (Nop)	Anesthesiologist (An)	Anesthesia assistant (Ana)
44/Male	I*	Removal of subdural hematoma	2008/11/16	2008/11/27	11	O1	R1, R2	Nwd1, Nwd2*, Nwd3	Nop1	An1	Ana1
20/Male	II#	Cranioplasty	2008/12/11	2008/12/18	7	O2†	R1	Nwd4, Nwd5#	Nop2#	An2#	Ana2#
54/Female	III#	Cranioplasty	2008/12/16	2008/12/30	14	O3	R1	Nwd4, Nwd5#	Nop2#	An3	Ana3
45/Male	IV†	Cranioplasty	2008/12/18	2009/01/05	17	O2†	R2†	Nwd6, Nwd7, Nwd8	Nop3	An3†	Ana4†
58/Female	V†	Ventriculoperitoneal shunt	2009/01/02	2009/01/06	4	O2†	R2†	Nwd9, Nwd10 Nwd11	Nop4	An4†	Ana5

*cluster 1, #cluster 2, †cluster 3.

8.3%, $p < 0.05$). In 160 environmental surveillance samples, only 1, from the computer keyboard in the operating room, was positive for MRSA. The environmental contamination rate was significantly lower than MRSA carriage in HCWs (12/107, 13.3% versus 1/160, 0.6%, $p < 0.05$) (Figure 1).

The phylogenic analysis by PFGE disclosed 4 clusters (Figure 2). In the first cluster, the isolate from patient I was similar to that from the in-charge nurse. The second cluster included patient II, patient III, and 5 HCWs including 1 nurse in the ward, 1 nurse in the operating room, a neurosurgeon, an anesthesiologist, and an anesthesia assistant. All the involved HCWs in this cluster were related to patient II. Patient III shared a nurse with patient II (Table 1). Patient IV and V belonged to cluster 3. Similarly, these 2 patients shared neurosurgeons and resident doctors (as a surgical team). Two anesthesiologists and an anesthesia assistant were also involved in this cluster. Finally, cluster 4 contained the only environmental MRSA isolate, from a keyboard to control the image system in the operation room, and a strain from 1 anesthesia assistant carriage.

The importance of hand hygiene was emphasized after the investigation, and more attention were paid to education and compliance. All HCWs with positive MRSA carriage were treated with mupirocin applied in their nostrils. Follow up culture repeated 1 week after the mupirocin treatment did not reveal any more colonization in the HCWs. Through improvement in the infection control policies, MRSA postoperative wound infection was interrupted. No new case was noted in the next 6 months.

Discussion

A review of prevalence of MRSA in HCWs showed colonization rates ranging from 1.6% to 15.5%. Some risk factors including work experience, area of service, close contact with patients, poor attention to infection control policy, and high

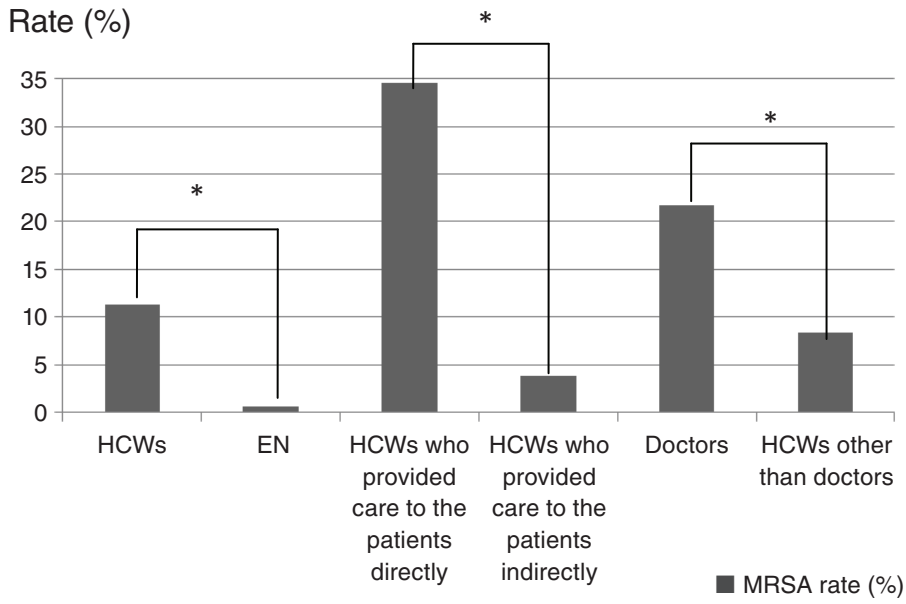

Figure 1. Rate of methicillin-resistant *Staphylococcus aureus* nasal carriage in healthcare workers (HCWs) was higher than the rate of environmental contamination (EN) (11.3% versus 0.6%). HCWs caring for patients directly had higher carriage rates than HCWs who were not involved directly (34.5% versus 3.8%). Doctors had higher rates than other HCWs (21.7% versus 8.3%). *p <0.05

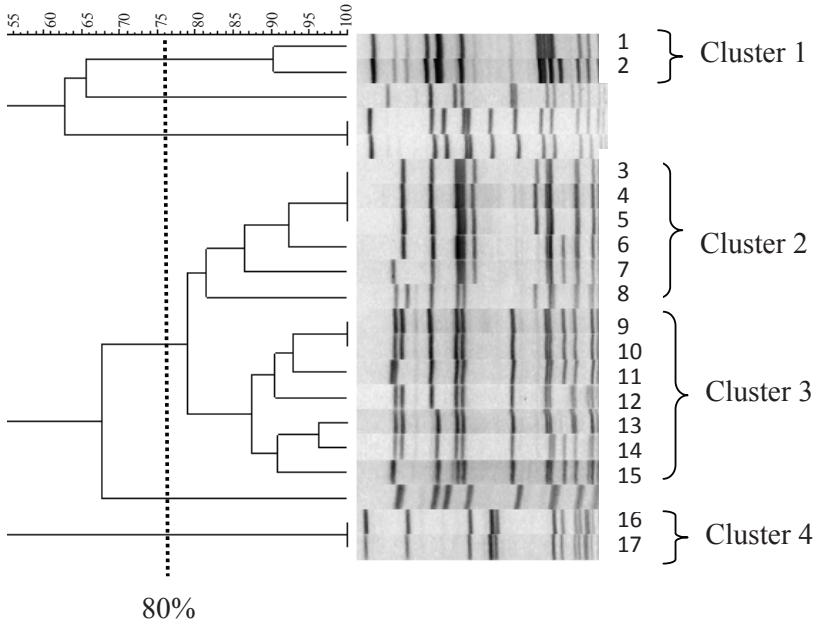

Figure 2. Four clusters in the pulsed-field gel electrophoresis analysis. Cluster 1 included a nurse (Nwd2) in the neurosurgery ward (lane1) and patient I (lane2). Cluster 2 included an anesthesia assistant (Ana2, lane 3), a nurse in the operating room (Nop2, lane 4), patient II (lane 5), an anesthesiologist (An2, lane 6), a nurse in the neurosurgery ward (Nwd5, lane 7), and patient III (lane 8). Cluster 3 included patient IV (lane 9), patient V (lane10), an anesthesia assistant (Ana4, lane11), two anesthesiologists (An3 and An4, lane 12 and 14), a neurosurgeon (O2, lane 13), and a surgery assistant (R2, lane15). Cluster 4 included a nurse in the operating room (lane 16) and MRSA isolated from a computer keyboard controlling the imaging system in the same operating room (lane 17). More than 80% similarity in the pulsed-field gel electrophoresis (PFGE) patterns was viewed as the same genotype.

work load were reported[10]. The colonization rate varied in different geographic regions, but was found to be higher in East Asia (13.1%) than that in other areas of the world. The prevalence of MRSA carriage among our HCWs was 11.2%. The rate was close to that in East Asia but higher than that in other regions such as Europe or America. Further, HCWs having close contact to the index patients in this investigation had a higher rate of MRSA carriage (34.5%). The HCWs with MRSA carriage included different professionals from the hospital such as surgeons, nurses, assistants, and anesthesiologists. This indicated that HCWs with different kinds of occupations were all at risk to receive or mediate MRSA in the hospital. However, doctors were observed to have the highest colonization rate in this study compared with the other professionals. This may be explained by long-term contact with the patient, especially with the wound. Furthermore, doctors have been reported as having lower compliance to infection control policies in many previous studies[11].

Several clusters were detected by using PFGE. Strictly, because the clinical isolates did not have identical genotypes, the cases involved in this study were not from an outbreak. However, all 5 clinical isolates were found to have identical strains to those isolated from at least 1 medical staff having direct contact with those patients. Although the transmission route could not defined in this point survey due to the lack of data on patient carriage status before operation, it was still proven that MRSA circulated between patients and HCWs. HCWs were likely not only a source but also a vector or victim of MRSA infection [10]. Furthermore, the carriage status could lead to household transmission if the home environment was contaminated[12-14].

A computer keyboard in the operating room was contaminated with MRSA, and the genotype was identical to 1 isolate from a nurse. This illustrates the relationship between human carriage and environmental contamination reported previously[15]. Although lower MRSA contamination over an inanimate surface was found in hospital, it still could serve as a transmission source[5]. The environmental contamination rate (0.6%) in our hospital was lower than that in a previous study (11%) by Bures et al, but similar to that in another study (1.1%) conducted in southern Taiwan[4,15]. It revealed that routine disinfection of environmental contamination eliminated MRSA effectively and that environmental disinfection was easier to achieve than hand hygiene. This could partially explain the lower environmental contamination rate but higher nasal colonization rate in the present study.

Universal MRSA screening for surgical patients on admission significantly reduces postoperative surgical site infections[6]. However, issues regarding the screening of HCWs for carriage are still debated because of the high cost, stigmatization, and uncertain effectiveness. In this study, we provided evidence of the roles of carriage among HCWs in the transmission of postoperative infection. Mupirocin treatment effectively decolonized all MRSA carriage among HCWs in this study. The screening and decolonization treatment in combination with hand hygiene eliminated the cross transmission between patients, HCWs, and the hospital environment. Hence, the role of selective screening for carriage and decolonization regimens for decreasing the rate of ongoing intra-hospital MRSA transmissions warrants more studies to evaluate its efficacy and cost-effectiveness.

There were some limitations. First, the carriage status of patients and HCWs before surgery was lacking. Even though identical strains were recovered from both HCWs and patients, we could not conclude that our staff members transmitted MRSA to the patients. Our staff may also have acquired MRSA during their service due to contact with patients. However, HCWs could still be the source of MRSA for other patients in this situation.

Second, culture from the hands of HCWs was not performed immediately to investigate transient colonization and poor hand hygiene compliance. Third, though negative conversion of the nasal carriage was noted in every treated HCW, we did not perform continuous monitoring after this investigation to evaluate the possibility of re-colonizing, and a longitudinal follow up was needed.

In summary, MRSA circulated in the hospital among patients, HCWs, and the environment. Hand hygiene is still the most important infection control policy for preventing cross transmissions in hospitals. The timing and cost-effectiveness of screening and decolonization of HCWs with MRSA nasal carriage deserves further attention.

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醫護人員金黃色葡萄球菌帶原調查及其和神經外科術後成傷口感染的關連性

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摘 要

抗藥性金黃色葡萄球菌仍是引起術後的傷口感染的主要致病菌之一。在某一神經內科加護病房同時發生抗藥性金黃色葡萄球菌術後傷口感染。因此我們執行了前瞻性的醫療照護相關人員鼻腔帶菌以及醫院環境細菌污染的調查來釐清可能的傳播途徑。對抗藥性金黃色葡萄球菌而言，醫療照護相關人員鼻腔帶菌的比率比環境汙染的比率高(11.3% versus 0.6%, $p<0.05$)。醫療照護相關人員有參與直接照顧群聚感染病患的帶菌比例比沒有參與直接照顧的高(31.0% versus 3.8%, $p<0.05$)，而就職業別來看的話醫師帶菌比率比其他醫師外的醫療照護相關人員高(21.7% versus 8.3%, $p<0.05$)。藉由利用脈衝式電泳的親源分析來分析所有的陽性菌株，所有病患分離出感染菌株均能分別找出一致的醫療照護相關人員的鼻腔帶菌。範圍包括主刀醫師，住院醫師，麻醉科醫師，病房及開刀房護理人員和助手。且在歸屬在同一族群的病患多半由相同的醫療照護人員照護。醫療照護相關人員，尤其是有鼻腔內帶菌者有潛在的危險會助長院內的抗藥性金黃色葡萄球菌傳播。藉由此次的帶菌調查，我們有更令人信服的證據來推廣洗手運動以阻絕接觸感染的院內水平傳播。此外鼻腔帶菌的清除，尤其在感染率高的時期，也可以是一個除了洗手以外值得考慮的輔助措施。