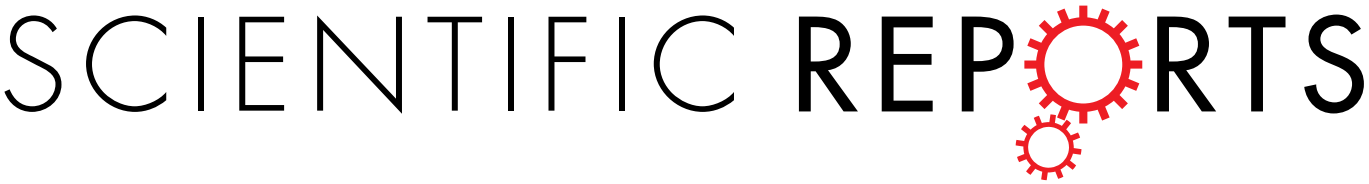

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Metro system in Guangzhou as a hazardous reservoir of methicillin-resistant *Staphylococci*: findings from a point-prevalence molecular epidemiologic study

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Yang Peng[1], Qianting Ou[1], Dongxin Lin[1], Ping Xu[1], Ying Li[2], Xiaohua Ye[1], Junli Zhou[1] & Zhenjiang Yao[1]

Staphylococci **are common causes of healthcare-associated and community-associated infections. However, limited data are available on the prevalence, phenotypes and molecular characteristics of *Staphylococci* in metro system around the world. 320 surface samples were collected from the Guangzhou metro system to isolate and characterize *Staphylococci* strains. Of the samples, 75.6% (242/320) were contaminated with *Staphylococci*. The *Staphylococci* isolates, especially the methicillin resistant isolates, were resistance to most of the antibiotics, with 79.8% (193/242) classified as multidrug resistant (MDR) strains. 8 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) carried a range of staphylococcal cassette chromosome *mec* (SCC*mec*) types [I (1), II (3), III (2) and NT (2)]. *Staphylococcus aureus* isolates were classified into several ST types and showed possible cross transmissions of strains from various sources. All MRSA strains were positive for the qac gene, and only one methicillin-susceptible *Staphylococci aureus* (MSSA) strain was positive for the Panton-Valentine leukocidin (PVL) genes. This study demonstrated that environmental surfaces in the Guangzhou metro system may be a hazardous reservoir for transmission of *Staphylococci* to passengers. The resistance to antibiotics and disinfectants observed among isolates was also noteworthy.**

Staphylococcus aureus is one of the common causes of serious healthcare associated and community associated infections[1,2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have spread in many countries and caused various life-threatening infections[3–5]. In addition, an increasing number of reports have shown that coagulase-negative *Staphylococcus* (CoNS), which is considered part of the normal flora in human bodies, has also become a significant conditional pathogen and has displayed a high rate of antibiotic resistance[6,7].

Even more striking is that *Staphylococci* have been isolated and characterized in several non-hospital settings such as buses[8,9], fire stations[10], universities[11], and marine beaches[12]. The astonishing survival time of *Staphylococci* on inanimate objects and the high volume of people in public areas facilitate the transmission of *Staphylococci* in such areas. However, there remains a paucity of data regarding the distribution and features of *Staphylococci* circulating in community settings.

[1]Department of Epidemiology and Health Statistics, Guangdong Pharmaceutical University, Guangzhou 510310, China. [2]Department of Environmental Health and Public Health Laboratory Center, Guangdong Pharmaceutical University, Guangzhou 510310, China. Correspondence and requests for materials should be addressed to Z.Y. (email: zhjyao2001@yahoo.com)

The Guangzhou metro system has an average of 5 million passengers daily and the environment is highly enclosed, which creates an ideal setting for the accumulation and transmission of *Staphylococci* among passengers. In this cross-sectional study, the aims were to elucidate the prevalence, antimicrobial susceptibilities, and molecular characteristics of *Staphylococci* strains contaminating metro surfaces in Guangzhou, China.

Methods

Environmental sample collection. Surface sampling in the metro system was conducted in November of 2013. Environmental samples were collected from five predetermined locations: hand rails, seats, stanchions, Ticket Vending Machines (TVMs), and escalators. These locations were chosen because they are frequently touched and are more likely to be contaminated with bacteria. The sampling points covered 32 metro stations in 7 lines (line 1, 2, 3, 4, 5, 8 and APM).

Swabs moistened with saline were used to sample surfaces, and the sample area of each swab was approximately 10 cm × 10 cm. To streamline the collection and processing, we used the composite surface sampling method. Each swab was placed into a sterile tube with 7.5% sodium chloride broth and the tubes were transported to the laboratory immediately after sampling.

Isolation and identification. After 24 hours of incubation at 37 °C, the swabs were transferred to mannitol salt agar plates for another 24 hours of incubation. Colonies were identified as *Staphylococci* through morphology, color and catalase reaction. All *Staphylococci* isolates were further screened for β-haemolysm and were verified as *S. aureus* by tube coagulase testing. The strains were regarded as CoNS if they were negative for coagulase testing. Those *Staphylococci* strains that were positive for the *mecA* gene and/or resistance to cefoxitin were labelled as methicillin-resistant.

Antimicrobial susceptibility testing. Antibiotic resistance testing was conducted using the Kirby-Bauer disk diffusion method, following the Clinical and Laboratory Standards Institute guidelines[13]. All *Staphylococci* isolates underwent phenotype analysis for antibiotic resistance to 11 antimicrobial agents from 9 antibiotic classes: penicillins (cefoxitin 30 μg, penicillin 10 units); lincosamides (clindamycin 2 μg); ansamycins (rifampicin 5 μg); fluoroquinolones (moxifloxacin 5 μg); aminoglycosides (tobramycins 10 μg, gentamicin 10 μg); sulfonamides (sulfamethoxazole-trimethoprim 25 μg); oxazolidones (linezolid 30 μg); glycopeptides (teicoplanin 30 μg); and macrolides (erythromycin 15 μg). Isolates were classified as multidrug resistant (MDR) if they were non-susceptible to ≥3 antibiotic classes[14].

Detection of *mec*A, qac, Panton-Valentine leukocidin, and SCC*mec* typing. All *Staphylococci* isolates were further tested to confirm the presence of the *mec*A gene[12]. *S. aureus* isolates were tested for the presence of the qac gene, an anti-disinfectant gene, and the Panton-Valentine leukocidin (PVL) genes using polymerase chain reaction (PCR) assays[15].

A multiplex PCR technique was used to confirm and type the staphylococcal cassette chromosome *mec* (SCC*mec*) gene[16]. The results were reported as types I–V, and those isolates that were not type I–V were deemed nontypeable (NT).

Multilocus sequence typing. The multilocus sequence typing (MLST) PCR assays were carried using previously published primers and conditions[17]. Allelic profiles and sequence types (ST) were assigned using MLST database (http://www. mlst.net). Dendrogram analysis was performed based on ST types to determine the clonal relatedness and potential epidemiologic origin.

Statistical analysis. Statistical analysis was conducted using Stata 13.0 (College Station, Texas, USA). Data were analyzed using descriptive statistics and χ^2 tests. A P value < 0.05 was considered statistically significant, and all statistical analyses were 2-sided.

Results

Identification of Staphylococci isolates. Of the 320 surface samples collected from the stations and carriages in the metro system, a total of 8 samples (2.5%) were MRSA-positive, 28 samples (8.75%) were methicillin-susceptible *Staphylococcus aureus* (MSSA)-positive, 21 samples (6.56%) were methicillin-resistant CoNS (MRCoNS)-positive, 185 samples (57.81%) were methicillin-susceptible CoNS (MSCoNS)-positive, and 78 samples (24.38%) were *Staphylococci*-negative. There were no statistically significant differences in the distributions of isolates between sample locations ($\chi^2 = 14.89$, $P = 0.53$) (Table 1).

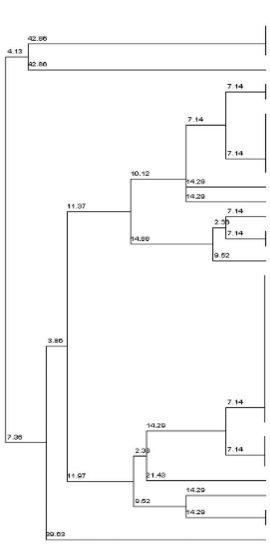
Antimicrobial susceptibility profiles. Of the 242 *Staphylococci* isolates, 228 (94.21%) displayed resistance to penicillin, 215 (88.84%) to erythromycin, 156 (64.46%) to rifampicin, 109 (45.04%) to trimethoprim, 99 (40.91%) to clindamycin, 76 (31.40%) to gentamicin, 33 (13.64%) to moxifloxicin, 30 (12.40%) to tobramycin, 26 (10.74%) to cefoxitin, 7 (2.89%) to linezolid, and 6 (2.48%) to teicoplanin. The details of the resistance rates among *Staphylococci* tested are summarized in Table 2. The overall MDR rate among *Staphylococci* was 79.75% (193/242). It was 100% for MRSA and MRCoNS isolates, 82.16% (152/185) for MSCoNS isolates, and 42.86% (12/28) for MSSA isolates.

Location	MRSA n (%)	MSSA n (%)	MRCoNS n (%)	MSCoNS n (%)	*Staphylococci*(−) n (%)	Total
Seats	1 (1.56)	7 (10.94)	3 (4.69)	33 (51.56)	20 (31.25)	64
Escalators	1 (1.56)	1 (1.56)	7 (10.94)	36 (56.25)	19 (29.69)	64
Hand rails	2 (3.13)	7 (10.94)	3 (4.69)	36 (56.25)	16 (25.00)	64
Stanchions	1 (1.56)	7 (10.94)	4 (6.25)	39 (60.94)	13 (20.31)	64
VTMs	3 (4.69)	6 (9.38)	4 (6.25)	41 (64.06)	10 (15.63)	64
Total	8 (2.50)	28 (8.75)	21 (6.56)	185 (57.81)	78 (24.38)	320

Table 1. Distribution of isolates among different sample locations. TVMs, Ticket Vending Machines.

Drug agent	MRSA n (%)	MSSA n (%)	MRCoNS n (%)	MSCoNS n (%)	Total n (%)
CEF	6 (75.00)	0 (0.00)	20 (95.24)	0 (0.00)	26 (10.74)
CLI	7 (87.50)	8 (28.57)	21 (100.00)	107 (57.84)	99 (40.91)
RIF	5 (62.50)	6 (21.43)	15 (71.43)	60 (32.43)	156 (64.46)
MOX	4 (50.00)	4 (14.29)	11 (52.38)	14 (7.57)	33 (13.64)
TOB	5 (62.50)	8 (28.57)	8 (38.10)	9 (4.86)	30 (12.40)
TRI	5 (62.50)	5 (17.86)	18 (85.71)	81 (43.78)	109 (45.04)
PEN	8 (100.00)	24 (85.71)	21 (100.00)	175 (94.59)	228 (94.21)
LIN	2 (25.00)	0 (0.00)	5 (23.81)	0 (0.00)	7 (2.89)
TEI	1 (12.50)	0 (0.00)	4 (19.05)	1 (0.54)	6 (2.48)
ERY	7 (87.50)	14 (50.00)	21 (100.00)	173 (93.51)	215 (88.84)
GEN	6 (75.00)	6 (21.43)	12 (57.14)	52 (28.11)	76 (31.40)

Table 2. Antibiotic resistance rates for tested *Staphylococci* [n (%)]. CEF, cefoxitin; CLI, clindamycin; RIF, rifampicin; MOX, moxifloxicin; TOB, tobramycin; TRI, trimethoprim; PEN, penicillin; LIN, linezolid; TEI, teicoplanin; ERY, erythromycin; GEN, gentamicin.



Isolate Number	ST Type	Station	Surface	SCCmec Type	PVL	qac	MDR
MRSA-146	398	Line2 #9	Seat	II	-	+	+
MRSA-161	398	Line8 #10	VTM	NT	-	+	+
MRSA-221	398	APM #1	Handrail	II	-	+	+
MRSA-201	30	Line3 #18	Stanchion	III	-	+	+
MRSA-13	125	Line5 #21	VTM	I	-	+	+
MRSA-118	125	Line3 #4	Handrail	III	-	+	+
MRSA-112	5	Line3 #4	VTM	II	-	+	+
MSSA-69	5	Line4 #10	Handrail		-	-	-
MSSA-73	5	Line4 #4	VTM		-	-	+
MSSA-192	5	Line3 #23	Stanchion		-	-	+
MSSA-204	5	Line3 #18	Handrail		-	-	-
MSSA-106	6	Line2 #24	Seat		-	-	-
MSSA-212	2605	APM #9	Stanchion		-	-	+
MSSA-216	1860	APM #9	Seat		-	-	+
MSSA-231	72	Line3 #13	Stanchion		-	-	-
MSSA-281	72	Line1 #13	Handrail		-	-	-
MSSA-282	1507	Line1 #13	Handrail		-	-	-
MSSA-9	188	Line5 #15	Seat		+	-	+
MSSA-10	188	Line5 #15	Seat		-	-	-
MSSA-16	188	Line5 #21	Stanchion		-	-	+
MSSA-60	188	Line4 #16	Seat		-	-	-
MSSA-61	188	Line4 #10	VTM		-	-	-
MSSA-87	188	Line8 #4	Handrail		-	-	+
MSSA-124	188	Line3 #1	Escalator		-	-	-
MSSA-127	188	Line3 #1	Handrail		-	-	+
MSSA-162	188	Line8 #10	VTM		-	-	-
MSSA-170	188	Line8 #10	Seat		-	-	+
MSSA-310	188	Line1 #5	VTM		-	-	-
MSSA-65	1462	Line4 #10	Stanchion		-	-	-
MSSA-155	1462	Line5 #1	Stanchion		-	-	+
MSSA-175	1462	Line8 #1	Seat		-	-	-
MSSA-110	1141	Line2 #24	Handrail		-	-	+
MSSA-42	2668	Line4 #1	VTM		-	-	-
MSSA-46	97	Line4 #1	Stanchion		-	-	-
MSSA-132	97	Line2 #5	VTM		-	-	+
MRSA-94	15	Line2 #20	Escalator	NT	-	+	+

Figure 1. Clonal dendrogram and detailed information on *Staphylococcus aureus* isolates.

Molecular characteristics of *S. aureus*. Detailed information regarding the molecular features of the *S. aureus* isolates is shown in Fig. 1. Of the eight MRSA isolates, three were classified as SCC*mec* typeII (37.5%), two were type III (25%), one was type I (12.5%), and two were NT (25%). The most predominant ST type among the MRSA strains was ST398 (3/8, 37.5%), followed by ST125 (2/8, 25%),

ST5 (1/8, 12.5%), ST15 (1/8, 12.5%) and ST30 (1/8, 12.5%). None of the environmental MRSA isolates carried the PVL genes, and all of them carried the qac gene.

Of the 28 MSSA isolates, ST 188 was the most prevalent ST type (11/28, 39.29%), followed by ST5 (4/28, 14.29%), ST1462 (3/28, 10.71%), ST72 (2/28, 7.14%), ST97 (2/28, 7.14%), ST6 (1/28, 3.57%), ST1141 (1/28, 3.57%), ST1507 (1/28, 3.57%), ST1860 (1/28, 3.57%), ST2605 (1/28, 3.57%) and ST2668 (1/28, 3.57%). Isolate MSSA-9 (ST188) was PVL positive, whereas the remaining MSSA isolates were PVL negative. Unlike the MRSA isolates, none of the MSSA isolate was positive for the qac gene.

Discussion

To the best of our knowledge, this is the first systematic study to report the occurrence and characteristics of *Staphylococci* strains from environmental surfaces in a metro system of the world. In the current study, 11.25% of the samples were MRSA-positive (2.5%) and/or MSSA-positive (8.75%), whereas the majority (>60%) of the environmental samples were positive for MRCoNS or MSCoNS. The MRSA isolation rate in our work is in similar to that from a Japanese study conducted on trains (2.5% vs 2.3%)[18]. However, considerably higher MRSA detection levels were noted in several previous studies. In two Portuguese studies, 26% (22/85) and 36% (72/199) of sampled buses were tested positive for MRSA contamination[19,20]. Additionally, an American study found that 14.8% (35/237) of the surfaces sampled from buses were contaminated with MRSA isolates[8]. The considerable differences in the reported value for MRSA prevalence could be affected by factors such as limited sampling locations, varying sampling techniques, and different regional hygiene measures.

The findings of drug resistance in this study are also noteworthy. The levels of resistance to some common antibiotics, such as penicillin, erythromycin and rifampicin, are alarming, especially among methicillin resistant strains. In addition, some isolates were even resistant to teicoplanin and linezolid, both of which are final effective agents against *Staphylococci* infections[21]. Even more intriguing is that most of the *Staphylococci* isolates displayed the characteristics of multidrug resistance, suggesting that they are more likely to have originated from healthcare-associated settings.

The results of the analyses of molecular features further broadened our insights into *Staphylococci* in a non-hospital environment. ST 188, the most predominant ST type of MSSA in our study, is widely disseminated in China and originated from community and hospital settings[22,23]. Some of the isolates, such as MSSA-16, MSSA-87, MSSA-127 and MSSA-170, have same molecular characteristics, suggesting they may represent a single clone. It is possible that the strains were transferred to the contaminated surfaces by discharged patients and/or healthcare workers who carried the recent epidemic strains in Guangzhou, although the sources of these strains are unclear. In contrast, ST398 and ST97 were also observed in the present study, and they have been reported as typical animal clones in many countries[24–27]. The appearance of these clones in an urban environment is rarely reported and, thus, we cannot rule out the possibility that those five ST398 and ST97 isolates were transmitted by passengers who have close contacts with animals or meats. Most of the MRSA isolates were typed as SCC*mec* I–III, and all of them were negative for the PVL genes; both of these features are indicators of hospital-acquired isolates. This phenomenon is in agreement with the findings of the drug resistance testing. A high frequency of the anti-disinfectant qac gene was discovered among all MRSA isolates in our study, and this gene was previously detected in airborne *Staphylococci* isolates from Shanghai metro stations[15]. Hence, the efficiency of sterilization processes in metro systems should be improved by reselection of suitable disinfectants.

In conclusion, our study demonstrated that the frequently touched surfaces in metro system may be a reservoir for *Staphylococci* transmissions and the resistance to antimicrobials and disinfectants is prevalent, especially among methicillin resistant strains. Cross transmissions of *Staphylococci* isolates from various sources, including hospitals, communities and livestock, are possible. More stringent infection control and surveillance measures are urgently needed.

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Author Contributions

Y.P. and Z.Y. designed the study and wrote the manuscript; Q.O., D.L., P.X. and J.Z. collected the samples and performed the experiments; X.Y. and Y.L. analysed the data.

Additional Information

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