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Note

Comparative analysis of methicillin-resistant *Staphylococcus aureus* isolated from outpatients of dermatology unit in hospitals and clinics☆

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ABSTRACT

Community-onset methicillin-resistant *Staphylococcus aureus* (CO-MRSA) is a causative agent of intractable skin infections. In general, clinical symptoms of hospital outpatients with skin infections are severer than those of clinic patients. Hence, molecular epidemiological features of the CO-MRSA strains from hospital outpatients are predicted to be different from those of clinic patients. Here, we conducted a comparative analysis for CO-MRSA isolates from outpatients with impetigo in hospitals and clinics located in the same district of Tokyo, Japan. Incidence of MRSA infection was higher in hospital outpatients (21.5%, 20/93 isolates) than in clinic patients (14.5%, 121/845 isolates). The resistance rate to clindamycin, which is a common topical antimicrobial agent in dermatology, in the isolates from hospital outpatients (60.0%) was higher than those from clinic patients (31.4%). Proportion of the staphylococcal cassette chromosome (SCC) *mec* type II, which is a representative type of hospital-acquired MRSA in Japan, in the isolates from hospital outpatients (65.0%) was significantly higher than those from clinic patients (30.6%) ($P < 0.01$). Multilocus sequence typing showed that the clonal complex 89-SCC*mec* type II (CC89-II) clone, which exhibits clindamycin resistance, was the most predominant (55.0%) in the isolates from hospital outpatients. On the other hand, all CC8-IV, CC121-V, and CC89-V clones accounted for 60% in clinic patients were susceptible to clindamycin. Our findings suggested that the clindamycin-resistant CC89-II CO-MRSA clone might be more related to skin infections in hospital outpatients than clinic patients.

Methicillin-resistant *Staphylococcus aureus* (MRSA) exhibits resistance to multiple antimicrobial agents; in addition, it is one of the most common nosocomial pathogens. MRSA strains are divided into two types: hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA), which are mainly isolated from hospitalized patients and outpatients, respectively [1]. In Japan, the main HA-MRSA strains comprise staphylococcal cassette chromosome (SCC) *mec* types I, II, and III, whereas the main CA-MRSA strains are SCC*mec* types IV and V [2]. In general, HA-MRSA strains exhibit high-level resistance to multiple non-β-lactam antimicrobial agents such as quinolones, aminoglycosides, and macrolides [3]. CA-MRSA strains are usually susceptible to non-β-lactams, whereas they produce various virulence and colonization factors, such as exfoliative toxin, Panton-Valentine leukocidin (PVL), and arginine catabolic mobile element (ACME) [4]. Thus, the pathogenicity of CA-MRSA strains is considered to be higher than HA-MRSA strains.

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In general, the symptoms of hospital outpatients with skin infections are severer than those of clinic patients. Hence, molecular epidemiological features of the community-onset MRSA (CO-MRSA) strains from hospital outpatients were predicted to be different from those of clinic patients. Here, we conducted a comparative analysis for CO-MRSA isolates from outpatients with impetigo in hospitals and clinics located in the same district of Tokyo, Japan.

The study protocol was approved by the Tokyo University of Pharmacy and Life Sciences Ethics Committee (16-12). Informed consent was not required from the original patients because the study did not involve clinical interactions with the patients. All 1469 outpatients who visited the dermatology unit of seven hospitals and seven clinics located in Tama district of Tokyo, Japan, between 2008 and 2015 were investigated. To normalize bias of disorder between hospitals and clinics, the samples were selected from only patients with impetigo. A total of 938 *S. aureus* (hospitals, 93 isolates; clinics, 845 isolates) were collected. All strains were isolated from different patients. All clinical isolates were identified as *S. aureus* by a positive Gram stain, proliferation on mannitol salt agar (Oxoid, Hampshire, UK), tests for coagulase production (PS LATEX; Eiken Chemical, Tokyo, Japan), and detection of the *nuc* gene [5]. MRSA isolates were identified using a polymerase chain reaction (PCR) -based detection of the *mecA* gene [6]. In this study, we defined all MRSA isolates as CO-MRSA, because all samples were isolated from outpatients [1]. PCR assays for the detection of *mecA*, the exfoliative toxin (ET) genes (*eta*, *etb*, and *etd*), the staphylococcal enterotoxin (SE) genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, and *sej*), the toxic shock syndrome toxin-1 (TSST-1) gene (*tst*), the PVL gene (*lukS/F-PV*), ACME genes (*arcA* and *opp*-3C), the fibrinogen-binding protein gene (*fib*), fibronectin-binding protein genes (*fnbA* and *fnbB*), clumping factor genes (*clfA* and *clfB*), macrolide resistance genes (*ermA* and *ermC*), and aminoglycoside resistance gene (*aacA-aphD*) were performed as described previously [7,8]. SCC*mec* typing was performed as described previously [6]. Isolates not identified as SCC*mec* types I to V were classified as nontypeable (NT). Minimum inhibitory concentrations (MICs) were determined by the agar doubling-dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The breakpoints of these antimicrobial agents were determined using the CLSI interpretation criteria [9]. MLST was performed as described previously [10]. Comparisons between isolates from outpatients of the dermatology unit of hospitals and clinics were evaluated using χ^2 or Fisher's exact tests. Results with $P < 0.05$ were considered statistically significant.

The median ages (interquartile range) of hospital and clinic outpatients were 5 (3-9) and 4 (2-6), respectively. Additionally, the male ratios of hospital and clinic outpatients were 51.6% and 56.6%, respectively. Thus, no significant differences were found in the patient's age and gender. When MRSA identification of *S. aureus* isolates from outpatients of hospitals and clinics was conducted, detection rate of MRSA in outpatients of hospitals (21.5%, 20/93 isolates) was higher than those of clinics (14.3%, 121/845 isolates) (Table 1). Among the outpatients of hospitals, MRSA isolates were found in only 2 of 7 hospitals, and 90% (18 isolates) of them were detected in Hospital C which is a tertiary care university hospital. Usually, patients with skin infections visit the dermatology unit of hospital when their symptoms could not be improved by antimicrobial therapy in clinic. The antimicrobial-resistant bacteria, such as MRSA, are frequently associated with intractable infections. Hence, we hypothesized that it is one of the reasons for the higher incidence of MRSA in hospital outpatients than clinic patients. However, the number of MRSA isolates from outpatients of hospitals was too small to prove our hypothesis. Further surveillance participating a greater number of hospitals is necessary to validate our data.

Table 1
Isolation rate of MRSA and proportion of 4 major clones identified in this study.

Facility (n[a])	No. (%) of MRSA	No. (%) of the following MRSA clone;			
		CC8-IV	CC89-II	CC89-V	CC121-V
Hospital A (1)	0	0	0	0	0
Hospital B (6)	0	0	0	0	0
Hospital C (72)	18 (25.0)	1 (5.6)	11 (61.1)	3 (16.7)	1 (5.6)
Hospital D (1)	0	0	0	0	0
Hospital E (2)	0	0	0	0	0
Hospital F (5)	2 (40.0)	0	0	0	0
Hospital G (6)	0	0	0	0	0
Total (93)	20 (21.5)	1 (5.0)	11 (55.0)	3 (15.0)	1 (5.0)
Clinic A (100)	16 (16.0)	2 (12.5)	8 (50.0)	4 (25.0)	0
Clinic B (3)	3 (100.0)	0	3 (100.0)	0	0
Clinic C (443)	54 (12.2)	7 (13.0)	17 (31.5)	16 (29.6)	8 (14.8)
Clinic D (93)	18 (19.4)	3 (16.7)	3 (16.7)	4 (22.2)	3 (16.7)
Clinic E (126)	14 (11.1)	1 (7.1)	3 (21.4)	5 (35.7)	3 (21.4)
Clinic F (6)	1 (16.7)	0	1 (100.0)	0	0
Clinic G (74)	15 (20.3)	0	2 (13.3)	13 (86.7)	0
Total (845)	121 (14.3)	13 (10.7)	37 (30.6)	42 (34.7)	14 (11.6)

[a] Number of *S. aureus* isolates collected in each facility.

Antimicrobial susceptibilities of CO-MRSA isolated from outpatients of hospitals and clinics were compared (Table S1). The MIC_{50} values of β-lactams, clindamycin, and gentamicin in the isolates from hospital outpatients were higher than those from clinic patients. The MIC_{50}/MIC_{90} values of cefdinir and faropenem, which are commonly used in department of pediatrics, in the isolates from hospital outpatients were higher than those from clinic patients. Further, the isolates from hospital outpatients showed significantly higher resistance rate to clindamycin, which is a common topical antimicrobial agent against in dermatology. By contrast, the resistance rate of gentamicin, which is also used as a topical antimicrobial agent in dermatology, was equivalent between the isolates from hospital and clinic outpatients. No great differences of susceptibilities were found in fosfomycin, levofloxacin, macrolides, arbekacin, minocycline, chloramphenicol, fusidic acid, and vancomycin between isolates from outpatients of hospitals and clinics. Among the tested antimicrobial agents, levofloxacin, arbekacin, minocycline, chloramphenicol, fusidic acid, and vancomycin were effective against the both CO-MRSA isolates from outpatients of hospitals and clinics.

To understand the genetic differences between CO-MRSA isolates from outpatients of hospitals and clinics, genotypes were determined using SCC*mec* typing and MLST. Proportion of the SCC*mec* type II, which is representative type of HA-MRSA in Japan [2], in the isolates from hospital outpatients (65.0%, 13/20 isolates) was significantly higher than clinic patients (30.6%, 37/121 isolates) ($P < 0.01$). By contrast, SCC*mec* type IV and V, which are mainly found in CA-MRSA in Japan [5], were the predominant in the isolates from clinic patients (20.7% and 46.3%, respectively). MLST analysis showed that the clonal complex 89-SCC*mec* type II (CC89-II) clone was the most predominant in the isolates from hospital outpatients (55.0%, 11/20 isolates), and the proportion of that was significantly higher than the isolates from clinic patients (30.6%, 37/121 isolates) ($P < 0.05$) (Table 1). By contrast, diverse genotypes were found in the isolates from clinic patients (Table 2). The CC89-II and CC89-V were equally found in the isolates from clinic patients. Moreover, CC8-IV and CC121-V clones were found in the isolates from clinic patients, but those clones were rarely found in the isolates from hospital outpatients (Table 1). Although the sample number of Clinics B and F were small, the incidence of CC89-II clones in Clinics A, B, and F was higher than that of the other

Table 2
Genetic features of MRSA isolates from outpatients of hospitals and clinics.

Genotype[a]	Hospital or Clinic (n)	No. (%) of isolates															
		ermA	*ermC*	*aacA-aphD*	*eta*	*etb*	*seb*	*sec*	*seg*	*sei*	*tst*	*cna*	*fib*	*fnbA*	*fnbB*	*clfA*	*clfB*
CC5-II	Hospital (1)	1 (100.0)											1 (100.0)	1 (100.0)		1 (100.0)	1 (100.0)
	Clinic (0)																
CC5-IV	Hospital (0)																
	Clinic (1)								1 (100.0)	1 (100.0)			1 (100.0)	1 (100.0)		1 (100.0)	1 (100.0)
CC8-II	Hospital (1)	1 (100.0)		1 (100.0)		1 (100.0)						1 (100.0)		1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
	Clinic (0)																
CC8-IV	Hospital (1)			1 (100.0)			1 (100.0)	1 (100.0)			1 (100.0)		1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
	Clinic (13)		3 (23.1)	7 (53.9)			8 (61.5)	7 (53.8)			11 (84.6)	2 (15.4)	12 (92.3)	12 (92.3)	10 (76.9)	12 (92.3)	11 (84.6)
CC8-NT	Hospital (0)																
	Clinic (1)							1 (100.0)			1 (100.0)		1 (100.0)	1 (100.0)		1 (100.0)	
CC121-V	Hospital (1)			1 (100.0)	1 (100.0)				1 (100.0)	1 (100.0)		1 (100.0)	1 (100.0)	1 (100.0)		1 (100.0)	1 (100.0)
	Clinic (14)	1 (7.1)	9 (64.3)	11 (78.6)	13 (92.9)	2 (14.3)			14 (100.0)	13 (92.9)		8 (57.1)	14 (100.0)	14 (100.0)		14 (100.0)	13 (92.9)
CC88-IV	Hospital (0)																
	Clinic (5)		3 (60.0)	1 (20.0)	3 (60.0)								4 (80.0)	4 (80.0)	4 (80.0)	3 (60.0)	3 (60.0)
CC89-I	Hospital (2)		1 (50.0)	1 (50.0)										2 (100.0)	1 (50.0)	2 (100.0)	2 (100.0)
	Clinic (1)		1 (100.0)	1 (100.0)		1 (100.0)								1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
CC89-II	Hospital (11)	11 (100.0)		9 (81.8)		11 (100.0)						5 (45.5)	1 (9.1)	11 (100.0)	11 (100.0)	11 (100.0)	11 (100.0)
	Clinic (37)	30 (81.1)		36 (97.3)		32 (86.5)			1 (2.7)	1 (2.7)		17 (45.9)	2 (5.4)	36 (97.3)	31 (83.8)	36 (97.3)	36 (97.3)
CC89-III	Hospital (0)																
	Clinic (1)	1 (100.0)		1 (100.0)		1 (100.0)						1 (100.0)		1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
CC89-IV	Hospital (0)																
	Clinic (3)	2 (66.7)	2 (66.7)	3 (100.0)	1 (33.3)	2 (66.7)						1 (33.3)	2 (66.7)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
CC89-V	Hospital (3)		1 (33.3)	3 (100.0)		2 (66.7)						2 (66.7)		3 (100.0)	2 (66.7)	3 (100.0)	3 (100.0)
	Clinic (42)		19 (45.2)	41 (97.6)		40 (95.2)						28 (66.7)	3 (7.1)	41 (97.6)	34 (80.1)	41 (97.6)	41 (97.6)
Other	Hospital (0)																
	Clinic (3)		1 (33.3)	2 (66.7)	1 (33.3)								2 (66.7)	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)

[a] Genotypes are shown by clonal complex (CC)-SCC*mec* type.

clinics. We could not analyze the reason due to the patient's background unavailable.

The antimicrobial susceptibilities of 4 major clones (CC89-II, CC89-V, CC8-IV, and CC121-V) of hospital outpatients and clinic patients were compared (Table 3). The both CC89-II clones of hospitals and clinics showed same patterns, and the resistance rates of clarithromycin, azithromycin, clindamycin were higher than the other clones. Although all CC89-V clones, which was the most predominant clone in clinic patients, showed resistance against gentamicin, they exhibited high susceptibility to other antimicrobial agents compared to the CC89-II clones. The CC8-IV clones showed similar antimicrobial susceptibilities to the CC89-V clones. The CC121-V clones were resistant to macrolides but susceptible to clindamycin.

To clarify the reason for clindamycin resistance in CC89-II clone, the macrolide resistance genes were detected (Table 2). The *ermA*, that confers constitutive resistance to the both macrolides and clindamycin [7], was detected in 100% and 81.1% of the CC89-II clones of hospital and clinic outpatients, respectively. By contrast, except for one CC121-V strain, no *ermA*-positive strain was found in the CC89-V, CC8-IV, and CC121-V clones. The *ermC*, that confers resistance to only macrolides [7], was detected in 45.2%, 23.1%, and 64.3% of the CC89-V, CC8-IV, and CC121-V clones, respectively. On the other hand, gentamicin resistance gene *aacA-aphD* was detected in 81.8%, 97.3%, 97.6%, 53.9%, and 78.6% of CC89-II (hospitals), CC89-II (clinics), CC89-V, CC8-IV, and CC121-V clones, respectively. To analyze the differences of the virulence for CO-MRSA isolates between outpatients of hospitals and clinics, detections of the virulence genes (ET genes, SE genes, TSST-1 gene, PVL gene, and ACME genes) and surface adhesion genes (*fib*, *fnbA*, *fnbB*, *clfA*, and *clfB*) were performed (Table 2). The CC89 clones mainly possessed *etb* gene, whereas other virulence genes were rare. The detection rates of *seb*, *sec*, and *tst* genes in CC8-IV clone were higher than those in the other clones. No *lukS/F-PV*-positive strain including USA300 clone was found in this study. The CC121-V clone frequently had *eta*, *seg*, and *sei* genes. However, notable difference was not observed in the profiles of the surface adhesion genes.

Although SCC*mec* type II is mainly found in a typical HA-MRSA in Japan [2], 65% of the CO-MRSA isolates from hospital outpatients were classified into SCC*mec* type II. As well as the previous reports, SCC*mec* type V was the most predominant in the CO-MRSA isolates from clinic patients [5]. Notably, ≥60% of the both CO-MRSA isolates from hospital and clinic outpatients were classified into CC89 clones. Kikuta and colleagues reported that the CC89-II was the predominant clone in patients with impetigo [11]. However, no report of the CC89-II clone prevalence in Japanese HA-MRSA was found. These data strongly suggest that the CC89-II clone found in this study has a genetic background of CA-MRSA. The CC89-II clone exhibited resistance to clindamycin which is usually effective for most CA-MRSA clones [5,7]. The clindamycin resistance transposon Tn*554* containing *ermA* is located on the type II SCC*mec* element [12]. Actually, the *ermA* was frequently detected in the both CC89-II clones of hospital and clinic outpatients. Topical clindamycin is frequently prescribed for the treatment of not only impetigo, but also acne vulgaris [7,13]. A hypothesis is strongly considered that the CC89 MSSA acquired *ermA*-including type II SCC*mec* and became clindamycin-resistant CC89-II CA-MRSA to survive against antimicrobial therapy. As a result, patients with intractable skin infections caused by the CC89-II clone were increased, these patients had high potential to visit the hospital. We reported a similar phenomenon in clindamycin-resistant *Propionibacterium acnes* isolated from hospitals, which was associated with severe patients [13]. Furthermore, the clindamycin resistance rate of *S. epidermidis* isolated from the same site of *P. acnes* was also high [13]. The results of the previous study strongly support the hypothesis.

The clindamycin-resistant CC89-II clone was detected in >30% of the isolates from clinic patients as well. The data suggest that the patients infected by the CC89-II clone have potential to fail antimicrobial therapy and to visit hospital. On the other hand, all CC8-IV, CC121-V, and CC89-V clones accounted for 60% in clinic patients were susceptible to clindamycin. Thus, a simplified prediction of CC89-II clone can be made by determination of clindamycin susceptibility.

Several limitations of this study should be acknowledged. First, because seven hospitals and seven clinics in this study is located at limited region of Japan, our data did not reflect conditions of whole regions of Japan. Second, we could not evaluate influence of previous antimicrobial therapy, because patient's drug history was unavailable. Third, severity of the patients was also unavailable. Fourth, the number of MRSA isolates from outpatients of hospitals was too small. Fifth, most MRSA isolates from outpatients of

Table 3
Comparison of the antimicrobial susceptibilities of 4 major clones identified in this study.

Antimicrobial agent	H[a]-CC89-II (n = 11)		C[b]-CC89-II (n = 37)		C[b]-CC89-V (n = 42)		C[b]-CC8-IV (n = 13)		C[b]-CC121-V (n = 14)	
	MIC_{50}/MIC_{90}	R (%)	MIC_{50}/MIC_{90}	R (%)	MIC_{50}/MIC_{90}	R (%)	MIC_{50}/MIC_{90}	R (%)	MIC_{50}/MIC_{90}	R (%)
Ampicillin	16/32	–	16/32	–	2/2	–	16/16	–	2/4	–
Cefdinir	32/64	–	16/64	–	0.5/1	–	8/16	–	0.5/2	–
Faropenem	4/64	–	8/64	–	0.13/0.25	–	1/2	–	0.25/0.5	–
Fosfomycin	2/≥256	–	16/≥256	–	≥256/≥256	–	4/8	–	8/64	–
Levofloxacin	0.25/0.25	0	0.13/0.25	0	0.25/0.25	0	0.13/0.5	7.7	0.13/0.25	7.1
Clarithromycin	≥256/≥256	100	≥256/≥256	100	0.25/≥256	47.6	0.13/≥256	30.8	≥256/≥256	85.7
Azithromycin	≥256/≥256	100	≥256/≥256	100	0.5/≥256	47.6	0.5/≥256	30.8	≥256/≥256	85.7
Clindamycin	≥256/≥256	90.9	≥256/≥256	94.6	≤0.06/0.13	0	≤0.06/0.13	0	≤0.06/≤0.06	0
Gentamicin	64/128	81.8	64/≥256	100	32/64	100	32/32	92.3	8/128	42.9
Arbekacin	1/1	–	0.5/2	–	0.5/1	–	0.25/1	–	0.25/1	–
Minocycline	≤0.06/≤0.06	0	0.13/0.25	0	0.13/0.25	0	≤0.06/0.25	0	≤0.06/0.13	0
Chloramphenicol	4/8	0	8/8	0	4/8	0	4/8	0	4/8	0
Fusidic acid	≤0.06/≤0.06	–	≤0.06/0.25	–	≤0.06/0.13	–	≤0.06/≤0.06	–	≤0.06/≤0.06	–
Vancomycin	0.5/1	0	1/1	0	1/1	0	1/1	0	0.5/0.5	0

MIC_{50}/MIC_{90}, the MICs (μg/ml) that inhibit the growth of 50%/90% of the strains; R, rate of resistant strains.
The resistance breakpoints of the following antimicrobial agents were defined according to criteria from the CLSI [9]: levofloxacin, ≥4 μg/ml; clarithromycin, ≥8 μg/ml; azithromycin, ≥8 μg/ml; clindamycin, ≥4 μg/ml; gentamicin, ≥16 μg/ml; minocycline, ≥16 μg/ml; chloramphenicol, ≥32 μg/ml; vancomycin, ≥16 μg/ml.
-, breakpoints were not defined.
[a] The isolates from hospital outpatients.
[b] The isolates from clinic patients.

hospitals were collected from Hospital C. Therefore, the possibility of hospital acquisition of the CC89-II clone in Hospital C could not be denied from our data. However, our findings would be considered as a valuable data, because this is the first report to focus the differences of CO-MRSA between outpatients of hospitals and clinics. Further study is necessary to demonstrate the CC89-II clone as the causative agent of intractable skin infections.

In conclusion, our findings suggested that the CC89-II CO-MRSA clone might be more related to skin infections in hospital outpatients than clinic patients. The CC89-II clone exhibited resistance to clindamycin which is usually effective for most CA-MRSA clones. Therefore, a simplified prediction of CC89-II clone can be made by determination of clindamycin susceptibility.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2018.09.002.

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