Title

Molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolated from skin and soft-tissue infections collected in the Japanese nationwide surveillance

Running Head

Molecular characteristics of MRSA from SSTI in Japan

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Abstract

Skin and soft tissue infections (SSTIs) are a common infection among both outpatients and inpatients. The most frequently isolated bacterium in SSTIs was *Staphylococcus aureus,* and quarter of which was methicillin-resistant *S. aureus* (MRSA). In this study, to investigate molecular epidemiology of the 141 MRSA strains collected in the Japanese nationwide surveillance, we performed multiplex real-time PCR to detect staphylococcal cassette chromosome *mec* (SCC*mec*) type and virulence genes. The percentage of SCC*mec* type I, II, III, and IV was 1.4%, 52.5%, 5.7%, and 40.4%, respectively. According to the SCC*mec* type, we classified the strains into HA-MRSA (n = 84) and CA-MRSA (n = 57). Among the virulence genes, the percentage of enterotoxin C gene-positive strains was significantly higher in CA-MRSA than in HA-MRSA. No significant differences were detected between the two groups in terms of antibiotic susceptibility and patients' background information, classification of SSTIs, or symptoms of SSTIs.

Key words

MRSA; SCC*mec*; surveillance; epidemiology; SSTI

Introduction

Skin and soft tissue infections (SSTIs) are common in both outpatient and inpatient. Although most MRSA infections are categorized as healthcare-associated infections, those caused by community-associated MRSA (CA-MRSA), which usually carries staphylococcal cassette chromosome *mec* (SCC*mec*) types IV or V, have been reported from all over the world for over 10 years.(1,2) However, the molecular characteristics of MRSA isolated from SSTIs in Japan remain unclear, because there are only a few multicenter studies on molecular epidemiology of MRSA isolated from SSTIs in Japan. (3–5)

To reveal the molecular epidemiology of MRSA isolated from patients with SSTIs in Japan, we performed genetic analysis of MRSA collected in the nationwide surveillance conducted by the Japanese Society of Chemotherapy, Japanese association for infectious diseases and Japanese society for Clinical Microbiology.(6) Additionally, we investigated the differences between HA-MRSA and CA-MRSA based on classification via genetic analysis.

Material and methods

Strains and patients' background

MRSA strains were collected throughout Japanese institutions included 30 dermatology departments within hospitals and 10 dermatology clinics (Supplementary Table 1) between January and October 2013, as described in a previous study.(6) Of the

141 strains, 7 strains were isolated from clinics and 134 strains were isolated from hospitals. Minimum inhibitory concentration (MIC) of MRSA strains was measured in the previous study. (6) Patients' background information was collected from all participants and anonymized for use in this study.

Real-time PCR assay

Bacterial DNA extraction and real-time PCR were performed as reported previously to amplify SCC*mec* I, SCC*mec* II-III, SCC*mec* I-II-IV, toxic shock syndrome toxin 1 genes (*tst*), enterotoxin C genes (*sec*), exfoliative toxin type b genes (*etb*), and *pvl*. (7) Based on the result of real-time PCR, the strains were determined as SCC*mec* type I, II, III, IV, and non-typeable. (2,7–9) Based on the SCC*mec* type, we classified the strains into HA-MRSA (SCC*mec* type I, II, and III) and CA-MRSA (SCC*mec* type IV).(8)

Ethics

This study followed the principles set forth in the Declaration of Helsinki and was approved by the ethics committee of Nagasaki University Hospital (approval number, 19012118).

Statistical analysis

In a comparative study, we used IBM SPSS version 25 (IBM Japan, Tokyo, Japan) for all statistical analyses, which were unpaired, two-tailed, and tests of significance. The statistically significant alpha level was set at ≤ 0.05. Fisher's exact test was used to

compare categorical variables. Continuous variables were expressed as mean ± standard deviation (SD), and compared using the Student t-test.

Results

Genetic analysis

Of the 141 strains, 2 (1.4%) carried SCC*mec* type I, 74 (52.5%) carried SCC*mec* type II, 8 (5.7%) carried SCC*mec* III, and 57 (40.4%) carried SCC*mec* type IV (Fig. 1A). There was no non-typeable strain. With regard to virulence genes, 114 strains (80.9%) were positive for *sec*, 132 (93.6%) were positive for *tst*, 14 (9.9%) were positive for *etb*, and 9 (6.4%) were positive for *pvl* (Fig. 1B).

According to the SCC*mec* type, we classified the strains into HA-MRSA (n = 84) and CA-MRSA (n = 57). The percentage of *sec* gene-positive strains was found to be significantly higher in CA-MRSA than in HA-MRSA (89.5%, CA-MRSA and 75.0%, HA-MRSA, $P = 0.048$) (Fig. 1C).

Comparison of patient background information between HA- and CA-MRSA

According to the patients' background information, the percentage of inpatients in the HA- and CA-MRSA groups was 36.9% and 38.6%, respectively. History of hospitalization within 1 year in the HA- and CA-MRSA groups was 45.2% and 42.1%, respectively. There were no significant differences between the two groups in patients' background (Table 1).

Differences in antibiotic susceptibility between HA-MRSA and CA-MRSA

The MICs of HA-MRSA and CA-MRSA are shown in Supplementary Table 2. There was no difference in MIC_{50} and MIC_{90} between HA-MRSA and CA-MRSA. Antibiotic susceptibility of HA-MRSA and CA-MRSA is shown in Figure 2. The susceptibility rate of ciprofloxacin, levofloxacin, and moxifloxacin was lower in HA-MRSA than in CA-MRSA. However, there was no significant difference in antibiotic susceptibility between the two groups.

Discussion

We investigated the molecular epidemiology of MRSA isolated from patients with SSTIs in the Japanese nationwide surveillance. From our genetic analysis, the percentage of SCC*mec* type II was higher than that of SCC*mec* type IV. On the other hand, in the previous nationwide surveillance of CA-MRSA isolated from skin and pus samples of outpatients in Japan, the most frequent SCC*mec* type was IV and the second was II.(4) However, there were some differences in study design between two nationwide surveillance. MRSA strains were collected from only outpatients in the first nationwide surveillance while MRSA strains were collected from both outpatients and inpatients in this study. In addition, MRSA strains were collected from many small hospitals that possible no microbiology laboratories in the first nationwide surveillance,(4) whereas MRSA strains were collected from many university hospitals. (6) Most of the MRSA strains (95.0%) in this study were isolated from hospitals. A previous multicenter study

of MRSA isolated from outpatients in Tama district of Tokyo revealed that the percentage of SCC*mec* type II in hospitals was higher than that in clinics.(3), which could explain why the most frequent SCC*mec* type was different between two nationwide surveillance.

A recent multicenter study on MRSA isolated from outpatients with impetigo in Kagawa reported that the most frequent SCC*mec* type was V.(5) The previous study in Tama also reported the percentage of SCC*mec* V in hospitals and clinics were 20.0% and 46.3%, respectively.(3) SCC*mec* V was determined as non-typeable in our method,(9) but there was no non-typeable strain in this study. There is a possibility that the difference in method between two previous studies and this study influenced the results. However, patients' background is markedly different between two previous studies and this study. The median age of patient in Kagawa was 12, (5) and that in hospitals and clinics in Tama was 5 and 4, respectively. (3) On the other hand, the mean age was 52.5 in this study. Moreover, 72.3% of the patients in this study had underlying diseases. Since SCC*mec* type V was generally seen in healthy children or young athletes,(10) these differences might influence the detection of SCC*mec* V. In addition, there is a possibility that epidemic SCC*mec* type vary depending on the region, because there was no participating institution located in Tama district or Kagawa in this study.

We compared virulence genes, patients' background, and antibiotic susceptibility between HA-MRSA and CA-MRSA groups in this study. For virulence factors, the percentage of *sec* gene-positive strains was significantly higher in the CA-MRSA group than in HA-MRSA as previously reported.(8,11) From a comparison of patient background information, we found no significant differences between HA-MRSA and

CA-MRSA groups. A percentage of inpatient in CA-MRSA group was almost as much as that in HA-MRSA. This means that hospital-acquired SSTIs was also caused by CA-MRSA strain. In this study, the susceptibility rate of fluoroquinolone was lower in HA-MRSA than in CA-MRSA, but there was no significant differences. A Previous studies reported that antibiotic susceptibility was different between HA-MRSA and CA-MRSA.(3,4,9)

There were some limitations to the current study. First, other than SCC*mec* typing, we did not perform a detailed molecular analysis, such as multi locus sequence typing (MLST). Recently, SCC*mec* type IV has been increasing in the hospital-acquired MRSA infections.(2,12) In addition, both SCC*mec* types II and IV were frequently found in the same clonal complex in the previous study.(5) Hence, in further nationwide surveillance, a performance of MLST is needed. Second, we analyzed MRSA strains isolated at a specific point in time. Since there is a possibility that the percentage of SCC*mec* type II and IV is different depending on the study period,(5) further study at other period is needed. Third, we were not able to investigate the effect of antibiotics. CA-MRSA tended to be sensitive to fluoroquinolones, but their effect remains unknown.

In conclusion, this study revealed that the percentage of SCC*mec* type II is higher than that of SCC*mec* type IV in MRSA strains isolated from patients with SSTIs in Japan. Additionally, there are no significant differences in patient background or antibiotic susceptibility between HA-MRSA and CA-MRSA in this study.

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Conflict of interest

The authors declare no conflict of interest.

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306 lookup/doi/10.1086/528716

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Supporting information

Supplementary Table 1. Participating institutions

Institutions
Hospitals
Akita University Hospital, Akita
Yamagata University Hospital, Yamagata
University of Tsukuba Hospital, Ibaraki
Yokohama City University Hospital, Kanagawa
Teikyo University Hospital, Tokyo
Tokyo Metropolitan Police Hospital, Tokyo
Center Hospital of the National Center for Global Health and Medicine, Tokyo
Saitama Cooperative Hospital, Saitama
Shinshu University School of Medicine, Nagano
University of Yamanashi Hospital
Toyama University Hospital, Toyama
Toyama Prefectural Central Hospital, Toyama
Gifu Prefectural General Medical Center, Gifu
Ishikawa Prefectural Central Hospital, Ishikawa
Shiga University of Medical Science Hospital, Shiga
Kyoto University Hospital, Kyoto
Nara Medical University Hospital, Nara
Wakayama Medical University Hospital, Wakayama
Meiwa Hospital, Hyogo
Okayama University Hospital, Okayama
Hiroshima University Hospital, Hiroshima
Yamaguchi University Hospital, Yamaguchi
Tottori University Hospital, Tottori
Shimane University Hospital, Shimane
Shimane Prefectural Central Hospital, Shimane
Kagawa University Hospital, Kagawa
Ehime University Hospital, Ehime
Kochi Medical School Hospital, Kochi

Kyushu University Hospital, Fukuoka

Kagoshima University Hospital, Kagoshima

Clinics

Tobudaishimae Clinic, Tokyo

Shinozaki Dermatology Clinic, Tokyo

Kobayashi Dermatology Clinic, Tokyo

Okuda Dermatology Clinic, Tokyo

Go Dermatology Clinic, Tokyo

Kikuchi Orthopedic Clinic, Tokyo

Okada Dermatology Clinic, Tokyo

Takeshima Dermatology Clinic, Tokyo

Kaneko Dermatology Clinic, Saitama

Fujimino Dermatology Clinic, Saitama

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Supplementary Table 2. Comparison of MICs between HA-MRSA and CA-MRSA

Antibiotic	HA-MRSA (n = 84)			CA-MRSA (n = 57)		
	50%	90%	range	50%	90%	range
PCG	8	32	0.125 to 64	8	32	0.5 to 32
MPIPC	64	128	4 to >128	64	> 128	4 to > 128
ABPC	8	32	0.25 to 128	16	32	1 to 64
SBT/ABPC	8	16	0.25 to 32	8	16	0.5 to 32
AMPC	16	32	1 to >64	16	32	1 to 64
CVA/AMPC	8	32	0.5 to 64	8	32	1 to 32
PIPC	64	128	2 to >128	64	128	2 to > 128
TAZ/PIPC-1	16	128	2 to >128	8	128	1 to > 128
TAZ/PIPC-2	32	128	2 to 128	16	128	2 to 128
CEZ	8	> 128	1 to > 128	8	> 128	1 to > 128

CTM	4	> 128	1 to > 128	8	> 128	1 to > 128
CFDN	4	> 64	0.5 to > 64	4	> 64	0.5 to > 64
CDTR	16	> 64	1 to > 64	16	> 64	2 to > 64
CFPN	16	> 128	2 to > 128	16	> 128	2 to > 128
CFX	64	> 128	4 to > 128	32	> 128	4 to > 128
CMZ	16	64	2 to 128	8	64	2 to 128
IPM	0.5	32	≦ 0.06 to > 64	0.5	32	≦ 0.06 to 64
MEPM	2	32	0.125 to 64	2	16	0.25 to 32
FRPM	1	> 128	0.25 to > 128	1	> 128	0.25 to > 128
CPFX	64	> 128	0.25 to > 128	16	> 128	0.125 to >128
TFLX	>16	> 16	≦ 0.06 to > 16	>16	> 16	≦0.06 to >16
NDFX	2	16	≦ 0.06 to 128	2	16	≦0.06 to 64
LVFX	16	> 128	0.125 to >128	8	>128	0.25 to >128
MFLX	2	64	≦0.06 to 128	2	64	≦0.06 to 128
GM	32	128	0.125 to > 128	32	64	0.125 to >128
ABK	0.5	1	0.25 to 8	0.5	1	0.25 to 8
EM	> 128	> 128	0.5 to > 128	>128	> 128	0.25 to > 128
CAM	> 64	> 64	0.25 to > 64	>64	> 64	0.25 to > 64
AZM	> 64	> 64	0.5 to > 64	>64	> 64	0.5 to > 64
CLDM	0.25	> 128	0.125 to > 128	0.25	>128	0.125 to > 128
MINO	0.125	16	≦ 0.06 to 32	0.125	16	≦ 0.06 to 32
VCM	1	1	0.5 to 2	1	1	0.5 to 2
TEIC	1	2	0.5 to 2	1	2	0.25 to 2
LZD	2	4	1 to 4	2	4	1 to 4

FOM	32	> 128	0.5 to > 128	8	>128	0.5 to > 128
ST	0.06	0.125	0.06 to > 8	0.06	0.125	0.06 to 0.25

MICs, minimum inhibitory concentrations; HA-MRSA, hospital associated MRSA; CA-MRSA, community associated MRSA

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312 **Figure legends**

313 **Figure 1. Genetic analysis of MRSA strains.**

314 A total of 141 strains were isolated from patients with SSTI, and identified as MRSA.

315 SCC*mec* type (A) and virulence genes (B) were identified using real-time PCR. We

316 compared the virulence genes between HA-MRSA and CA-MRSA (C).

317 *sec*, enterotoxin type C; *tst*, toxic shock syndrome toxin 1; *pvl*, Panton-Valentine

318 Leucocidin; *etb*, exfoliative toxin type b; HA-MRSA, healthcare-associated MRSA;

319 CA-MRSA, community-associated MRSA; NS, not significant in Fisher's exact test.

320

321 **Figure 2. Comparison of antibiotic susceptibility of HA-MRSA and CA-MRSA.**

322 Resistance breakpoints were defined according to criteria from the CLSI M100-S22.

323 CPFX, ciprofloxacin; LVFX, levofloxacin; MFLX, moxifloxacin; GM, gentamicin; EM,

324 erythromycin, CAM, clarithromycin; AZM, azithromycin; CLDM, clindamycin; MINO,

325 minocycline; ST, sulfamethoxazole / trimethoprim; VCM, vancomycin; TEIC,

326 teicoplanin; LZD, linezolid.

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329 **Tables**

Table 1. Comparison of patients' background information between HA-MRSA and CA-MRSA

Patients' background	HA-MRSA (n = 84)		CA-MRSA (n = 57)		*P* value
	n	(%)	n	(%)	
Age					
mean ± SD	52.5	± 27.5	52.4	± 29.9	
≦ 15	14	(16.7)	12	(21.1)	NS
16 - 64	35	(41.7)	17	(29.8)	NS
≧ 65	33	(39.3)	39	(49.1)	NS
Gender, female	40	(47.6)	21	(36.8)	NS
Outpatient	53	(63.1)	35	(61.4)	NS
Complicated underlying disease	62	(73.8)	40	(70.2)	NS
History of antibiotics within 4 weeks	36	(42.9)	23	(40.4)	NS
History of hospitalization within 1 year	38	(45.2)	24	(42.1)	NS
Classification of SSTI					NS
Superficial SSTI	38	(45.2)	28	(49.1)	NS
Deep-seated SSTI	35	(41.7)	19	(33.3)	NS
Unknown	11	(13.1)	10	(17.5)	NS
Symptoms of SSTI					NS
Redness	65	(77.4)	39	(68.4)	NS
Swelling	46	(54.8)	23	(40.4)	NS
Local heat	30	(35.7)	17	(29.8)	NS

Pain	32	(38.1)	14	(24.6)	NS
Fever	14	(16.7)	6	(10.5)	NS
Pus / discharge	28	(33.3)	20	(35.1)	NS

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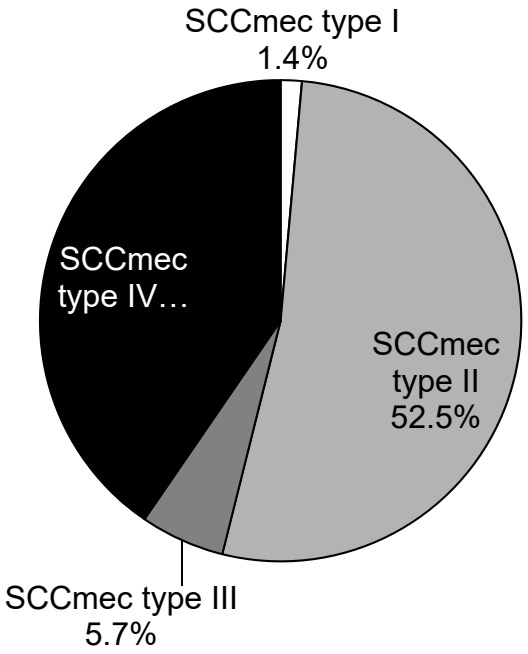
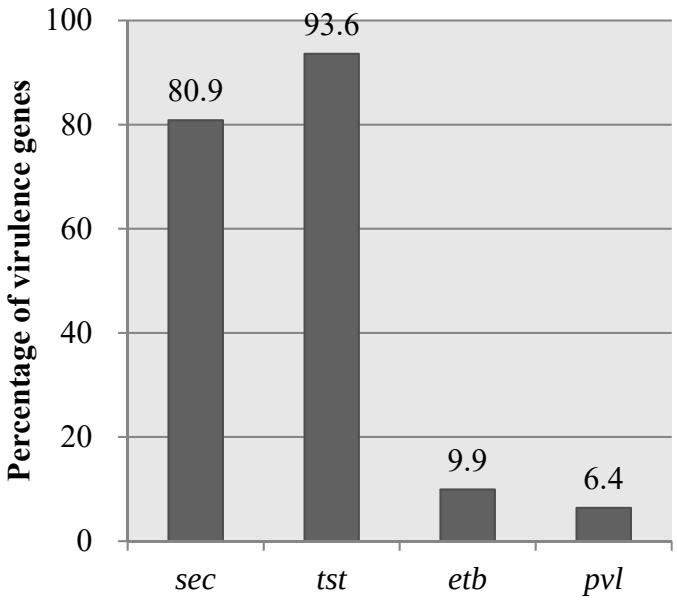
(A)



(B)



(C)

Virulence genes	HA-MRSA (n = 84)		CA-MRSA (n = 57)		*P* value
	n	(%)	n	(%)	
sec	63	(75.0)	51	(89.5)	0.048
tst	78	(92.9)	54	(94.7)	NS
etb	10	(11.9)	4	(7.0)	NS
pvl	5	(6.0)	4	(7.0)	NS

Figure 1. Genetic analysis of MRSA strains.

A total of 141 strains were isolated from patients with SSTI, and identified as MRSA. SCC*mec* type (A) and virulence genes (B) were identified using real-time PCR. We compared the virulence genes between HA-MRSA and CA-MRSA (C).

sec, enterotoxin type C; *tst*, toxic shock syndrome toxin 1; *pvl*, Panton-Valentine Leucocidin; *etb*, exfoliative toxin type b; HA-MRSA, healthcare-associated MRSA; CA-MRSA, community-associated MRSA; NS, not significant in Fisher's exact test.

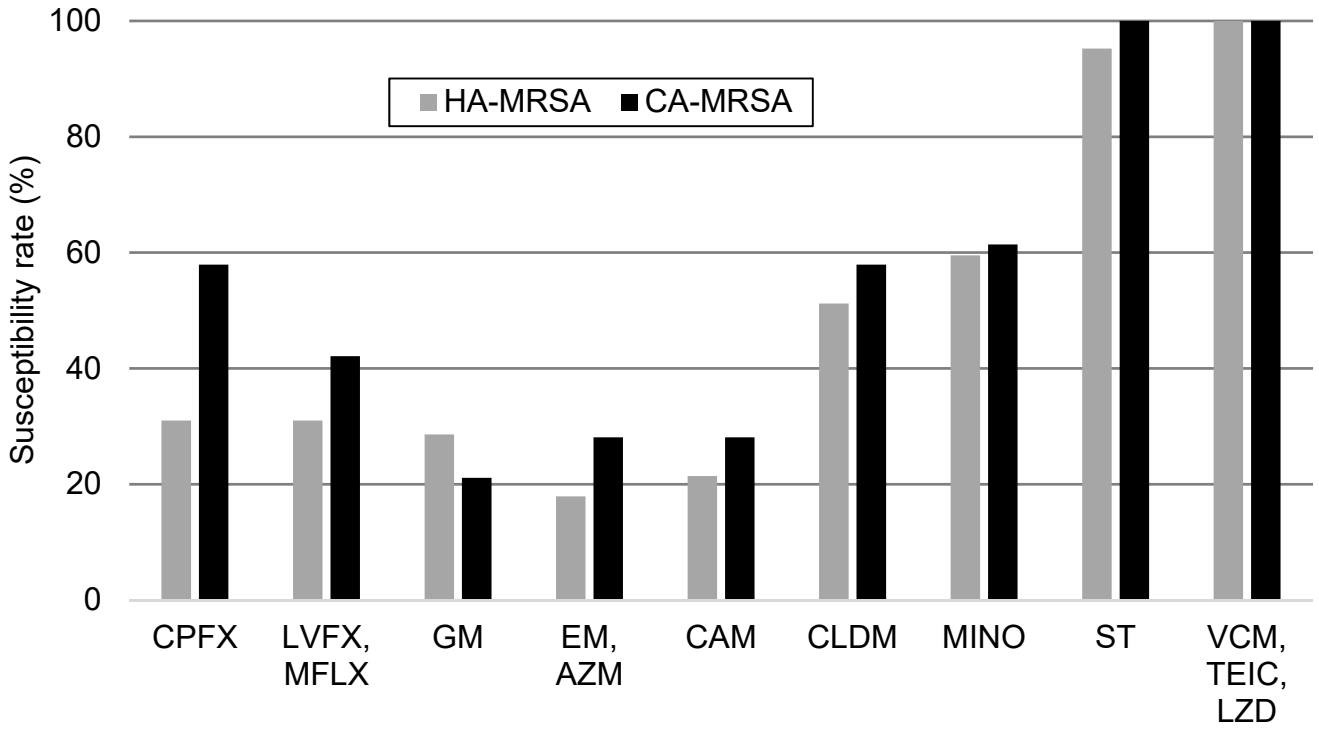


Figure 2. Comparison of the antibiotic susceptibility of HA-MRSA and CA-MRSA
Resistance breakpoints were defined according to criteria from the CLSI M100-S22. CPFX, ciprofloxacin; LVFX, levofloxacin; MFLX, moxifloxacin; GM, gentamicin; EM, erythromycin, CAM, clarithromycin; AZM, azithromycin; CLDM, clindamycin; MINO, minocycline; ST, sulfamethoxazole / trimethoprim; VCM, vancomycin; TEIC, teicoplanin; LZD, linezolid.