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Original Article

Prevalence of Inducible Macrolide, Lincosamide, and Streptogramin B (inducible MLS$_B$) Resistance in Clindamycin-Susceptible *Staphylococcus aureus* at Okayama University Hospital

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Inducible resistance to the macrolide, lincosamide, and streptogramin B (iMLS$_B$) antibiotic family is a latent mechanism for antimicrobial resistance in *Staphylococcus aureus*. We here investigated the frequency and genotypic profiles of iMLS$_B$ resistance in clindamycin (CLDM)-susceptible *S. aureus* isolated in Okayama University Hospital from June 2020 to June 2021. We phenotypically screened the iMLS$_B$ resistance via D-zone test and performed PCR testing for the erythromycin ribosomal methylase (*erm*) genes: *ermA* and *ermC*. Among 432 CLDM-susceptible *S. aureus* isolates, 138 (31.9%) exhibited an iMLS$_B$-resistance phenotype, with methicillin-resistant *S. aureus* isolates (MRSA; 61 isolates: 58.6%) exhibiting higher positivity than methicillin-sensitive *S. aureus* isolates (MSSA; 77 isolates: 23.5%) ($p < 0.001$). Male patients had a higher frequency of iMLS$_B$ resistance than females (OR [95%CI]: 1.8 [1.2-2.8]; $p = 0.007$). Genotypically, *ermA* predominated in both MSSA (70.1%) and MRSA (86.9%) compared to *ermC* (14.3% in MSSA and 11.5% in MRSA). A single strain of MRSA possessed both *ermA* and *ermC*, while 12 (15.6%) MSSA isolates were negative for both *ermA* and *ermC*, suggesting the presence of other genetic mechanisms. Collectively, these results show that approximately 33% of CLDM-susceptible *S. aureus* isolates at our university hospital exhibited iMLS$_B$ resistance, predominantly caused by *ermA* in both MSSA and MRSA.

Key words: antimicrobial resistance, clindamycin, *erm*, D-zone test, inducible MLSB

The emergence of antimicrobial resistance (AMR) pathogens is an increasing problem worldwide [1]. Among various AMR pathogens, *Staphylococcus aureus* is a leading cause of nosocomial and community-acquired infections [2]. Methicillin-sensitive *S. aureus* (MSSA) has traditionally been susceptible to beta-lactams, but the emergence of methicillin-resistant *S. aureus* (MRSA) has restricted the number of effective antibiotics. The development of alternative therapeutic agents and guidelines for the judicious use of existing antibiotics are increasingly required [3,4].

Clindamycin (CLDM) is a widely-available antibiotic drug with good pharmacokinetics and pharmacodynamics properties, and it has long been recommended to treat various infectious diseases caused by diverse organisms, such as *Staphylococci, Streptococci*, and anaerobic bacteria [5,6]. *S. aureus* can develop resistance to CLDM via ribosomal methylases encoded by erythromycin ribosomal methylase (*erm*) genes;

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these *erm* methylases induce ribosomal methylation at the binding site of *S. aureus* for macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics. MLS_B resistance is classified into constitutive and inducible phenotypes [7]. Isolates with the constitutive phenotype exhibit resistance to both erythromycin (EM) and CLDM, which can be simply identified by antimicrobial susceptibility testing. In cases where an isolate shows a pattern of EM resistance with CLDM susceptibility, inducible MLS_B ($iMLS_B$) resistance may exist. Thus, single susceptibility testing for CLDM may fail to detect $iMLS_B$ resistance, resulting in ineffective treatment [8]. In such cases, the double disk method, or D-zone test, is applied to confirm or exclude the presence of $iMLS_B$ resistance [7, 9].

The prevalence of $iMLS_B$ resistance in *S. aureus* has been investigated worldwide, with high incidences observed in developing countries, such as Nepal [10], India [11], and Pakistan [12]. However, relatively few studies have been performed in Japanese clinical settings. Moreover, more data are needed regarding $iMLS_B$ resistance among clinical isolates of *S. aureus* worldwide. In this study, we uncovered the presence of $iMLS_B$ resistance in CLDM-susceptible *S. aureus* at our medical institute by means of a genotype analysis.

Materials and Methods

Study design. This study was conducted at Okayama University Hospital, an 865-bedded, tertiary care, national university hospital in Japan, from June 24, 2020 to June 26, 2021. During the one-year study period, we prospectively collected CLDM-susceptible MSSA and MRSA isolates from clinical samples. Ethical approval was obtained from the institutional review board of Okayama University Hospital (approval no. 2009-048). The requirement for informed consent was waived as all samples and data were anonymized.

Phenotypic study. We performed D-zone testing for all the collected isolates according to the Clinical & Laboratory Standards Institute 2016 guidelines [13]. Briefly, CLDM-susceptible *S. aureus* isolates were adjusted to a McFarland turbidity level of 0.5 and inoculated onto cation-adjusted Mueller Hinton II agar plates (Becton Dickinson and Co., Sparks, MD, USA). Antibiotic disks (Eiken Chemical Co., Tokyo) for EM (15 μg) and CLDM (2 μg) were placed 15 mm apart on the agar plate. Following overnight incubation at 37°C for 16 to 18 h, the inhibition zone around the CLDM disk was measured, revealing a flattening at the side adjoined to the EM disk (a "D" shape) (Fig. 1). Isolates showing these changes on the agar plate were determined to be D-zone test-positive and were considered to have $iMLS_B$ resistance. Those with a smooth, round inhibition zone were defined as D-zone test-negative (truly CLDM-susceptible). All D-zone test-positive isolates were preserved for further genotypic analysis of $iMLS_B$ resistance.

Genotypic study. The frozen samples were subcultured for nucleic acid separation. After overnight incubation, bacterial colonies suspended in 1.5 mL tubes containing 0.5 mL of 0.25% Triton X-100 (Nakalai Tesque, Kyoto, Japan) were incubated in a heat block for 15 min at 94°C [14]. Immediately after cooling, samples were centrifuged at 10,000 g for 5 min at 4°C. A 10-fold dilution of the supernatant was then used as the DNA template for the PCR assay. For the detection of *erm* genes, we performed 35-cycle PCR using Quick TaqTM HS Dye Mix (Toyobo, Osaka, Japan) and GeneAtlas G02 (Astec, Fukuoka, Japan) under the conditions recommended by the manufacturers. Each 20 μL of PCR reaction mixture contained 1 μL of DNA template. We designed PCR primers for *ermA* based on the deposited nucleotide sequences of the representa-

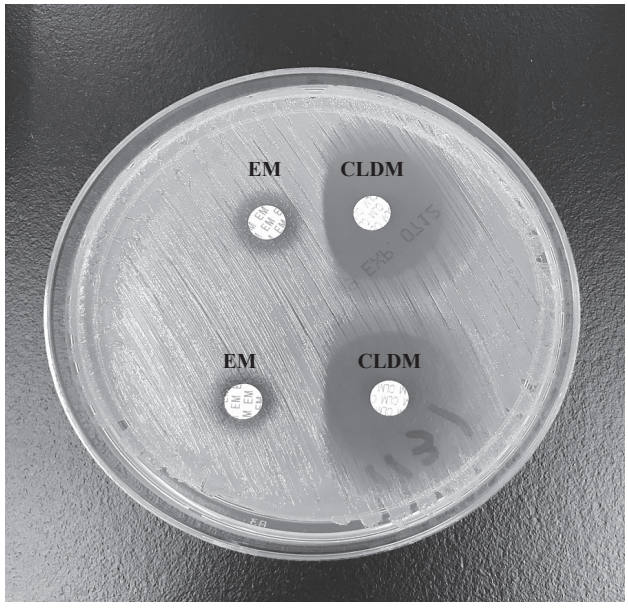

Fig. 1 A positive example of D-zone testing. A cation-adjusted Mueller Hinton II agar plate was used. EM, erythromycin; CLDM, clindamycin.

tive *S. aureus* strain N315 (NCBI RefSeq accession number: NC_002745.2), which is confirmed to harbor the *ermA* gene [15]. PCR primers for *ermC* were designed from the sequence of another registered strain (NCBI RefSeq accession number: NG_055988.1). Table 1 lists the primer sequences. We used two positive control strains to confirm the validity of our primers. *S. aureus* N315 was used for *ermA*, and *S. aureus*, which is a clinical strain confirmed to harbor *ermC* in the previous study [16], was used for *ermC*. We repeatedly confirmed that our original primer pairs could detect *ermA* and *ermC* in the positive control strains in preliminary experiments (Fig. 2). When applied to the clinical strains, universal primers for the 16S rRNA gene were also used to confirm the successful extraction of the bacterial DNA [17]. After electrophoresis at 100 V for 30 min using 1.5% agarose gel containing Gel Red™ (Biotium, Fremont, CA, USA), the sizes of the PCR products were analyzed by standard molecular weight markers (Fig. 3).

Statistical analysis. Statistical analyses were performed using Pearson's chi-square test for categorical variables. Odds ratios (OR) with 95% confidential intervals (CI) and p values were calculated using EZR, a graphical user interface for R 4.0.3 software (The R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $p < 0.05$.

Table 1 Details data of primer pairs used in this study

Genes	Primer sequence		Product size
ermA	Forward	5'-AGCGGTAAACCCCTCTGAGA-3'	220 bp
	Reverse	5'-ACCCAAAGCTCGTTGCAGAT-3'	
ermC	Forward	5'-ACAGAAAATAAACTTGTTGATCACGA-3'	468 bp
	Reverse	5'-ATCGTCAATTCCTGCATGTTTT-3'	
16S rRNA	27F	5'-AGAGTTTGATCMTGGCTCAG-3'	1,523 bp
	1494R	5'-TACGGTTACCTTGTTACGAC-3'	

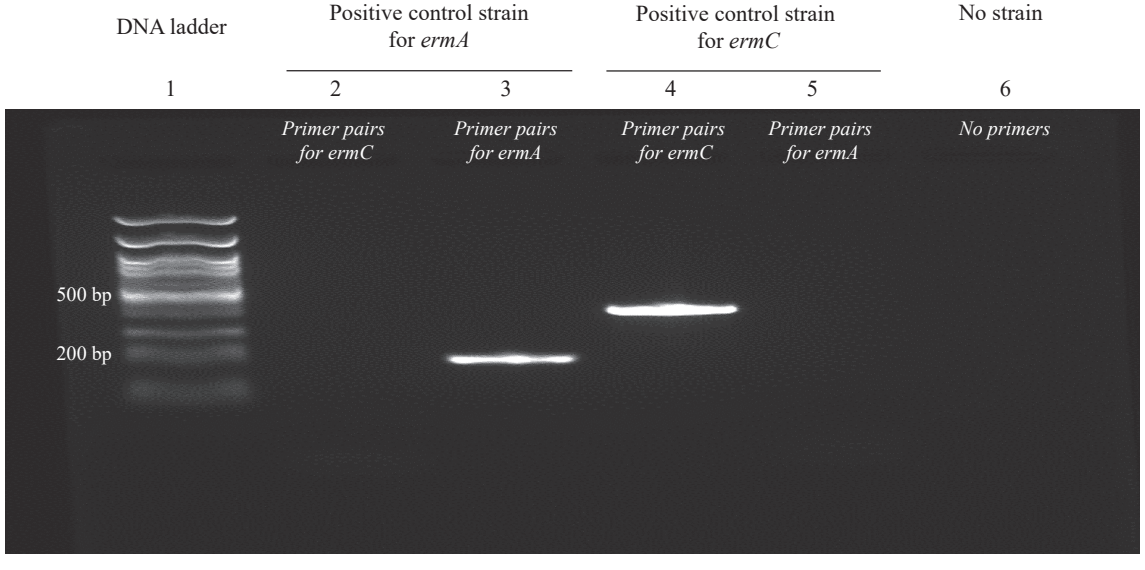

Fig. 2 Confirmation of validity for originally designed primer pairs by agarose gel electrophoresis. *Staphylococcus aureus* N315 [15] and a clinical strain of *S. aureus* [16] were used as positive control strains for *ermA* and *ermC*, respectively. In 1.5% agarose gel electrophoresis, the amplicons of *ermA* (220 bp) and *ermC* (468 bp) genes were detected in Lane 3 and Lane 4, respectively.
Lane 1, DNA molecular size marker; Lane 2, no primers; Lane 3, primer pairs for *ermA*; Lane 4, primer pairs for *ermC*; Lane 5, no primers; and Lane 6, negative control.

Results

During the one-year study period, a total of 432 CLDM-susceptible *S. aureus* isolates were examined, comprising 328 (75.9%) MSSA and 104 (24.1%) MRSA isolates. Overall, 138 (31.9%) CLDM-susceptible *S. aureus* isolates were determined to be iMLS$_B$-resistant via D-zone test. Table 2 shows the frequency of iMLS$_B$ resistance in MSSA and MRSA isolates according to categories of sex and age variables. The proportion of MRSA isolates with iMLS$_B$ resistance was significantly higher than that of MSSA isolates with iMLS$_B$ resistance (58.6% vs 23.5%; OR [95% CI]: 4.6 [2.8-7.6]; $p<0.001$). Compared to female patients, male patients had a higher frequency of iMLS$_B$-resistant infection (37.2% vs 24.7%; OR [95% CI]: 1.8 [1.2-2.8]; $p=0.007$). The

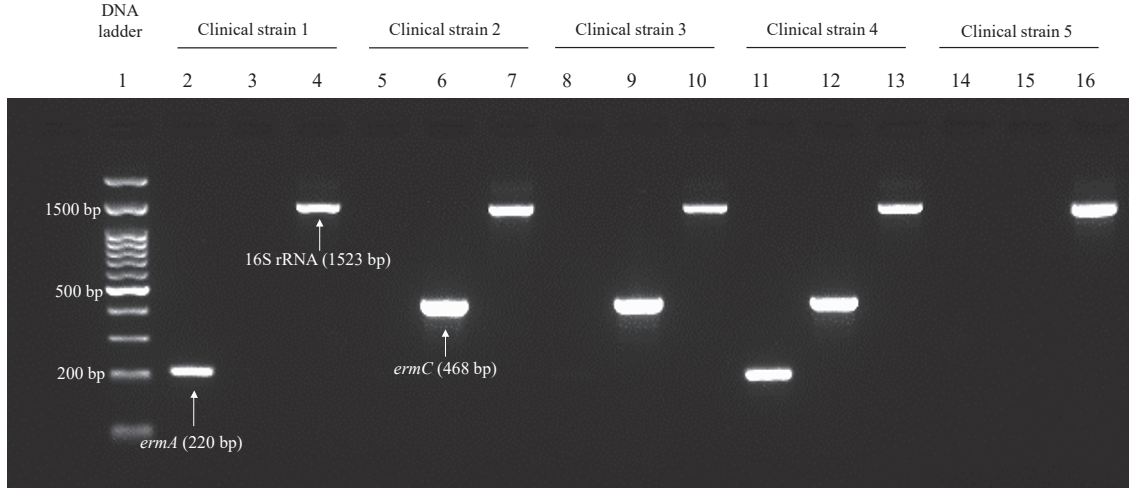

Fig. 3 Agarose gel electrophoresis of PCR products for *ermA*, *ermC*, and 16S rRNA genes to confirm inducible macrolide, lincosamide, and streptogramin B (iMLS$_B$)-resistant *Staphylococcus aureus*. PCR assay results for the *ermA*, *ermC*, and 16S rRNA genes for five representative clinical strains are shown as examples. Strain 1 is positive for *ermA* alone. Strains 2 and 3 are positive for *ermC* alone. Strain 4 is positive for both *ermA* and *ermC*. Strain 5 is negative for both *ermA* and *ermC*.
Lane 1, DNA molecular size marker; Lanes 2, 5, 8, 11, and 14, primer pairs for *ermA*; Lanes 3, 6, 9, 12, and 15, primer pairs for *ermC*; Lanes 4, 7, 10, 13, and 16, primer pairs for the 16S rRNA gene.

Table 2 Backgrounds of iMLS$_B$ resistance in clindamycin-susceptible *Staphylococcus aureus*

	Samples size	iMLSB resistance		OR [95% CI]	*P*-value
		Overall	MSSA vs. MRSA		
Total	432	138 (31.9%)	77/328 (23.5%) vs. 61/104 (58.6%)	4.6 [2.8 to 7.6]	<0.001
Sex					
Male	250	93 (37.2%)	48/187 (25.7%) vs. 45/63 (71.4%)	7.2 [3.7 to 14.5]	<0.001
Female	182	45 (24.7%)	29/141 (20.6%) vs. 16/41 (39.0%)	2.5 [1.1 to 5.5]	0.02
Age					
Under 10 years	60	24 (40.0%)	–	–	–
11–20 years	22	5 (22.7%)	–	–	–
21–40 years	43	7 (16.2%)	–	–	–
41–60 years	112	38 (33.9%)	–	–	–
61–80 years	160	46 (28.7%)	–	–	–
≥81 years	35	18 (51.4%)	–	–	–

iMLS$_B$, inducible macrolide, lincosamide, and streptogramin B; OR, odds ratio; CI, confidential interval; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

higher proportions of iMLS$_B$ resistance in MRSA compared to MSSA were observed in both male (OR [95% CI]: 7.2 [3.7-14.5]; $p<0.001$) and female patients (OR [95% CI]; 2.5 [1.1-5.5]; $p=0.02$). Among those aged under 10 or over 80 years, the proportions of iMLS$_B$ resistance were relatively higher (40.0% and 51.4%, respectively).

The percentages of iMLS$_B$-resistant isolates by sample sources are summarized in Table 3. The iMLS$_B$-resistance rate was the highest in isolates from blood (64.7%), followed by isolates from pleural fluid (61.5%) and intravascular catheters (44.4%).

Among the 138 iMLS$_B$-resistant *S. aureus* isolates, 126 expressed the *ermA* gene, the *ermC* gene, or both (Table 4). In total, the positivity rates for *ermA*, *ermC*, and both genes were 77.5%, 13.0%, and 0.7%, respectively. The positivity rate for *ermA* was significantly different between MSSA (70.1%) and MRSA (86.9%) (OR [95% CI]: 3.3 [1.2-9.8]; $p=0.01$). Conversely, no statistically significant difference was found for *ermC*. All the MRSA isolates were positive for either *ermA* or *ermC*, whereas 12 isolates (15.6%) of MSSA were negative for these resistance genes.

Table 3 Sources of the clinical samples isolating the iMLS$_B$ resistance *Staphylococcus aureus* and its positivity rates

Sources	Number	iMLS$_B$ resistance
Sputum	99 (22.9%)	35 (35.4%)
Abscess	77 (17.8%)	23 (29.9%)
Skin swab	77 (17.8%)	15 (19.5%)
Eye mucous	38 (8.8%)	8 (21.1%)
Nasal or oral swab	28 (6.5%)	10 (35.7%)
Ear mucous	22 (5.1%)	7 (31.8%)
Blood	17 (3.9%)	11 (64.7%)
Urine	14 (3.2%)	3 (21.4%)
Pleural fluid	13 (3.0%)	8 (61.5%)
Intravascular catheter	9 (2.1%)	4 (44.4%)
Bronchoalveolar lavage	3 (0.7%)	0
Others	35 (8.1%)	14 (40.0%)

iMLS$_B$, inducible macrolide, lincosamide, and streptogramin B.

Discussion

Our study revealed that the overall rate of iMLS$_B$ resistance among CLDM-susceptible *S. aureus* was 31.9%, with a significantly higher isolation rate in MRSA. This clearly indicates the importance of performing the D-zone test for CLDM-susceptible *S. aureus* to confirm the latent resistance mechanism. Notably, the *ermA* gene was dominant in both MSSA (70.1%) and MRSA (86.9%) with the iMLS$_B$-resistance phenotype. Twelve isolates (15.6%) of MSSA were negative for both *ermA* and *ermC*, suggesting the presence of another genetic mechanism for the iMLS$_B$ resistance.

In our study, approximately one-third of CLDM-susceptible *S. aureus* isolates were phenotypically determined to have iMLS$_B$ resistance. Previous studies, primarily performed in Asian and developing countries, have reported widely differing prevalences of iMLS$_B$ resistance in CLDM-susceptible *S. aureus* (Table 5) [12,18-40]. Some reported remarkably higher rates. Thus, the percentage of iMLS$_B$-resistant isolates in CLDM-susceptible *S. aureus* was 94.0% in Jordan [21], 88.9% in Turkey [33], 80.0% in Egypt [22], 81.6% in Nepal [26], 71.7% in Pakistan [12], and 65.4% in Iran [39]. Like our present study, most of these reports found that the rates of MRSA were equivalent to or higher than those of MSSA. These studies have revealed that the prevalence of iMLS$_B$ resistance in *S. aureus* differs depending on several clinical factors, including age distribution, geographical difference, patient population, hospital characteristics, sample source (commu-

Table 4 Numbers and proportions of *erm* gene family detected in the iMLS$_B$ resistance *Staphylococcus aureus*

Genes	Total	MSSA	MRSA	OR [95% CI]	*P*-value
Number of iMLSB resistance	138	77	61	–	–
ermA	107 (77.5%)	54 (70.1%)	53 (86.9%)	3.3 [1.2 to 9.8]	0.01
ermC	18 (13.0%)	11 (14.3%)	7 (11.5%)	0.9 [0.3 to 2.7]	1
ermA + *ermC*	1 (0.7%)	0	1 (1.6%)	–	–
Both negative	12 (8.7%)	12 (15.6%)	0	–	–

iMLS$_B$, inducible macrolide, lincosamide, and streptogramin B; OR, odds ratio; CI, confidential interval; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*.
An isolate with double positive for *ermA* and *ermC* was incorporated to the statistical calculation of each gene.

Table 5 Previous studies of $iMLS_B$ resistance in clindamycin-susceptible *Staphylococcus aureus*

			Numbers (percentages) of iMLSB		
Authors name, Published year	Country	Total isolates tested	Overall	MSSA	MRSA
Levin et al., 2005	United States	91	25 (27.5%)	17 (68%)	8 (12.3%)
Lim et al., 2006	Korea	778	94 (12.1%)	58 (9.8%)	36 (19.2%)
Mama et al., 2019	Ethiopia	77	19 (24.6%)	3 (21.4%)	16 (25.4%)
Zorgani et al., 2009	Libya	116	43 (37.0%)	0	43 (70.5%)
Pereira et al., 2016	Brazil	38	5 (13.2%)	4 (12.5%)	1 (14.3%)
Jarajreh et al., 2017	Jordan	35	33 (94.0%)	0	33 (94%)
Nashwa & Noha, 2017	Egypt	35	28 (80.0%)	13 (72.2%)	15 (88.2%)
Kishk et al., 2020	Egypt	108	24 (22.2%)	4 (7.1%)	20 (38.5%)
Cetin et al., 2010	Turkey	31	24 (77.4%)	9 (69.2%)	15 (83.3%)
Aksu et al., 2012	Turkey	45	40 (88.9%)	6 (75%)	34 (91.9%)
Rahbar & Hajia, 2007	Iran	26	17 (65.4%)	5 (83.3%)	12 (60%)
Seifi et al., 2012	Iran	150	24 (16.0%)	6 (5.5%)	18 (43.9%)
Mansouri & Sadeghi, 2014	Iran	112	14 (12.5%)	3 (4.6%)	11 (23.4%)
Saffar et al., 2016	Iran	33	13 (39.4%)	6 (no data)	7 (no data)
Fasih et al., 2010	Pakistan	138	99 (71.8%)	39 (73.6%)	60 (70.5%)
Angel et al., 2008	India	185	43 (23.2%)	6 (4.7%)	37 (63.8%)
Deotale et al., 2010	India	238	36 (15.1%)	2 (1.61%)	34 (29.8%)
Dubey et al., 2013	India	236	140 (59.3%)	23 (48.9%)	117 (61.9%)
Mokta et al., 2015	India	290	48 (16.5%)	25 (10.8%)	23 (39.6%)
Abbas et al., 2015	India	442	54 (12.2%)	8 (2.9%)	46 (27.2%)
Maijhi et al., 2016	India	87	46 (52.9%)	14 (53.8%)	32 (52.4%)
Kavitha et al., 2020	India	425	76 (17.9%)	27 (14.8%)	49 (20.2%)
Thapa & Sapkota, 2016	Nepal	109	89 (81.6%)	36 (76.6%)	53 (85.5%)
Baral, 2014	Nepal	284	33 (11.6%)	3 (1.71%)	30 (27.5%)
Shoji et al., 2015	Japan	1,941	533 (27.4%)	397 (24.3%)	136 (44.3%)
Present study	Japan	432	138 (31.9%)	77 (23%)	61 (58.6%)

$iMLS_B$, inducible macrolide, lincosamide, and streptogramin B.

nity or nosocomial), methicillin-susceptibility, study period, and prior antibiotic exposures [18,25,41-43]. Thus, it is important to understand the epidemiology of $iMLS_B$ resistance in *S. aureus* in each clinical setting and to generate local antibiogram. In addition to these factors, our results indicated that *S. aureus* strains isolated from aseptic samples, such as blood, pleural fluid, and intravascular catheters, showed a relatively higher $iMLS_B$ resistance rate. Although the generalizability of these findings should be clarified by a future study, the importance of monitoring for the presence or absence of $iMLS_B$ resistance, particularly in these clinical samples, should be stressed and is worthy of being shared among clinicians and microbiology laboratories.

An analysis of the prior literature revealed that the dominant genotypes in the *erm* gene family differed greatly among the reports. In the present study, we examined the positivity rates for *ermA* and *ermC*, which are considered the most prevalent genes in $iMLS_B$-resistant strains [44], and found that *ermA* was frequently observed in both MSSA (70.1%) and MRSA isolates (88.5%, including double-positive strains). Table 6 presents the positivity rates for *ermA* and *ermC* in $iMLS_B$-resistant MSSA and MRSA in previous studies. These studies show conflicting results, with some indicating higher rates of *ermA* positivity [22,28,33,35,42] and others reporting the dominance of *ermC* [10,21,44,45]. Our data suggested that the $iMLS_B$ resistance rate in MRSA (58.6%) was approximately two times higher than that in MSSA (23.5%), which could be explained by the higher positivity rate for *ermA* in MRSA.

We should also consider the sex- and age-related differences. First, the $iMLS_B$ resistance in CLDM-susceptible *S. aureus* was detected more frequently in men than women (37.2% vs. 24.7%). However, there is no clear explanation for this difference. Moreover, some previous studies reported no sex difference in the

Table 6 Positivity rates of *ermA* and *ermC* in the $iMLS_B$ resistance *Staphylococcus aureus* in previous studies

Reference	Place of study	Organism	*ermA*	*ermC*	*ermA* and *ermC*
Lina et al., 1999	France	MSSA	16%	83.8%	0
		MRSA	60%	40%	0
Otsuka et al., 2007	Japan	MSSA	56%	43%	0.7%
		MRSA	76%	12.8%	11%
Cetin et al., 2010	Turkey	MSSA	55%	22.2%	0
		MRSA	60%	26.7%	3%
Aksu et al., 2012	Turkey	MSSA	50%	33.3%	11.1%
		MRSA	88%	6%	0
Jarajreh et al., 2017	Jordan	MSSA	n.p.	n.p.	0
		MRSA	51.5%	84.8%	15.1%
Nashwa & Noha, 2017	Egypt	MSSA	69.2%	7.7%	7.7%
		MRSA	66.6%	20%	0
Khashei et al., 2018	Iran	MSSA	0%	100%	0
		MRSA	50%	50%	0
Timsina et al., 2020	Nepal	MSSA	4.2%	0	0
		MRSA	58.8%	70.5%	17.6
Present study	Okayama, Japan	MSSA	70.1%	14.3%	0
		MRSA	86.9%	11.5%	1.6%

$iMLS_B$, inducible macrolide, lincosamide, and streptogramin B; n.p., not performed

$iMLS_B$ resistance rate [34,46]. Thus, future studies will be needed to definitively clarify the influence of sex, if any, on $iMLS_B$ resistance in CLDM-susceptible *S. aureus*. Second, our data suggested that age may be associated with the $iMLS_B$-resistance rate. Patients aged under 10 or over 80 years showed higher positivity rates (40.0% and 51.4%, respectively) in comparison with other age groups. However, there were too few cases to discuss this potential association in detail, and in any case, there was no clear rationale for this finding. Thus, a further, preferably multi-centered, study is warranted to confirm the difference in the distribution of $iMLS_B$ resistance by age.

The main strength of the present study compared with the preceding report based on a multi-centered investigation [42] is that we stratified the data by various clinical variables, including sex, age, and sample source. However, our study also had several notable limitations. First, we targeted CLDM-susceptible *S. aureus*, rather than all *S. aureus* isolates. This was because our primary aim was to uncover the prevalence of $iMLS_B$ resistance in CLDM-susceptible *S. aureus* at our hospital, where the D-zone test was not routinely implemented. Thus, we could not determine the overall positivity rates of $iMLS_B$ resistance in *S. aureus* isolates. Second, we examined only *ermA* and *ermC* as associated genetic factors underlying the latent resistance mechanism. However, prior studies have revealed other possible genetic variants, such as *ermB* and *msrA* [18]. The *ermB* is another gene in the *erm* gene family with target site modification, and *msrA* provides an efflux-mediated antimicrobial resistance. Indeed, the 12 MSSA isolates that were found to be negative for both *ermA* and *ermC* may have harbored other such relevant genes. Third, we could not differentiate the source of the isolates (the community or hospital) owing to difficulty in accessing the data. Despite these limitations, our results are valuable in that the data were prospectively and consecutively collected and were genetically analyzed to uncover the hospital epidemiology of $iMLS_B$ resistance in CLDM-susceptible *S. aureus*.

In conclusion, we determined that nearly one-third of CLDM-susceptible *S. aureus* strains at our university hospital showed $iMLS_B$ resistance, and the majority of these $iMLS_B$-resistant strains were positive for *ermA*. The epidemiology of $iMLS_B$ resistance may vary by geographical location, hospital background, isolation settings, and patient characteristics. To better understand the national prevalence of $iMLS_B$-resistant *S. aureus*, a multi-centered study that includes detailed clinical information and genetic examinations for a wide range of associated genes will be needed.

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