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Investigation of the cefazolin inoculum effect in blood culture-isolated methicillin-susceptible *Staphylococcus aureus* strains: A Japanese multicenter study

Shinnosuke Fukushima [a,b,c], Shuma Tsuji [d], Kazuyoshi Gotoh [d], Koji Iio [e], Sakura Ogawa [a], Norihito Koyanagi [f], Yuji Ito [g], Hiroshi Koganemaru [h], Atsushi Yoshida [h], Hideharu Hagiya [c,*]

[a] Department of General Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[b] Department of Bacteriology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[c] Department of Infectious Diseases, Okayama University Hospital, Okayama, Japan
[d] Department of Medical Laboratory Science, Okayama University Graduate School of Health Sciences, Okayama, Japan
[e] Microbiology Division, Clinical Laboratory, Okayama University Hospital, Okayama, Japan
[f] Department of Clinical Laboratory, Chutoen General Medical Center, Kakegawa, Shizuoka, Japan
[g] Department of General Internal Medicine, Chutoen General Medical Center, Kakegawa, Shizuoka, Japan
[h] Department of Infectious Diseases, Tokyo Metropolitan Institute for Geriatrics and Gerontology, Itabashi, Tokyo, Japan

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ABSTRACT

Background: Cefazolin inoculum effect (CInE) is a microbiological phenomenon where the MIC of cefazolin against methicillin-susceptible *Staphylococcus aureus* (MSSA) strains increases with higher bacterial volumes.
Method: We retrospectively investigated the prevalence and characteristics of the CInE among MSSA strains isolated from blood cultures at three Japanese hospitals. The collected isolates were screened for *blaZ* using PCR, and the cefazolin minimum inhibitory concentration (MIC) for the *blaZ*-positive MSSA isolates was measured at standard and high inoculum volumes. CInE-positive MSSA strains were defined as those with a cefazolin MIC ≥16 μg/mL at 10^7 CFU/mL and ≤8 μg/mL at 10^5 CFU/mL. In these *blaZ*-positive strains, we performed *blaZ* typing and tested a modified nitrocefin-based rapid examination to detect the CInE.
Results: We collected 329 MSSA strains isolated from blood cultures. Of these, 96 (29.2%) were positive for the *blaZ* gene, with the following genotypes: type A (15, 15.6%), type B (3, 3.1%), type C (77, 80.2%), type D (0, 0.0%), and non-type (1, 1.0%). Among 96 *blaZ*-positive MSSA isolates, 11 exhibited the CInE, all of which harbored *blaZ* type A. The rapid nitrocefin test detected CInE positivity with high sensitivity (100%), specificity (94.1%), and diagnostic accuracy (94.8%).
Conclusion: This study highlighted the low prevalence of CInE-presenting MSSA isolates in Japan. When the cefazolin MIC is ≥1 μg/mL or the penicillin G MIC is ≥0.25 μg/mL, the rapid nitrocefin test may be useful for considering the CInE in patients with high bacterial volume MSSA infections.

1. Introduction

Staphylococcus aureus is one of the most prevalent and clinically significant pathogens, potentially causing fatal diseases in both community and healthcare settings [1,2]. While the clinical burden of methicillin-resistant *S. aureus* infections has been declining in recent years, the impact of methicillin-susceptible *S. aureus* (MSSA) infections has inversely become a growing concern [3,4]. Cefazolin has long served as the first-line antimicrobial agent for various types of MSSA infections worldwide [5,6]; however, the potential involvement of the cefazolin inoculum effect (CInE) needs to be addressed when administering the drug to patients, especially in severe clinical conditions.

The CInE is an *in vitro* phenomenon in which the minimum inhibitory concentration (MIC) of cefazolin against an MSSA strain increases markedly in an environment with a high bacterial volume [7–9]. Thus, clinically, the validity of cefazolin treatment for MSSA infections should be considered in patients with presumably high bacterial inocula, such as those with endocarditis, undrained abscesses, and device-associated

* Corresponding author.
E-mail address: hagiya@okayama-u.ac.jp (H. Hagiya).

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infections [7,10,11].

Notably, previous overseas studies have shown that CInE-positive MSSA strains are associated with increased mortality when compared with CInE-negative strains [12,13]. The CInE is caused by β-lactamase overproduction in MSSA, with which the *blaZ* gene is reportedly associated [14,15]. The *blaZ* gene is classified into four main variants—type A, type B, type C, and type D. Of these variants, type A and type C have been reported more frequently in CInE-positive MSSA strains [15]. The CInE positivity rates in MSSA clinical isolates vary worldwide, with 54.5% in Argentina, 40.0% in Latin America, 18.6% in North America, 11.8% in Korea, and 5.8% in Japan [13,16–19]. In North America, the positivity rate varies by region, both between and within countries, ranging from 0% to 27.9% [17].

Understanding the local prevalence of CInE-positive MSSA strains is crucial for optimizing treatment options for patients with MSSA infections. However, little is known about the detailed molecular epidemiology of CInE-positive MSSA in Asia, including in Japan. In 2021, a nitrocefin-based diagnostic method for CInE detection was developed, with a sensitivity and specificity of 82.5% and 88.9%, respectively [20]. Later, in 2024, a modified technique was introduced, demonstrating improved sensitivity and specificity [21]. However, the clinical utility of this rapid diagnostic method has not been well validated since then.

In this study, we aimed to evaluate the CInE positivity rate, characterize the *blaZ* types, and investigate the rapid diagnostic approach in MSSA clinical isolates in Japan.

2. Materials and methods

2.1. Study design and settings

We collected MSSA clinical isolates from patients with bacteremia from the following three hospitals in Japan: Okayama University Hospital (865 beds; 2021–2023), located in Okayama Prefecture; Tokyo Metropolitan Institute for Geriatrics and Gerontology (550 beds; 2021–2023), located in Tokyo Metropolitan City; and Chutoen General Medical Center (500 beds; 2013–2023), located in Shizuoka Prefecture. The collected isolates did not include isolates repeatedly identified from a single patient. These hospitals were equipped with in-house microbiology laboratories containing automated blood culture systems, including the BD BACTEC™ FX system (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) at Okayama University Hospital and Tokyo Metropolitan Institute for Geriatrics and Gerontology and the VersaTREK™ (Thermo Fisher Scientific, Waltham,MA, USA) at Chutoen General Medical Center.

Ethical approval was obtained from the Institutional Review Board of Okayama University Hospital (No. 2208-021). Since this was a retrospective analysis of routinely collected fully anonymized data, the requirement for informed consent was waived.

2.2. Isolates identification and antimicrobial susceptibility testing

All isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS Biotyper; Bruker Daltonics Inc., Billerica, MA, USA) or the VITEK® mass spectrometry system (bioMérieux, Marcy-l'Étoile, France) according to the manufacturers' instructions. MICs were measured using the Dry Plate "Eiken" (Eiken Chemical Co., Ltd., Tokyo, Japan) or VITEK 2™ system (bioMérieux, Marcy-l'Étoile, France) and interpreted according to the Clinical and Laboratory Standards Institute guidelines (M100, 32nd edition) [22]. The susceptibility and MIC for penicillin G (PCG) were evaluated retrospectively.

2.3. Investigation of the blaZ gene

Since an association has been observed between CInE positivity and *blaZ* gene possession, which encodes class A β-lactamase (penicillinase) [23], we screened all the collected MSSA isolates for *blaZ* using PCR based on previously published primers and conditions [8,20]. The primer sequences employed were *blaZ* 1F 5′-TACAACTGTAAT ATCGGAGGG-3′ and *blaZ* 1R 5′-CATTACACTCTTGGCGGTTTC-3′, which were used to amplify an approximately 850-bp region of the structural gene [24]. PCR products were purified and subjected to Sanger sequencing to determine the four different variants (types A, B, C, and D) of the *blaZ* gene, which were classified according to Ambler positions 128 and 216 as follows: type A, threonine at position 128 and serine at position 216; type B, lysine at position 128 and asparagine at position 216; type C, threonine at position 128 and asparagine at position 216; and type D, alanine at position 128 and serine at position 216 (**Supplementary Table 1**) [15,19,25]. Quality control testing was performed for each assay using two control strains: ATCC 29213 (*blaZ*-positve, CInE-negative) and ATCC 25923 (*blaZ*-negative, CInE-negative) [20,24]. No CInE-positive control strain was available in our country; therefore, this was not applied in the present study. DNA sequencing was performed at the Central Research Laboratory of Okayama University Medical School using an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.4. Definitions

For the *blaZ*-positive MSSA isolates, we measured the cefazolin MIC at two different inocula: a standard inoculum at 10^5 CFU/mL and a high inoculum at 10^7 CFU/mL. In this study, the CInE was defined as a situation where the cefazolin MIC was ≥16 μg/mL at the high inoculum and ≤8 μg/mL at the standard inoculum [13]. The MIC measurements for CInE confirmation were performed twice separately. The overall study flowchart for CInE-positive MSSA strain identification is depicted in Fig. 1.

2.5. Rapid nitrocefin test protocol to identify CInE-positive MSSA strains

We performed modified rapid nitrocefin tests using 10 μg ampicillin disks (BBL; Becton, Dickinson and Company, Cockeysville, MD, USA) [21]. First, 1 mL of brain heart infusion broth and an ampicillin disk were combined and incubated at room temperature. Bacterial colonies from brain heart infusion agar were selected using a 1-μL sterile loop and resuspended in ampicillin-supplemented broth. After vortex mixing and incubation, the suspension was centrifuged and 25 μL of the supernatant was tested with nitrocefin solution. Chromogenic changes were assessed at 30 minutes, 1 h, and 2 hours post-incubation. The rapid nitrocefin test for *blaZ*-positive strains was performed in a duplicate manner. We defined the level of reaction in the nitrocefin test based on the degree of color change: a weakly positive reaction was defined as a pink color change, whereas a strongly positive reaction was defined as a red color change.

3. Results

3.1. Characteristics of isolates

During the study period, a total of 329 blood-origin MSSA strains were collected and analyzed, with 71 isolates from Okayama (2021 to 2023), 81 isolates from Tokyo (2021 to 2023), and 177 isolates from Shizuoka (2013 to 2023).

Table 1 summarizes the phenotypic and genotypic characteristics of MSSA isolates in each region. The PCG MIC for MSSA was ≤0.12 μg/mL in 163 isolates (49.5%) and ≥0.25 μg/mL in 166 isolates (50.5%). Among 329 MSSA isolates, 96 (29.2%) were identified to harbor *blaZ* genes, with *blaZ* positivity rates of 22.5%, 34.6%, and 29.4% in Okayama, Tokyo, and Shizuoka, respectively. The *blaZ* positivity rate among MSSA isolates exhibiting a PCG MIC ≥0.25 μg/mL was 55.4% (92/166 isolates), while that in those with PCG MIC ≤0.12 μg/mL was considerably lower at 2.5% (4/163 isolates). Notably, 95.8% (92

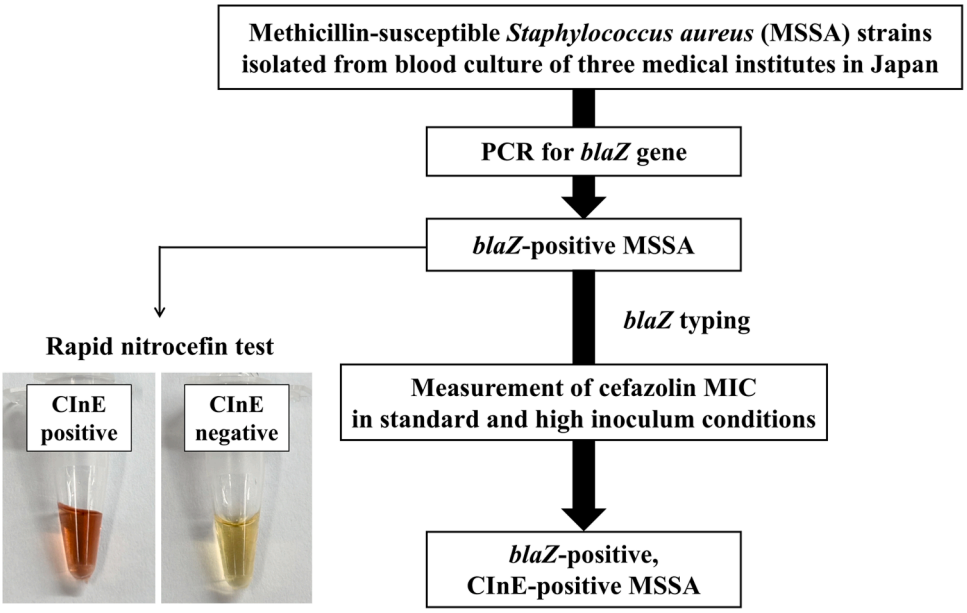

Fig. 1. Study Flowchart. Of the 329 MSSA clinical strains, 96 (29.2%) were positive for the *blaZ* gene. Among *blaZ*-positive MSSA strains, 11 (11.5%) demonstrated CInE positivity. The overall CInE positivity among the clinical strains was 3.3%. Abbreviations: CInE, cefazolin inoculum effect; MIC, minimum inhibitory concentration.

Table 1
Phenotypic and genotypic characteristics of the MSSA isolates and the positivity of cefazolin inoculum effect (CInE).

	Total	Okayama	Tokyo	Shizuoka
Period (year)	2013-2023	2021–2023	2021–2023	2013–2023
Number of MSSA strains	329	71	81	177
MIC of PCG				
≤0.12 μg/mL	163 (49.5)	41 (57.7)	33 (40.7)	89 (50.3)
≥0.25 μg/mL	166 (50.5)	30 (42.3)	48 (59.3)	88 (49.7)
Positivity for *blaZ* gene				
Overall (N = 329)	96 (29.2)	16 (22.5)	28 (34.6)	52 (29.4)
PCG MIC ≤0.12 μg/mL (N = 163)	4 (2.5)			
PCG MIC ≥0.25 μg/mL (N = 166)	92 (55.4)			
***blaZ* gene variant**				
Type A	15 (15.6)	2 (12.5)	0 (0.0)	13 (25.0)
Type B	3 (3.1)	2 (12.5)	0 (0.0)	1 (1.9)
Type C	77 (80.2)	12 (75.0)	28 (100)	37 (71.2)
Type D	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Non-type	1 (1.0)	0 (0.0)	0 (0.0)	1 (1.9)
Positivity of CInE	11 (3.3)	2 (2.8)	0 (0.0)	9 (5.1)

MSSA, methicillin-susceptible *Staphylococcus aureus*; MIC, minimum inhibitory concentration; PCG, penicillin G. Percentages are denoted in parentheses.

isolates) of *blaZ*-positive isolates demonstrated a PCG MIC ≥0.25 μg/mL. Based on the specific amino acid positions (128 and 216 residues) in the *bla*Z protein sequence, type A, type B, and type C were identified in 15 (15.6%), 3 (3.1%), and 77 (80.2%) isolates, respectively, while 1 isolate (1.0%) was identified as non-typable and none were identified as type D. In the non-typable strain, serine and lysine were found at positions 128 and 216, respectively.

Overall, the number of CInE-positive MSSA isolates was 11 (3.3%). The positivity rates were 2.8% (2/71 isolates) in Okayama and 5.1% (9/177 isolates) in Shizuoka, while no CInE-positive MSSA isolates were found in Tokyo. All 11 CInE-positive MSSA isolates were identified to harbor *blaZ* type A. All 11 isolates were sporadically identified without temporal or spatial clustering, with no accumulation in the same ward within three months and only 0–2 cases per year detected from 2013 to 2023.

The MICs of cefazolin in *blaZ*-positive MSSA isolates (96 isolates) ranged from ≤0.5 to 2 μg/mL in the standard inoculum and ≤0.5 to 32 μg/mL in the high inoculum (Fig. 2). The cefazolin MICs of the CInE-positive strains in the standard inoculum were all ≥1 μg/mL (nine isolates at 1 μg/mL and two isolates at 2 μg/mL), while those in the high inoculum were ≥16 μg/mL (ten isolates at 16 μg/mL and one isolate at 32 μg/mL). In the standard inoculum, the proportion of isolates with cefazolin MICs ≥1 μg/mL was higher in CInE-positive strains than in CInE-negative strains (100% vs. 44.7%) (**Supplementary Table 2**). Importantly, CInE-positive strains were exclusively observed among isolates with cefazolin MIC ≥1 μg/mL under standard inoculum conditions, demonstrating that higher cefazolin MICs are strongly associated with the CInE phenotype.

3.2. Rapid nitrocefin test

We performed the rapid nitrocefin test on 96 *blaZ*-positive strains (Table 2). The rapid test was positive in 16 strains (16.7%) and negative in 80 strains (83.3%). Among the 16 rapid test-positive strains, 10 were strongly positive, all of which were determined to be CInE-positive. In addition, six strains were weakly positive, including one CInE-positive strain and five CInE-negative *blaZ* strains (**Supplementary Figure**). The rapid test showed a sensitivity of 100%, specificity of 94.1%, accuracy of 94.8%, positive predictive value (PPV) of 68.8%, and negative predictive value (NPV) of 100% for detecting CInE-positive MSSA strains. When focusing on strongly positive rapid tests, the sensitivity, specificity, and accuracy for identifying the CInE were 90.9%, 100%, and 99.0%, respectively.

4. Discussion

We conducted a multicenter retrospective study in Japan to evaluate the CInE positivity rates and *blaZ* genotypes in MSSA strains isolated from blood cultures. Of 329 MSSA strains evaluated, 96 (29.2%) were confirmed to harbor *blaZ* genes, with 95.8% (92 strains) demonstrating PCG MICs of ≥0.25 μg/mL. Their genotypic distribution was as follows: type A, 15.6%; type B, 3.1%; type C, 80.2%; type D, 0.0%; and non-type,

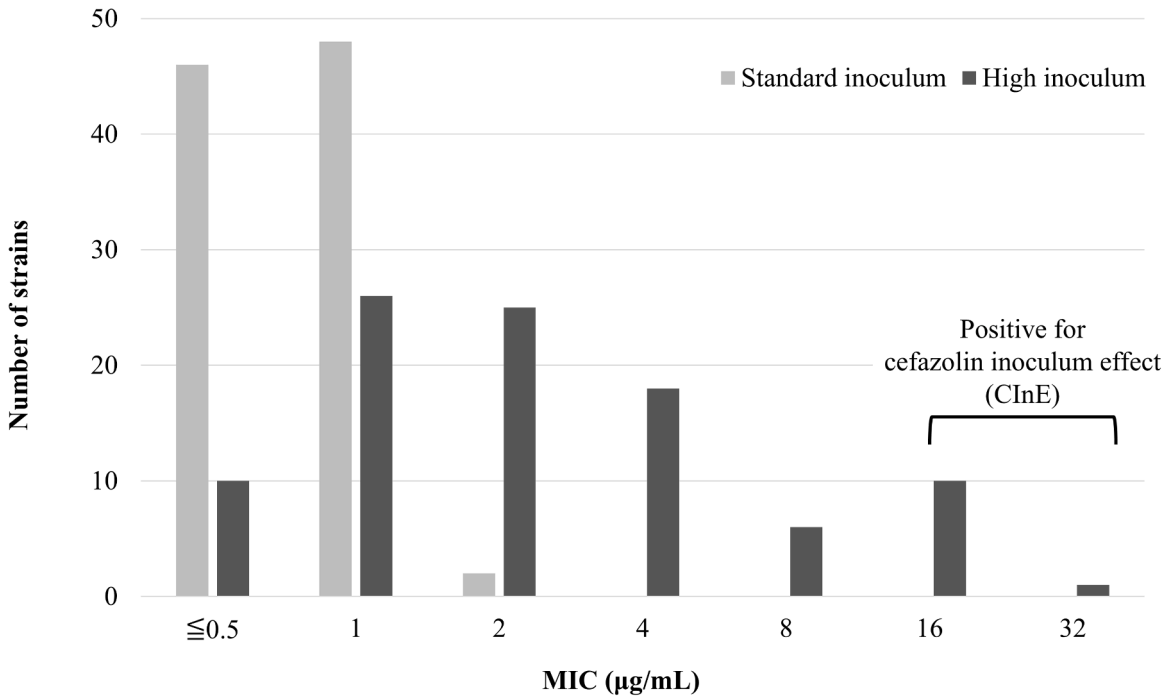

Fig. 2. Distribution of the Cefazolin MICs by Inoculum Volume in *blaZ*-positive MSSA strains. In the high inoculum, 11 strains were positive for the cefazolin inoculum effect at ≥16 μg/mL (ten strains at 16 μg/mL and one strain at 32 μg/mL). Abbreviations: MSSA, methicillin-susceptible *Staphylococcus aureus*; MIC, minimum inhibitory concentration.

Table 2
The results of the rapid nitrocefin test for 96 *blaZ*-positive MSSA strains.

			Rapid nitrocefin test	
			Positive	Negative
CInE	***blaZ* type**	**Total (*N* = 96)**	16 (16.7)	80 (83.3)
Positive	A	11 (11.5)	11	0
	B	0	-	-
	C	0	-	-
	D	0	-	-
Negative	A	4 (4.2)	4	0
	B	3 (3.1)	0	3
	C	77 (80.2)	0	77
	D	0	-	-
	Non-type	1 (1.0)	1	0

MSSA, methicillin-susceptible *Staphylococcus aureus;* CInE, cefazolin inoculum effect.
The rapid nitrocefin test showed a sensitivity of 100%, specificity of 94.1%, positive predictive value of 68.8%, negative predictive value of 100%, and accuracy of 94.8% for detecting CInE-positive MSSA strains. Percentages are denoted in parentheses.

1.0%. The overall CInE-positivity rate was 3.3% (11 isolates), all of which were positive for the *blaZ* type A gene. Rapid nitrocefin testing showed high accuracy in detecting CInE-positive MSSA strains and could be adopted in clinical laboratories owing to its simple methodology.

The *blaZ* gene encodes β-lactamases, which potentially inactivate penicillins [23]. The *blaZ* prevalence in Japan has been reported to range from 1.0% to 73.1%, varying considerably depending on the region and time of investigation [8,19,26]. Other previous studies have shown that the *blaZ* positivity rates in MSSA isolates were much higher in France (91.7%), Latin America (80.7%), and South Korea (80.0%) when compared with Japan, including the rates found in the present study (29.2%) [15,18,27]. In contrast, the proportions of penicillin-susceptible *S. aureus* (PSSA) among MSSA are reportedly equivalent across the countries, with 26.4% in Singapore, 32.4% in the United States of America, and 49.3% in Japan (49.5% in the present case) [28–30]. Domestic data suggest that 108 MSSA strains with a PCG MIC ≤0.06 μg/mL did not harbor *blaZ* genes [31], while our study identified that 2.5% (4/163) of MSSA strains with a PCG MIC ≤0.12 μg/mL were positive for *blaZ*. Of the four *blaZ*-positive PSSA strains identified in this study, one was type B and three were type C, and the cefazolin MIC was ≤0.5 μg/mL in both the standard and high inocula. The carriage of *blaZ* is rare in PSSA isolates, and no strains appearing with the CInE were identified in PSSA isolates in the present study. All CInE-positive MSSA strains identified in this study had PCG MIC ≥0.25 μg/mL, suggesting the possibility of CInE positivity in penicillin-resistant *S. aureus*.

The CInE represents a significant clinical consideration that must be addressed when treating patients with severe infectious diseases, particularly those involving a high bacterial burden. The cefazolin MIC distributions of CInE-positive isolates range from 0.25 to 2 μg/mL under the standard inoculum conditions, with a significantly higher prevalence of MICs at 1 μg/mL when compared with CInE-negative isolates (29.8% vs 3.2%) ($p < 0.001$) in North America [17]. Under the high inoculum conditions, 70% of CInE-positive strains show MIC values ≥32 μg/mL, with a MIC_{50} of 64 μg/mL [16,17]. In the present study, all CInE-positive MSSA strains exhibited a cefazolin MIC of 1 or 2 μg/mL under the standard inoculum conditions, whereas the MIC values increased up to 16 or 32 μg/mL under the high inoculum conditions. Although the MIC values under the high inoculum conditions were lower in this study than those previously reported, a cefazolin MIC ≥1 μg/mL under the standard inoculum conditions was strongly predictive of the presence of the CInE (CInE positivity rate of 22.0% for a cefazolin MIC ≥1 μg/mL vs 0% for a cefazolin MIC <1 μg/mL). Collectively, these findings indicate that the CInE may be present when the cefazolin MIC under the standard inoculum conditions is ≥1 μg/mL. However, a previous North American study reported that 39 of 57 MSSA isolates with cefazolin MIC ≤0.5 μg/mL under standard inoculum conditions still exhibited CInE [17], indicating that screening based solely on cefazolin MIC may fail to identify a substantial proportion of CInE-positive isolates. Furthermore, in routine clinical laboratories in Japan, commonly used commercial dry plate susceptibility testing systems have a lower limit of cefazolin MIC at

0.5 μg/mL, making it difficult to apply a cutoff value of ≤0.5 μg/mL for screening purposes. Therefore, universal screening for CInE among all MSSA isolates may not be necessary in routine, particularly in regions with a low prevalence of this phenotype. Instead, a targeted screening strategy focusing on isolates with cefazolin MIC ≥1 μg/mL or PCG MIC ≥0.25 μg/mL, as well as on patients with high-risk clinical conditions characterized by a high bacterial burden, may be more practical and clinically meaningful.

Globally, the most prevalent *blaZ* gene types among CInE-positive MSSA strains are type A and type C [12,17,18]. However, detailed distribution patterns exhibit considerable regional variation: in North America, type A (33.3–38.2%), type C (36.8–59.2%), type B (1.2–22.8%), and type D (1.0–1.2%) show predominance, with 0.2% being non-typeable [15,17]; in France, type A (56.8%) and type B (29.5%) are most common; and in Korea, type B (36.5%) and type C (42.9%) represent the dominant variants [18,27]. When stratified by the *blaZ* gene type, the CInE positivity rate is higher for type A (73.9–77.9%) than that for type C (52.6–67.4%), with type A demonstrating the highest CInE prevalence [17,32]. A previous Japanese study examining 52 MSSA isolates identified the *blaZ* gene in 38 strains, with the following genotype distribution: type A, 31.6%; type B, 34.2%; type C, 31.6%; and type D, 2.6%. Among these, type A was identified in three CInE-positive strains (5.8%) [19]. In the present study, type C was the most common genotype ($n = 77$, 80.2%), followed by type A ($n = 15$, 15.6%). Moreover, of the 15 type A strains, 11 (73.3%) exhibited a CInE-positive phenotype. These findings suggest that type A is the predominant genotype among CInE-positive isolates in Japan. Inter-facility differences were observed in both *blaZ* genotype distribution and the prevalence of CInE-positive isolates, suggesting that local epidemiological characteristics may influence the occurrence of CInE. Similar regional variation has been reported in international studies [17], highlighting the importance of considering local epidemiology when interpreting CInE prevalence. Unlike the previous single-center Japanese study [19], which primarily described *blaZ* genotypes and antimicrobial susceptibility patterns, the present study is the first multicenter investigation in Japan to evaluate both the prevalence of CInE-positive MSSA isolates and a practical screening approach using a modified nitrocefin-based assay. By including isolates from multiple institutions, our findings provide more generalizable epidemiological data and extend prior observations by addressing the feasibility of CInE detection in routine clinical microbiology laboratories. However, further comprehensive epidemiological studies are required to investigate regional variations in *blaZ* genotypes within the country.

Detection of the CInE phenotype remains challenging in routine clinical microbiology laboratories. To address this, a rapid-detection method for CInE has been developed, with the modified nitrocefin-based rapid test demonstrating a sensitivity of 96–100% and specificity of 92–94%, with the overall diagnostic accuracy ranging from 94% to 98% [21,32]. However, these findings were derived from studies conducted in regions with a CInE positivity rate exceeding 50%, and data from low-prevalence settings remain limited. In our study, the prevalence of CInE-positive MSSA strains was determined to be comparatively low, at 3.3%, with all positive isolates identified as *blaZ* type A. Under these conditions, the rapid nitrocefin assay for CInE-positive strains showed high sensitivity (100%), specificity (94.1%), and diagnostic accuracy (94.8%), which is consistent with the findings of previous studies. Although the modified assay demonstrated 100% sensitivity, specificity, and overall diagnostic accuracy for detecting CInE-positive strains in MSSA strains with *blaZ* type A [21,32], the PPV for the CInE in this study was 68.8%, with some false positives observed for type A strains. Previous experimental studies have demonstrated that the hydrolytic activity of β-lactamase varies by *blaZ* genotype. Notably, type A β-lactamase potentially hydrolyzes cefazolin and penicillin more rapidly than other *blaZ* types, providing a mechanistic explanation for the strong association between *blaZ* type A and the CInE observed in our study [25]. The clinical significance of weakly positive results in the modified nitrocefin-based assay remains unclear. In our study, several CInE-negative isolates showed weak positivity, possibly reflecting early or partial hydrolytic activity of *blaZ* type A β-lactamase rather than true CInE, indicating that weakly positive reactions should be interpreted with caution. Although the CInE positivity rate among type A strains was high (73.3%), the suboptimal PPV observed in this low-prevalence setting highlights the limitations of this screening approach. Furthermore, currently available screening methods, including nitrocefin-based assays, have practical limitations related to reagent handling complexity and turnaround times exceeding two hours. These findings underscore the need for simpler and more rapid screening tools for CInE that can be implemented in routine clinical microbiology laboratories.

The CInE has been considered clinically relevant in high-inoculum infections, such as infective endocarditis, deep-seated abscesses, and device-associated infections, in which reduced efficacy of cefazolin has been reported [7,10,11]. Previous clinical studies have suggested poorer outcomes among patients with infective endocarditis caused by CInE-positive MSSA isolates [33]. Experimental and clinical data indicate that antistaphylococcal penicillins (e.g., nafcillin) are less affected by the inoculum effect than cefazolin, and broad-spectrum β-lactams such as ceftriaxone and cefepime show a much lower frequency of this phenomenon, with no inoculum effect observed for meropenem [9,34,35]. Although vancomycin may be considered when CInE is suspected, multiple studies have demonstrated inferior outcomes compared with β-lactams for definitive therapy of MSSA bacteremia [36]. Daptomycin has shown comparable efficacy to β-lactams but has been associated with prolonged hospitalization in a cohort study [37]. The role of ceftriaxone remains controversial, as retrospective studies have reported both noninferior and inferior outcomes compared with cefazolin or antistaphylococcal penicillins [38,39]. In Japan, where antistaphylococcal penicillins are not available, the optimal antimicrobial regimen for CInE-positive MSSA infections has not been established. Although CInE-positive strains are rare, our findings suggest that a subset of patients with severe MSSA bacteremia before adequate source control or surgical intervention may be at risk of receiving suboptimal therapy with cefazolin. When CInE is suspected or confirmed, alternative agents such as ceftriaxone or daptomycin may be considered, particularly during the early phase of treatment until adequate source control and reduction of bacterial burden are achieved. Further prospective studies are warranted to determine the optimal therapeutic strategy for CInE-positive MSSA infections.

Our study yielded important findings that verified the CInE positivity rate and validated rapid testing performance for blood culture-isolated MSSA strains in Japan, where the CInE prevalence is lower than that in other regions. However, this study had several limitations. First, there was a potential for selection bias toward facilities with CInE-positive strains. Although no temporal clustering was observed, clonal analysis of positive isolates was not performed. Furthermore, differentiation between hospital-acquired and community-acquired origin was not evaluated in this study. Second, clinical evaluation was not performed in patients from whom MSSA was isolated. Therefore, the actual clinical impact of CInE-positive MSSA strains on patient prognoses remains unknown; future studies are warranted to accumulate clinical data on CInE-positive isolates. Third, the nitrocefin reaction was assessed qualitatively based on visible color change. Although this approach is consistent with previous studies [20,21], standardized quantitative criteria to distinguish weak and strong reactions have not been established. Fourth, this study evaluated only *blaZ*-positive MSSA isolates, as CInE has been mainly linked to *blaZ*-mediated β-lactamase production. Nevertheless, the presence of CInE-like behavior among *blaZ*-negative MSSA isolates cannot be completely excluded and warrants further investigation. Fifth, the rapid assay has not been fully validated due to the limited distribution of *blaZ* genotypes and the low number of CInE-positive strains. In the present study, the majority of isolates tested carried type C, whereas all CInE-positive strains carried *blaZ* type A.

Therefore, further investigations examining additional MSSA isolates are required to verify the epidemiology of the *blaZ* genotypes and the clinical utility of nitrocefin-based rapid assays.

In conclusion, the prevalence of the CInE among blood culture-derived MSSA isolates appears to be lower in Japan than in other countries. Our data suggest that MSSA strains with a cefazolin MIC ≥1 μg/mL or PCG MIC ≥0.25 μg/mL may possess the potential to exhibit the CInE phenotype, particularly in patients with high-risk clinical conditions associated with a high bacterial burden, for whom rapid testing may be clinically beneficial. Furthermore, the distributions of *blaZ* genotypes and CInE positivity rates identified in this study may provide invaluable regional epidemiological insights that could impact on the clinical management of MSSA infections.

Data availability

The datasets used during the current study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Shinnosuke Fukushima: Writing – original draft, Investigation, Conceptualization. **Shuma Tsuji:** Investigation. **Kazuyoshi Gotoh:** Investigation. **Koji Iio:** Resources, Investigation. **Sakura Ogawa:** Investigation. **Norihito Koyanagi:** Resources. **Yuji Ito:** Resources. **Hiroshi Koganemaru:** Resources. **Atsushi Yoshida:** Resources. **Hideharu Hagiya:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2026.117345.

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