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Short communication

Genotype-specific prevalence of heterogeneous vancomycin-intermediate *Staphylococcus aureus* in Asian countries☆

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ABSTRACT

Although heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) has been increasingly reported, the true prevalence of hVISA is unclear, especially in Asia. In this study, the genotype-specific prevalence of hVISA among meticillin-resistant *S. aureus* (MRSA) clinical isolates collected from Asian countries was determined. Among MRSA collections from South Korea, Taiwan, Hong Kong, Thailand, the Philippines, Vietnam, India and Sri Lanka in the ANSORP surveillance study during 2004 and 2006, isolates with a vancomycin minimum inhibitory concentration of ≥0.25 mg/L were randomly selected. After screening by macro Etest, hVISA was confirmed using the modified population analysis profile method. MRSA isolates were typed by *spa* tying and multilocus sequence typing (MLST). Among 462 MRSA isolates, 16 (3.5%) were confirmed as hVISA. The proportion of hVISA was highest in South Korea and Vietnam (both 7.0%), followed by Thailand (3.2%) and Taiwan (1.9%). *spa* type t601 belonging to clonal complex (CC) 5 showed the highest proportion of hVISA (33.3%), and hVISA accounted for 6.9% among isolates of t002 belonging to CC5. Among CC239 isolates, only those of t037 were hVISA (1.6%). Among isolates of community-associated MRSA genotypes, hVISA was found only in those of t437 (4.8%) belonging to CC59, and no hVISA was found among those of CC30 or CC72. The prevalence of hVISA in the Asian region differed by country and was dependent upon the genotype of MRSA strains. It suggests that differences in hVISA prevalence between countries can be affected by the genotype distribution of MRSA strains.

1. Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) is highly prevalent in hospitals worldwide [1] and the emergence of community-associated (CA) MRSA has added another serious concern. Furthermore, vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) have increased, posing challenges in treatment. Although previous reports have shown variable hVISA prevalence rates by country [2], the true prevalence of hVISA is still unclear and the hVISA prevalence has not been determined for each MRSA clone. In this study, the genotype-specific hVISA prevalence among MRSA clinical isolates collected from Asian countries was determined.

2. Materials and methods

MRSA isolates from the Asian Bacterial Bank (Seoul, South Korea), which were part of collections of the Asian Network for Surveillance of Resistant Pathogens (ANSORP) surveillance study [1] from eight Asian countries, were included in this study. Antimicrobial susceptibility was determined by the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [3]. A total of 462 MRSA isolates were randomly selected among the vancomycin-susceptible isolates with a vancomycin minimum inhibitory concentration (MIC) ≥0.25 mg/L and were screened for hVISA by the macro Etest (AB BIODISK, Solna, Sweden). A strain was considered as possible hVISA if microcolonies were detected at ≥8 mg/L for both vancomycin and

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Table 1
Characteristics of heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) and all meticillin-resistant *S. aureus* (MRSA) clinical isolates.

Country	No. of MRSA tested No. (%) of hVISA	Specimens (*n*)	Vancomycin MIC in mg/L (*n*)	*spa* type (*n*)[a]	MLST (*n*)[b]
South Korea	114	Blood (29), sputum (24), pus (18), urine (9), ascites (8), wound swab (6), joint fluid (4), CSF (3), ear discharge (3), pleural fluid (2), catheter tip (2), others (5)	2 (2) 1 (47) 0.5 (64) 0.25 (1)	t002 (34), t037 (21), t601 (15), t324 (15), t2460 (7), t148 (5)	
	8 (7.0)	Blood (1), sputum (2), pus (1), urine (1), ascites (1), pleural fluid (1), catheter tip (1)	1 (4) 0.5 (4)	t002 (3), t601 (5)	ST5 (8)
Taiwan	104	Pus (50), blood (18), sputum (18), urine (5), ear discharge (4), others (9)	2 (2) 1 (49) 0.5 (53)	t437 (47), t654 (13), t037 (12), t002 (9), t44 (6), t019 (3), t1751 (3)	
	2 (1.9)	Pus (1), blood (1)	1 (2)	t437 (1), t002 (1)	ST59 (1), ST5 (1)
Vietnam	71	Pus (47), blood (10), sputum (7), urine (7)	2 (6) 1 (45) 0.5 (20)	t037 (39), t437 (5), t189 (1), t324 (1)	
	5 (7.0)	Pus (3), blood (1), sputum (1)	2 (1) 1 (4)	t437 (2), t324 (1), t037 (1), t189 (1)	ST188 (3), ST239 (1), ST63 (1)
Sri Lanka	57	Pus (49), blood (3), sputum (1), others (4)	2 (1) 1 (16) 0.5 (40)	t425 (33), t002 (8), t127 (4), t021 (2), t437 (1), t084 (1), t1081 (1)	
	0 (0)				
The Philippines	46	Pus (22), blood (8), sputum (2), others (14)	1 (5) 0.5 (41)	t019 (30), t002 (4), t2670 (3), t037 (1), t267 (1), t1081 (1)	
	0 (0)				
Thailand	31	Pus (10), blood (8), sputum (6), urine (4), CSF (2), others (1)	1 (3) 0.5 (25) 0.25 (3)	t037 (24), t654 (5)	
	1 (3.2)	Urine (1)	1 (1)	t037 (1)	ST239 (1)
Hong Kong	26	Blood (15), pus (9), sputum (1), joint fluid (1)	1 (7) 0.5 (18) 0.25 (1)	t037 (13), t437 (3), t002 (2), t019 (1), t494 (1), t1081 (1)	
	0 (0)				
India	13	Pus (10), pleural fluid (1), others (2)	1 (8) 0.5 (5)	t037 (6), t657 (2), t064 (2)	
	0 (0)				

[a] *spa* typing was performed for only some of the isolates.
[b] MLST was performed for hVISA isolates only.
MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; CSF, cerebrospinal fluid.

teicoplanin or at ≥12 mg/L for teicoplanin alone. For confirmation of hVISA, the modified population analysis profile (PAP) method was used by calculating the area under the curve (AUC) of the PAP graph and comparing with reference strain Mu3 (ATCC 700698) [4]. hVISA was defined as a PAP-AUC ratio of ≥0.9. *spa* typing was performed on some of the isolates as previously described [5] and the *spa* type was determined using the Ridom SpaServer (http://spaserver2.ridom.de/spatypes.shtml). Multilocus sequence typing (MLST) was carried out by PCR amplification and sequencing of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) [6]. This study was approved by the Institutional Review Board of Samsung Medical Center (Seoul, South Korea).

3. Results

Among the 462 MRSA isolates, 16 (3.5%) were confirmed as hVISA. The proportion of hVISA was highest in South Korea and Vietnam (both 7.0%), followed by Thailand (3.2%) and Taiwan (1.9%) (Table 1). No hVISA was found among the isolates from other countries. The hVISA prevalence differed by genotype (Fig. 1). *spa* type t601 belonging to clonal complex (CC) 5 showed the highest hVISA frequency (33.3%), and hVISA accounted for 6.9% among the isolates of t002 belonging to CC5. Among CC239, which is a widespread hospital MRSA clone in Asia, only isolates of t037 were hVISA (1.6%). Among the CA-MRSA genotypes, hVISA was found only in isolates of t437 (4.8%) belonging to CC59, and no hVISA was found in CC30 or CC72. Isolates of t189 showed a high hVISA proportion (9.1%).

The frequency of hVISA showed an increasing trend as the vancomycin MIC increased. Among 5 isolates with vancomycin MICs of 0.25 mg/L, hVISA was not found, and the proportion of hVISA was only 1.5% among 266 isolates with vancomycin MICs of 0.5 mg/L. In contrast, among 180 isolates with vancomycin MICs of 1 mg/L and 11 isolates with vancomycin MICs of 2 mg/L, hVISA accounted for 6.1% and 9.1%, respectively.

4. Discussion

This study revealed that the hVISA prevalence among MRSA isolates from Asian countries differed by country and genotype. hVISA was most frequent in MRSA isolates belonging to CC5, which is a major hospital clone in South Korea and Japan. Another hospital MRSA clone (CC239) showed a low frequency of hVISA. This contrasts with the results of the previous MRSA bacteraemia study in Australia which showed that hVISA predominantly occurred in ST239-like MRSA isolates [7]. Interestingly, the current study

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