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Short report

Chlorhexidine sensitivity in staphylococci isolated from patients with central line-associated bloodstream infection

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SUMMARY

Since 2011, 2% chlorhexidine in 70% isopropyl alcohol (2% chlorhexidine tincture) has been widely used in Korea. To investigate changes in chlorhexidine sensitivity of staphylococci causing central line-associated bloodstream infections, 264 blood culture isolates from adult patients treated in intensive care units of five university hospitals between 2008 and 2016 were analysed. We observed no significant changes in chlorhexidine minimum inhibitory and bactericidal concentrations, or in the prevalence of resistance-associated genes before and after introduction of 2% chlorhexidine tincture. Thus, there was no evidence of increased resistance to chlorhexidine in staphylococci causing central line-associated bloodstream infections.

Introduction

Central line-associated bloodstream infection (CLABSI) is one of the most common healthcare-associated infections

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(HCAIs). Data collected from 2006 to 2013 by the Korean National Healthcare-associated Infections Surveillance System (KONIS) indicated that CLABSI occurred in 1.1–2.5% of patients with central venous catheters (CVCs) and in 2.3–3.4 cases per 1000 catheter-days, and that *Staphylococcus aureus* was the most common causative agent of CLABSI [1].

To reduce CLABSI, the so-called 'bundle approach', including hand hygiene, maximal barrier precautions, optimal catheter site selection, and chlorhexidine skin antiseptic treatment has been implemented in intensive care units (ICUs) in Korea since 2011 [2]. Chlorhexidine is a cationic disinfectant providing versatile broad-spectrum antibacterial protection in various clinical situations such as CVC insertion and is widely used to reduce HCAIs. It is known that extensive application of antimicrobial agents increases the prevalence of nosocomial antibiotic-resistant strains within hospital settings, which is a serious healthcare concern [3]. However, there are insufficient data on the emergence of bacterial strains with decreased sensitivity to common antiseptics used in hospitals, including chlorhexidine, although it has been reported that exposure to chlorhexidine increases the risk of resistance to certain antimicrobial agents [4]. In particular, it is unclear whether chlorhexidine application during insertion and maintenance of CVCs promotes the resistance of *S. aureus* to this common hospital disinfectant [5].

The present study evaluated the distribution of *qacA/B* and *qac*C (*smr*) genes implicated in the development of resistance to cationic antiseptic agents, and determined minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of chlorhexidine against staphylococci isolated from patients with CLABSIs before and after introduction of 2% chlorhexidine tincture for central line management in Korea.

Methods

Patients

Among the 15 medical centres participating in the intervention study to reduce CLABSI, five hospitals stored blood culture isolates identified from patients with CLABSIs in ICUs before and after the application of 2% chlorhexidine tincture was introduced [6]. Staphylococcal isolates from adult patients treated in these five hospitals were analysed.

Microbiological analysis

There are no specific Clinical and Laboratory Standards Institute (CLSI) guidelines for testing MICs and MBCs of chlorhexidine; therefore, we determined MICs and MBCs using the broth microdilution method recommended by CLSI for testing antibiotic resistance. Each experiment was repeated twice under the same conditions; where the two results were significantly different, an additional experiment was performed and two identical values among the three results were considered as the MIC.

The presence of *qacA/B* and *smr* was determined by polymerase chain reaction as previously described, and *spa* genotyping was performed as reported earlier [7,8]. Specific primers are listed in Table A.1. *S. aureus* strains TS77 and L20A were used as positive controls for *qacA/B* and *smr* genes and *S. aureus* ATCC 29213 was used as negative control.

Statistical analysis

Categorical data such as positivity for *qacA/B* and *smr* genes were analysed by the binomial logistic regression test. Continuous data such as chlorhexidine MICs and MBCs were expressed as the mean ± standard deviation and evaluated by Student's *t*-test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with IBM SPSS statistics 24 (IBM Corp., Armonk, NY, USA).

Ethical considerations

The study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (B-1612-376-303); it was subject to consent exemption because the experiments were conducted only with stored bacterial isolates.

Results

A total of 890 CLABSI cases were identified in adult patients of ICUs; among them, 339 (38%) were due to staphylococci. Among the 264 isolates stored and finally included in this study, 185 (70.1%) were *S. aureus* and 79 (29.9%) were coagulase-negative staphylococci. Table A.2 shows the numbers of strains isolated before and after the introduction of 2% chlorhexidine tincture and Figure A.1 illustrates compliance with the use of 2% chlorhexidine tincture for CVC insertion in each hospital.

The mean chlorhexidine MIC and MBC were 3.15 ± 1.01 mg/L and 8.08 ± 5.01 mg/L, respectively. However, we did not detect statistically significant differences in chlorhexidine MIC ($P = 0.765$) or MBC ($P = 0.945$) before and after the introduction of 2% chlorhexidine tincture. Furthermore, frequent use of 2% chlorhexidine did not affect chlorhexidine MICs and MBCs for isolates in each hospital (Figure 1A) and there were no significant changes in MIC ($MIC_{50} = 2$ mg/L; $MIC_{90} = 4$ mg/L) and MBC ($MIC_{50} = 8$ mg/L; $MIC_{90} = 8$ mg/L) over the whole study period (Figure 1B).

The positivity rates for *qacA/B* and *smr* disinfectant-resistant genes were not increased after the introduction of 2% chlorhexidine tincture; moreover, in hospitals B and D the prevalence of *smr* and *qacA/B* genes, respectively, was significantly decreased by the use of chlorhexidine ($P < 0.007$ and $P < 0.02$, respectively) (Table I). Chlorhexidine MICs and MBCs were significantly higher for strains with the *qacA/B* gene than for those without it (mean MIC: 3.60 vs 2.52 mg/L, $P < 0.001$; mean MBC: 9.17 vs 6.51 mg/L, $P < 0.001$). However, there were no statistically significant differences in chlorhexidine MIC and MBC between *smr*-positive and -negative strains (mean MICs: 3.14 and 3.18 mg/L, respectively, $P = 0.827$; mean MBCs: 7.96 and 8.75 mg/L, respectively, $P = 0.375$).

Typing of the *spa* gene revealed that the most common *S. aureus spa* type was t2460 (74/264, 28.0%), followed by t9353 (27/264, 10.2%), t002 (13/264, 4.9%), t037 (10/264, 3.8%), and t324 (10/264, 3.8%); cumulatively, these five types were detected in 72.4% of isolates. The distribution of chlorhexidine MICs, MBCs, and carriage of *qacA/B* and *smr* gene according to each *spa* type are shown in Table A.3.

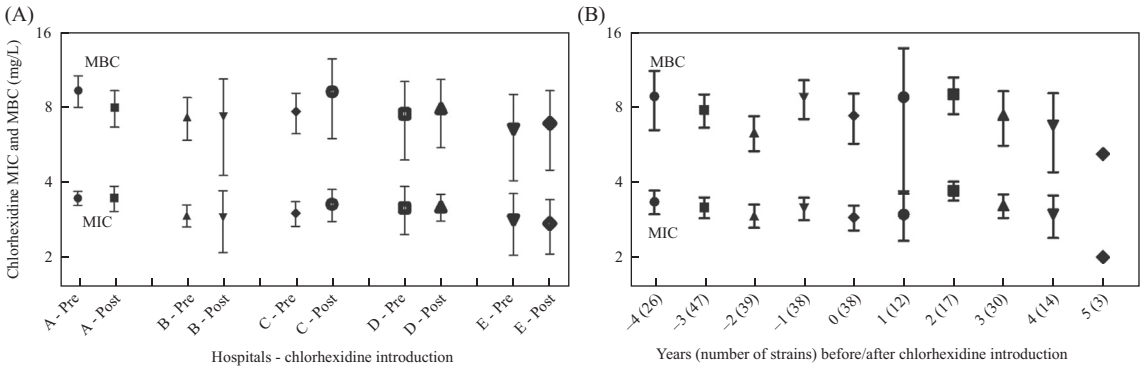

Figure 1. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of chlorhexidine before (Pre) and after (Post) the introduction of chlorhexidine tincture. (A) Changes in chlorhexidine MICs and MBCs in hospitals A–E. (B) Time-dependent changes in chlorhexidine MICs and MBCs. Numbers of staphylococcus isolates are presented in parentheses.

Table I
Positive rates of *qacA/B* and *smr* genes before and after introduction of 2% chlorhexidine tincture

Hospital	Before 2% chlorhexidine tincture introduction		After 2% chlorhexidine tincture introduction		*P*-values		Total	
	qacA/B	*smr*	*qacA/B*	*smr*	*qacA/B*	*smr*	*qacA/B*	*smr*
A	41/62 (66.1%)	53/62 (85.5%)	13/22 (59.1%)	21/22 (95.5%)	0.554	0.215	54/84 (64.3%)	74/84 (88.1%)
B	21/51 (41.2%)	43/51 (84.3%)	3/9 (33.3%)	4/9 (44.4%)	0.658	0.007	24/60 (39.7%)	47/60 (78.3%)
C	27/39 (69.2%)	36/39 (92.3%)	13/19 (68.4%)	15/19 (78.9%)	0.95	0.143	40/58 (68.9%)	51/58 (87.9%)
D	12/13 (92.3%)	12/13 (92.3%)	15/27 (55.6%)	21/27 (77.8%)	0.02	0.257	27/40 (67.5%)	33/40 (82.5%)
E	4/11 (36.4%)	11/11 (100%)	6/11 (54.6%)	11/11 (100%)	0.392	–	10/22 (46.4%)	22/22 (100%)
Total	105/176 (59.7%)	155/176 (88.1%)	50/88 (56.8%)	72/88 (81.8%)	0.659	0.168	155/264 (58.2%)	227/264 (86.0%)

–, incalculable.

Discussion

Chlorhexidine tincture is widely used to prevent HCAIs. Therefore, there is a concern regarding possible development of resistance to chlorhexidine similar to that observed for overused antibiotics. In this study, we investigated whether there was an increasing trend for chlorhexidine MICs and MBCs as well as for the prevalence of disinfectant-resistance genes depending on the use of 2% chlorhexidine tincture for eight years in Korea. Our results indicate that despite the overall high prevalence of *qacA/B* and *smr* genes, 2% chlorhexidine did not promote resistance of staphylococcus isolates from patients with CLABSI: chlorhexidine MICs and MBCs calculated for strains from individual hospitals were unchanged and their cumulative average values did not depend on the time of 2% chlorhexidine application. Furthermore, 2% chlorhexidine did not increase the proportion of *qacA/B*- and *smr*-positive isolates.

Because methods to determine chlorhexidine MICs and MBCs in staphylococci have not been standardized, it was difficult to directly compare our results with those obtained in other studies. Therefore, we repeatedly measured chlorhexidine MICs and MBCs to ensure experimental reproducibility. As a result, our measurements of chlorhexidine MICs (mean: 3.15 mg/L; range: 1–4 mg/L) and MBCs (mean: 8.08 mg/L; range: 2–32 mg/L) are comparable with those of previous studies showing MICs in the range of 0.5–8 mg/L and MBCs in the range of 0.5–64 mg/L [9].

The prevalence of *qacA/B* and *smr* genes in *S. aureus* has been typically investigated in cross-sectional studies performed in a single hospital and the results varied significantly. Thus, the rate for the *qnrA/B* gene ranged from as low as 0.5% up to as high as 64.5% depending on the country, hospital, or patient group, whereas that of the *smr* gene varied from zero to 70% [10]. In this study, positive rates for *qacA/B* and *smr* genes (58.2% and 86%, respectively) were relatively high compared with previous reports. High prevalence of disinfectant resistance-related genes might be attributed to the inclusion of isolates from ICUs. In addition to chlorhexidine resistance, the *qacA/B* genes also mediate the export of various negatively charged small molecules, including quaternary ammonium compounds, intercalating dyes, and diaminidines [5]. Therefore, exposure to other antibiotics or disinfectants such as quaternary ammonium compounds and 0.5% chlorhexidine aqueous solutions, which were previously used in Korean hospitals, may be a reason for high *qacA/B* positivity before the introduction of 2% chlorhexidine tincture for use as disinfectant. As the *smr* gene is also known to be involved in the export of various chemicals, its high prevalence could be due to the same factors [5].

This study has some limitations. First, we analysed only strains isolated from ICUs and there may be a possibility that an epidemic clone in each hospital could affect the results. However, since the distribution of *spa* types was relatively uniform in different hospitals and over the time course, it is unlikely that certain *S. aureus* types were indigenous and influenced the outcome. Second, we examined only the presence of *qacA/B* and *smr* genes, but not their activities. The *qacA/B* and *smr* genes have distinct functions; furthermore,

they are not the only genes associated with chlorhexidine resistance. Therefore, it cannot be unequivocally concluded that the presence of *qacA/B* and *smr* is responsible for chlorhexidine resistance. Finally, it was difficult to determine whether the increase in compliance was directly correlated with increased chlorhexidine use.

In conclusion, we did not detect a trend for increase in chlorhexidine MICs and MBCs or in the prevalence of *qacA/B*- or *smr*-positive staphylococcal strains despite wide application of 2% chlorhexidine tincture in recent years. However, it is necessary to be vigilant about the disinfectant resistance problem, because genes mediating chlorhexidine resistance are prevalent among staphylococci isolated from ICUs.

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Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2019.07.009.

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