American Journal of Infection Control 47 (2019) e27–e29

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Brief Report

Vancomycin-resistant *Enterococcus faecium* sensitivity to isopropyl alcohol before and after implementing alcohol hand rubbing in a hospital

Consuelo G. Tinajero MD [a], Miriam Bobadilla-Del Valle PhD [b], Jose A. Alvarez MD, PhD [a,c], Juan L. Mosqueda MD, PhD [a], Alfredo Ponce De Leon MD [b], Alejandro E. Macias MD [a,*]

[a] *Microbiology Laboratory, Department of Medicine, University of Guanajuato, León, Mexico*
[b] *National Institute of Medical Sciences and Nutrition Salvador Zubiran, Mexico City, Mexico*
[c] *Research Department, Bajio Regional High Specialty Hospital, Secretariat of Health, León, Mexico*

Key Words:
hand hygiene
hand antisepsis
disinfectants

A recent study reported enterococci that developed alcohol tolerance. We measured minimum inhibitory concentrations (MICs) of isopropyl alcohol against 55 vancomycin-resistant *Enterococcus faecium*. We did not find an increase in MICs when comparing the periods before and after the use of alcohol for hand hygiene in a hospital, and we did not find a single isolate with a MIC higher than 11.5%. We consider alcohol to still be an effective measure for hand antisepsis.

Enterococci are part of the normal gastrointestinal tract microbiota; however, they are currently important pathogens in health care–associated infections, such as those related to intravascular and urinary catheters.[1,2] Health care–associated infections are a global problem; bloodstream infections in the United States generate costs estimated at approximately $6.25 billion a year.[3] Washing hands with soap and water is a good strategy to prevent these infections, but the use of alcohol-based hand sanitizers containing at least 60% alcohol has been a good alternative because washing requires more time and sanitary facilities.[4,5] Ethyl and isopropyl alcohol have an inhibitory effect against *Enterococcus* spp even at reported concentrations as low as 11%.[6] A recent report, however, showed that some enterococci may be tolerant to alcohol at concentrations as high as 23%.[7] Therefore, the objective of the present study was to determine whether exposure to alcohol used for hand antisepsis has induced susceptibility changes to isopropyl alcohol in strains of vancomycin-resistant *Enterococcus faecium* (VRE) in a health care institution.

METHODS

We measured the minimum inhibitory concentration (MIC) of isopropyl alcohol (Sigma-Aldrich; St. Louis, MO) against 55 vancomycin-resistant *E faecium* clinical isolates. The strains were isolated from patients hospitalized at a single hospital in Mexico, the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran (INNSZ). At the INNSZ, the institutional policy for hand cleaning was based on soap and water until 2009, when the use of alcohol for hand antisepsis was formally implemented in all areas of the hospital, concomitantly with the influenza A (H1N1) pandemic; the use of alcohol with 0.5% chlorhexidine was implemented in 2012.

For the purposes of this study we selected VRE strains from 2 different periods: 1) before the systematized use of alcohol for hand antisepsis (2002 and 2007), and 2) after alcohol hand antisepsis was systematized at the INNSZ (2010 and 2018). The procedure was performed by microdilution in 96-well, round-bottom plates (Corning; Corning, NY). The isolates were grown on sheep blood agar plates with overnight incubation at 35°C. Subsequently, a suspension of 2 or 3 colonies in 0.9% saline was performed until obtaining the turbidity of 0.5 McFarland (1.5×10^8 CFU/mL), which was measured in a DensiCHEK Plus nephelometer (Biomerieux; Durham, NC). Tubes were prepared with 10 mL of BD Tryptic Soy Broth (BST) (Becton, Dickinson and Company; Franklin Lakes, NJ) with the following final alcohol concentrations (v/v): 36.8 mg/mL (46%), 18.4 mg/mL (23%), 9.2 mg/mL (11.5%), 4.6 mg/mL (5.75%), and 2.3 mg/mL (2.87%). In the wells, 100 μL of the BST mixture were added to each of the concentrations of isopropyl alcohol to be tested, and then 10 μL of each of the strains diluted 1:20 in saline were added. A well with 100 μL of BST,

* Address correspondence to Alejandro E. Macias, MD, Universidad de Guanajuato, 20 de Enero 929, León, Guanajuato, México 37000.
E-mail address: aaeemmhh@yahoo.com (A.E. Macias).
Conflicts of interest: None to report.

https://doi.org/10.1016/j.ajic.2019.03.027

Table 1
Clinical origin of strains of vancomycin-resistant *Enterococcus faecium* (vanA+) isolated and tested against isopropyl alcohol

Specimen	Frequency	%
Urine	29	52.7
Abscess	6	10.9
Blood	5	9.1
Endotracheal aspirate	3	5.5
Biopsy	3	5.5
Ascites fluid	2	3.6
Other	7	12.7
Total	55	100

a well with isopropyl alcohol (92%), and a well with BST plus the strain were included as controls for each strain tested.

The plates were incubated at 35°C for 18 to 24 hours, and the MICs of each strain tested were read. The MIC was determined as the concentration at which 99.9% of the bacteria did not grow. An inferential and correlational analysis for qualitative variables was performed using the online software package VassarStats (http://vassarstats.net). A P value < .05 was considered significant.

RESULTS

A total of 55 strains of vancomycin-resistant *E faecium* were evaluated. From these, 29 (52.7%) were isolated before the systematized use of alcohol for hand antisepsis (2002 and 2007), and 26 (47.3%) were isolated during the period when alcohol hand antisepsis was systematized at the INNSZ (2010 and 2018). The strains were obtained from different sources, as described in Table 1; most of them (29, 52.7%) were isolated from urine, and 5 (9%) were isolated from blood.

Overall, of the 55 strains studied, 12 (21.8%) had a MIC of 2.87%, 26 (47.3%) had a MIC of 5.75%, and 17 (30.9%) had a MIC of 11.5%. When comparing the proportion of MICs identified among the isolates from the 2 time frames (before and after the systematized use of alcohol for hand antisepsis), there was no statistically significant difference ($\chi^2 = 1.386$; degrees of freedom = 2; $P = .5$). Figure 1 compares the 2 periods related to the MICs identified for *E faecium*. The correlation analysis between the periods of alcohol hand antisepsis and the observed MICs showed a low correlation coefficient (Spearman's rho) of -0.105 ($P = .387$).

DISCUSSION

The present study found no significant increase in the MICs of isopropyl alcohol for vancomycin-resistant *E faecium* strains when comparing the period before and after the systematized use of alcohol for hand hygiene in a medical institution. These results contrast with those published by Pidot et al,[7] who reported that recent strains of vancomycin-resistant *E faecium* (isolated from 2010 to 2015)

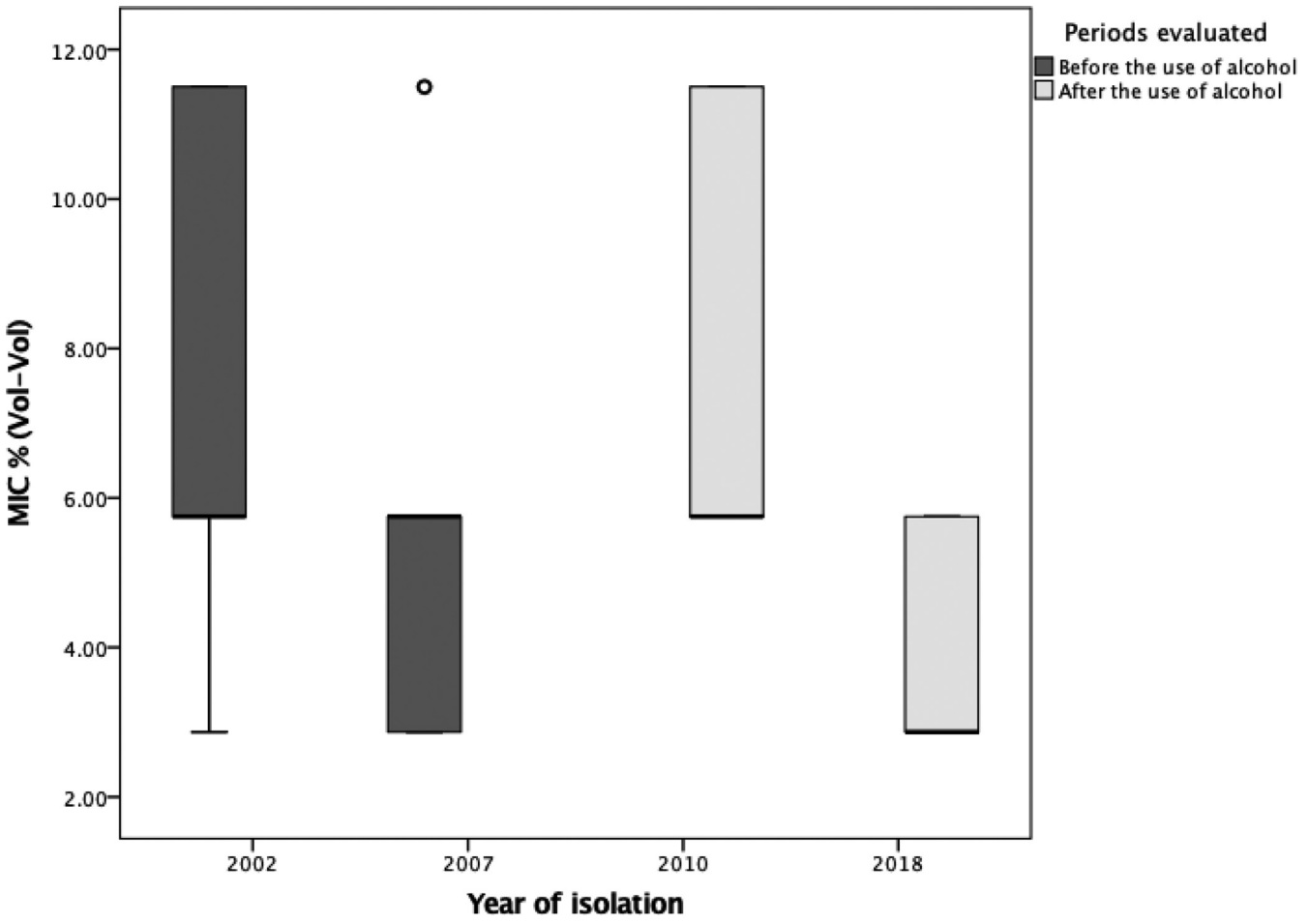

Fig 1. Minimum inhibitory concentrations (MICs) of isopropyl alcohol against vancomycin-resistant *Enterococcus faecium* strains (vanA+) isolated in specific years before and after the use of alcohol for hand antisepsis in a hospital.

tolerated substantially higher concentrations of isopropyl alcohol, up to 10 times more than strains isolated earlier (1997); the authors hypothesized that the increased tolerance was a consequence of adaptation of the organisms. Many journalistic notes based on these conclusions followed, warning about a loss of efficacy of alcohol for hand hygiene.[8] Our study has methodological differences, such as a smaller sample size and a shorter period of exposure of *Enterococci* to alcohol in a single institution. Thus, these studies may not be comparable; however, we did not find a single isolate showing an isopropyl alcohol MIC higher than 11.5% (post hoc statistical power [1 - β]: 0.83), which is a strong observation, as hand antisepsis uses alcohol concentrations at least 5 times higher (60%-70%).

These results are concordant with several reports in the literature describing the sensitivity of VRE isolates to alcohol.[6,9] Thus, based on these reports and on our results, we consider that alcohol at the appropriate concentrations should still be considered an effective measure for hand antisepsis in healthcare institutions.

CONCLUSIONS

We did not find an increase in tolerance to isopropyl alcohol among vancomycin-resistant *E faecium* isolated during 2 different periods. We think that the use of alcohol is still an adequate measure for hand hygiene in clinical environments, as it is used at concentrations much higher than those that are required to inhibit the growth in in vitro tests.

Acknowledgment

We thank Estrella Tovar-Calderon for her invaluable technical assistance.

References

1. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. Infect Control Hosp Epidemiol 2016;37:1288-301.
2. Pinholt M, Østergaard C, Arpi M, Bruun NE, Schønheyder HC, Gradel KO, et al. Incidence, clinical characteristics and 30-day mortality of enterococcal bacteraemia in Denmark 2006-2009: a population-based cohort study. Clin Microbiol Infect 2014;20:145.
3. Pathak R, Gangina S, Jairam F, Hinton K. A vascular access and midlines program can decrease hospital-acquired central line-associated bloodstream infections and cost to a community-based hospital. Ther Clin Risk Manag 2018;14:1453-6.
4. Rutala WA, Weber DJ. HICPAC. Guideline for disinfection and sterilization in healthcare facilities, 2008. Atlanta, GA: Centers for Disease Control and Prevention; 2008.
5. WHO. WHO guidelines on hand hygiene in health care: First Global Patient Safety Challenge Clean Care is Safer Care. Geneva: World Health Organization; 2009.
6. Mazzola PG, Jozala AF, Novaes LCDL, Moriel P, Penna TCV. Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. Braz J Pharm Sci 2009;45:241-8.
7. Pidot SJ, Gao W, Buultjens AH, Monk IR, Guerillot R, Carter GP, et al. Increasing tolerance of hospital *Enterococcus faecium* to handwash alcohols. Sci Transl Med 2018;10:eaar6115.
8. Pittet D, Peters A, Tartari E. *Enterococcus faecium* tolerance to isopropanol: from good science to misinformation. Lancet Infect Dis 2018;18:1065-6.
9. Kampf G, Hollingsworth A. Comprehensive bactericidal activity of an ethanol-based hand gel in 15 seconds. Ann Clin Microbiol Antimicrob 2008;7:2.