

# Alcohol-based Hand Rub as a Prophylactic Measure against SARS-COV-2 Infection: Can it Reduce the Infection with Vancomycin-Resistant *Enterococcus faecalis*?

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**Background and study aim:** As part of the intestinal microbiota, *Enterococcus faecalis* is the most prevalent enterococcal species in the human gastrointestinal tract and performs vital physiological activities. It has become a common cause of nosocomial infections in critically ill patients, resulting in life-threatening bacteremia and endocarditis. Alcohol-based hand washes have been shown to prevent hospital-acquired infections with multidrug-resistant pathogens. The aim of the study is to assess the efficacy of alcohol-based hand rub on protection against Vancomycin-resistant *Enterococcus faecalis*.

**Materials and Methods:** In the current prospective cohort study, we collected samples from patients presenting with gastrointestinal symptoms. The patients were grouped into frequent (cases group) and infrequent users (control group) of alcohol-based hand rub as a prophylactic

measure for SARS-CoV-2 infection. The alcohol and vancomycin susceptibility were examined over three months.

**Results:** Thirty patients were included in each group. Alcohol resistance was significantly higher in samples of group 1 compared to group 2 \_the control group\_ (36.7% compared to 3.3%, with  $P = 0.01$ ) at the first month. Same findings were observed at the second (46.7% vs. 3.3%, respectively) and third month (73.3% vs. 3.3%, respectively). Similarly, vancomycin resistance was significantly higher in 33.3% of group 1, while group 2 showed 3.3% of drug resistance ( $P < 0.001$ ).

**Conclusion:** *Enterococcus faecalis* resistance to alcohol and vancomycin was found to be considerably increased in people who often used alcohol-based hand rub .

## INTRODUCTION

Enterococci are gastrointestinal (GI) commensals of healthy humans that belong to gram-positive anaerobes. Currently, there are more than 50 identified enterococcal species [1]. *Enterococcus faecalis* is the most abundant enterococcal species within the human GI tract and exerts critical physiological functions as a part of intestinal microbiota [2]. Despite serving as a commensal organism, the *Enterococcus faecalis* has emerged as a prevalent cause of opportunistic nosocomial infections in critically ill patients, leading to life-threatening bacteremia and endocarditis [3]. Besides, *Enterococcus faecalis* infection may occur within the community, and it has been reported in urinary tract infections [4]. With

the advance of antibiotic regimens, enterococcal species have demonstrated genetically-driven intrinsic resistance to a wide range of antimicrobial agents. The Enterococci also exhibit acquired, sometimes a rapid-onset, multidrug resistance determinants [5]. Since early reports of vancomycin-resistant enterococci (VRE), growing evidence has highlighted the significant impact of these species on the outcomes of critically ill patients and the healthcare system. Previous reports demonstrated that enterococcal species usually develop resistance to vancomycin through various mechanisms, including dysregulated glycopeptide synthesis and the emergence of *van* gene clusters [5,6]. Previous reports showed that nearly

one-third of isolated *Enterococcus faecalis* were resistant to vancomycin [4].

On the other hand, alcohol-based disinfectants are one of the cornerstones for infection-control strategies in various healthcare settings. The current body of evidence demonstrated that alcohol-based hand washes have significantly reduced hospital-acquired infections with multidrug-resistant organisms [7]. Enterococcal species generally show susceptibility to alcoholic agents, even at a concentration as low as 11% [8]. Nonetheless, increased tolerance of Enterococcal species to alcohol-based disinfectants was recently reported following the alcohol hand rubbing policy, with equivocal results [7,8].

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), primarily reported in China in December 2019, is the cause of a worldwide pandemic, infecting more than 200 million persons, with a mortality rate of 1.4-6.9% [9]. Prophylactic measures -such as social isolation, mask-wearing, and handwashing- have been widely recommended and implemented amongst the general population in combat against the COVID-19 pandemic [10]. Particularly, handwashing with alcohol-based disinfectants is prompted as an effective strategy for hand decontamination, preventing the spread of SARS-CoV-2 [11,12]. However, little is known about the impact of frequent handwashing with alcohol-based disinfectants on the alcohol sensitivity of commensals presenting naturally in the environment, such as Enterococci species. To our knowledge, the current study is the first study to assess the resistance of *Enterococcus faecalis* to alcohol and vancomycin among frequent users of alcohol-based hand rub presenting with gastrointestinal symptoms.

## MATERIALS AND METHODS

### Setting and patients:

The current study protocol was coordinated in concordance with the guidelines established declaration of Helsinki, and it was approved by the ethics committee at Masr University for Science and Technology (MUST) hospital. Patients were recruited from the outpatient clinic of internal medicine departments, MUST hospital. In this observational study, samples were collected from patients presenting with GI symptoms including abdominal pain, nausea,

vomiting, and diarrhea. The patients were grouped into frequent alcohol users \_more than 3 times per day and after any activity they do\_ (group 1) and infrequent users \_less than 3 times per day\_ (group 2) of alcohol-based hand rub as a prophylactic measure from SARS-CoV-2 infection.

### Sample collection:

We included subjects who did not receive any antibiotic therapy for the past three days to prevent any false-negative results. Anal samples were collected using sterile swabs during the visit to the outpatient clinic. Samples were washed in distilled water and transported to the laboratory for further analysis. Samples were stored on sheep blood agar culture media at 35°C. After overnight incubation, the cultured plates were assessed. First, slides were developed from the structured colonies. The *Enterococcus faecalis* were identified by the standard methods.

### Alcohol and vancomycin susceptibility testing:

A suspension of 2 or 3 colonies in 0.9% saline was performed until obtaining the turbidity of 0.5 McFarland (1.5 × 10<sup>8</sup> 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 mL of the BST mixture were added to each of the concentrations of isopropyl alcohol to be tested, and then 10 mL of each of the strains diluted 1:20 in saline were added. A well with 100 mL of BST, a well with isopropyl alcohol (92%), and BST plus the strain were included as controls for each strain tested. The vancomycin susceptibility was tested according to the Clinical and Laboratory Standards Institute (CLSI) standards.

### Statistical analysis:

Data were analyzed using SPSS software, version 26 (Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, New York: IBM Corp). Chi-square was used for comparison between cases and controls regarding gender, alcohol resistance, and vancomycin resistance. T-test student was used for comparison between groups regarding age.  $P < 0.05$  was considered statistically significant.

## RESULTS:

During the period of this study, thirty patients were included in group 1. In group 1, the average age was  $29.3 \pm 5.1$  years old. 40% of the included patients were males, and 60% were females. In group 2, the average age was  $29.1 \pm 4.3$  years old. 46.7% of the included patients were males, and 53.3% were females (**Table 1**). There was no significant difference between both groups regarding age and gender distribution ( $P = 0.84$  and  $P = 0.432$ , respectively).

Alcohol resistance was significantly higher in samples of group 1 compared to the control group (36.7% compared to 3.3% , with  $P = 0.01$ ) at the first month . (**Table 2**)

Same findings were observed between the cases group and the control group at the second (46.7% vs. 3.3%, respectively) and third month (73.3% vs. 3.3%, respectively). Vancomycin resistance was significantly higher in group 1 (33.3%), while group 2 showed 3.3% of drug resistance ( $P < 0.001$ ).

**Table 1.** Demographics for the study cohort.

Parameters	Cases (n= 30)	Controls (n= 30)	P-value
Age, mean ( $\pm$ SD)	$29.3 \pm 5.1$	$29.1 \pm 4.3$	0.84
Gender			
Male, N (%)	12 (40)	14 (46.7%)	0.432
Female, N (%)	18 (60)	16 (53.3%)	

SD: Standard Deviation

**Table 2.** Clinical Data of the study cohorts

Parameters	1 month		P-value	2 months		P-value	3 months		P-value
	Cases (n= 30)	Controls (n= 30)		Cases (n= 30)	Controls (n= 30)		Cases (n= 30)	Controls (n= 30)	
<b>Alcohol</b>									
Sensitive, N (%)	19(63.3%)	29 (96.7%)	0.001	16(53.3%)	29 (96.7%)	0.001	8 (26.7%)	29 (96.7%)	0.001
Resistant, N (%)	11(36.7%)	1 (3.3%)		14(46.7%)	1 (3.3%)		22(73.3%)	1 (3.3%)	
<b>Vancomycin</b>									
Sensitive, N (%)	20(66.7%)	29 (96.7%)	0.003	18 (60%)	30 (100%)	0.001	18 (60%)	27 (90%)	0.001
Resistant, N (%)	10(33.3%)	1 (3.3%)		12 (40%)	0		12 (40%)	3 (10%)	

## DISCUSSION

Bloodstream infections in the United States account for an estimated \$6.25 billion in annual healthcare-associated infection expenses [13]. While soap and water washing is an effective method of preventing these infections, alcohol-based hand sanitizers containing at least 60% alcohol have been shown to be an effective substitute since washing needs more time and hygienic facilities [14,15]. *Enterococcus spp.* are inhibited by Ethyl and isopropyl alcohol even at low concentration levels as 11% [16]. Alcohol resistance in certain enterococci was shown to be 23% [7]. In a tertiary care hospital in Northern India, investigators reported that vancomycin-resistant strains were found in 7.9% out of 1488 isolates; of them, 61% were *E. faecalis* and 39% were *E. faecium*. Ventilator support and hospitalization for more than 48 hours were shown to be independent risk factors for VRE. *faecalis* infection [17]. A systematic review of

four studies suggested that the prolonged use of hand hygiene products may cause antimicrobial resistance (AMR) in health care settings [18]. However, the included studies of this systematic review were not homogenous conducted in US between the period 1986 and 2015.

In this study, our findings showed a significant difference between the two groups in terms of alcohol and vancomycin resistance. At the end of the first month, samples from group 1 had considerably higher alcohol resistance than those from the control group (36.7% vs. 3.3%,  $P = 0.01$ ). The same findings were observed at the second (46.7% vs. 3.3%, respectively) and third month (73.3% vs. 3.3%, respectively). Similarly, vancomycin resistance was significantly higher in the 33.3% of the cases group, while the control group showed 3.3% of drug resistance ( $p < 0.001$ ).

With the increasing use of alcohol-based hand sensitizers, *E. faecium* isolates obtained between

1997 and 2015 were tested for alcohol tolerance, and it was found that those obtained after 2010 were 10 times more resistant to alcohol than those obtained before that year, according to Pidot and his colleagues [7]. However, many researchers disagreed with the findings of this study. For example, Pittet and his team commented on this study, they said [19] "As experts in hand hygiene, we feel these misinterpretations could lower healthcare worker compliance with hand hygiene practices and put patients at risk. The study compared older and newer *E faecium* isolates (obtained between 1997 and 2015) and their tolerance to a 23% alcohol solution and found that some newer strains were more tolerant to alcohol. Hospital alcohol-based hand rub formulations contain 60–90% alcohol. There is no evidence of resistance at these concentrations, thus tolerance to a 23% alcohol solution is not clinically relevant. The study itself states that there was no difference in the bacterial log reduction between human isolates exposed to 70% isopropanol solution (similar to what might be used in an alcohol-based hand rub), regardless of those strains' tolerance to alcohol. Hospitals must select high-quality, validated alcohol-based hand rub formulations and encourage high rates of hand hygiene compliance among healthcare workers to lower rates of health-care-associated infections and the spread of antimicrobial resistance. Hospital environmental disinfection for VRE is a comprehensive protocol, not a quick wipe. Furthermore, alcohol is not recommended for VRE environmental control."

In contrast, in the study of Tinajero et al., they isolated a set of vancomycin-resistant *Enterococcus faecium* before and after using alcohol-based hand sensitizers. They found no significant rise in the minimum inhibitory concentrations (MICs) of isopropyl alcohol for vancomycin-resistant *Enterococcus faecium* strains [20]. Some studies tried to test the efficacy of increasing the concentration and volume of isopropanol to compact the alcohol-resistant *Enterococcus*. Gebel et al. [21] demonstrated that isopropanol alcohol 60% or 70% was effective against all *Enterococcus* strains, while 23% isopropanol was ineffective against all *Enterococcus* strains. In the four-field test, 70% isopropanol was shown to be effective against *E. faecium*. Gebel et al. concluded that *E. faecium* may be effectively treated with 60% and 70% isopropanol with the correct amount [21].

To the best of our knowledge, this is the first study that investigates the resistance of *Enterococcus faecalis* to alcohol and vancomycin among frequent users of alcohol-based hand rub presenting with gastrointestinal symptoms. However, our study has some limitations including the small sample size and single-center setting.

In conclusion, the frequent use of alcohol-based hand rub was associated with significantly elevated *Enterococcus faecalis* resistance against alcohol and vancomycin. Further studies with a larger sample size and longer follow-up period are required to investigate the impact of prolonged use of alcohol-based hand rub and other disinfectants on bacteria and its relation with antimicrobial resistance.

#### Conflict of Interest:

All authors confirm no financial or personal relationship with a third party whose interests could be positively or negatively influenced by the article's content.

#### Funding Source:

None (authors confirm they did not receive any funding to do this work)

#### Ethical consideration:

The work was done according to the declaration of Helsinki and the sound practice. IRB approval was obtained.

#### HIGHLIGHTS

- Alcohol-based hand rub is associated with significantly elevated *Enterococcus faecalis* resistance.
- Alcohol-based hand rub should be generalized for antimicrobial resistance.
- *Enterococcus faecalis* resistance to alcohol and vancomycin was found to be considerably increased in people who often used alcohol-based hand rub.

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