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Brief Report

Does chlorhexidine mouthwash reduce the rate of oral colonization by gram-negative bacteria in patients with chemotherapy? A placebo-controlled trial

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The presence of gram-negative bacteria in the oral cavity is an undesirable occurrence in patients undergoing chemotherapy. Our aim was to investigate the antibacterial effect of 0.12% chlorhexidine mouthwash in chemotherapy patients with a randomized, double-blind, placebo-controlled trial. There were no significant differences between oral colonization rates; there may be local factors that interfere with chlorhexidine activity.

BACKGROUND

Patients who receive chemotherapy are at risk of developing bacterial infections.[1] Gram-negative bacteria are a common causative agent of complications in these patients and can colonize the oral cavity, creating a source of systemic infection.[2] Colonization typically begins within the first few hours of hospitalization; within 7 days of hospitalization, the rate of colonization nears 25%.[3]

The addition of mouthwash containing an effective antiseptic to the dental hygiene routine may be an appropriate strategy for reducing colonization by gram-negative organisms. Mouthwashes containing chlorhexidine are currently recommended for the treatment of gingivitis and other periodontal infections.[4] Results of studies on chlorhexidine mouthwashes compared with diverse agents in different clinical scenarios are controversial.[5-7]

There are no standard guidelines on the use of antiseptic mouthwashes to reduce gram-negative bacterial colonization in patients receiving chemotherapy. The aim of this study was to determine whether a 0.12% chlorhexidine mouthwash reduces the rate of oral colonization by gram-negative bacteria in chemotherapy patients.

METHODS

This randomized, double-blind clinical trial was conducted at the Bajio Regional High Specialty Hospital between April 2015 and May 2016. The protocol was approved by the hospital's Ethics Committee, and the study has been registered at ClinicalTrials.gov (NCT 02414581). The eligibility criteria were patients aged 18 years or older with hematologic malignancies whose treatment included cycles of chemotherapy and hospitalization with an expected stay of at least 10 days. Each eligible patient was assigned at random to receive either mouthwash containing 0.12% chlorhexidine in 7% ethyl alcohol (Pisa, Guadalajara, Mexico) or 7% ethyl alcohol placebo mouthwash (Pisa). All study patients were assigned to a standard oral care plan, involving toothbrushing using neutral toothpaste with a soft-bristle toothbrush. The mouthwashes were used 30 minutes after brushing, to prevent interaction with toothpaste ingredients. The mouthwash regimen was performed twice daily, with 15 mL of the solution swished in the mouth for 30 seconds at each use.

Oral swab samples were obtained at the start of cytotoxic chemotherapy and 10 days after. Swab samples were immediately inoculated on MacConkey agar (BD, Mexico city, Mexico) and in brain heart infusion broth and transported for processing at the Microbiology Laboratory at the University of Guanajuato. Inoculated media were incubated, and the organisms isolated were identified with conventional biochemical assays. An antimicrobial susceptibility test was performed to identify the production of extended-spectrum β-lactamase and carbapenem-resistant gram-negative bacteria. At

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the end of the study, selected organisms isolated from patients using chlorhexidine were tested to determine their minimum bactericidal concentration (MBC) with a microdilution method.

We determined the need for a minimum sample of 30 patients per group. Inferential analysis for quantitative and qualitative variables was performed using the online software package Vassar Stats (http://vassarstats.net). A P value $< .05$ was considered significant.

RESULTS

Between April 2015 and August 2016, 63 patients who met the eligibility criteria were recruited. A CONSORT flow chart of the study patients is shown in Figure 1. The patients' baseline clinical characteristics are presented in Table 1. The final colonization rate was 47% (95% confidence Interval, 30%-64%) in the chlorhexidine group and 40% (95% confidence interval, 25%-58%) in the placebo group ($P = .79$). The other colonization rates are shown and compared in Figure 2.

Globally, we isolated 34 gram-negative organisms, 17 in each study arm, at the end of the study. *Klebsiella pneumoniae* and *Pantoea agglomerans* were the most common isolates. Only 3 organisms were extended-spectrum β-lactamase producers, and none of the organisms was carbapenem resistant.

In the chlorhexidine group, severe neutropenia was observed in 22 patients (73%), 12 of whom (54.5%) were colonized at the completion of the study ($P > .05$). In the placebo group, 24 patients (80%) had severe neutropenia, of whom 9 (37.5%) were colonized at the trial completion ($P > .05$). Of 60 patients included in the study, 46 had severe neutropenia, of whom 21 (46%) were colonized at trial completion ($P > .05$). Febrile neutropenia was found in 17 patients (57%) in the chlorhexidine group and in 15 patients (50%) in the placebo group ($P > .05$). Third- or fourth-degree mucositis was observed in 4 patients (13%) in the chlorhexidine group and in 5 patients (17%) in the placebo group ($P > .05$).

We evaluated a total of 6 bacteria species isolated from the oral cavities of patients from the chlorhexidine group at the end of the

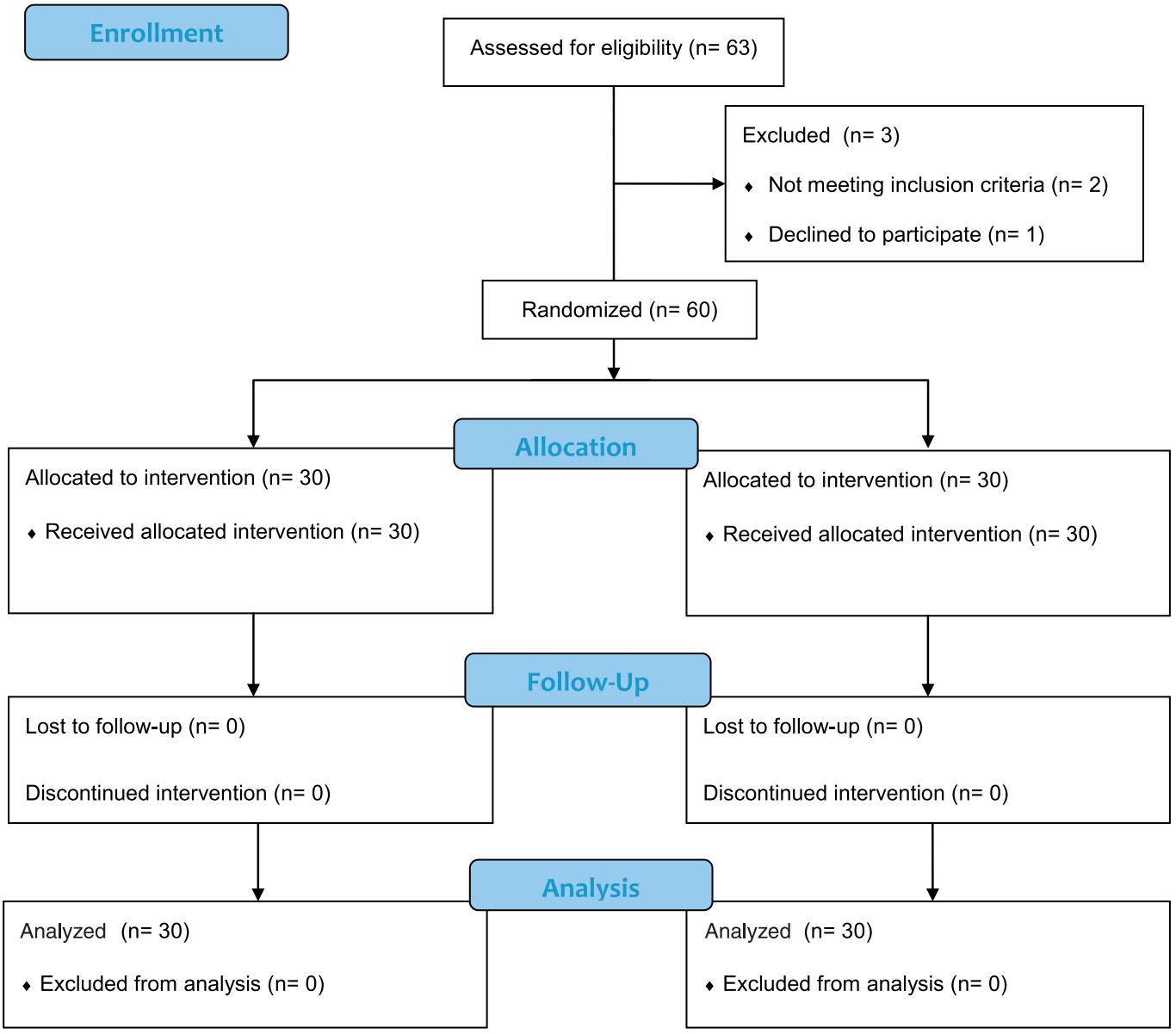

Fig 1. CONSORT flowchart of the study patients.

Table 1
Patient demographic and clinical characteristics

Characteristic	Chlorhexidine group (n = 30)	Placebo group (n = 30)	*P* value
Sex, n (%)			
Female	10 (33)	10 (33)	.78*
Male	20 (67)	20 (67)	
Age, y, median (IQR)	26 (18-45)	25.5 (18-56)	1.0†
Hemoglobin, g/dL,median (IQR)	9.4 (7.7-11.2)	8.9 (7.5-11.3)	.72†
Leukocytes, $\times 10^3$/mm^3, median (IQR)	6.9 (4.4-13.9)	6.9 (4-12.5)	.99†
Absolute neutrophil count, cells/mm^3, median (IQR)	2,878 (820-4,892)	2,334 (606-3,572)	.42†
Platelets, x 10^3/mm^3, median (IQR)	102 (33-109)	94 (23-169)	.46†
Diagnosis, n (%)			
Lymphoblastic leukemia	18 (60)	23 (77)	.25‡
Myeloblastic leukemia	9 (30)	4 (13)	
B-cell non-Hodgkin lymphoma	3 (10)	2 (7)	
T-cell non-Hodgkin lymphoma	0 (0)	1 (3)	
Previous chemotherapy, n (%)			
Yes	18 (60)	15 (50)	.60*
No	12 (40)	15 (50)	
Oral colonization at start, n (%)			
Colonized	10 (33)	7 (23)	.57*
Not colonized	20 (67)	23 (77)	

IQR, interquartile range.
*χ^2 test.
†Mann-Whitney U test.
‡Fisher exact test.

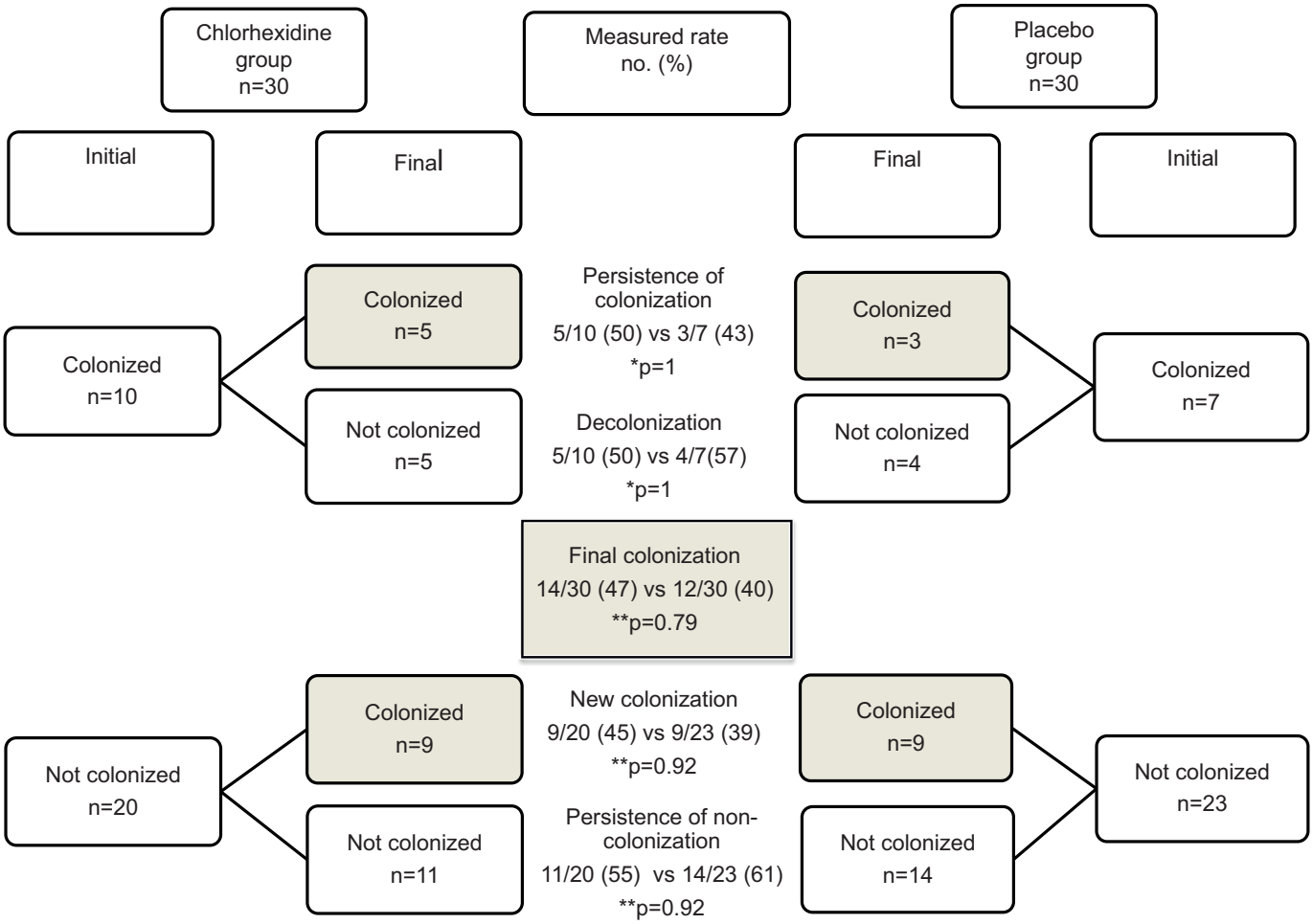

Fig 2. Colonization stages and their effect on rates in the 2 groups. Colonization was defined as the isolation of gram-negative bacilli from oral swabbing in MacConkey agar, either directly or indirectly after enrichment in brain-heart infusion broth. *Fisher exact test; **χ^2 test.

intervention, to determine the MBC of chlorhexidine using the microdilution method. *Stenotrophomonas maltophilia* showed an MBC of 0.060%, whereas the MBC for the rest of the organisms was 0.015% or lower.

RESULTS

When comparing 0.12% chlorhexidine mouthwash with a placebo mouthwash in patients receiving chemotherapy, no differences were observed in the rates of oral colonization by gram-negative bacteria, and no significant differences were found in the other rates assessed: persistence of colonization, decolonization, new colonization, and persistence of noncolonization. We carried out in vitro testing on gram-negative bacteria isolated from the mouths of patients in the chlorhexidine arm that thus could be considered resistant a priori. Testing for the in vitro MBC of chlorhexidine showed that concentrations $<0.12\%$ inhibited bacterial growth. This indicates that both intrinsic (eg, concentration, duration of rinsing, temperature) and extrinsic (eg, presence or absence of teeth, dentures, or organic material; food or beverage consumption; saliva pH; and electrolytes) factors in the oral cavity inactivated chlorhexidine, and that the possibility of bacterial resistance can be ruled out. We used 0.12% chlorhexidine because this is the most widely available concentration and the most frequently used in studies; there is contradictory evidence about the usefulness of mouthwashes with chlorhexidine compared with mouthwashes of herbal extracts.[8-10]

CONCLUSIONS

Based on these results, we consider that the absence of a statistically significant difference may be owing to a small sample size; however, we cannot reject the hypothesis of the inactivation of chlorhexidine inside the oral cavity. It will be desirable to evaluate chlorhexidine at higher concentrations to avoid possible inactivation and achieve a significant clinical effect.

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