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ORIGINAL ARTICLE

The effect of chlorhexidine on *Acinetobacter baumannii* in intensive care units

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ABSTRACT

Background and Objectives: Measures to prevent the emergence of hospital-acquired infections (HAIs) include a daily bath with chlorhexidine gluconate (CHG). The aim of this study was to determine the effect of patients bathing daily with CHG on the bacterial colonization on patient surfaces, environmental surrounding areas, and attending healthcare workers (HCWs).

Materials and Methods: Patients were randomized by a 1:1 in two groups. Patients in group 1 were bathed daily with CHG; patients in group 2 were bathed with a placebo. Microbiological sampling of patients, environment, and HCWs were carried out on days 0, 3, and 10. The clonal relatedness of selected isolates collected was determined through pulsed-field gel electrophoresis. Clinical and demographic data were obtained from medical files.

Results: Thirty-three patients were included (18 in group 1 and 15 in group 2). The more common species was *Acinetobacter baumannii* (n=144), followed by *Klebsiella pneumoniae* (n=81). *A. baumannii* was isolated more frequently on environmental surfaces in group 2 than group 1 (day 0 vs. day 3 vs. day 10; p = 0.0388). Twelve clones of *A. baumannii* were detected, with predominant clone A detected in patients and environmental surfaces. No pathogens were detected in HCWs.

Conclusion: Our data support that CHG bathing decreases *A. baumannii* surviving on the environmental surfaces of critically ill patients.

Keywords: Infection disease transmission; Chlorhexidine; Decontamination; *Acinetobacter baumannii*; Gram-negative bacteria

INTRODUCTION

Hospital-acquired infections (HAIs) cause significant morbidity and mortality in hospitalized patients (1), primarily those considered to be critically ill. For this reason, the study and control of HAIs have been deeply examined, with particular emphasis on epidemiological surveillance methods (2).

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About 25% of all HAIs develop in critically ill patients. This population is associated with several risk factors for these infections, including the use of invasive devices, the improper use of broad-spectrum antibiotics, as well as prolonged hospital stay (3). Furthermore, almost 40% of HAIs are caused by multidrug-resistant microorganisms, most of which are grouped by the acronym ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*) (1, 4, 5).

Measures to prevent the emergence of HAIs include a daily bath with chlorhexidine gluconate (CHG), which has been shown to reduce overall infection rates (6-8). In addition, previous studies have demonstrated that patients bathed using the traditional method had a higher risk of colonization by Gram-negative bacteria than those who bathed with CHG (6, 9).

To date, limited studies have evaluated whether the decrease in HAIs in critically ill patients bathed with CHG is associated with less colonization on their skin, in their immediate environment, and among healthcare personnel.

This study aimed to determine the effect of patients bathing daily with CHG on the colonization of the ESKAPE group on patient surfaces, environmental surrounding areas, and attending healthcare workers (HCWs).

MATERIALS AND METHODS

Study setting. The study was conducted at the University Hospital "Dr. José Eleuterio González", a third-level hospital with 600 beds in Monterrey, Mexico. This hospital has two intensive care units (ICU) for adults: one with 9 beds, surgical, and 11 medical beds. Both ICUs have approximately 500 hospitalizations per year.

Study design and criteria for inclusion, exclusion, and elimination. This was a prospective, experimental, randomized, double-blind study. All patients admitted to ICU with less than 48 h of hospital stay were included. Patients were excluded when they met one of the following criteria: younger than 18 years, pregnant, burns on more than 20% of total body surface area, or history of a CHG allergy. Patients with an adverse reaction to CHG, defined as the appearance of rash or pruritus, were eliminated. An established hand hygiene program was continued throughout the study.

Ethics approval. This protocol was approved by the Ethical Committee in Investigation at the School of Medicine, Autonomous University of Nuevo León (IF16-00003). Informed consent was requested from patients, patients' relatives, and HCWs who agreed to participate in the study.

Randomization and intervention. Patients were randomized by a 1:1 closed envelope method and separated into two bath groups by a person outside the study. Patients assigned to group 1 were bathed daily with 2% CHG-impregnated wipes (Clorhexi-Wipes One Step; G70 Antisepsis, Leon, Mexico), and hair was washed with no-rinse 0.12% CHG foam shampoo (Chlorhexidine Shampoo One Step; G70 Antisepsis) in addition to a 0.12% chlorhexidine hydrochloride oral solution. CHG products were used according to the supplier's specifications.

Patients assigned to group 2 were bathed daily with towels made of the same components as the CHG wipes but without CHG, and standard shampoo without CHG was used to wash the scalp. Products for body and hair wash had the same labels, color, and smell as the CHG products. Baths were initiated from the day of admission until discharge from the ICU.

Decontamination procedures were daily done according to hospital protocols using 0.5% sodium hypochlorite impregnated microfibers. Between patients, an intensive cleaning process, including walls and ceiling, was undertaken.

Sampling of patients, environment, and health care workers. Sampling was carried out on days 0, 3, and 10 using the swabbing method as previously described (Williamson-Kligman 24 and HMP Protocol #07-001, version number 12.0) with a few modifications.

For environmental surfaces (bed rails, ventilators, and tables), a delimitation of an approximate area of 3 cm^2 was made for sampling. Then, 1 mL non-ionized solution was applied, and the area was gently scraped with a sterile cotton swab.

Nasal, cubital fossa, retro auricular region, and anorectal region samples from patients and palms and nasal samples of HCWs were obtained by rubbing and rotating the swab premoistened with sterile SCF-1 buf-

fer (50 mM Tris buffer, 1 mM EDTA, 0.5% Tween 20) for 30 s on each site. One swab for each site was used. All samples were placed directly in agar plates.

Microbiological methods. Cultures were processed by standard methods. Samples were cultured on 5% blood agar and eosin-methylene blue agar. All plates were incubated at 37°C for 48 h. All isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system (Bruker Daltonics, Billerica, MA).

Clonal relatedness. The clonal relatedness of isolates collected was determined through pulsed-field gel electrophoresis (PFGE) on representative isolates (10, 11). Tenover criteria were used for interpretation using the Jaccard coefficient (10). SPSS version 21 was used for analysis (IBM Corp., Amonk, N.Y.).

Data collection. Clinical and demographic data were obtained from medical files. Retrieved data included age, gender, length of stay, vital signs, urinary output, laboratory, and outcome. In addition, the following severity scores were used: acute physiology and chronic health evaluation (APACHE) II, Glasgow coma scale, simplified acute physiology score (SAPS) II, and Kirby index (PaO_2/FiO_2).

Statistical analysis. Continuous variables were analyzed as the means and standard deviation. For categorical variables, percentages and frequencies were used. Numerical variables were compared with Student's t-test or with the Wilcoxon test. Categorical variables were analyzed using the chi-square test or Fisher's exact test. Statistical analysis was performed with SPSS version 21.

RESULTS

Study population and clinical data. A total of 1,743 patients were admitted to the ICU from September 2017 to September 2019, and 63 met the inclusion criteria. From these patients, 30 were excluded from the analysis: 8 died, and 22 were transferred outside the ICU before the second swabbing.

Thirty-three patients were included (18 in group 1 and 15 in group 2). Both groups were paired among clinical, laboratory, and severity scores data. The median length of stay was 11 days (IQR, 6.0-15.2) in group 1 and 12 days (IQR, 8.0–19.0) in group 2. Both groups had more surgical patients than medical patients (66.7% vs. 33.3% in group 1 and 60% vs. 40% in group 2, respectively) (Table 1). In total, 33 nurses were sampled.

Three deaths were reported in group 1 (due to abdominal sepsis, meningitis, and cranial trauma) and one in group 2 (due to epidural hematoma). In group 1, 10 (55.5%) patients presented HAIs; 9 ventilator-associated pneumonia (VAP) (*A. baumannii*, n=6; *K. pneumoniae*, n=2; *P. aeruginosa*, n=1), and one urinary tract infection (*P. aeruginosa*). In group 2, 12 (80.0%) patients presented HAIs; 10 VAP (*A. baumannii*, n=5; *K. pneumoniae*, 4; and *P. aeruginosa*, 1); 1 catheter line-associated bloodstream infection (*K. pneumoniae*) and one surgical site infection (*P. aeruginosa*).

Microbiology data. Most isolates were Gram-negative bacilli species (354 Gram-negative and 82 Gram-positive), including *A. baumannii* (n = 144), *K. pneumoniae* (n = 81), and *P. aeruginosa* (n = 20), both from the patient´s body surfaces and from the environment. None of these pathogens were detected in HCWs. Other species of the ESKAPE group were *E. cloacae* (n = 44), *Escherichia coli* (n = 40), *S. aureus* (n = 14), and *E. faecium* (n =14).

Other non-ESKAPE potential pathogens detected were *Enterococcus faecalis* (n = 54), *Klebsiella aerogenes* (n = 9), *Stenotrophomonas maltophilia* (n = 4), *Klebsiella oxytoca* (n = 3), *Serratia marcescens* (n = 2), *Proteus mirabilis, Morganella morganii, Citrobacter koseri Citrobacter freundii, Pantoea calida, Pantoea septica*, and *Achromobacter insolitus* (n = 1 from each).

For *A. baumannii*, group 2 exhibited more isolates on environmental surfaces (bedrails, worktables, and ventilators) than group 1 (day 0 vs. day 3 vs. day 10; p = 0.038). No differences were detected for *K. pneumoniae* and *P. aeruginosa* or other members of the ESKAPE group. When *A. baumannii, K. pneumoniae*, and *P. aeruginosa* bacilli were combined, group 2 had a higher number of isolates from patient bedrails than group 1 (day 0 vs. day 3 vs. day 10; $p = 0.097$). However, these findings were not statistically significant (Table 2). A total of 231 surfaces related to each patient included were sampled at day day 0; *A. baumannii* and *K. pneumoniae* rates colonization on sampled surfaces were 68/231 and 34/231, respectively at day 0 (before bath application).

Clonal diversity. Randomly selected most common species *A. baumannii* and *K. pneumoniae* isolates (in-

Table 1. Clinical and demographic characteristics of the study population

	CHG (n = 18)	No CHG (n = 15)	*p* value
Demographic			
Age, median (IQR)	40.5 (25.2-56.0)	43.0 (37.0-53.0)	0.468
Male, n (%)	13 (72.2)	8 (53.3)	0.261
Length of stay, days, median (IQR)	11.0 (6.0-15.2)	12.0 (8.0-19.0)	0.318
Treatment			
Medical, n (%)	6 (33.3)	6 (40.0)	0.691
Surgical, n (%)	12 (66.7)	9 (60.0)	
Clinical data			
APACHE II score, median (IQR)	12.0 (8.0-15.2)	12.0 (10.0-16.0)	0.716
SAPS II score, median (IQR)	41.0 (32.2-47.5)	46.0 (37.0-51.0)	0.262
Glasgow coma scale, median (IQR)	10.0 (8.0-11.0)	10.0 (8.0-11.0)	0.950
Temperature (°C), median (IQR)	37.2 (26.7-37.8)	37.6 (36.8-38.0)	0.496
Systolic blood pressure (mmHg), median (IQR)	110.0 (93.7-132.5)	100.0 (85.0-120.0)	0.607
Mean arterial pressure (mmHg), median (IQR)	80.0 (70.0-96.2)	82.0 (72.0-98.0)	0.852
PaO_2/FiO_2, median (IQR)	188.5 (139.5-214.0)	197.0 (145.0-214.0)	0.973
FiO_2, median (IQR)	75.0 (60.0-85.0)	75.0 (60.0-85.0)	0.889
pH, median (IQR)	7.41 (7.38-7.43)	7.40 (7.28-7.43)	0.465
Heart rate, median (IQR)	83.0 (60.0-103.5)	78.0 (64.0-103.0)	0.739
Respiratory rate, median (IQR)	17.0 (13.0-21.0)	16.0 (11.0-22.0)	0.834
Urinary output (mL/day), median (IQR)	1043 (721.0-1208.0)	963.0 (794.0-1175.0)	0.768
Laboratories			
Sodium (mEq/L), median (IQR)	139.0 (132.0-144.2)	142.0 (132.0-149.0)	0.685
Potassium (mEq/L), median (IQR)	3.8 (3.2-4.9)	4.2 (3.6-5.0)	0.506
Creatinine (mg/dL), median (IQR)	1.1 (0.9-1.6)	1.2 (0.9-1.5)	0.779
Hematocrit (%), median (IQR)	41.0 (33.0-47.2)	36.0 (29.0-43.0)	0.216
Leucocytes ($\times 10^6$ cells/mm^3), median (IQR)	10.5 (6.2-14.7)	11.4 (5.7-15.3)	0.838
BUN (mg/dL), median (IQR)	26.0 (14.2-64.7)	28.0 (16.0-59.0)	0.861
Bicarbonate (mEq/L), median (IQR)	19.0 (16.7-21.0)	19.0 (16.0-22.0)	0.629
Total bilirubin (mg/dL), median (IQR)	4.1 (0.9-4.9)	3.8 (1.9-5.2)	0.809
Outcome			
In-hospital mortality, n (%)	3 (16.67)	1 (6.6)	*

APACHE: chronic health evaluation; SAPS: simplified acute physiology score; IQR: interquartile range.

cluding isolates form all kinds of specimens) were analyzed by PFGE (89 *A. baumannii* and 49 *K. pneumoniae*), and 22 distinct PFGE patterns were observed for *K. pneumoniae* and 41 for *A. baumannii*. Similarity percentages ranged from 75% to 100%.

For *A. baumannii*, 12 clones were detected (Fig. 1, clones A-L), with predominant clone A detected in nostrils, retro auricular, and antecubital fossae of 3 patients, as well as on Tables, bed rails, and ventilator surfaces. For *K. pneumoniae*, nine clones were detected (Fig. 2, clones A-I). *K. pneumoniae* isolates were recovered from the rectum, nostrils, and retro auricular fossae of 2 patients.

DISCUSSION

In this study, we assessed the effect of bathing patients daily with CHG on colonization on patients, in their environments, and among healthcare personnel caring for them. Our results showed that CHG baths significantly decreased the amount of *A. baumannii* in the nearby surrounding areas of patients, and this may reduce the risk of HAIs.

A. baumannii is one of the most important causative agents of VAP (12), and the reduction of this bacterial species may lead to less VAP cases. In our study, HAIs were more common in patients with a placebo

Table 2. Distribution of *K. pneumoniae, A. baumannii* and *P. aeruginosa* isolates among patients and environmental surfaces

		Day			*p* value			
K. pneumoniae	**Group**	**0**	**3**	**10**	**0 vs. 3**	**0 vs. 10**	**3 vs. 10**	**0 vs. 3 vs. 10**
Tables (n)	1	0	2	1	1.000	1.000	0.583	1.000
	2	0	5	1				
Bedrails (n)	1	0	0	0	NE	NE	NE	NE
	2	0	1	2				
Ventilators (n)	1	2	6	2	0.594	0.628	0.685	0.202
	2	2	4	1				
Nasal (n)	1	3	4	2	0.689	0.500	0.500	0.181
	2	2	3	3				
Retro auricular (n)	1	1	0	2	0.400	0.714	0.300	0.660
	2	1	3	3				
Cubital fossa (n)	1	0	0	0	NE	NE	NE	NE
	2	0	2	4				
Anorectal (n)	1	2	5	2	0.296	0.651	0.412	0.370
	2	4	3	3				
All environmental surfaces (n)	1	2	8	3	0.631	0.651	0.648	0.582
	2	2	10	4				
All corporal surfaces (n)	1	6	9	6	0.613	0.320	0.298	0.431
	2	7	11	13				
A. baumannii								
Tables (n)	1	2	4	5	0.666	0.500	0.500	0.537
	2	1	3	6				
Bedrails (n)	1	4	2	3	0.156	0.181	0.617	0.113
	2	2	6	7				
Ventilators (n)	1	5	7	3	0.267	0.157	0.418	0.169
	2	2	8	6				
Nasal (n)	1	1	1	3	0.523	0.618	0.545	0.541
	2	1	4	6				
Retroauricular (n)	1	3	3	1	0.484	0.666	0.706	0.474
	2	4	7	2				
Cubital fossa (n)	1	2	3	4	0.713	0.383	0.328	0.523
	2	4	6	3				
Anorectal (n)	1	1	1	1	0.642	0.700	0.523	0.708
	2	2	4	1				
All environmental surfaces (n)	1	11	13	11	0.090	0.038	0.396	0.038
	2	5	17	19				
All corporal surfaces	1	7	8	9	0.311	0.530	0.205	0.461
	2	11	21	12				
P. aeruginosa								
Tables (n)	1	0	1	0	1.000	NE	1.000	1.000
	2	0	0	0				
Bedrails (n)	1	0	0	1	NE	1.000	1.000	1.000
	2	0	0	0				
Ventilators (n)	1	0	3	1	1.000	1.000	0.800	1.000
	2	0	1	0				
Nasal (n)	1	0	1	1	1.000	1.000	0.700	1.000
	2	0	1	2				

Table 2. Continuing...

Retroauricular (n)	1	0	0	1	1.000	1.000	0.500	1.000
	2	0	1	0				
Cubital fossa (n)	1	0	2	1	1.000	1.000	1.000	1.000
	2	0	0	0				
Anorectal (n)	1	0	1	0	1.000	1.000	0.333	1.000
	2	0	0	2				
All environmental surfaces (n)	1	0	4	2	1.000	1.000	0.714	1.000
	2	0	1	0				
All corporal surfaces	1	0	4	3	1.000	1.000	0.383	1.000
	2	0	2	4				
All three species								
Tables	1	2	7	6	0.500	0.500	0.637	0.475
	2	1	8	7				
Bedrails	1	4	2	4	0.118	0.165	0.523	0.097
	2	2	7	9				
Ventilators	1	7	16	6	0.453	0.265	0.333	0.346
	2	4	13	8				
Nasal	1	4	6	6	0.438	0.296	0.475	0.316
	2	3	8	11				
Retroauricular	1	4	3	4	0.238	0.681	0.238	0.362
	2	5	11	5				
Cubital Fossa	1	2	5	5	0.621	0.572	0.595	0.573
	2	4	8	7				
Anorectal	1	3	7	3	0.363	0.690	0.363	0.455
	2	6	7	6				
All environmental surfaces (n)	1	13	25	21	0.136	0.155	0.559	0.117
	2	7	28	23				
All corporal surfaces	1	13	21	18	0.536	0.464	0.466	0.496
	2	18	30	29				

(80%) than in patients with CHG bath (55%), and the most frequent pathogen associated with HAIs was *A. baumannii* (n=11) and in all cases was associated to VAP.

Multiple studies have demonstrated that daily bathing with CHG is a cost-benefit approach in terms of nursing time that may reduce infections caused by Gram-negative bacteria, including *A. baumannii* (13, 14). However, two meta-analyses have shown the opposite. The first included 34,895 patients and demonstrated that the CHG bath does not lower the risk of Gram-negative infections (including *Acinetobacter* spp.) in the ICU compared with traditional bathing method (p = 0.24) (15). The second study included 22,850 patients and showed similar results (16).

It has been reported that exposure to sublethal CHG levels may enhance resistance to this antiseptic. Indeed, a pan-resistant isolate of *A. baumannii* that was able to grow in 1% CHG has been reported (17). For this reason, it is essential to survey the compliance with CHG bathing to reduce the exposure to suboptimal concentrations of CHG and the risk of acquiring CHG resistance. In our study, the compliance was 100%; thus, a low generation of resistance was expected.

Our findings show a lower number of isolates from patient bedrails was detected when patients were bathed with CHG, and although this result was not statistically significant (p = 0.097), it underscores that bedrails deserve special attention when cleaning and disinfecting because patients and HCWs frequently touch it.

Concerning Gram-positive bacteria, CHG has been reported to prevent colonization by *Enterococcus* spp. resistant to vancomycin in critically ill patients (7, 8). In our study, *Enterococcus* spp. was isolated in

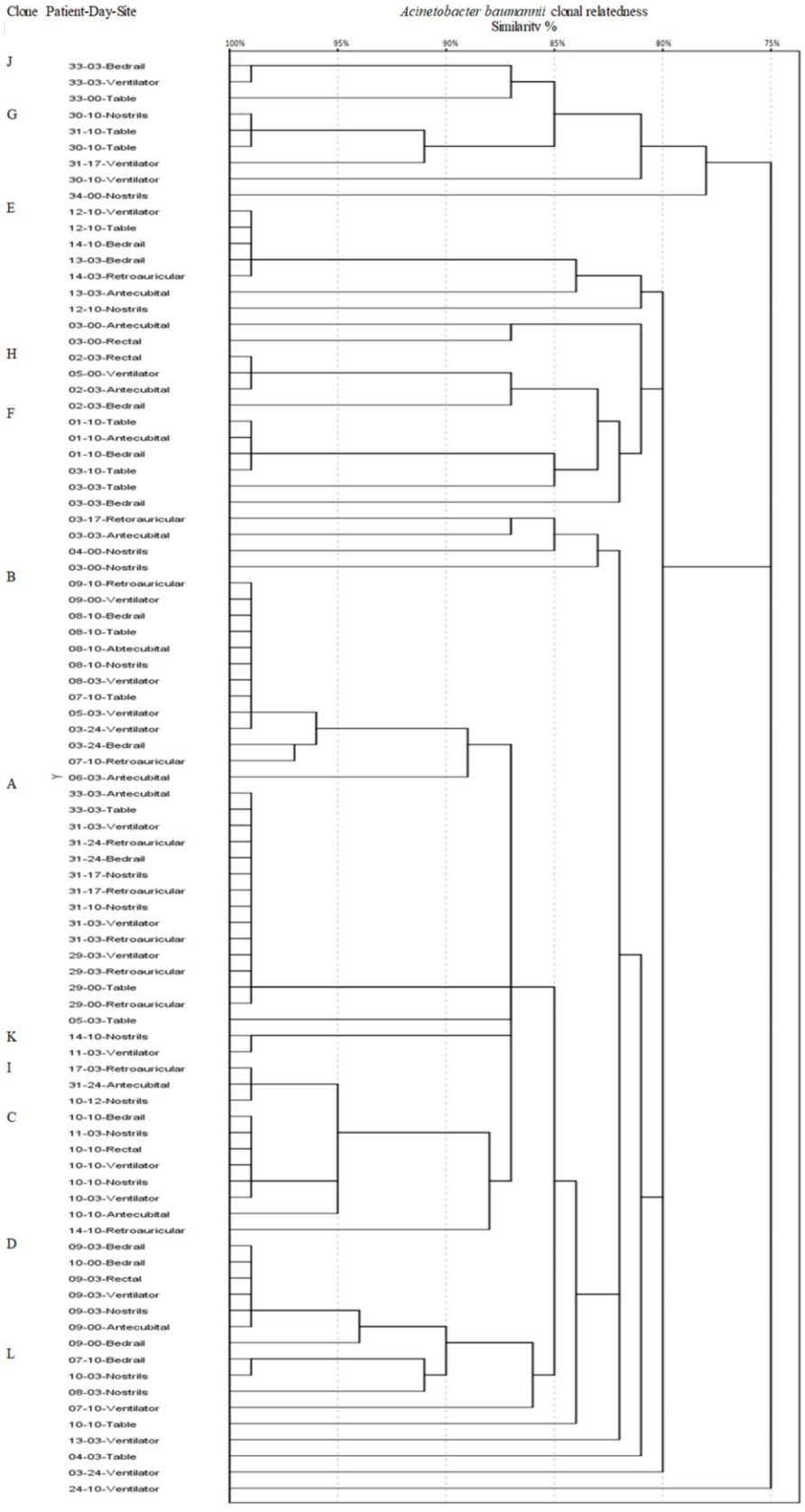

Fig. 1. Clonal relatedness of selected *A. baumannii* isolates. Tenover criteria were used for interpretation using the Jaccard coefficient

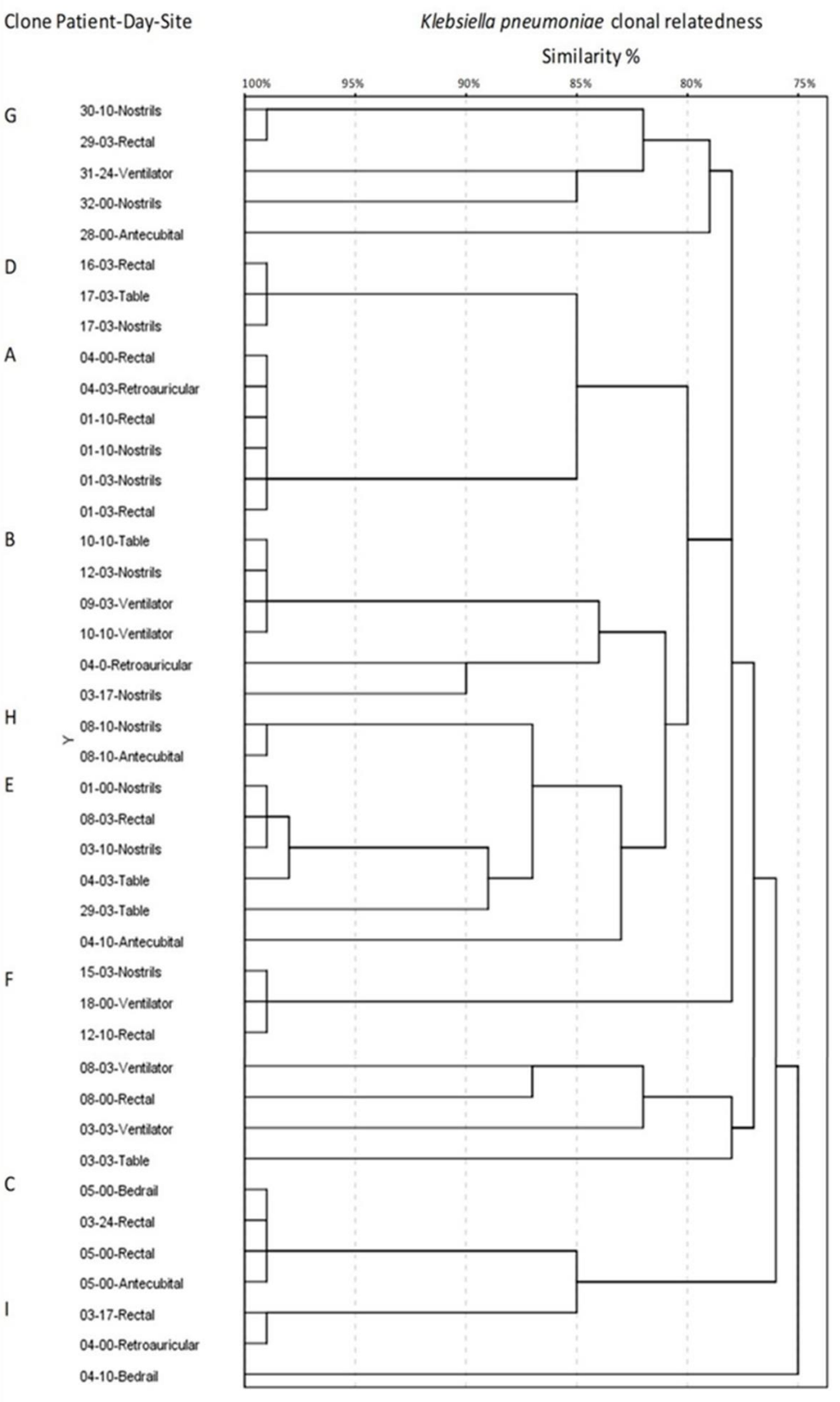

Fig. 2. Clonal relatedness of selected *K. penumoniae* isolates. Tenover criteria were used for interpretation using the Jaccard coefficient

several patients; however, no significant differences were detected.

Our study has some limitations. We did not evaluate the skin concentrations of CHG after bathing to ensure compliance. In addition, minimal inhibitory concentrations to CHG to evaluate the potential generation of resistance was not determined.

In conclusion, our data support that bathing patients with CHG decreases *A. baumannii* surviving on environmental surfaces surrounding of critically ill patients and that the bedrails of patients deserve special attention when cleaning and disinfecting patients' beds.

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