SHORT COMMUNICATION

Microbial diversity and colonization patterns of two step-down care units from a tertiary care hospital

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Nosocomial surfaces are potential pathogen reservoirs. Our aim was to describe the microbial diversity and analyze microbial patterns of healthcare-associated pathogens in two step-down-care-units at a tertiary care hospital. We monitored infected patients over 45 days to describe microbial diversity and colonization patterns. A total of 2762 isolates were recovered from the sampled sites, coagulase-negative staphylococci represented 44.64% (1233/2762) of the isolates. The most frequently recovered ESKAPE species (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter cloacae*) were *A. baumannii* (7.53%; 208/2762 isolates) and *E. faecium*/*Enterococcus faecalis* (5.18%; 143/2762). We recovered a high diversity of species, including potential pathogens. *A. baumannii* was detected more frequently on diverse surfaces and persisted in patients' nostrils during the hospital stay.

Key words: colonization, ESKAPE, hospital microbiota, nosocomial surface contamination, pathogen dissemination, transmission pathogens

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INTRODUCTION

The development of healthcare-associated infections (HAI) is associated with patients' colonization by pathogens from the hospital environment.[1-3] The ESKAPE group members (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter cloacae)* are identified as frequent causative agents of HAI that rapidly disseminate through the nosocomial environment and have high drug resistance profiles.[4,5] In a previous study, the possible transmission routes of HAI causative agents in patients of the two step-down care units (SDCUs) were investigated. Causative agents were present on environmental surfaces and medical devices before and after HAI onset.[6] Here, we describe an additional analysis concerning all isolates; our aim was to analyze the microbial diversity on surfaces, nurses, visitors, and infected patients over time in two SDCU.

MATERIALS AND METHODS

This study was prospectively conducted in two 40-bed SDCUs at Hospital Civil de Guadalajara "Fray Antonio Alcalde," a tertiary care hospital in Mexico. Recently admitted adult patients (18 years or older, no recent hospitalizations during 30 days previous to hospital

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admission, without evidence of infection at admission or during 48 h after admission) were included in the study. Recently, cleaned high contact areas (bedrail and table near patients) were also included in the study.

Samples were obtained according to the Human Project Protocol #07-001, version number 12.0. Samples from nostrils, antecubital, and retroauricular crease were obtained from patients, their relatives, and SDCU primary caretaker nurses. Hand swabs were collected only from the patients' relatives and nurses. Environmental surfaces near the patient and medical devices (exposed mechanical ventilation tubes, central venous, and urinary catheters) were also sampled.

Samples were collected at admission to SDCU, on day 3, and every 5 days until the patient left the unit. Samples from nurses were collected once during the 1st week of the study. All samples were cultured by standard methods, and recovered species were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry system (Bruker Daltonics, Billerica, MA, USA).

This study was approved by the Research Ethical Committee of the Hospital Civil de Guadalajara "Fray Antonio Alcalde" (research registration no. 118/17). Informed consent was obtained from all patients, patient's relatives, and nurses who agreed to participate in the study.

The frequency of all recovered species per sampled sites was determined. The association between clinically relevant species and sampled sites was determined using the Chi-square test or Fischer's exact test. According to the length of stay (LOS), colonization differences between patients were determined by the Kruskal–Wallis test. All statistical analysis was performed with IBM SPSS Statistics 25 (Armonk, NY, USA).

RESULTS

Eight patients and their surrounding areas, eight patient relatives, and 35 SDCU primary caretaker nurses were included in this study. All patients included were men, with an average LOS of 24 ± 15 days. Four hundred and twenty-six samples were collected.

A total of 2868 isolates were recovered from the sampled sites (914 from nurses, 679 from patients, 502 from patient relatives, 465 from environmental surfaces, and 308 from medical devices); only 2762 were identified by MALDI-TOF.

Among the ESKAPE group, the most frequent species recovered was *A. baumannii* (7.53%; 208/2762 isolates), followed by *Enterococcus* faecium/*Enterococcus faecalis* (5.18%; 143/2762), *K. pneumoniae*/*Klebsiella aerogenes* (3.4%; 94/2762), *S. aureus* (1.52%; 42/2762), *E. cloacae*/*Enterobacter asburiae* (1.44%; 40/2762), and *P. aeruginosa* (0.51%; 14/2762).

Coagulase-negative staphylococci (CoNS) represented 44.64% of the isolates (1233/2762); *Staphylococcus epidermidis* (54.17%; 668/1233) was the most frequent species, followed by *Staphylococcus hominis* (26.76%; 330/1233).

Commensal microorganisms, including staphylococci, decreased over time, and a high Gram-negative diversity was observed on day 8 in all patients [Figure 1a-h]. *Acinetobacter* spp. was recovered from the environmental surfaces of seven patients. High microbial diversity was observed among patients' relatives over time, including CoNS, *A. baumannii, K. pneumoniae, K. aerogenes, Stenotrophomonas maltophilia, and E. faecalis* [Figure 1a-h]. In addition, *A. baumannii*, *E. cloacae, K. pneumoniae, Pseudomonas* spp., and *S. aureus* were recovered from ten nurses [Figure 1i].

Diverse associations between recovered species and sampled sites were found, including *A. baumannii* in patients' antecubital crease ($P = 0.045$), bedrail ($P = 0.000$), table ($P = 0.016$), and mechanical ventilation tube ($P = 0.005$) [Table 1], and all sample sites from nurses ($P < 0.05$) [Table 2]. In addition, *A. baumannii* persisted in nostrils patients ($P = 0.007$).

DISCUSSION

Nosocomial surfaces act as reservoirs of potential pathogens and may contribute to the patient's colonization and subsequent HAI development.[3,7,8] In our study, the high contact surfaces near the hospitalized patients were colonized by skin microbiota and were gradually colonized over time by potential pathogens. *S. epidermidis* was the most frequently recovered CoNS species from all the environmental and corporal surfaces; this species is described as a significant nosocomial pathogen.[9]

Gram-negative pathogens were recovered from patients' corporal surfaces, increasingly recovered over time, and inversely associated with microbiota proportion in patients. Gram-negative pathogens on patient relatives were recovered mainly from the palmar surface. This result could be an aspect for hospitals to consider to prevent the spread of microorganisms in the hospital environment and reduce infection risk in the community.

A correlation between positive cultures from infected patients and high environmental contamination has been previously reported.[8] In our study, diverse associations between bacterial species and environmental samples were found. In addition, all monitored patients developed at least

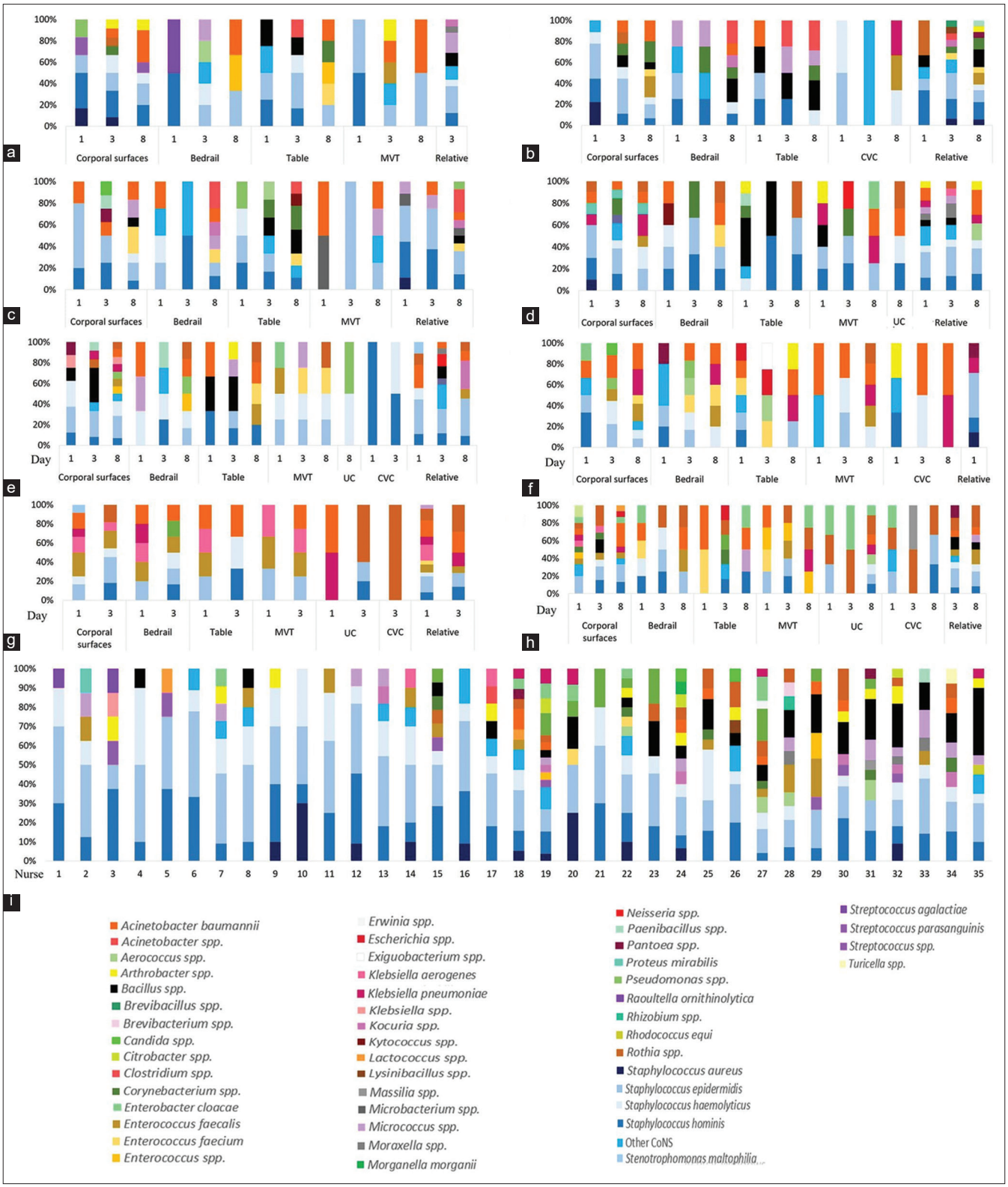

Figure 1: Frequency of recovered species from patient-related surfaces, patients' relatives, and nurses. CoNS=Coagulase-negative staphylococci, CVC=Central venous catheter, MVT=Mechanical ventilation tube, UC=Urinary catheter. (a) Patient 4, (b) Patient 5, (c) Patient 6, (d) Patient 7, (e) Patient 8, (f) Patient 9, (g) Patient 10, (h) Patient 11, (i) Nurses. P10 died before day 8

one HAI,[6] and the presence of some causative agents on the sampled surfaces (bedrail and table) was demonstrated in patients who subsequently developed an infection.

Some aspects, such as prolonged LOS and increased mechanical ventilation time, are risk factors for developing ventilator-associated pneumonia.[10] In this study,

Table 1: Distribution of clinical importance microbiota isolates among patients, environmental surfaces, and medical devices

	Patients			Environmental surfaces		Medical devices		
	AC (*n*=29), *n* (%); *P*	Nostrils (*n*=29), *n* (%); *P*	RC (*n*=29), *n* (%); *P*	Bedrail (*n*=29), *n* (%); *P*	Table (*n*=29), *n* (%); *P*	CVC (*n*=18), *n* (%); *P*	MVT (*n*=18), *n* (%); *P*	UC (*n*=17), *n* (%); *P*
Enterococcus faecium	1 (3); 0.470	3 (10); 0.414	3 (10); 0.414	5 (17); 0.018*	8 (28); 0.000*	0; 0.622	3 (12); 0.404	0; 0.618
Enterococcus faecalis	11 (38); 0.001*	5 (17); 0.838	8 (28); 0.075	6 (21); 0.461	4 (14); 0.748	1 (6); 0.320	7 (27); 0.160	2 (12); 1.000
Staphylococcus aureus	0; 0.143	4 (14); 0.096	1 (3); 0.496	0; 0.144	0; 0.143	0; 0.620	1 (4); 1.000	0; 0.616
Klebsiella pneumoniae	5 (17); 0.670	10 (35); 0.002*	9 (31); 0.009*	5 (17); 0.666	3 (10); 0.506	4 (22); 0.314	8 (31); 0.038*	4 (24); 0.289
Klebsiella aerogenes	1 (3); 0.944	4 (14); 0.003*	0; 0.275	1 (3); 0.946	1 (3); 0.944	0; 1.000	2 (8); 0.248	0; 1.000
Acinetobacter baumannii	14 (48); 0.045*	12 (41); 0.239	12 (41); 0.239	18 (62); 0.000*	15 (52); 0.016*	5 (28); 0.802	15 (58); 0.005*	7 (41); 0.427
Pseudomonas aeruginosa	3 (10); 0.003*	3 (10); 0.003*	1 (3); 0.671	0; 0.393	0; 0.392	0; 1.000	2 (8); 0.115	1 (6); 0.332
Enterobacter cloacae	1 (3); 0.470	6 (21); 0.002*	2 (7); 0.962	1 (3); 0.472	2 (7); 0.962	2 (11); 0.342	4 (15); 0.086	3 (18); 0.097
Escherichia spp.	2 (7); 0.036*	0; 0.445	1 (3); 0.506	1 (3); 0.504	4 (14); 0.000*	0; 1.000	0; 1.000	0; 1.000
Staphylococcus epidermidis	19 (66); 0.446	23 (79); 0.331	26 (90); 0.025*	18 (62); 0.257	13 (45); 0.001*	6 (33); 0.001*	20 (77); 0.656	6 (35); 0.002*
Staphylococcus hominis	21 (72); 0.006*	4 (14); 0.000*	15 (52); 0.653	18 (62); 0.106	18 (62); 0.109	6 (33); 0.237	6 (23); 0.014*	7 (41); 0.629
Staphylococcus haemolyticus	13 (45); 0.070	4 (14); 0.049*	12 (41); 0.164	16 (55); 0.002*	7 (24); 0.479	5 (28); 1.000	9 (35); 0.659	7 (41); 0.293
Proteus mirabilis	0; 0.509	4 (14); 0.000*	0; 0.509	0; 0.510	0; 0.509	1 (6); 0.226	0; 1.000	0; 1.000
Stenotrophomonas maltophilia	1 (3); 0.998	6 (21); 0.000*	1 (3); 0.998	1 (3); 1.000	1 (3); 0.998	1 (6); 0.476	2 (8); 0.225	0; 1.000
Pantoea spp.	2 (7); 0.160	0; 0.347	0; 0.347	1 (3); 0.814	0; 0.347	0; 1.000	1 (4); 0.528	0; 1.000

*Statistically significant. AC=Antecubital crease; CVC=Central venous catheter; MVT=Mechanical ventilation tube; RC=Retroauricular crease; UC=urinary catheter

Table 2: Distribution of clinical importance microbiota isolates among nurses and patient's relatives

	Nurses				Patients' relatives			
	AC (*n*=35), *n* (%); *P*	Nostrils (*n*=35), *n* (%); *P*	RC (*n*=35), *n* (%); *P*	PS (*n*=35), *n* (%); *P*	AC (*n*=22), *n* (%); *P*	Nostrils (*n*=22), *n* (%); *P*	RC (*n*=22), *n* (%); *P*	PS (*n*=22), *n* (%); *P*
Enterococcus faecium	2 (6); 0.811	0; 0.099	0; 0.099	0; 0.099	2 (9); 0.642	1 (5); 0.680	1 (5); 0.680	0; 0.198
Enterococcus faecalis	2 (6); 0.086	4 (11); 0.451	4 (11); 0.451	4 (11); 0.451	7 (32); 0.036*	1 (5); 0.135	1 (5); 0.135	2 (9); 0.370
Staphylococcus aureus	3 (9); 0.594	8 (23); 0.000*	1 (3); 0.367	6 (17); 0.007*	0; 0.206	(18); 0.022*	0; 0.206	0; 0.206
Klebsiella pneumoniae	2 (6); 0.122	2 (6); 0.122	1 (3); 0.041*	0; 0.011*	3 (14); 0.901	4 (18); 0.620	2 (9); 0.456	1 (5); 0.192
Klebsiella aerogenes	0; 0.227	2 (6); 0.507	0; 0.227	0; 0.227	1 (5); 0.826	1 (5); 0.826	1 (5); 0.826	2 (9); 0.167
Acinetobacter baumannii	2 (6); 0.001*	1 (3); 0.000*	1 (3); 0.000*	5 (14); 0.022*	10 (46); 0.150	9 (41); 0.333	6 (27); 0.656	5 (23); 0.360
Pseudomonas aeruginosa	0; 0.343	0; 0.343	0; 0.343	0; 0.343	0; 0.460	0; 0.460	0; 0.460	0; 0.460
Enterobacter cloacae	1 (3); 0.345	3 (9); 0.641	2 (6); 0.811	2 (6); 0.811	0; 0.198	0; 0.198	0; 0.198	0; 0.198
Escherichia spp.	0; 0.398	0; 0.398	0; 0.398	0; 0.398	0; 0.509	0; 0.509	0; 0.509	0; 0.509
Staphylococcus epidermidis	17 (49); 0.002*	34 (97); 0.000*	33 (94); 0.002*	28 (80); 0.242	14 (64); 0.406	21 (96); 0.010*	20 (91); 0.038*	12 (55); 0.072
Staphylococcus hominis	30 (86); 0.000*	4 (11); 0.000*	21 (60); 0.129	20 (57); 0.243	13 (59); 0.272	2 (9); 0.000*	6 (27); 0.049*	16 (73); 0.016*
Staphylococcus haemolyticus	15 (43); 0.082	4 (11); 0.013*	7 (20); 0.180	20 (57); 0.000*	7 (32); 0.845	1 (5); 0.008*	1 (5); 0.008*	2 (9); 0.028*
Proteus mirabilis	0; 0.465	1 (3); 0.436	0; 0.465	0; 0.465	0; 0.569	0; 0.569	0; 0.569	0; 0.569
Stenotrophomonas maltophilia	0; 0.243	0; 0.243	0; 0.243	0; 0.243	1 (5); 0.774	0; 0.362	0; 0.362	1 (5); 0.774
Pantoea spp.	0; 0.298	0; 0.298	1 (3); 0.972	1 (3); 0.972	1 (5); 0.601	1 (5); 0.601	3 (14); 0.001*	1 (5); 0.601

*Statistically significant. AC=Antecubital crease; PS=Palmar surface; RC=Retroauricular crease

A. baumannii was the only species with statically significant persistence throughout LOS in patients' nostrils, which would suggest that LOS is related to the probability of colonization, and therefore, to a higher risk of developing ventilator-associated pneumonia in SDCU.

CONCLUSION

To the best of our knowledge, this is the first study carried out in SDCUs at a tertiary care hospital in Mexico to analyze microbial diversity and colonization patterns. The number of patients included is a limitation in our study. Nevertheless, we recovered a high microbial diversity; potential pathogens (such as ESKAPE group and CoNS) colonized environmental surfaces, patients, nurses, and patients' relatives, suggesting that nosocomial surfaces are reservoirs for pathogens. Further studies are needed to clarify colonization's contribution by these pathogens in developing HAIs in SDCUs.

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Conflicts of interest

There are no conflicts of interest.

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