Multidrug-Resistant Microorganisms Colonizing Lower Extremity Wounds in Patients in a Tertiary Care Hospital, Lima, Peru

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Abstract. Multidrug-resistant organism (MDRO) infections cause high morbidity and mortality, and high costs to patients and hospitals. The study aims were to determine the frequency of MDRO colonization and associated factors in patients with lower-extremity wounds with colonization. A cross-sectional study was designed during November 2015 to July 2016 in a tertiary care hospital in Lima, Peru. A wound swab was obtained for culture and susceptibility testing. MDRO colonization was defined if the culture grew with methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and/or extended spectrum beta-lactamase (ESBL) microorganisms. The frequency of MDRO wound colonization was 26.8% among the 97 patients enrolled. The most frequent MDRO obtained was ESBL-producing *Escherichia coli*, which was significantly more frequent in chronic wounds versus acute wounds (17.2% versus 0%, $P < 0.05$). Infection control measures should be implemented when patients with chronic lower-extremity wounds are admitted.

INTRODUCTION

Infections caused by multidrug-resistant microorganisms (MDROs) are a public health concern as they are mainly associated with health-care settings, cause high mortality rates, have a limited number of therapeutic options for treatment, and increase costs to patients and hospitals.¹ Although patients carrying these MDROs should be under adequate infection control measures to prevent transmission in hospital settings, it is difficult to comply among hospitals from low- or middle-income settings, as investment in human and economic resources for implementing infection control programs at a national or local level in these areas remains low.²

MDRO-colonized patients act as reservoirs and become potential sources of transmission in hospital settings. The most frequent MDROs seen in hospital settings are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended spectrum beta-lactamase (ESBL) producing enterobacterial microorganisms, and multidrug resistant non-fermenting gram-negative bacilli.³

Staphylococcus aureus may colonize skin and mucous membranes of humans, but nasal mucosa is the most commonly colonized area. MRSA nasal colonization prevalence ranged from 0.45% to 28% in in- and outpatient populations.^{4,5} VRE colonization is mainly isolated in rectal and/or perianal regions, but can also colonize other skin areas.⁶ The prevalence of VRE colonization ranged between 11.3% and 94% in patients from high-risk units, such as intensive care units (ICUs).^{7,8} In Peru, VRE colonization was found in 11.5% of patients in high-risk units of a tertiary care hospital.⁹ Few data about skin or wound colonization are available regarding ESBL-producing enterobacterial microorganism colonization in Peru. Previous studies have reported frequencies of

ESBL-producing enterobacterial microorganisms causing rectal colonization in a wide range from 2% to 49% in ICUs.^{10,11} Different studies have shown that ESBL-producing enterobacterial microorganisms are an alarming problem in Peru; more than 70% of ESBL production had been seen among *Klebsiella* sp. and *Escherichia coli* isolated from blood cultures.¹²

The study aims were to determine the frequency of colonization of multidrug-resistant microorganisms in patients with lower extremity wounds and to describe associated factors of colonization.

METHODS

A cross-sectional study was performed during November 2015 to July 2016. All recruited patients were 18 years or older with lower-extremity skin wounds. These patients came from the outpatient clinics and wards of Hospital Cayetano Heredia, a 420-bed, third-level, public hospital in Lima, Peru. Wounds were classified according to duration; acute, if the duration was less than 4 weeks and chronic, if it was more than 4 weeks. The Hospital Ethics Committee approved the study and informed consent was obtained from all study participants.

Data collection included a questionnaire with demographic and clinical data, including the use of antimicrobials. One swab was obtained from the patient after the surface of the wound was vigorously washed by saline. Swabs were transported to the laboratory and were inoculated into four media cultures: blood agar, mannitol agar, McConkey agar, and bile esculin agar to obtain the isolation of the *S. aureus*, *Enterococcus* sp., and gram-negative microorganisms. Bacterial identification and susceptibility testing by disk diffusion method were done following standard procedures,¹³ except for vancomycin susceptibility of *Enterococcus* sp. for which the E-test method (bioMérieux, Marcy-L'Etoile, France) was used.

For statistical analysis, qualitative variables were analyzed by frequencies. Discrete quantitative variables with normal distribution were analyzed by mean and standard deviation;

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for those without normal distribution, median and interquartile ranges were used. Student *t* and χ^2 tests were performed to compare continuous and categorical variables with normal distribution, respectively, and Mann-Whitney *U* test for quantitative variables without normal distribution, using Stata 14 (College Station, TX); *P* < 0.05 was defined as significant.

RESULTS

Ninety-seven patients were recruited. Of these, 64 (66%) had chronic wounds, 71.1% were male, and the median age was 58 years (interquartile range [IR]: 51–64). Overall, 85.6% of patients had received antibiotics in the last 3 months. Ciprofloxacin and clindamycin were the most frequently prescribed antibiotics with a frequency of 56/97 (57.7%) for each (Table 1).

At least one MDRO was found to be colonized in 26.8% (26/97) of lower limb wounds. A higher frequency of history of hospitalization in the past 3 months was found among patients carrying a MDRO (53.8%) compared with those noncolonized (32.4%), but this difference did not reach statistical significance (*P* = 0.05). More frequently, patients with chronic wounds were found to be carrying a MDRO (80.8%), compared with those noncolonized (60.6%), but this difference was not significant (*P* = 0.06). No major differences were found regarding age, sex, presence of diabetes, arterial hypertension, peripheral neuropathy, and history of hospitalization in the previous year (Table 1).

At least one gram-positive coccus and gram-negative bacillus were detected in 70.1% and 62.9% of patients, respectively. The frequency of detection of 1, 2, and 3 MDROs was 16.5%, 9.3%, and 1.0%, respectively. Overall, at least one ESBL-producing enterobacterial microorganisms was detected in 23.7% of patients. However, ESBL-producing *E. coli* was isolated only in patients with chronic wounds (17.2% versus 0%, *P* < 0.05). MRSA was detected in 4.1% of isolates. No VRE isolates were detected (Table 2).

A total of 184 isolates were found in the 97 patients enrolled, of which 103 (56%) were gram-negative bacilli. *Escherichia coli* (*N* = 22, 12%) and *Klebsiella pneumoniae* (*N* = 19, 10.3%) were the most frequent microorganisms isolated. Overall, the

frequency of ESBL detection among *E. coli*, *Klebsiella*, *Proteus*, and *Enterobacter* ranged between 26.7% and 63.2%. Among 81 (44%) gram-positive cocci, *Enterococcus* sp. (*N* = 31, 16.8%) and *S. aureus* (*N* = 29, 15.8%) were the most frequently isolated, four (13.8%) of *S. aureus* isolates were MRSA (Table 3).

DISCUSSION

The overall frequency of MDRO colonization in lower-extremity wounds was 26.8%. A higher frequency was found among patients with chronic wounds and with a previous history of hospitalization in the last 3 months. Almost 90% of MDRO colonized patients carried one ESBL-producing enterobacterial microorganism. Additionally, a significant difference was found among ESBL-producing *E. coli* colonization based on wound duration, as only patients with chronic wounds carried these microorganisms. ESBL-producing microorganisms are an overwhelming problem in Latin America compared with other regions of the world. One study showed that the frequency of ESBL production among *Klebsiella* in Latin America was 34% compared with 10% in North America, and 20% in Europe (Hawser). In Peru, ESBL production among *E. coli* and *Klebsiella* is even higher. More than 70% of *Klebsiella* and *E. coli* blood isolates produced ESBLs.¹⁴ This may explain why ESBL-producing microorganism colonizing wounds was the most common MDRO detected in this study.

MRSA colonization was low, 4.1%. In Peru, most MRSA isolates have shown molecular characteristics of hospital-acquired infections. Community-associated MRSA infections are emerging in Latin America but are still considered rare in Peru.¹⁵ Since most study patients came from the outpatient clinics, MRSA colonization rate was low. No cases of VRE colonization were found. This finding was comparable with prior literature that found a low prevalence of VRE skin colonization that ranged from 0% to 2.7% in two long-term care facilities located in Germany and Italy, respectively.^{16,17} A potential explanation may be that perianal and/or rectal colonization is a better environment for isolating VRE. However, VRE infections are not as significant of a problem in the Latin American region (3.0%) as compared with the United States (9.9%).¹⁸

TABLE 1
Clinical and epidemiologic characteristics of patients of whom lower-limb wounds were colonized with at least one MDRO or not colonized

Epidemiologic variable	All (<i>N</i> = 97)	Colonized by at least one MDRO (<i>N</i> = 26)	Noncolonized by MDRO (<i>N</i> = 71)	<i>P</i> value
Duration of wound days, median (interquartile range)	56 (15–90)	60 (30–114)	42 (14–90)	0.06
Age-year, mean (standard deviation)	57.9 (±1.06)	56.2 (±1.77)	58.5 (±1.29)	0.93
Male sex, <i>n</i> (%)	69 (71.1)	18 (69.2)	51 (71.8)	0.80
Outpatient, <i>n</i> (%)	70 (72.2)	17 (65.4)	53 (74.6)	0.37
Chronic wound, <i>n</i> (%)	64 (66.0)	21 (80.8)	43 (60.6)	0.06
Presence of diabetes mellitus type II, <i>n</i> (%)	85 (87.6)	24 (92.3)	61 (85.9)	0.40
Presence of arterial hypertension, <i>n</i> (%)	29 (29.9)	7 (26.9)	22 (31.0)	0.70
Presence of peripheral neuropathy, <i>n</i> (%)	69 (71.1)	19 (73.1)	50 (70.4)	0.80
Hospitalization period during the previous year, <i>n</i> (%)	45 (46.4)	15 (57.7)	30 (42.3)	0.18
Hospitalization period during the previous 3 months, <i>n</i> (%)	37 (38.1)	14 (53.8)	23 (32.4)	0.05
Hospitalization at the day of enrolment, <i>n</i> (%)	27 (27.8)	9 (34.6)	18 (25.4)	0.37
Antibiotic used in the last 3 months, <i>n</i> (%)	83 (85.6)	23 (88.5)	60 (84.5)	0.62
Use of clindamycin, <i>n</i> (%)	56 (57.7)	15 (57.7)	41 (57.7)	1.00
Use of ciprofloxacin, <i>n</i> (%)	56 (57.7)	17 (65.4)	39 (54.9)	0.36

MDRO = multidrug-resistant microorganism.

TABLE 2

Multidrug-resistant microorganisms isolated in patients with lower-extremity wound based on duration of the wound

Isolates	Acute wound (N = 33)		Chronic wound (N = 64)		Total	
	n	%	n	%	n	%
At least one bacteria	30	90.9	64	100	94	96.9
Mean of isolates per patient	2.0		1.9		2.0	
At least one gram-negative bacilli	20	60.6	41	64.1	61	62.9
Mean of gram-negative bacilli	1.4		1.8		1.7	
At least one gram-positive coccus	26	78.8	42	65.6	68	70.1
Mean of gram-positive coccus	1.2		1.2		1.2	
At least one MDRO	5	15.2	21	32.8	26	26.8
One MDRO	3	9.1	13	20.3	16	16.5
Two MDRO	2	6.1	7	10.9	9	9.3
Three MDRO	0	0.0	1	1.6	1	1.0
At least one ESBL-producing enterobacterial microorganisms	5	15.2	18	28.1	23	23.7
ESBL-producing <i>Escherichia coli</i>	0*	0.0	11*	17.2	11	11.3
MRSA	1	3.0	3	4.7	4	4.1

ESBL = extended spectrum β lactamase; MDRO = multidrug-resistant microorganism; MRSA = methicillin-resistant *S. aureus*.

* P value < 0.05.

A main limitation of this study is that a cross-sectional survey cannot determine causal relationships. Also, recall bias may be present in the questionnaire completed by each patient. A higher number of cases would be desirable to do additional analysis of association between clinical and epidemiological factors.

In conclusion, in this patient population, at least one of four patients with lower-extremity wounds had MDROs present within their wounds, and nearly all carried at least one ESBL-producing microorganism. It is important to implement infection control measures to limit the transmission of MDRO to susceptible patients who are at high risk to develop severe infections, especially when patients with chronic lower-extremity wounds are admitted to hospital settings.

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TABLE 3

Gram-negative and gram-positive microorganism distribution in patients with lower-extremity wounds based on duration of the wound

Isolates	Acute wound		Chronic wound		Total	
	n	%	n	%	n	%
Gram-negative bacilli	28	15.2	75	40.8	103	56.0
<i>Escherichia coli</i>	4	2.2	18	9.8	22	12.0
% ESBL	0	0.0	11	61.1	11	50.0
<i>Klebsiella pneumoniae</i>	5	2.7	14	7.6	19	10.3
% ESBL	2	40.0	10	71.4	12	63.2
<i>Proteus</i> sp.	5	2.7	11	6.0	16	8.7
% ESBL	2	40.0	3	27.3	5	31.3
<i>Morganella morganii</i>	6	3.3	9	4.9	15	8.2
% ESBL	0	0.0	1	11.1	1	6.7
<i>Enterobacter cloacae</i>	4	2.2	11	6.0	15	8.2
% ESBL	2	50.0	2	18.2	4	26.7
Others	2	1.1	1	0.5	3	1.6
% ESBL	0	0.0	0	0.0	0	0.0
Nonfermenters*	1	0.5	11	6.0	12	6.5
Gram-positive coccus	32	17.4	49	26.6	81	44.0
<i>Staphylococcus aureus</i>	13	7.1	16	8.7	29	15.8
% MRSA	1	7.7	3	18.8	4	13.8
<i>Enterococcus</i> sp.	11	6.0	20	10.9	31	16.8
% VRE	0	0.0	0	0.0	0	0.0
Others	8	4.3	13	7.1	21	11.4
Total	60	32.6	124	67.4	184	100.0

ESBL = extended spectrum β lactamase producing; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant enterococci.

* *Acinetobacter* spp. and *Pseudomonas* spp.

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