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Original Article

Evolution of Antimicrobial Resistance Levels of ESKAPE Microorganisms in a Peruvian IV-Level Hospital

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ABSTRACT

Background: The members of the so-called ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) are a frequent cause of severe infection, ranking among the most relevant causes of hospital infections. In Peru, few studies, often focused in a single ESKAPE microorganism, have been performed, but none providing an overall and comprehensive long-time analysis of the antibiotic resistance of ESKAPE microorganisms. In the present study, the evolution of antimicrobial resistance levels of ESKAPE microorganisms isolated during 2009 - 2010 (Period 1) and 2012 - 2014 (Period 2) in a IV-level hospital in Lima was analyzed.
Materials and Methods: ESKAPE microorganisms were isolated from inpatients clinical samples. Bacterial identification, as well as antimicrobial susceptibility levels for up to 29 antimicrobial agents and presence of Extended-Spectrum β-Lactamases (only established in *K. pneumoniae*) were determined using automatic methods.
Results: Of 9,918 clinical isolates, 1,917/3,777 (50.8%) [JAN/2009-JUN/2010 (Period 1)] and 4764/6141 (46.4%) [JAN/2012-DEC/2014 (Period 2)] belonged to the ESKAPE group (P <0.0001). ESKAPE were more frequent in the intensive care unit (ICU) (P <0.0001). *E. faecium* decreased from 5.1% to 4.1% (P <0.5), *S. aureus* from 10.5% to 7.0% (P <0.05), and *P. aeruginosa* from 12.9% to 11.6% (P <0.05), while, *A. baumannii* increased from 5.0% to 6.7% (P <0.05), mainly related to an increase in ICU isolates (8.4% *vs.* 17.1%; P <0.05). Overall, high levels of antimicrobial resistance were detected, but with few exceptions (*e.g.* vancomycin in *E. faecium*), antibiotic resistance levels remained stable or lower in Period 2. Contrarily, *A. baumannii* showed significantly increased resistance to different cephalosporins, carbapenems and amoxicillin plus sulbactam.
Conclusion: The introduction of a successful extensively drug-resistant *A. baumannii* clone in the ICU is suspected. The isolation of ESKAPE and levels of antibiotic resistance levels have reduced over time.

Keywords: Multidrug resistance; Intensive Care Unit; *Acinetobacter baumannii*; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*

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INTRODUCTION

The description of infections related to pathogenic microorganisms showing resistance to various antimicrobial agents is continuously rising. Accordingly, multidrug resistant (MDR) and extensively drug-resistant microorganisms (XDR) are often described as a cause of infections [1-3], with pan-resistant microorganisms also being reported as a cause of severe hospital-acquired infections [4]. These finding have led to antibiotic resistance currently being considered as one of the most relevant challenges in public health [5].

Antibiotic resistance has a direct impact on human health and behavior. Recent data on the impact of antibiotic resistance in the European Union (EU) have shown that >33,000 deaths and 874,541 disability-adjusted life-years (DALYS) were attributed to infections caused by antibiotic-resistant microorganisms in 2015 [6] and these antibiotic-resistant microorganisms have a direct impact on treatment costs. In the EU, in 2007, these costs were estimated at more than 1.5 billion euros/year [7]. Regarding future antibiotic resistance scenarios, the World Bank has predicted the risk of a deficit in the annual gross domestic product of $3.4 trillion by 2030 rising to $6.1 trillion annually by 2050 [8]. Indeed, antibiotic resistance is considered one of the most relevant health challenges of the present century, directly threating the Sustainable Development Goals [4, 8, 9].

In this context, a series of pathogenic microorganisms are especially notorious for both their extremely high levels of antibiotic resistance, arriving to the above-mentioned status of pan-resistance and their ability to survive in hospital environments [4, 9]. Among these pathogenic microorganisms, members of the so-called ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) are of special concern [4, 9], resulting in worse patient outcomes and increased treatment costs [4, 6, 8, 10]. This problem is further compounded by ESKAPE microorganisms often accounting for more than 50% of hospital acquired infections [11].

Peru is a middle-income country with over-the-counter access to medications, including antibiotics [12, 13]. Although data on antibiotic resistance in Peru are scarce, the panorama is discouraging, with high levels of antibiotic resistance to carbapenems, third-generation cephalosporins and fluoroquinolones, among others having been reported [1-3, 14], in addition to emerging resistance to last-resort antibiotics such as colistin or tigecycline [2, 15]. Despite this, there is practically no comprehensive surveillance of the evolution of antibacterial resistance of microorganisms, such as those of the ESKAPE group, over different time periods.

In this context, the evolution of antimicrobial resistance levels of ESKAPE microorganisms isolated during 2009 - 2010 and 2012 - 2014 in a IV-level hospital in Lima using data from the internal database was analyzed.

MATERIAL AND METHODS

1. Study area

All isolates belonging to one of the ESKAPE species isolated from patients admitted to the medicine and surgery departments, and the intensive care unit (ICU) of the Hospital Nacional Guillermo Almenara Irigoyen (HNGAI) of Lima (Peru) during 2009 - 2010 (Period 1)

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and 2012 - 2014 (Period 2) were included in the study. The study did not involve humans and no personal data was accessible for the researchers.

The HNGAI is a IV-level hospital with 1,051 beds in the departments studied: Medicine (477 beds), Surgery (453 beds) and ICU (21 beds). Samples from the Gynecology, Psychiatry and the Emergency and Outpatient (mainly involving community-acquired infections) Departments (overall accounting for 100 beds) were excluded from the analysis.

2. Microorganisms

The microorganisms were isolated from different clinical samples following standard microbiology protocols at the HNGAI Microbiology Laboratory. The identification, antibiotic susceptibility and presence of Extended-Spectrum β-Lactamases (ESBL) (only *K. pneumoniae*) were performed using the automatic MicroScan system (Siemens Medical Solutions Diagnostics, Camberley, UK) in Period 1 and with Microscan and Vitek 2 (BioMérieux, Marcy l'Etoile, France) in Period 2.

The antibiotics in each analysis varied according to the bacterial species and included: ampicillin, penicillin, oxacillin, amoxicillin plus clavulanic acid, piperacillin-tazobactam (TZP), ampicillin-sulbactam (SAM), cefazolin, cefotaxime, ceftriaxone, ceftazidime, cefepime, imipenem, meropenem, ertapenem, aztreonam, trimethoprim-sulfamethoxazole (TMP/SMX), amikacin, gentamicin, streptomycin, tetracycline, ciprofloxacin, vancomycin, rifampicin, erythromycin and clindamycin. Susceptibility patterns were established in agreement with the Clinical and Laboratory Standards Institute [16]. A minimal inhibitory concentration ≤2 μg/ml was considered the tigecycline breakpoint in accordance with the US Food and Drug Administration (https://www.fda.gov/drugs/development-resources/tigecycline-injection-products) and Nicolau et al. [17].

In addition, chloramphenicol was included in the determination of *S. aureus* susceptibility during Period 1, while linezolid was tested in 39 *E. faecium* and 54 *S. aureus* in Period 2. Similarly, in Period 2, tigecycline susceptibility was also tested in 177 *K. pneumoniae* and 37 *A. baumannii*, while colistin susceptibility levels were established in 31 *P. aeruginosa* and 20 *A. baumannii*.

3. Statistical analysis

The Fisher exact test was used to determine statistical associations. A *P*-value <0.05 was considered significant.

In all cases intermediate and resistant isolates were classified together as “non-susceptible”. Coagulase negative *Staphylococcus* (CoNS) were considered as contamination [18] and were not included in the statistical analyses.

4. Ethics Approval and Consent to Participate

The data extracted and the manuscript was reviewed with Scientific and Ethics Committee. No experimental intervention was performed. It did not require any specification of guidelines, legislations, or permissions.

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RESULTS

1. Analysis of the time and ESKAPE isolation according to hospital department

Overall, 10,948 isolates were recovered from clinical samples of admitted patients. Of these, 1,030 belonged to CoNS and were classified as sample contamination (contamination rate 9.4%). Excluding CoNS, 3,777 bacterial isolates were recovered in Period 1 (2,227, 887, 663 from medicine, surgery and ICU, respectively) and 6,141 in Period 2 (3,271, 1,860, 1,010 from medicine, surgery and ICU respectively). Of these, 4,764 (48.0%) microorganisms belonged to the ESKAPE group (1,917 - Period 1 and 2,848 - Period 2), representing 50.8% of the isolates of Period 1 and 46.4% of Period 2 (P <0.05). Analysis of the evolution of ESKAPE isolates in Periods 1 and 2 showed a reduction in the isolation of *E. faecium* from 5.1% to 4.1% (P <0.05), *S. aureus* from 10.5% to 7.0% (P <0.05), and *P. aeruginosa* from 12.9% to 11.6% (P <0.05), and an increase in *A. baumannii* from 5.0% to 6.7% (P <0.05) (**Table 1**).

In Periods 1 and 2, ESKAPE microorganisms were more frequently isolated as a cause of infection in the ICU (62.3% and 61.3%, respectively; P <0.05) (**Table 1**).

K. pneumoniae was the most common member of ESKAPE group, accounting for 1,313 isolates (497 and 816 in the period 1 and 2 respectively; 13.2% of total isolates). Accordingly, the analysis by hospital areas, showed that *K. pneumoniae* was the most frequent ESKAPE microorganism in medicine and surgery departments (749 and 353 isolates respectively). The microorganisms most frequently isolated in the ICU in Period 1 were *P. aeruginosa* (112 isolates) followed by *S. aureus* (99 isolates), being *A. baumannii* (173 isolates) and *P. aeruginosa* (152 isolates) in Period 2 (**Table 1**). Among the ESKAPE group *Enterobacter* spp. was the least frequently isolated accounting for 3.7% of all the microorganisms analyzed and 7.8% of the ESKAPE group.

On comparing the evolution of the microorganisms isolated in the two study periods, the most relevant finding was the significant increase in *A. baumannii* isolates (5.0% *vs.* 6.7%; P <0.05) and the parallel decrease in *E. faecium* (5.1% *vs.* 4.1%; P <0.05), *P. aeruginosa* (12.9% *vs.* 11.6%; P <0.05) and *S. aureus* (10.5% *vs.* 7.0%; P <0.05). (**Table 1**).

2. Analysis of antimicrobial resistance

In general, we found high levels of antimicrobial resistance to most of the antibiotics tested (**Fig. 1**, **Table 2** - **4**). Fortunately, although only a reduced number of microorganisms

Table 1. ESKAPE isolates, 2009 - 2010 and 2012 - 2014

ESKAPE	Medicine			Surgery			ICU			Overall		
	2009 - 2010 (2,227)	2012 - 2014 (3,271)	*P*	2009 - 2010 (887)	2012 - 2014 (1,860)	*P*	2009 - 2010 (663)	2012 - 2014 (1,010)	*P*	2009 - 2010 (3,777)	2012 - 2014 (6,141)	*P*
	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
Enterococcus faecium	119 (5.3)	153 (4.7)	0.3101	40 (4.5)	71 (3.8)	0.4075	35 (5.3)	31 (3.1)	0.0289	194 (5.1)	255 (4.1)	0.0251
Staphylococcus aureus	193 (8.7)	214 (6.5)	0.0039	104 (11.7)	117 (6.3)	<0.0001	99 (14.9)	101 (10.0)	<0.0001	396 (10.5)	432 (7.0)	<0.0001
Klebsiella pneumoniae	301 (13.5)	448 (13.7)	0.8728	114 (12.8)	239 (12.8)	0.8557	82 (12.4)	129 (12.8)	0.8218	497 (13.2)	816 (13.3)	0.8788
Acinetobacter baumannii	100 (4.5)	147 (4.5)	1.000	33 (3.7)	95 (5.0)	0.1212	56 (8.4)	173 (17.1)	<0.0001	189 (5.0)	415 (6.7)	0.0004
Pseudomonas aeruginosa	271 (12.2)	357 (10.9)	0.0038	106 (11.9)	201 (10.8)	0.3999	112 (16.9)	152 (15.0)	0.3372	489 (12.9)	710 (11.6)	0.0423
Enterobacter	92 (4.1)	118 (3.6)	0.3515	31 (3.5)	69 (3.7)	0.8281	29 (4.4)	33 (3.3)	0.2300	152 (4)	220 (3.6)	0.2764
ESKAPE	1,076 (48.3)	1,437 (43.9)	0.0014	428 (48.2)	792 (42.6)	<0.0001	413 (62.3)	619 (61.3)	0.6813	1,917 (50.8)	2,848 (46.4)	<0.0001
$P_{M+S/I}$							<0.0001	<0.0001				

ESKAPE, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.; ICU, intensive care unit; M, medicine; S, surgery; I, intensive care unit.

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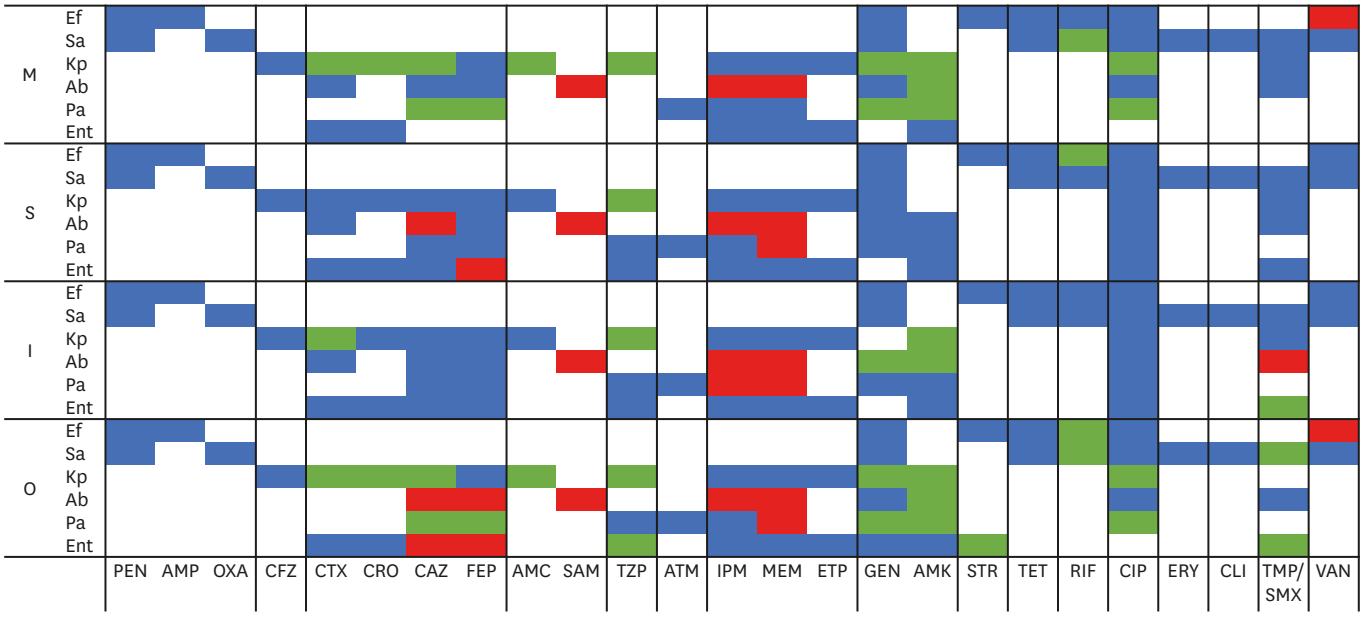

Figure 1. Evolutive trends between the two study periods.
Blue, No significant differences; Green, Significant decrease; Red, Significant increase.
M, medicine department; S, surgery department; I, intensive care unit; O, overall data; Ef, *Enterococcus faecium*; Sa, *Staphylococcus aureus*; Kp, *Klebsiella pneumoniae*; Ab, *Acinetobacter baumannii*; Pa, *Pseudomonas aeruginosa*; Ent, *Enterobacter* spp.; PEN, penicillin; AMP, ampicillin; OXA, oxacillin; CFZ, cefazolin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; AMC, amoxicillin-clavulanic; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; ATM, aztreonam; IPM, imipenem; MEM, meropenem; ETP, ertapenem; GEN, gentamicin; AMK, amikacin; STR, streptomycin; TET, tetracycline; RIF: rifampicin; CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; TMP/SMX, trimethoprim-sulfamethoxazole; VAN, vancomycin.

were tested, the isolates of the ESKAPE group showed high susceptibility to last-resort antibacterial agents. Thus, *E. faecium* was only susceptible to linezolid, *S. aureus* to linezolid and vancomycin, *A. baumannii* to colistin and tigecycline, *P. aeruginosa* to colistin. Regarding *Enterobacteriaceae,* both *K. pneumoniae* and *Enterobacter* spp. were highly susceptible to carbapenems (**Table 2** - **4**). Surprisingly, the antibiotic resistance levels tended to remain stable or decrease in Period 2, except for *A. baumannii,* in which resistance to different cephalosporins, carbapenems and SAM significant increased over time (**Fig. 1**).

Regarding other antibacterial agents, the antibiotic resistance levels of *E. faecium* ranged from 48% (vancomycin) to 94% (ciprofloxacin). Overall, the antibiotic resistance levels remained unaltered during the two periods, except for resistance to vancomycin, which significantly increased, and rifampicin which significantly decreased. In addition, a non-significant increase in tetracycline resistance was observed. Analysis by hospital department showed that resistance to vancomycin was higher in isolates from the ICU in both periods, being significant when all the isolates were considered together. Meanwhile the resistance of high level to gentamicin was significantly lower among ICU isolates while that to β-lactam tend to be lower in ICU isolates (**Table 2A**).

Regarding *S. aureus*, resistance levels to TMP/SMX (13%), tetracycline (18%) and rifampicin (12%) were low irrespective of the period or department. In addition, while the levels of resistance to most of the antibiotics tested remained unaltered in both periods, resistance to TMP/SMX and rifampicin decreased over time. In the case of TMP/SMX, a trend to decreasing levels of resistance was observed in all departments, while a decrease in rifampicin resistance was observed in isolates from the medicine department and the ICU. The presence of methicillin-resistant *S.*

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Table 2. Antimicrobial resistance levels of *Enterococcus faecium* and *Staphylococcus aureus* isolates

A) *Enterococcus faecium*

		N	PEN		AMP		VAN		GEN[a]		STR[a]		TET		CIP		RIF	
			%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*
M+S+I	P1	194	90	NS	90	NS	42	<0.05	74	NS	67	NS	56	NS	93	NS	88	<0.05
	P2	255	93		91		53		74		68		63		95		78	
	O	449	91		90		48		74		67		60		94		82	
M	P1	119	93	NS	92	NS	36	<0.05	80	NS	67	NS	60	NS	96	NS	86	NS
	P2	153	95		93		52		76		72		65		95		79	
	O	272	94		92		45		78		70		62		95		82	
S	P1	40	90	NS	90	NS	47	NS	72	NS	75	NS	47	NS	95	NS	95	<0.05
	P2	71	89		88		46		71		60		59		94		77	
	O	111	89		89		46		71		66		55		94		84	
I	P1	35	80	NS	80	NS	54	NS	54	NS	57	NS	49	NS	93	NS	86	NS
	P2	31	94		90		74		71		71		58		97		74	
	O	66	86		85		64		62		64		53		95		80	
P1	$P_{M+S/I}$			0.06		0.07		NS		<0.05		NS		NS		NS		NS
P2	$P_{M+S/I}$			NS		NS		NS		NS		NS		NS		NS		NS
O	$P_{M+S/I}$			NS		NS		<0.05		<0.05		NS		NS		NS		NS

B) *Staphylococcus aureus*

		N	Antibiotic resistance levels															
			PEN		OXA		ERY		GEN		CIP		CLI		TMP/SMX		TET		RIF	
			%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*
M+S+I	P1	396	97	NS	77	NS	80	NS	78	NS	78	NS	80	NS	16	<0.05	20	NS	15	<0.05
	P2	432	98		76		81		75		76		78		11		16		10	
	O	828	98		76		81		76		77		79		13		18		12	
M	P1	193	96	NS	66	NS	73	NS	69	NS	68	NS	70	NS	15	NS	20	NS	14	<0.05
	P2	214	98		65		71		65		64		66		10		18		7	
	O	297	97		65		72		67		66		68		12		19		10	
S	P1	104	98	NS	83	NS	86	NS	85	NS	83	NS	85	NS	14	NS	21	NS	13	NS
	P2	117	97		85		87		83		86		87		11		15		15	
	O	221	97		84		87		84		85		86		12		18		14	
I	P1	99	99	NS	91	NS	88	NS	90	NS	93	NS	92	NS	21	0.09	20	NS	19	0.08
	P2	101	99		90		94		84		90		90		12		15		10	
	O	200	99		90		91		86		91		91		16		17		14	
P1	$P_{M+S/I}$			NS		<0.05		<0.05		<0.05		<0.05		<0.05		NS		NS		
P2	$P_{M+S/I}$			NS		<0.05		<0.05		<0.05		<0.05		<0.05		NS		NS		NS
O	$P_{M+S/I}$			NS		<0.05		<0.05		<0.05		<0.05		<0.05		NS		NS		

[a]High level resistance.
In the Table are only reported significant differences (*P* <0.05), as well as those *P*-values ≤0.09.
In addition, susceptibility to linezolid was tested in 39 *E. faecium* and 53 *S. aureus* isolates from Period 2 with all being susceptible.
All *S. aureus* isolates were susceptible to vancomycin. In addition, chloramphenicol was tested in *S. aureus* from Period 1 showing an overall resistance rate of 16% with a maximum among intensive care unit isolates of 21%.
M, medicine; S, surgery; I, intensive care unit; P1, Period 1 (2009 - 2010); P2, Period 2 (2012 - 2014); O, overall; PEN, penicillin; AMP, ampicillin; VAN, vancomycin; GEN, gentamicin; STR, streptomycin; TET, tetracycline; CIP, ciprofloxacin; RIF, rifampicin; NS, non significant; OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; TMP/SMX, trimethoprim-sulfamethoxazole.

aureus (MRSA) was higher among ICU isolates in Periods 1 and 2, being lower in the Medicine Department. In general, antibiotic resistance was higher among ICU isolates (**Table 2B**).

In addition to carbapenems, tigecycline (for which only partial data from Period 2 were available) and amikacin showed good activity against *K. pneumoniae* (12% and 14%, of antibiotic resistance respectively. Unexpectedly, a trend towards higher levels of susceptibility to all the antibacterial agents tested, except cefepime was observed, being especially notable in the Medicine Department and the ICU. This finding was especially relevant in regard to TZP, with overall resistance levels decreasing from 48% to 22%, related to a significant decrease in resistance levels in all the departments analyzed. Regarding cephalosporins, a slight overall, albeit non-significant, decrease was observed in the frequency of ESBL in Periods 1 and 2 (80% *vs.* 76%), being especially notable among ICU isolates (81% *vs.* 74%) (**Table 3A**).

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Table 3. Antimicrobial resistance levels of *Klebsiella pneumoniae* and *Enterobacter* spp. isolates

A) *Klebsiella pneumoniae*

		N	Antibiotic resistance levels																					
			AMC		CFZ		CTX		CRO		CAZ		FEP		ETP		TZP		AMK		GEN		CIP		TMP/SMX	
			%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*
M+S+I	P1	497	76	<0.05	86	NS	84	<0.05	83	<0.05	83	<0.05	78	NS	3	NS	48	<0.05	21	<0.05	66	<0.05	81	<0.05	69	0.06
	P2	816	68		86		76		78		77		74		2		22		10		54		73		74	
	O	1,313	71		86		79		80		79				2		32		14		59		76		72	
M	P1	301	76	<0.05	87	NS	85	<0.05	84	<0.05	84	<0.05	78	NS	3	NS	49	<0.05	24	<0.05	67	<0.05	80	<0.05	67	NS
	P2	448	65		87		75		77		75		73		2		24		10		52		71		72	
	O	749	69		87		79		80		79		75		2		34		16		58		75		70	
S	P1	114	69	NS	82		81	NS	78	NS	80	NS	77	NS	2	NS	46	<0.05	19	NS	64	NS	79	NS	69	NS
	P2	239	71		86		80		80		79		75		3		20		14		57		75		76	
	O	353	70		84		80		79		79		76		3		29		16		59		77		74	
I	P1	82	85	0.09	89		85	<0.05	85	0.09	85	NS	80	NS	3	NS	45	<0.05	13	<0.05	65	0.06	85	NS	76	NS
	P2	129	75		82		71		75		76		73		3		22		4		55		76		74	
	O	211	79		85		77		79		80		76		3		31		8		59		80		75	
P1	$P_{M+S/I}$			<0.05		NS		NS		NS		NS		NS		NS		NS		0.08		NS		NS		NS
P2	$P_{M+S/I}$			0.08		NS		NS		NS		NS		NS		NS		NS		<0.05		NS		NS		NS
O	$P_{M+S/I}$			<0.05		NS		NS		NS		NS		NS		NS		NS		<0.05		NS		NS		NS

B) *Enterobacter* spp.

		N	Antibiotic resistance levels																					
			CTX		CRO		CAZ		FEP		ETP		IPM		MEM		TZP		AMK		GEN		CIP		TMP/SMX	
			%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*
M+S+I	P1	152	58	NS	54	NS	45	<0.05	32	<0.05	1	NS	0	NS	0	NS	45	<0.05	16	NS	50	NS	62	0.09	63	<0.05
	P2	220	57		62		57		46		3		1		1		31		17		42		53		51	
	O	372	57		59		52		40		2		1		1		37		17		45		57		56	
M	P1	92	60	NS	54	NS	42	0.06	33	0.09	0	NS	0	NS	0	NS	46	0.07	16	NS	50	0.07	66	0.07	64	0.07
	P2	118	58		61		57		45		4		3		0		33		16		37		53		51	
	O	210	59		58		51		40		2		1		0		39		16		43		59		57	
S	P1	31	61	NS	58	NS	55	NS	29	<0.05	3	NS	0	NS	0	NS	52	NS	16	NS	55	NS	58	NS	55	NS
	P2	69	65		72		61		55		2		0		3		34		21		52		58		58	
	O	100	64		68		59		47		2		0		2		39		19		53		58		57	
ICU	P1	29	48	NS	48	NS	41	NS	31	NS	0	NS	0	NS	0	NS	34	NS	14	NS	45	NS	52	NS	66	<0.05
	P2	33	40		44		47		31		3		0		0		22		9		34		40		31	
	O	62	44		46		45		31		2		0		0		27		11		39		45		47	
P1	$P_{M+S/I}$			NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS
P2	$P_{M+S/I}$			<0.05		<0.05		NS		0.06		NS		NS		NS		<0.05		NS		NS		NS		<0.05
O	$P_{M+S/I}$			0.08		<0.05		NS		NS		NS		NS		NS		NS		NS		NS		<0.05		NS

In the Table are only reported significant differences (P <0.05), as well as those P-values ≤0.09.
All *K. pneumoniae* isolates were susceptible to meropenem and imipenem. In the second period was established the tigecycline susceptibility in 117 *K. pneumoniae* isolates, with an overall resistance of 12% being observed (Medicine 18%; Surgery: 0%; Intensive Care Unit: 12%).
M, medicine; S, surgery; I, intensive care unit; P1, Period 1 (2009 - 2010); P2, Period 2 (2012 - 2014); O, overall; AMC, amoxicillin-clavulanic acid; CFZ, cefazolin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ETP, ertapenem; TZP, piperacillin-tazobactam; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; TMP/SMX, trimethoprim-sulfamethoxazole; NS, non significant; IPM, imipenem; MEM, meropenem.

A. baumannii isolates presented high levels of antimicrobial resistance, with a trend to an increase in Period 2, except for aminoglycosides (amikacin and gentamicin) which showed a significant antimicrobial resistance levels decrease. These trends were observed in the three departments analyzed, with most antibiotics reaching resistance levels higher than 90%. In general, isolates from the ICU showed significantly higher levels of resistance to the antimicrobial agents analyzed, being greater than 80% to all antibiotics (except colistin and tigecycline) (**Table 4A**).

In contrast to *A. baumannii,* the overall levels of antibiotic resistance of *P. aeruginosa* tended to decrease or remain unaltered, with most of the antibiotics showing levels of resistance of around 50%. The most notable exception was the level of resistance to carbapenems which increased in both periods analyzed and were significant with meropenem, largely fueled by the increase in carbapenem resistance levels in ICU isolates (**Table 4B**).

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Table 4. Antimicrobial resistance levels of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates

A) *Acinetobacter baumannii*

		N	Antibiotic resistance levels																			
			SAM		CTX		CAZ		FEP		IPM		MEM		AMK		GEN		CIP		TMP/SMX	
			%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*
M+S+I	P1	189	65	<0.05	91	0.06	85	<0.05	85	<0.05	63	<0.05	61	<0.05	84	<0.05	78	NS	89	NS	86	NS
	P2	414	91		97		93		93		90		88		69		72		93		90	
	O	603	83		95		91		91		81		80		74		74		92		89	
M	P1	100	61	<0.05	87	NS	81	NS	81	NS	61	<0.05	61	<0.05	81	<0.05	78	NS	87	NS	84	NS
	P2	147	89		92		88		88		86		78		59		71		88		84	
	O	247	78		90		85		85		76		72		68		74		88		84	
S	P1	33	68	<0.05	88	NS	82	<0.05	82	0.08	63	<0.05	64	<0.05	82	NS	73	NS	88	NS	91	NS
	P2	94	91		95		94		93		87		90		67		76		94		89	
	O	127	86		93		91		90		81		83		71		75		93		90	
I	P1	56	61	<0.05	100	NS	95	NS	93	NS	67	<0.05	71	<0.05	91	<0.05	80	<0.05	93	NS	86	<0.05
	P2	173	91		99		96		98		95		95		74		72		97		96	
	O	229	83		99		96		97		88		89		78		76		95		93	
P1	$P_{M+S/I}$			NS		<0.05		<0.05		<0.05		NS		NS		NS		NS		NS		NS
P2	$P_{M+S/I}$			NS		<0.05		<0.05		<0.05		<0.05		<0.05		NS		<0.05		<0.05		<0.05
O	$P_{M+S/I}$			NS		<0.05		<0.05		<0.05		<0.05		<0.05		NS		<0.05		<0.05		<0.05

B) *Pseudomonas aeruginosa*

		N	Antibiotic resistance levels																	
			CAZ		FEP		ATM		IPM		MEM		TZP		AMK		GEN		CIP	
			%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*	%	*P*
M+S+I	P1	489	59	<0.05	64	<0.05	62	NS	53	NS	47	<0.05	48	NS	54	<0.05	67	<0.05	69	<0.05
	P2	710	53		56		63		58		57		49		44		56		58	
	O	1,199	55		59		63		56		53		49		49		48		62	
M	P1	271	58	<0.05	64	<0.05	62	NS	53	NS	45	NS	48	0.06	57	<0.05	71	<0.05	69	<0.05
	P2	357	49		50		57		50		49		40		39		51		56	
	O	628	53		56		59		51		47		43		47		60		62	
S	P1	106	61	NS	65	NS	66	NS	52	0.09	46	<0.05	48	NS	57	NS	65	NS	72	NS
	P2	201	57		62		71		62		61		59		51		63		63	
	O	307	58		63		69		59		56		55		53		64		66	
I	P1	112	58	NS	62	NS	61	NS	54	<0.05	51	<0.05	46	NS	45	NS	62	NS	65	NS
	P2	152	57		60		65		70		67		52		47		59		58	
	O	264	57		61		63		63		60		49		46		60		61	
P1	$P_{M+S/I}$			NS		NS		NS		NS		NS		NS		NS		NS		NS
P2	$P_{M+S/I}$			NS		NS		NS		<0.05		<0.05		NS		NS		NS		NS
O	$P_{M+S/I}$			<0.05		NS		NS		<0.05		<0.05		NS		NS		NS		NS

In the Table are only reported significant differences (*P* <0.05), as well as those *P*-values ≤0.09.
In Period 2 susceptibility to colistin was established in 20 *A. baummanni* and 31 *P. aeruginosa* isolates, and that of tigecycline was established in 37 *A. baumanni* isolates, all being susceptible.
M, medicine; S, surgery; I, intensive care unit; P1, Period 1 (2009 - 2010); P2, Period 2 (2012 - 2014); O, overall; SAM, ampicillin-sulbactam; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP: ciprofloxacin; TMP/SMX, trimethoprim-sulfamethoxazole; NS, non significant; ATM, aztreonam; TZP, piperacillin-tazobactam.

Finally, *Enterobacter* spp. showed an overall trend to decreased resistance to antibiotics tested, achieving significance in relation to TZP and TMP/SMX. On the other hand, cephalosporins tended to show higher levels of antibiotic resistance, with ceftazidime and cefepime presenting significant increases. This increase in cephalosporin resistance was mainly found in isolates from medicine and surgery departments (**Table 3B**).

DISCUSSION

Antibiotic resistance is a growing problem worldwide [5, 6, 19, 20]. This phenomenon is fueled by different factors beyond the obligation to adequately treat human or animal infections and includes social, economic, and cultural factors, which severely impact the final

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levels of antibiotic consumption and subsequent selective pressure of antibiotic-resistant microorganisms [20]. Furthermore, current trade and travel facilities play a key role in the rapid dispersion of antibiotic-resistant microorganisms which may be visible and produce pathogenic processes, or silent and occult, when related to commensal or non-pathogenic microorganisms [20-22].

1. Analysis of the period and ESKAPE isolation according to clinical department

In the departments analyzed, the role of ESKAPE microorganisms as infectious agents was relevant, overall accounting for ~50% of all recorded microorganisms isolated as a cause of infection in the HNGAI. This high relevance is in accordance with that described in other reports, in which ESKAPE rates in different clinical settings were around 40 - 60%, being together *Escherichia coli* the most frequent pathogen involved in hospital-acquired infections [2, 10, 11]. It was of note that while the number of *A. baumannii* increased, ESKAPE were significantly less frequently recovered during Period 2. There is no clear explanation for the reduction in ESKAPE. A Program for Optimization of Antibiotic use (PROA) was not established until 2018, and there is no information about changes in internal procedures. The increase of *A. baumannii* might be related to the presence of a successful hospital clone causing an undetected outbreak. This suggestion is supported by the increase in cephalosporin, carbapenem and SAM resistance levels detected, and with the overall increase mainly in the ICU. Inter-hospital dissemination of successful *A. baumannii* clones exhibiting high levels of antibiotic resistance, similar to those reported in the present study, has been previously described in Lima [2], the most relevant difference being the lower levels of resistance to SAM (62.5%) compared to our study (91%).

2. Analysis of antimicrobial resistance

While the present data showed a clear trend to maintained or reduced antibiotic resistance levels, overall, high levels of antibiotic resistance were detected in both study periods similar to previous reports in Peru [1-3, 14]. This trend to decreasing resistance levels is intriguing, and taking into account the lack of specific antibiotic management related to PROA, it is not the result of a series of structured countermeasures.

High levels of antibiotic resistance were especially notable among ICU isolates. The fragility of ICU patients usually requires greater use of antibiotics [23], resulting in enhanced pressure towards the selection of antibiotic-resistant microorganisms, which may explain this phenomenon. As mentioned above, in the ICU, the most serious concerns were related to the increasing presence of vancomycin-resistant *Enterococcus faecium* (VRE) and the high and increasing levels of resistance to antibiotics shown by *A. baumannii*.

The presence of VRE is of increasing concern. In 2017, VRE were identified as causing 54,500 infections in the United States, with a lethality rate of ~10% (5,400 deaths), leading the Centers for Disease Control and Prevention (CDC) to classify this pathogen as a serious threat [19]. Another relevant concern is the possible transfer of genetic material from VRE to *S. aureus*. Thus, while infrequent [24], and to our knowledge, has not been reported in health care settings in Peru, the potential risk of transfer of vancomycin-resistant determinants from VRE to *S. aureus* cannot be ruled out [24].

Meanwhile, *A. baumannii* isolates showed antibiotic resistance levels >80% to most of the agents tested, with carbapenems and extended spectrum cephalosporins resistance levels being >90%. This finding indicates the *de facto* lack of utility of these latter antibiotics for

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the treatment of *A. baumannii* infections and the scarcity of treatment options out of the last-resort antibiotics. Thus, the present study showed that in a small subset of isolates, *A. baumannii* presented 100% of susceptibility to colistin and tigecycline and could therefore, potentially, be useful for treatment. Nevertheless, colistin has not been used in the clinical setting for years because its high levels of toxicity [25], and should be used with caution. Regarding tigecycline, the literature is controversial. Thus, while several studies have reported a higher risk of death in patients receiving this antibiotic [26], other data suggest that is a good candidate for the treatment of infection by *A. baumannii* [27]. Considering that carbapenem-resistant *A. baumannii* has been classified as an urgent threat [19], other alternative treatments such as sulbactam/avibactam [28] or cefiderocol [29] might be useful in the treatment of MDR and XDR *A. baumannii*.

As with other ESKAPE members, *S. aureus* from the ICU showed higher levels of antibiotic resistance, in agreement with the significantly higher presence of MRSA, which often presents higher levels of antibiotic resistance in ICUs [30]. While several antibiotics, including vancomycin and linezolid, showed excellent activity, the current data are concerning. The prevalence of MRSA in this study is higher than that of other studies performed in Peru. Thus, while Schwalb et al. reported that 65% of *S. aureus* acquired in hospital settings were MRSA, on analyzing samples from 7 hospitals in Peru, Garcia et al., reported 72.5% of MRSA in ICUs and 45% in the remaining hospital wards [30, 31]. Of note, Garcia *et al.* also reported worrisome levels of antibiotic resistance >90% in several cases, such as ciprofloxacin or erythromycin [30].

Carbapenem-resistant and ESBL-producing *Enterobacteriaceae* are considered urgent and serious threats, respectively [19]. In the present study carbapenem-resistant *K. pneumoniae* was practically absent. While different carbapenemases including KPC, NDM, and IMP have been detected among *K. pneumoniae* from Peru [32, 33], other reports described a low prevalence of carbapenem-resistant *K. pneumoniae* [1, 34], in agreement with our results. Notwithstanding, local carbapenem-resistant *K. pneumoniae* outbreaks have been described in recent years [33, 35]. On the other hand, while tending to decrease over time, high levels of ESBL-producer *K. pneumoniae* were detected. In this sense, ESBL-producer *K. pneumoniae* are frequent in Peru, accounting for >70% of *K. pneumoniae* isolates in different studies [1, 34]. Of note, a great variety of ESBLs, including different CTX-M and SHV have been described among Peruvian *K. pneumoniae*, with CTX-M-15 being the most prevalent [1]. Regarding tigecycline, 18% and 16% of resistant isolates were detected in the Medicine Department and ICU. While these are low levels of resistance, alert about the emergence of resistance to most modern agents.

P. aeruginosa is an opportunistic pathogen frequently involved in nosocomial infections, often showing high levels of antibiotic resistance [14, 36]. In this sense, the levels of resistance ranging from ~50 to ~75% (except colistin) found in the present study were to be expected and are in the line of previous reports in Peru [14]. The unexpected result was the trend to maintained or decreased antibiotic resistance levels extended to almost all antibiotics, except carbapenems and TZP, which showed an inverse trend in the ICU and Surgery Department. While increasing levels of carbapenems and TZP might be related to the pressure exerted by these agents, resulting in the selection of *P. aeruginosa* resistant isolates, we have no explanation for the decreasing levels of resistance to other agents, including aminoglycosides, cephalosporins and fluoroquinolones.

In our study, *Enterobacter* spp. was the least frequent of the ESKAPE members, and this finding may have affected the detection of significant differences between the two study periods

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analyzed, and probably underlies several differences which were borderline significant. While data for resistance levels of clinical isolates of *Enterobacter* spp. from Peru are rare, the low levels of carbapenem resistance found agree with the scarcity of carbapenamases detected in *Enterobacter* spp. isolates from Peru. Thus, a recent systematic review only recorded 7 carbapenemase-producing *Enterobacter* isolates reported from a total of 313 microorganisms in 2000 - 2019 [37]. Regarding other antimicrobial agents, the present data showed higher overall resistance levels than those found among 433 *Enterobacter* spp. analyzed in Latin America from 2013 - 2015 [38].

In addition to the long analysis of the evolution of antibiotic resistance levels of ESKAPE microorganisms in a Peruvian clinical setting, the present data are of special relevance within the context of the coronavirus disease 2019 (COVID-19) pandemic, in which the use of antibiotics and biocides has been extreme in both hospital and community settings, likely contributing to the emergence of XDR and pan-resistant clinical isolates in different areas [13, 39]. Thus, these data could contribute to future analyses of the collateral impact of the COVID-19 pandemic on antibiotic resistance levels, providing retrospective pre-pandemic baseline data and trends in the evolution of drug resistance.

The lack of information about clinical outcomes as well as the uncertain role of CoNS as infective pathogens in a small fraction of patients are the main limitations of the present study [11,34]. It should be noted that in 2017 *Enterobacter aerogenes* was confirmed to be identical to *Klebsiella mobilis*, was thereby unified as a single species under the genus *Klebsiella*, and named *Klebsiella aerogene*s [40]. As databases record isolates pre-2017, it is likely that a number of *K. aerogenes* were categorized as *Enterobacter* spp.

In summary, the present study found high levels of antibiotic resistance among ESKAPE microorganisms. While the establishment of a successful XDR *A. baumannii* clone is suspected in the ICU, the present data provide two intriguing results which are a clear trend to a reduction in the isolation of ESKAPE microorganisms as well as a generalized decrease in the levels of antibiotic resistance. Further studies are needed to determine the evolution of resistance, which is especially important to evaluate the impact of the COVID-19 pandemic on the evolution of antibiotic resistance levels.

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