doi: Epub ahead of print

ORIGINAL ARTICLE

EVOLUTION OF THE PREVALENCE AND ANTIMICROBIAL RESISTANCE AMONG *Escherichia coli* ISOLATED AS A CAUSE OF INFECTION IN PATIENTS ADMITTED TO A IV-LEVEL HOSPITAL IN LIMA, PERU

Wilfredo Flores-Paredes[1], Nestor Luque[2*], Roger Albornoz[2], Nayade Rojas[3], Manuel Espinoza[4], Joaquim Ruiz[5] and Maria J. Pons[5]*

ABSTRACT

The levels and evolution of antimicrobial resistance of *Escherichia coli* during 01/2009-06/2010 (Period 1), 01/2012-06-2013 (Period 2) and 07/2013-12/2014 (Period 3) were analyzed. Identification, susceptibility levels to 13 antibiotics and the presence of extended-spectrum β-lactamases (ESBLs) were determined. Overall, 9,918 microorganisms were isolated as a cause of infection. Of these 3,016 (30.4%) were *E. coli,* with 1,770 (59%), 992 (33%) and 254 (8%), from the Medicine and the Surgery Departments and the Intensive Care Unit (ICU), respectively. There was a significant increase (p=0.0002) of *E. coli* throughout considered periods. The isolates presented high levels of resistance (>60%) to cephalosporins, ciprofloxacin and cotrimoxazole, being only susceptible to imipenem (0.3% of resistance) and tigecycline. Overall the analysis of evolution of antimicrobial resistance showed that resistance to cephalosporins and amikacin significantly increased, while, the ones of piperacillin-tazobactam, cotrimoxazole and gentamicin had significantly decreased. Nevertheless, the ICU isolates showed an inverse scenario for cephalosporins. These findings agree with an increase of ESBLs on the Medicine (56% to 66%; p<0.0001) and on the Surgery (54% to 62%; p=0.0197) departments, with a parallel decrease in the ICU (76% to 68%). In summary, high levels of antimicrobial resistance have been reported among *E. coli*, with worrisome levels of ESBL. A continuous surveillance of antimicrobial resistance levels in the area is needed.

KEY WORDS: *Escherichia coli*; antimicrobial resistance; cephalosporins.

1. Hospital Nacional Guillermo Almenara Irigoyen, Servicio de Microbiología, Lima, Peru.
2. Universidad Peruana Union (UPeU), Escuela de Medicina Humana, Facultad de Ciencias de la Salud, Lima, Peru.
3. Ministerio de Salud, Lima, Peru.
4. Instituto Nacional de Salud, Lima, Peru.
5. Grupo de Investigación en Dinámicas y Epidemiología de la Resistencia a Antimicrobianos - "One Health", Universidad Científica del Sur, Lima, Peru.
*Wilfredo Flores-Paredes and Nestor Luque contributed equally to this work.

Wilfredo Flores-Paredes: ORCID: https://orcid.org/0000-0002-4824-7956; Nestor Luque: ORCID: https://orcid.org/0000-0002-6192-4392; Roger Albornos: ORCID: https://orcid.org/0000-0001-6155-0848; Nayade Rojas: ORCID: https://orcid.org/0000-0003-0213-1410; Manuel Espinoza: ORCID: https://orcid.org/0000-0003-1283-2253; Joaquim Ruiz: ORCID: https://orcid.org/0000-0002-4431-2036; Maria J Pons: ORCID: https://orcid.org/0000-0001-8384-2315

Corresponding author: Joaquim Ruiz, Grupo de Investigación en Dinámicas y Epidemiología de la Resistencia a Antimicrobianos - "One Health", Universidad Científica del Sur, Panamericana Sur Km 19, Lima, Perú. E-mail: joruiz.trabajo@gmail.com; jruizb@cientifica.edu.pe

Received for publication: 21/3/2022. Reviewed: 27/6/2022. Accepted: 14/9/2022.

INTRODUCTION

Antimicrobial resistance is an increasing problem worldwide, affecting all microorganisms regardless of source or whether they are or they are not pathogenic (Bumbangi et al., 2022; Palma et al., 2017; Pons & Ruiz, 2019; Ymaña et al., 2022). This scenario is challenging to medical practices because of the increasing ineffectiveness of the current antibacterial armamentarium. While often under the radar, the development of antimicrobial resistance also has a direct impact on medical practices beyond treatment of infectious diseases. Thus, surgery, transplants, immunosuppressed patients´ prophylaxis among other are directly impaired (Cervera et al., 2014; Teillant et al., 2015). Furthermore, the economy, environment and global development are affected by antimicrobial resistance, with serious concerns related to its negative effect on Sustainable Development Goals (Jasovský et al., 2016). This problem is clearly visualized in the economical- and life-costs related to infections by these pathogens (Cassini et al., 2019; CDC, 2019).

In this scenario, the so-called ESKAPE group of microorganisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) are considered of special concern because of the severity of infections and the increasing treatment difficulties related to their high levels of antimicrobial resistance (Pons & Ruiz, 2019; De Oliveira et al., 2020).

In addition to these microorganisms, *Escherichia coli* is one of the microorganisms most frequently isolated as one of the causes of human infections (Pons & Ruiz, 2019; Vila et al., 2016). Thus, while being a common commensal inhabitant of the human gut, several virulent pathotypes of *E. coli* play a main role as a cause of infectious diarrhea, leading to different diarrheogenic pathotypes of *E. coli* being considered one of the most relevant causes of children death (Khalil et al., 2018; Levine et al., 2020). Moreover, extraintestinal *E. coli* are amid the most frequent pathogens involved in other types of infections, such as urinary, wound or systemic infections (Medina & Castillo-Pino, 2019; Poolman & Wacker, 2016).

In Perú, reports focused on antimicrobial resistance show a discouraging panorama, with both commensal and clinical isolates of *E. coli* presenting high levels of antimicrobial resistance to agents such as cephalosporins or fluoroquinolones, and others (Alcedo et al., 2022; Palma et al., 2017; Yabar et al., 2017). Furthermore, *E. coli* resistance to last-resort antimicrobial agents, such as colistin, has been reported in Peru (Ugarte Silva et al., 2018). Nonetheless, there is a lack of studies focused on the comprehensive evolution of antimicrobial resistance in Peruvian hospital settings. Thus, this present study focuses on the evolution of resistance levels observed in *E. coli* in a IV-level hospital in Lima, Peru.

MATERIAL AND METHODS

Microorganisms

All *E. coli* isolates isolated were included in the study from hospitalized patients [on the Medicine and the Surgery Departments and the Intensive Care Unit (ICU)] from January, 2009 to June, 2010 also from January, 2012 to December, 2014 in the *Hospital Nacional Guillermo Almenara Irigoyen* (HNGAI) of Lima (Peru). Information about samples was extracted from databases of HNGAI. The analysis of data was performed considering 3 periods of 18 months each: Period 1 (January, 2009 - June, 2010), Period 2 (January, 2012 - June, 2013) and Period 3 (July, 2013 - December, 2014).

Bacterial identification and antimicrobial susceptibility

The different samples were processed according to the standard microbiology protocols at the Microbiology Laboratory of HNGAI. Bacterial identification, antibiotic susceptibility and the presence of Extended-Spectrum β-Lactamases (ESBLs) were performed using the automatic MicroScan system (Siemens Medical Solutions Diagnostics, Camberley, UK) in Period 1 and MicroScan and Vitek 2 system (BioMérieux, Marcy l'Etoile, France) in Period 2 and 3.

The antibiotics included in the study were ampicillin, piperacillin-tazobactam, cefazolin, cefotaxime, ceftriaxone, ceftazidime, cefepime, imipenem, cotrimoxazole, amikacin, gentamicin and ciprofloxacin. In addition, susceptibility to tigecycline was established in a subset of 124 isolates from Period 2 and Period 3. Antimicrobial susceptibility levels to all antimicrobial agents, except tigecycline, were established according to Clinical and Laboratory Standards guidelines (CLSI, 2014). Tigecycline susceptibility was determined according to the US Food and Drug Administration criteria (https://www.fda.gov/drugs/development-resources/tigecycline-injection-products)

Statistical analysis

The Chi-squared test was used to analyze statistical associations. A p-value <0.05 was considered as significant.

In all cases intermediate and resistant isolates were classified together for analysis purposes. Coagulase negative Staphylococcus (CoNS) were considered as contamination and were not included in the statistical analysis (Dargere et al, 2018).

RESULTS

Overall, 10,948 microorganisms were recovered from clinical samples from admitted patients: 1,030 were CoNS and were excluded from further analyses. Thus, a total of 9,918 microorganisms were considered for analysis. From these, 3,016 isolates (30.4%) were *E. coli*, with 1,770 (59%) and 992 (33%) being from the Medicine and the Surgery Departments, respectively, and 254 (8%) from the ICU. Most of those *E. coli* were recovered from urine samples (2,188, 72.5% of total *E. coli*), with 287 (9.5%), 201 (6.7%) and 194 (6.4%) from intrabdominal, wounds and blood samples as the most frequent sources. Other sources accounting together for the remaining 4.9% of isolates (Table 1).

Analysis by periods showed that the number of *E. coli* varied from 1,060 (28.1% of the microorganisms considered in Period 1) to 1,123 (31.8% of microorganisms considered in Period 2) and 833 (32.8% of microorganisms considered in Period 3) (p=0.0002). This increase in the number of *E. coli* was observed on the three departments analyzed, being only significant on the Medicine department (Table 2). Nevertheless, considering the overall number of microorganisms recovered/month and the *E. coli*/month, the proportion of *E. coli* from ICU varied around a half and a third of the ones observed on the Medicine and on the Surgery Departments (Table 3).

Analysis of the levels of antimicrobial resistance showed that, overall, *E. coli* isolates from the ICU showed higher levels of antimicrobial resistance. The highest resistance rates found were to ampicillin (90.7%) ciprofloxacin (79.4%) and cefazolin (75.2%). All isolates, with the exception of 8 recovered in the Period 2 from urine samples, were susceptible to imipenem. Similarly, the subset tested for tigecycline showed 0% of resistance (Table 4; Figure 1). When antimicrobial resistance was analyzed considering the original sample, significant differences in the resistance levels of all tested antimicrobial agents, excepting amikacin and imipenem was observed (Table 1). Thus, the isolates from lower respiratory tract samples showed the higher levels of resistance to cephalosporins and aminoglycosides, while those from wounds showed the higher levels of resistance to ciprofloxacin and cotrimoxazole (Table 1). As predictable, significant differences in the origin of isolates were observed among those proceeding from each one of the wards (the Medicine and the Surgery department and ICU) (Table 1).

Table 1. Samples analyzed and antimicrobial resistance

Sample	*Escherichia coli* (N=3016)						Percentage of antimicrobial resistance											
	Overall		M	S	ICU													
Sample	N	%	(1770)	(992)	(254)	P	Amp	PTZ	Cfz	Ctx	Cro	Caz	Fep	Imp	Ak	Gm	Cip	Sxt
Urine	2188	72.5	83.2	62.0	39.4	<0.0001	90.6	8.0	72.3	62.1	62.9	61.9	59.5	0.3	11.0	47.3	79.0	74.9
IA	287	9.5	2.0	19.4	23.2	<0.0001	86.4	12.9	67.9	61.7	62.4	59.6	57.5	0.0	7.3	38.3	77.0	71.4
Wounds	201	6.7	4.7	10.4	5.5	<0.0001	95.0	11.9	77.7	71.6	72.6	70.6	67.6	0.0	11.9	52.2	88.1	90.5
Blood	194	6.4	5.8	4.5	19.7	<0.0001	91.8	12.9	76.3	71.6	73.7	72.2	70.1	0.0	7.2	38.1	75.2	74.7
LRT	98	3.2	3.1	2.0	9.1	<0.0001	92.9	12.2	92.9	80.1	81.6	80.6	77.6	0.0	12.2	54.1	82.7	67.3
Other[1]	48	1.6	1.1	2.3	11.4	<0.0001												
p							0.0155	0.0157	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS	NS	0.0110	0.0112	<0.0001

Table 2. Number of *Escherichia coli* by ward and period analyzed.

	Medicine N (%)			Surgery N (%)			ICU N (%)			Overall N (%)		
	Period 1 N=2227	Period 2 N=1900	Period 3 N=1371	Period 1 N=887	Period 2 N=1084	Period 3 N=776	Period 1 N=663	Period 2 N=615	Period 3 N=395	Period 1 N=3777	Period 2 N=3599	Period 3 N=2542
E. coli	672 (30.2)	620 (32.6)	478 (34.9)	296 (33.4)	403 (37.2)	293 (37.8)	92 (13.9)	100 (16.3)	62 (15.7)	1060 (28.1)	1123 (31.2)	833 (32.8)
P		0.0122			0.1146			0.4691			0.0002	

ICU: Intensive Care Unit; Period 1: January 2009-June 2010; Period 2: January 2012-June 2013; Period 3: July 2013-December 2014
Significant differences referring to the number of *E. coli* isolated in each ward in each Period analyzed.

Overall, significant increases were observed in the resistance to cephalosporins and amikacin, with piperacillin-tazobactam, and gentamicin showing significant reductions in resistance, and imipenem and ciprofloxacin showing no relevant differences between this study periods (Table 4, Figure 2). Regarding cotrimoxazole levels of resistance decreased between Periods 1 and 2, however they tend to recover the original values in Period 3. Nevertheless, the evolution differed when analyzed by department. Isolates from the Medicine and the Surgery Departments tended to agree with above-mentioned data, with some exceptions, as amikacin in the Surgery Department, with the resistance levels remaining unaltered among the 3 analyzed periods. Meanwhile, isolates from the ICU showed a different scenario when tested for cephalosporins. Thus, in the Period 2 amid the ICU isolates the resistance rates of all cephalosporins decreased towards values similar or slightly over to those observed in the other two departments. They tend to increase again in the Period 3 over values detected on the Medicine and on the Surgery Departments, with these fluctuations being significant in the case of cefotaxime (p=0.0047) and ceftriaxone (p=0.0161), (Table 4; Figure 2).

Likewise, the overall prevalence of ESBLs has increased in the three periods analyzed, from 57.1% (Period 1) to 67.1% (Period 3) (p<0.0001), with an increase of 56.1% (Period 1) to 67.8% (Period 3) (p=0.0001) on the Medicine Department and 54.1% (Period 1) to 64.8% (Period 3) (p=0.0278) on the Surgical Department. Meanwhile, the prevalence of ESBLs on the ICU showed a non-significant reduction between the first and second period from 76.1% to 65.0%, returning to values of 74.2% in the Period 3.

Table 3. Differences in positivity among Periods.

	Medicine			Surgery			ICU			Overall		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
MM	123.7	105.3	76.1	49.3	60.2	43.1	36.8	40.8	21.8	209.8	199.3	141.1
EcM	37.3	34.4	26.5	16.4	22.4	16.3	5.1	5.5	3.4	58.9	62.4	46.3
EcM/ MM	0.30	0.33	0.35	0.33	0.37	0.38	0.14	0.13	0.16	0.28	0.31	0.33

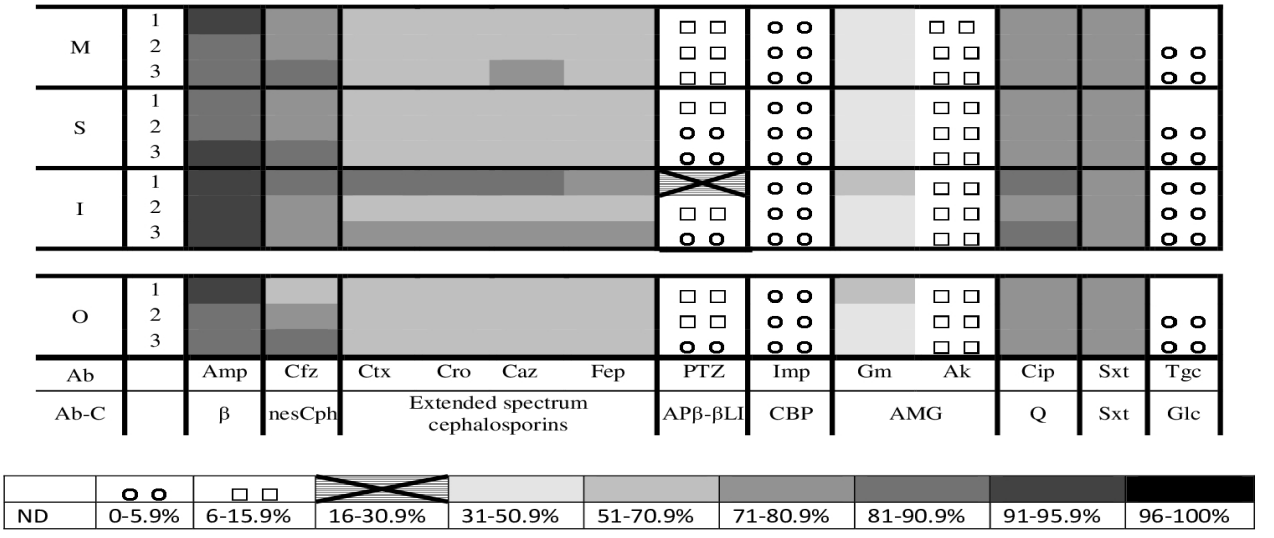
ICU: Intensive Care Unit; P1: Period 1 (January 2009-June-2010); P2: Period 2 (January 2012-June-2013); P3: Period 3 (July-2013-December-2014); EcM: *E. coli* per month; MM: Microorganisms per month.

Table 4. Antimicrobial resistance levels

			Percentage of Antimicrobial Resistance												
	N	Period	Amp	PTZ	Cfz	Ctx	Cro	Caz	Fep	Imp	Ak	Gm	Cip	Sxt	Tgc[a]
HNGAI	3016	Overall	90.7	8.7	75.2	64.5	65.2	64.0	61.4	0.3	10.5	47.2	79.4	74.7	---
	1060	01/2009-06/2010	92.0	13.7	66.8	61.0	61.6	59.6	57.2	0.0	8.3	51.0	80.7	77.1	---
	1123	01/2012-06/2013	89.7	6.1	74.6	65.3	65.7	64.2	61.3	0.5	10.5	46.7	78.1	72.0	0.0
	833	07/2013/12/2014	90.9	5.9	86.8	67.8	69.1	69.4	67.1	0.2	13.2	43.2	79.6	75.3	0.0
		P	NS	<0.0001	<0.0001	0.0072	0.0026	<0.0001	<0.0001	NS	0.0025	0.0029	NS	0.0233	NS
Medicine	1770	Overall	91.1	8.9	73.6	64.1	65.4	64.4	61.9	0.3	10.8	48.0	79.6	75.8	---
	672	01/2009-06/2010	92.1	12.5	75.5	59.5	60.0	58.6	56.1	0.0	7.7	50.7	80.8	79.0	---
	620	01/2012-06/2013	90.8	6.5	73.0	66.1	67.6	66.3	63.4	0.5	10.8	48,2	79.8	73.2	0.0
	478	07/2013/12/2014	90.4	7.2	89.1	68.0	70.1	69.9	68.0	0.4	15.3	43.9	77.6	74.7	0.0
		P	NS	0.0002	<0.0001	0.0056	0.0006	0.0002	0.0002	NS	0.0003	BS	NS	0.0416	NS
Surgery	992	Overall	89.1	7.6	73.4	62.8	62.5	61.4	58.1	0.2	9.6	45.6	78.2	72.9	---
	296	01/2009-06/2010	89.9	15.2	62.8	57.1	58.1	55.4	53.0	0.0	9.5	49.7	78.7	73.6	---
	403	01/2012-06/2013	86.8	4.5	73.0	64.3	62.5	61.0	57.1	0.5	9.4	44.9	75.2	70.5	0.0
	293	07/2013/12/2014	91.1	4.1	84.6	66.6	66.9	67.9	64.5	0.0	9.9	42.3	81.9	75.4	0.0
		P	NS	<0.0001	<0.0001	0.0437	0.0885	0.0076	0.0164	NS	NS	NS	NS	NS	NS
ICU	254	Overall	94.1	11.4	79.9	73.6	74.8	72.0	71.7	0.4	11.4	48.4	82.7	74.0	---
	92	01/2009-06/2010	95.0	17.4	84.8	84.8	84.8	80.4	78.3	0.0	8.7	57.6	85.8	73.9	---
	100	01/2012-06/2013	93.0	10.0	76.0	64.0	67.0	64.0	65.0	1.0	10.0	44.0	79.0	71.0	0.0
	62	07/2013/12/2014	93.5	4.8	79.0	72.6	72.6	72.6	72.6	0.0	12.9	41.9	83.9	79.0	0.0
		P	NS	0.0474	NS	0.0047	0.0161	NS	NS	NS	NS	BS	NS	NS	NS

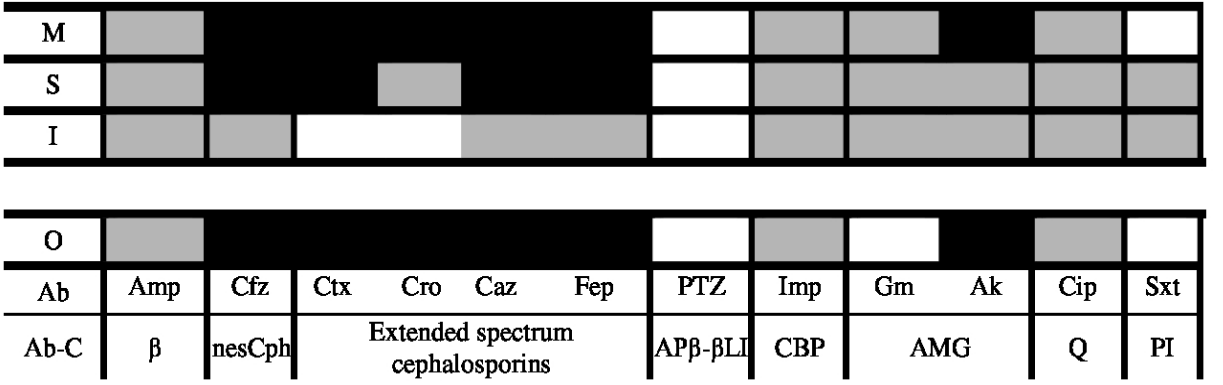
N: Number; Amp: ampicillin; PTZ: piperacillin-tazobactam; Cfz: cefazolin; Ctx: cefotaxime; Cro: ceftriaxone; Caz; ceftazidime; Fep: cefepime; Imp: imipenem; Ak: amikacin; Gm: gentamicin; Cip: ciprofloxacin; Sxt: cotrimoxazole; Tgc: tigecycline; HNGAI: *Hospital Nacional Guillermo Almenara Irigoyen*; ICU: intensive care unit; BS: bordering significance (0.05<p<0.09); NS: no significant; --: No data.
Significant differences established among levels of resistance of each antibiotic in each ward among the analyzed periods.
[a] Tested in 124 isolates.

Figure 1: Heat map of antimicrobial resistance by period and department analyzed.



1: Period 01/2009-06/2010; 2: Period 01/2012-06/2013; 3: Period 07/2013-12/2014; M: Medicine; S: Surgery; I: Intensive Care Unit; O: Overall; Amp: ampicillin; Cfz: cefazolin; Ctx: cefotaxime; Cro: ceftriaxone; Caz; ceftazidime; Fep: cefepime; PTZ: piperacillin-tazobactam; Imp: imipenem; Gm: gentamicin; Ak: amikacin; Cip: ciprofloxacin; Sxt: cotrimoxazole; Tgc: Tigecycline Ab: Antibiotic; Ab-C: Antibiotic category defined by Magiorakos et al (2012); β: β-lactam antibiotics (penicillins); nesCph: non extended-spectrum cephalosporins; APβ: anti-Pseudomonal β-lactam; βLI: β-lactam plus β-lactamase inhibitors; CBP: carbapenems; AMG: aminoglycosides; Q: quinolones; PI: pholate inhibitors; GLC: glycylcyclines. ND: No data

Figure 2: Evolutive trends in antimicrobial resistance between the study periods.



M: Medicine; S: Surgery; I: Intensive Care Unit; O: Overall; Amp: ampicillin; Cfz: cefazolin; Ctx: cefotaxime; Cro: ceftriaxone; Caz; ceftazidime; Fep: cefepime; PTZ: piperacillin-tazobactam; Imp: imipenem; Gm: gentamicin; Ak: amikacin; Cip: ciprofloxacin; Sxt: cotrimoxazole; Tgc: Tigecycline; Ab: Antibiotic; Ab-C: Antibiotic categories defined by Magiorakos et al (2012); β: β-lactam antibiotics (penicillins); nesCph: non extended-spectrum cephalosporins; APβ: anti-Pseudomonal β-lactam; βLI: β-lactam plus β-lactamase inhibitors; CBP: carbapenems; AMG: aminoglycosides; Q: Quinolones; PI: pholate inhibitors.
Grey: No significant differences; White: Significant decrease; Black: Significant increase

DISCUSSION

While *E. coli* is a common inhabitant of the human gut, a series of these microorganisms are relevant human pathogens and they are frequently isolated as a cause of severe life-threatening infections (Simonsen et al., 2014). In those cases, an early and adequate antibiotic treatment is the key for good patient outcomes (Bonine et al., 2019). Nevertheless, the increasing levels of antimicrobial resistance are challenging the current antibiotic schedules, having a direct impact on fatal outcomes (ARC, 2022; Cassini et al., 2019; CDC, 2019). In most low- and middle-income countries, this scenario is aggravated by socioeconomic and cultural factors such as lack of adequate and sufficient health infrastructures, the treatment costs, the over-the-counter access to antibiotics or (especially in low-income countries) a limited number of antibiotic alternatives, and others (Mandomando et al., 2010; Mills, 2014; Nusair et al., 2021; Worku et al., 2003)

Antimicrobial resistance differs among isolates from different samples. Of note, as the burden of each sample within each ward differs, this finding probably contributes to the differences in the levels of resistance observed among isolates recovered from the ICU, the Medicine and the Surgery departments.

Overall, the present data shows high rates of resistance to β-lactams excepting carbapenems and piperacillin-tazobactam. These data are directly related to the high levels of ESBLs found in our study also in accordance with the frequency of ESBLs in *E. coli* and other pathogenic microorganisms reported in previous studies in Peru (Alcedo et al., 2022; García et al., 2016; Palma et al., 2017; Castillo-Tokumori et al., 2017; Granda et al., 2019).

In addition to older β-lactam agents (ampicillin and cefazolin), the highest levels of antimicrobial resistance were to ciprofloxacin and cotrimoxazole, with ESBL prevalence being >50% in all Departments and periods analyzed. For years, quinolones have been a commonly used agent to treat the community and hospital infections and it can be easily obtained over-the-counter in Peru (Rojas-Adrianzén et al., 2018; Ruiz, 2019). Furthermore, quinolones, such as enrofloxacin, have been largely used in veterinary health for therapeutic, prophylaxis or growth promoter purposes (Ruiz, 2019). The levels of quinolone resistance found in this study agree with previous data in the country. Thus, Palma et al (2017), reported 71% of ciprofloxacin non-susceptible *E.coli* isolated from children with bacteremia, and García et al. (2012) reported 85.6% of ciprofloxacin resistance by bacteremic *E. coli*. Quinolone resistance is mainly mediated by chromosomal mechanisms, mainly mutations in quinolone targets, in addition to chromosomal efflux pumps, cell wall alterations and native chromosomal qnr genes (Ruiz, 2003, 2019). Additionally, a series of transferable genes able to produce low-level quinolone resistance and favoring further selection of full quinolone-resistance have been described (Ruiz, 2019). Of these, while scarcely sought, previous reports have shown at least the

circulation of aac (6') Ib-cr, qnrB, qnrS and qnrVC resistance genes among *E. coli* isolated in Peru (Palma et al., 2017; Pons et al., 2014; Tamariz et al., 2018). Meanwhile, for many years cotrimoxazole has been largely used in the treatment of community-acquired infections, with *E. coli* usually presenting high levels of resistance worldwide (Benmessaoud et al., 2016: Mandomando et al., 2010; Ruiz et al., 2007; Yabar et al., 2017).

The description of a high prevalence of ESBLs is not a surprising fact. Thus, different reports showed a high prevalence of ESBL-producing *E. coli* in Peru, which can be up to 76.8% reported by Garcia et al (2012). Most recent reports in the country described around 40% of ESBL-producing *E. coli* around 40%. Thus, Palma et al. (2017), showed a 43.5% of ESBL-producing *E. coli* in the blood samples. In the same line, Castillo-Tokumori et al. (2017) and Ormeño et al. (2022) reported a 40.8% and 42.5%, respectively, of ESBL-producing *E. coli* among uropathogenic *E. coli*. In Peru, the presence of ESBLs has also been reported among healthy people, as well as in non-human samples, from the environment, the food, the livestock and from wild animals (Alcedo et al., 2022; Castillo et al., 2022).

Descriptions of antimicrobial-resistant microorganisms have expanded from all Latin America, including microorganisms from the environment, animals, food and human origins (Castillo et al., 2022; García-Betancur et al., 2021; Medina-Pizzalli et al., 2021). Regarding *E. coli*, similar data have been reported in different countries, with descriptions of high level of antimicrobial resistance (Finello et al., 2021; Garza-González et al., 2019), which includes the description of *E. coli* isolates showing resistance to last resort antibacterial agents (Paiva et al., 2021) and high levels of ESBLs (Ponce-de-Leon et al., 2018).

Of note, these antimicrobial resistance values, with the exception of those for ciprofloxacin, are also in accordance with most modern CLSI guidelines (CLSI, 2021), as no change in breakpoints have been done. Regarding ciprofloxacin, as current breakpoints are more restrictive, it is predictable that under current considerations ciprofloxacin resistance levels will be higher. Unfortunately, in the database no exact minimal inhibitory concentration values were recorded.

The evolution of antimicrobial resistance over time has shown a general trend to increase, especially when regarding cephalosporins. This finding is not so surprising, as this trend has been reported worldwide which lead the World Health Organization to issue an alert to this trend (WHO, 2019). Of note, albeit modest, an overall significant increase in the levels of resistance to amikacin has been observed. Amikacin resistance is mostly associated with the presence of transferable genes (Ruiz et al., 2005; Seward et al., 1998), and then with the potential to be horizontally disseminated (Seward et al., 1998). This finding highlights the need to preserve functional antibiotics to avoid the presence of untreatable infections. When the evolution was analyzed by the

hospital departments, an unexpected scenario was observed in which *E. coli* isolates from the ICU showed a decrease in resistance levels and the prevalence of ESBLs in Period 2 towards values present in the Medicine and the Surgery Departments, then increasing again in Period 3. There is no clear explanation for this phenomenon, other than a possible increase in fluxes of microorganisms between different hospital departments resulting in the mix of bacterial populations, thus leading to increasing antimicrobial resistance levels in the Medicine and the Surgery Departments and an inverse phenomena in the ICU. While this scenario may favor a transient decrease of antimicrobial resistance in the ICU, over medium or long term it may lead to a rise in antimicrobial resistance in all hospital settings.

While their use is well established, the fact that two different automated methods were used during the time of the study may be considered as a limitation. Other limitations on the present study are the lack of data about patient outcomes and the uncertain role of CoNS as a cause of infection. Nevertheless, different reports have described a series of negative impacts of antibiotic resistance on patient outcomes, including the prolonged hospitalization, the expensive treatment costs as well as an effect on poor outcomes increasing (Cassini et al., 2019; CDC, 2019; Pons & Ruiz, 2019). Meanwhile, CoNS were classified as contaminants (Dargère et al., 2018), however a small fraction of them might act as a true infective agent (Quispe et al., 2020). In addition, no data on colistin resistance was available, with colistin being considered as the last resort treatment (Thomas et al., 2019). Finally, while antimicrobial resistance levels were established using the CLSI (2014) criteria, these breakpoints, except for ciprofloxacin, have not been modified (CLSI, 2021), thereby ciprofloxacin resistance might be underestimated as for current breakpoints.

In summary, high levels of resistance have been reported among *E. coli,* with worrisome levels of ESBL. While the levels of cephalosporin resistance tended to increase, those from the ICU isolates showed a transient inverse trend, suggesting a reduction on the controlling measures between different departments, which favors a higher circulation of isolates between departments. A close control of antibiotics used together with a continuous and an active surveillance on the antibiotic resistance´s development is needed to preserve the activity of these antibiotics.

ACKNOWLEDGMENTS

We thank Donna Pringle for the language correction. Funding: JR was supported by Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (FONDECYT - Perú) and Universidad Científica del Sur within the "Proyecto de Mejoramiento y Ampliación de los Servicios del Sistema Nacional de Ciencia, Tecnología e Innovación Tecnológica" [contract: 08-2019-FONDECYT-BM-INC-INV"].

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests to disclose.

REFERENCES

1. Alcedo K, Ruiz J, Ochoa TJ, Riveros M. High prevalence of blaCTX-M in fecal commensal *Escherichia coli* from healthy children. *Infect Chemother 54*: 59-69, 2022.
2. ARC. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet 399*: 629-655, 2022.
3. Benmessaoud R, Nezha M, Moraleda C, Jroundi I, Tligui H, Seffar M, Pons MJ, Alvarez MJ, Chaacho S, Vila J Alonso PL, Bassat Q, Ruiz J. Antimicrobial resistance levels among diarrhoeagenic microorganisms recovered from children under 5 with acute moderate-to-severe diarrhoea in Rabat, Morocco. *J Global Antimicrob Resist 7*: 34-36, 2016.
4. Bonine NG, Berger A, Altincatal A, Wang R, Bhagnani T, Gillard P, Lodise T. Impact of delayed appropriate antibiotic therapy on patient outcomes by antibiotic resistance status from serious Gram-negative bacterial infections. *Am J Med Sci 357*: 103-110, 2019.
5. Bumbangi FN, Llarena AK, Skjerve E, Hang'ombe BM, Mpundu P, Mudenda S, Mutombo PB, Muma JB. Evidence of community-wide spread of multi-drug resistant *Escherichia coli* in young children in Lusaka and Ndola districts, Zambia. *Microorganisms 10*: 1684, 2022.
6. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, Colomb-Cotinat M, Kretzschmar ME, Devleesschauwer B, Cecchini M, Ouakrim DA, Oliveira TC, Struelens MJ, Suetens C, Monnet DL; Burden of AMR Collaborative Group. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis 19*: 56-66, 2019.
7. Castillo AK, Espinoza K, Guibert F, Chaves AF, Ruiz J, Pons MJ. Antibiotic susceptibility levels among non-clinical *Escherichia coli* as a marker of antibiotic pressure in Peru (2009-2019): one health approach. *Heliyon 8*: e10573, 2022.
8. Castillo-Tokumori F, Irey-Salgado C, Málaga G. Worrisome high frequency of extended-spectrum beta-lactamase-producing *Escherichia coli* in community-acquired urinary tract infections: a case-control study. *Int J Infect Dis 55*: 16-19, 2017.
9. CDC. Center for Disease Control and Prevention. *Antibiotic resistance threats in the United States, 2019*. Department of Health and Human Services, CDC Atlanta, GA, 2019.
10. Cervera C, van Delden C, Gavaldà J, Welte T, Akova M, Carratalà J; ESCMID Study Group for Infections in Compromised Hosts. Multidrug-resistant bacteria in solid organ transplant recipients. *Clin Microbiol Infect 20*: 49-73, 2014.
11. CLSI. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing.* Document M100 - S24. CLSI, Wayne, PA, 2014.
12. CLSI. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing.* Document M100 – S31. CLSI, Wayne, PA, 2021.
13. Dargère S, Cormier H, Verdon R. Contaminants in blood cultures: importance, implications, interpretation and prevention. *Clin Microbiol Infect 24*: 964-969, 2018.
14. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ. Antimicrobial resistance in ESKAPE pathogens. *Clin Microbiol Rev 33*: e00181-19, 2020.

15. Finello M, Suasnabar DF, García MJ, Díaz MV, Richetta L, Toranzo A, Hernández D, Cometto MA, Vázquez SM, Caeiro JP, Sierra J, Saad EJ. Características clínicas y microbiológicas de infecciones del torrente sanguíneo en pacientes adultos neutropénicos. *Rev Argent Microbiol 53:* 183-193, 2021.

16. García C, Astocondor L, Rojo-Bezares B, Jacobs J, Sáenz Y. Molecular characterization of extended-spectrum β-lactamase-producer *Klebsiella pneumoniae* isolates causing neonatal sepsis in Peru. *Am J Trop Med Hyg 94*: 285-288, 2016.

17. García C, Horna G, Linares E, Ramírez R, Tapia E, Velásquez J, Medina V, Guevara J, Urbina M, Zevallos S, Espinoza N, Samalvides F, Jacobs J. Antimicrobial drug resistance in Peru. *Emerg Infect Dis 18*: 520-521, 2012.

18. García-Betancur JC, Appel TM, Esparza G, Gales AC, Levy-Hara G, Cornistein W, Vega S, Nuñez D, Cuellar L, Bavestrello L, Castañeda-Méndez PF, Villalobos-Vindas JM, Villegas MV. Update on the epidemiology of carbapenemases in Latin America and the Caribbean. *Expert Rev Anti Infect Ther 19:*197-213, 2021.

19. Garza-González E, Morfín-Otero R, Mendoza-Olazarán S, Bocanegra-Ibarias P, Flores-Treviño S, Rodríguez-Noriega E, Ponce-de-León A, Sanchez-Francia D, Franco-Cendejas R, Arroyo-Escalante S, Velázquez-Acosta C, Rojas-Larios F, Quintanilla LJ, Maldonado-Anicacio JY, Martínez-Miranda R, Ostos-Cantú HL, Gomez-Choel A, Jaime-Sanchez JL, Avilés-Benítez LK, Feliciano-Guzmán JM, Peña-López CD, Couoh-May CA, Molina-Jaimes A, Vázquez-Narvaez EG, Rincón-Zuno J, Rivera-Garay R, Galindo-Espinoza A, Martínez-Ramirez A, Mora JP, Corte-Rojas RE, López-Ovilla I, Monroy-Colin VA, Barajas-Magallón JM, Morales-De-la-Peña CT, Aguirre-Burciaga E, Coronado-Ramírez M, Rosales-García AA, Ayala-Tarín MD, Sida-Rodríguez S, Pérez-Vega BA, Navarro-Rodríguez A, Juárez-Velázquez GE, Cetina-Umaña CM, Mena-Ramírez JP, Canizales-Oviedo J, Moreno-Méndez MI, Romero-Romero D, Arévalo-Mejía A, Cobos-Canul DI, Aguilar-Orozco G, Silva-Sánchez J, Camacho-Ortiz A. A snapshot of antimicrobial resistance in Mexico. Results from 47 centers from 20 states during a six-month period. *PLoS One 14*: e0209865, 2019.

20. Granda A, Riveros M, Martínez-Puchol S, Ocampo K, Laureano-Adame L, Corujo A, Reyes I, Ruiz J, Ochoa TJ. Presence of extended-spectrum β-lactamase, CTX-M-65, in *Salmonella enterica* serovar Infantis isolated from children with diarrhea in Lima, Peru. *J Pediatric Infect Dis 14:* 194-200, 2019.

21. Jasovský D, Littmann J, Zorzet A, Cars O. Antimicrobial resistance-a threat to the world's sustainable development. *Ups J Med Sci 121*: 159-164, 2016.

22. Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE, Brewer TG, Engmann CM, Houpt ER, Kang G, Kotloff KL, Levine MM, Luby SP, MacLennan CA, Pan WK, Pavlinac PB, Platts-Mills JA, Qadri F, Riddle MS, Ryan ET, Shoultz DA, Steele AD, Walson JL, Sanders JW, Mokdad AH, Murray CJL, Hay SI, Reiner RC Jr. Morbidity and mortality due to *Shigella* and enterotoxigenic *Escherichia coli* diarrhoea: the Global Burden of Disease Study 1990-2016. *Lancet Infect Dis 18*: 1229-1240, 2018.

23. Levine MM, Nasrin D, Acácio S, Bassat Q, Powell H, Tennant SM, Sow SO, Sur D, Zaidi AKM, Faruque ASG, Hossain MJ, Alonso PL, Breiman RF, O'Reilly CE, Mintz ED, Omore R, Ochieng JB, Oundo JO, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ahmed S, Qureshi S, Quadri F, Hossain A, Das SK, Antonio M, Saha D, Mandomando I, Blackwelder WC, Farag T, Wu Y, Houpt ER, Verweiij JJ, Sommerfelt H, Nataro JP, Robins-Browne RM, Kotloff KL. Diarrhoeal disease and subsequent risk of death in infants and children residing in low-income and middle-income countries: analysis of the GEMS case-control study and 12-month GEMS-1A follow-on study. *Lancet Glob Health 8:* e204-e214, 2020.

24. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect 18*: 268-281, 2012.

25. Mandomando I, Sigaúque B, Morais L, Espasa M, Vallès X, Sacarlal J, Macete E, Aide P, Quintò L, Nhampossa T, Machevo S, Bassat Q, Menéndez C, Ruiz J, Roca A, Alonso PL. Antimicrobial drug resistance trends of bacteremia isolates in a rural hospital in southern Mozambique. *Am J Trop Med Hyg 83*: 152-157, 2010.

26. Medina M., Castillo-Pino E. 2019. An introduction to the epidemiology and burden of urinary tract infections. *Ther Adv Urol 11:*175628721983217, 2019.

27. Medina-Pizzali ML, Hartinger SM, Salmon-Mulanovich G, Larson A, Riveros M, Mäusezahl D. Antimicrobial resistance in rural settings in Latin America: a scoping review with a One Health lens. *Int J Environ Res Public Health 18:*9837, 2021.

28. Mills A. Health care systems in low- and middle-income countries. *N Engl J Med 370*: 552-557, 2014.

29. Nusair MB, Al-Azzam S, Alhamad H, Momani MY. The prevalence and patterns of self-medication with antibiotics in Jordan: A community-based study. *Int J Clin Pract 75:* e13665, 2021.

30. Ormeño MA, Ormeño MJ, Quispe AM, Arias-Linares MA, Linares E, Loza F, Ruiz J, Pons MJ. Recurrence of urinary tract infections due to *Escherichia coli* and its association with antimicrobial resistance. *Microb Drug Resist 28*: 185-190, 2022.

31. Palma N, Pons MJ, Gomes C, Mateu J, Riveros M, García W, Jacobs J, García C, Ochoa TJ, Ruiz J. Resistance to quinolones, cephalosporins and macrolides in *Escherichia coli* causing bacteraemia in Peruvian children. *J Glob Antimicrob Resist 11*: 28-33, 2017.

32. Paiva Y, Nagano DS, Franco Cotia AL, Guimarães T, Ruedas Martins RC, Perdigão Neto LV, Côrtes MF, Marchi AP, Corscadden L, Machado AS, de Paula AI, Moyses Franco LA, Neves PR, Levin AS, Costa SF. Colistin-resistant *Escherichia coli* belonging to different sequence types: genetic characterization of isolates responsible for colonization, community- and healthcare-acquired infections. *Rev Inst Med Trop Sao Paulo 63*: e38, 2021.

33. Ponce-de-Leon A, Rodríguez-Noriega E, Morfín-Otero R, Cornejo-Juárez DP, Tinoco JC, Martínez-Gamboa A, Gaona-Tapia CJ, Guerrero-Almeida ML, Martin-Onraët A, Vallejo Cervantes JL, Sifuentes-Osornio J. Antimicrobial susceptibility of Gram-negative bacilli isolated from intra-abdominal and urinary-tract infections in Mexico from 2009 to 2015: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). *PLoS One 13*: e0198621, 2018.

34. Pons MJ, Mosquito S, Gomes C, del Valle LJ, Ochoa TJ, Ruiz J. Analysis of quinolone-resistance in commensal and diarrheagenic *Escherichia coli* isolates from infants in Lima, Peru. *Trans R Soc Trop Med Hyg 108*: 22-28, 2014

35. Pons MJ, Ruiz J. Current trends in epidemiology and antimicrobial resistance in Intensive Care Units. *J Emerg Crit Care Med 3*: 5, 2019.

36. Poolman JT, Wacker M. Extraintestinal pathogenic *Escherichia coli*, a common human pathogen: challenges for vaccine development and progress in the field. *J Infect Dis 213*: 6-13, 2016.

37. Quispe AM, Soza G, Ramos Chirino M, Quiroz D, Pons MJ. Multidrug resistance bacteremia in neonates and its association with late-onset sepsis and Coagulase-negative *Staphylococci. J Infect Dev Ctries 30:* 1256-1263, 2020.

38. Rojas-Adrianzén C, Pereyra-Elias R, Mayta-Tristán P. Prevalencia y factores asociados a la compra de antimicrobianos sin receta médica, Perú 2016. *Rev Peru Med Exp Salud Publica 35:* 400-408, 2018.

39. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother 51*: 1109-1117, 2003.

40. Ruiz J. Transferable mechanisms of quinolone resistance from 1998 onward. *Clin Microbiol Rev 32*: e0007-19, 2019.

41. Ruiz J, Bertran S, Sauca G, Julià A, Vila X, Gómez E, Jiménez de Anta MT, Vila J. Isolation of an amikacin-resistant *Escherichia coli* strain after tobramycin treatment of previous recurrent episodes of respiratory tract infections caused by *Pseudomonas aeruginosa. Clin Microbiol Infect. 11*: 71-73, 2005.

42. Ruiz J, Mensa L, O'Callaghan C, Pons MJ, González A, Vila J, Gascón J. *In vitro* antimicrobial activity of rifaximin against enteropathogens causing traveler's diarrhea. *Diagn Microbiol Infect Dis 59*: 473-475, 2007.

43. Seward RJ, Lambert T, Towner KJ. Molecular epidemiology of aminoglycoside resistance in *Acinetobacter* spp. *J Med Microbiol 47:* 455-462, 1998.

44. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. *Clin Microbiol Rev 27*: 21-47, 2014.

45. Tamariz J, Llanos C, Seas C, Montenegro P, Lagos J, Fernandes MR, Cerdeira L, Lincopan N. Draft genome sequence of the first New Delhi metallo-β-lactamase (NDM-1)-producing *Escherichia coli* strain isolated in Peru. *Genome Announc 6*: e00199-18, 2018.

46. Teillant A, Gandra S, Barter D, Morgan DJ, Laxminarayan R. Potential burden of antibiotic resistance on surgery and cancer chemotherapy antibiotic prophylaxis in the USA: a literature review and modelling study. *Lancet Infect Dis 15:* 1429-1437, 2015.

47. Thomas R, Velaphi S, Ellis S, Walker AS, Standing JF, Heath P, Sharland M, Dona D. The use of polymyxins to treat carbapenem resistant infections in neonates and children. *Expert Opin Pharmacother 20*: 415-422, 2019.

48. Ugarte Silva RG, Olivo López JM, Corso A, Pasteran, F, Albornoz E, Sahuanay Blácido Z P. Resistencia a colistín mediado por el gen mcr-1 identificado en cepas de *Escherichia coli* y *Klebsiella pneumoniae*: primeros reportes en el Perú. *An Fac Med. 79*: 213-217, 2018.

49. Vila J, Sáez-López E, Johnson JR, Römling U, Dobrindt U, Cantón R, Giske CG, Naas T, Carattoli A, Martínez-Medina M, Bosch J, Retamar P, Rodríguez-Baño J, Baquero F, Soto SM. *Escherichia coli:* an old friend with new tidings. *FEMS Microbiol Rev 40*: 437-463, 2016.

50. WHO. World Health Organization. *No time to wait: securing the future from drug-resistant infections*. Report to the Secretary-General of the United Nations, 2019. Available at: https://www.who.int/antimicrobial-resistance/interagency-coordination-group/IACG_final_report_EN.pdf?ua=1. Accessed at 16 march 2021.

51. Worku S, Mariam AB. Practice of self-medication in Jimma Town. *Ethiop J Health Dev 17:* 111-116, 2003.

52. Yábar MN, Curi-Pesantes B, Torres CA, Calderón-Anyosa R, Riveros M, Ochoa TJ. Multirresistencia y factores asociados a la presencia de betalactamasas de espectro extendido en cepas de *Escherichia coli* provenientes de urocultivos. *Rev Peru Med Exp Salud Publica 34:* 660-665, 2017.

53. Ymaña B, Luque N, Ruiz J, Pons MJ. Worrying levels of antimicrobial resistance in Gram-negative bacteria isolated from cell phones and uniforms of Peruvian intensive care unit workers. *Trans R Soc Trop Med Hyg 116*: 676-678, 2022