

Original Article

## Multidrug resistance bacteremia in neonates and its association with late-onset sepsis and Coagulase-negative *Staphylococci*

Antonio M Quispe<sup>1</sup>, Gabriela Soza<sup>2</sup>, Maria Ramos Chirinos<sup>2</sup>, Danny Quiroz<sup>2</sup>, Maria J Pons<sup>3</sup>

<sup>1</sup> Universidad Continental, Huancayo, Peru

<sup>2</sup> Instituto Nacional Materno Perinatal, Lima, Peru

<sup>3</sup> Universidad Científica del Sur, Lima, Peru

### Abstract

**Introduction:** This study aimed to assess the association between multidrug resistance (MDR) and late-onset sepsis (LOS) among newborns with bloodstream infection (BSI).

**Methodology:** In this cross-sectional study, we routinely tested every newborn with a presumptive diagnosis of sepsis admitted to the largest reference maternity hospital in Lima, Peru for BSI over an 18-month period. We tested every isolate for MDR by using the disk-diffusion method and assessed its associated factors by using a robust Poisson regression analysis with a particular focus on its association with LOS (vs. early-onset sepsis, EOS).

**Results:** We analyzed a total of 489 subjects, including 340 (69%) newborns with LOS, and estimated an MDR rate of 80% (95% confidence interval, CI: 76%-83%), which was significantly higher (p-value < 0.001) among LOS (85%; 95% CI: 81%-89%) than EOS cases (67%; 95% CI: 59%-75%). The primary isolate was coagulase-negative *Staphylococci* (*CoNS*) (60%), which exhibited a limited subset of antibiotic MDR patterns, most of which were characterized by their resistance to ceftazidime, gentamicin, and clindamycin and levofloxacin. Overall, the prevalence of MDR was higher among LOS compared to EOS cases (adjusted prevalence ratio [aPR] = 1.28; 95% CI: 1.14-1.45), and among BSI due to *CoNS* compared to other bacteria (Apr = 1.10; 95% CI: 1.01-1.20).

**Conclusions:** MDR among newborns with sepsis is exceptionally high, being even higher among those with LOS than newborns with EOS, and among those infected with *CoNS* compared to other bacteria. Furthermore, *CoNS* exhibited a limited subset of MDR patterns, which could be used to guide therapeutic decisions.

**Key words:** Neonatal sepsis; drug resistance, microbial; anti-bacterial agents; bacteremia.

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### Introduction

Neonatal sepsis is a significant cause of neonatal morbidity and mortality in Latin America and worldwide [1]. Clinically, neonatal sepsis can be classified as early-onset sepsis (EOS) or late-onset sepsis (LOS) depending on whether the sepsis clinically manifests within or after the first 72 hours of life, respectively [2]. Regardless of the type, this disease is difficult to diagnose since it often presents with nonspecific signs and symptoms, making it essential to procure proper medical care and effective antibiotic treatment as soon as possible [3].

Currently, the increasing levels of antibiotic resistance are a public health concern worldwide. Neonatal sepsis is increasingly more frequently associated with bacteria showing multidrug-resistance (MDR), such as extended-spectrum beta-lactamase (ESBL), and carbapenemases producers [4]. This fact

makes it difficult to implement adequate empirical antimicrobial therapy in newborns, thereby extending the length of hospital stay [5] and increasing the risk of mortality [6]. To prevent such outcome, it is crucial to determine the epidemiology of EOS and LOS, which seems to be related to bacterial colonization by vertical transmission [7] and direct contact with contaminated environments by horizontal transmission [8], respectively.

As in other low-middle income countries, in Peru, antibiotic resistance levels continue to rise, becoming an every emerging threat to health [9]. Different reports have shown that bacterial antibiotic resistance levels are changing, varying by age, sample origin, geographical distribution, and several other not necessarily modifiable factors [10-12]. The present study aimed to assess the epidemiology of neonatal sepsis and its potential association between multidrug resistance and

its classification (EOS/LOS) in the Peruvian context, in order to identify modifiable risk factors to help reduce both.

## Methodology

### *Study design and Population*

Following a cross-sectional study design, we analyzed every case of neonatal sepsis with positive bacteremia diagnosed at the National Maternal Perinatal Institute (INMP) of Lima during the period January 2017-June 2018. At the INMP, which is the largest maternity reference hospital in Peru (> 20,000 births annually), every newborn has full insurance coverage, including critical care if needed. We aimed to enroll every eligible subject, and thus, the study inclusion criteria were newborns (< 28 days of life) with a diagnosis of neonatal sepsis confirmed with positive bacteremia tested by blood culture. Furthermore, to prevent information bias, the exclusion criteria included: contaminated blood cultures and samples collected from patients receiving antibiotic therapy during sample collection. Appropriate ethical approvals was obtained by National Maternal Perinatal Institute, in Peru.

### *Strain identification*

We cultured every blood sample in bottles which were incubated in a BD BACTEC automated blood culture system for seven days before reporting no growth. Then, we identified the etiological agent by performing Gram stains and sub-cultures in selective media, both according to conventional microbiology protocols [13]. During this process, we considered coagulase-negative *Staphylococci* (*CoNS*) as a plausible etiological agent only when patients tested *CoNS* positive in two separate blood cultures.

### *Antimicrobial susceptibility testing*

We assessed the antimicrobial susceptibility of each isolate using the standard disk- diffusion method in Mueller–Hinton agar plates as well as the Kirby Bauer method with *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 as quality control. Then we interpreted the results as resistant, intermediate, or sensitive according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Bacteria resistant to at least one antibiotic in three or more drug classes were considered to be MDR [15]. When requested, we assessed ESBL expression using a conventional ESBL disk synergy test, containing

cefotaxime, amoxicillin plus clavulanic acid, and ceftazidime in Mueller–Hinton agar [16].

### *Data Registry and Quality Control*

Data registry was conducted using the WHONET software, which is a free Windows-based database software developed by the World Health Organization Collaborating Centre for Surveillance of Antimicrobial Resistance to facilitate the registry and analysis of antimicrobial susceptibility data [17]. With this software, we incorporated several quality control procedures, including specific categories, range values, double-check, and two-pass verification with an independent reviewer (GS).

### *Statistical Analysis*

First, we performed a descriptive data analysis summarizing each categorical variable by its absolute and relative frequencies and every numerical variable by its mean and standard deviation. Second, we performed a bivariate comparison between newborns with EOS vs. LOS using the Chi-square test and the Student's t-test for proportion and mean comparisons, respectively. Third, we explored the antibiotic susceptibility of the top five most frequent etiological agents responsible for EOS and LOS cases separately, considering as resistant any isolate tested as either resistant or intermediate in the antimicrobial susceptibility test. Fourth, we assessed the rates of isolates with a positive ESBL phenotype, carbapenemases, methicillin-resistant *Staphylococcus aureus* (MRSA), and MDR. Fifth, we estimated the MDR rate for each etiological agent and compared MDR rates between EOS and LOS cases. Sixth, we performed a sub-analysis among newborns infected with *CoNS* to assess the main antibiotic-resistant patterns using only those antibiotics with levels of resistance over 50% per antibiotic tested. Finally, we used a by generalized linear model with a Poisson distribution, link log, and robust error variance to model the prevalence of MDR and identify its main associated factors, especially focused on its association with LOS. We conducted these analyses using STATA™ MP version 14.0 (Stata Corp., College Station, TX) and a 95% confidence interval (95% CI).

## Results

### *Study population*

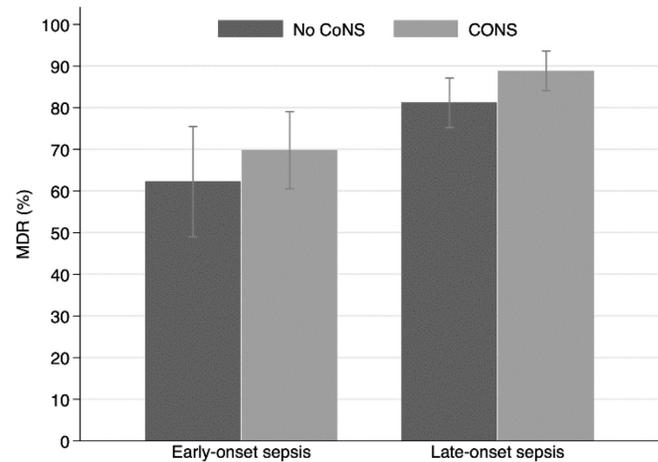
We analyzed a total of 489 newborns diagnosed with sepsis: 340 (69%) classified as LOS (13.5 ± 7.3 days of age) and 149 as EOS (1.5 ± 1.0 days of age) (Table 1). Overall, we tested most newborns (96%)

hospitalized at the INMP, including 38% from the intensive care unit, and only a small fraction of newborns (4%) were tested upon transfer from other hospitals. When comparing the demographic data and the clinical characteristics of the EOS vs. LOS groups, we only found significant differences in terms of preterm birth (22% vs. 34%, p-value = 0.011).

*Etiological strains among newborns with EOS or LOS*

Among newborns with EOS, the leading etiological agent identified in the blood cultures was by far *CoNS* (64%) (Figure 1). Other etiological agents included other *Staphylococcus* spp. (10%), *Enterococcus* spp. (6%), *Streptococcus* spp. (6%), *Escherichia coli* (5%), *Acinetobacter* spp. (3%), *Klebsiella pneumoniae* (3%), *Serratia* spp. (1%), and two other agents (*Staphylococcus aureus* and *Stenotrophomonas maltophilia*), each with a frequency of less than 1%. Among newborns with LOS, the principal etiological agent identified in blood cultures was by far *CoNS* (50%). Other etiological agents included *Acinetobacter* spp. (13%), *K. pneumoniae* (11%), *Escherichia coli* (7%), other *Staphylococcus* spp. (6%), *S. aureus* (4%), *Serratia* spp. (3%), *Streptococcus* spp. (3%), *S.*

**Figure 1.** MDR rates among newborns with EOS vs. LOS, infected with CoNS vs. no CoNS.



MDR: Multidrug-resistant; EOS: Early-onset sepsis; LOS: Late-onset sepsis; CoNS: *Coagulase-negative Staphylococci*.

*maltophilia* (2%), and three other bacteria (*Enterococcus* spp., and *P. aeruginosa* and *Enterobacter*), each with a frequency of 1% or less (Table 1).

**Table 1.** General characteristics of the study subjects.

Characteristic	EOS		LOS		Total	
	No-MDR N = 49	MDR N = 100	No-MDR N = 51	MDR N = 289	No-MDR N = 100	MDR N = 389
Age, days, Mean ± SD	1.4 ± 1.0	1.5 ± 1.0	14.2 ± 7.6	13.3 ± 7.3	8.0 ± 8.4	10.3 ± 8.1
Preterm birth	10 (20.4%)	24 (24.0%)	12 (24%)	105 (36.3%)	22 (22%)	129 (33.2%)
Male gender	22 (44.9%)	40 (40.0%)	23 (45%)	121 (41.9%)	45 (45%)	161 (41.4%)
Outpatient	2 (4.1%)	1 (1.0%)	3 (5.9%)	15 (5.2%)	5 (5.0%)	16 (4.1%)
<b>Bacteria Genera</b>						
<i>Staphylococcus</i>	35 (71.4%)	77 (77.0%)	28 (54.9%)	176 (60.9%)	63 (63.0%)	253 (65.0%)
<i>Enterobacteria</i>	0 (0.0%)	13 (13.0%)	10 (19.6%)	61 (21.5%)	10 (10.0%)	74 (19.0%)
<i>Acinetobacter</i>	1 (2.0%)	4 (4.0%)	0 (0.0%)	43 (14.9%)	1 (1.0%)	47 (12.1%)
<i>Streptococcus</i>	4 (8.2%)	5 (5.0%)	3 (5.9%)	6 (2.1%)	7 (7.0%)	11 (2.8%)
<i>Enterococcus</i>	8 (16.3%)	1 (1.0%)	3 (5.9%)	2 (0.7%)	11 (11.0%)	3 (0.8%)
<i>Stenotrophomonas</i>	1 (2.0%)	0 (0.0%)	6 (11.8%)	0 (0%)	7 (7.0%)	0 (0.0%)
<i>Pseudomonas</i>	0 (0.0%)	0 (0.0%)	1 (2.0%)	1 (0.4%)	1 (1.0%)	1 (0.3%)
<b>Bacteria specie</b>						
<i>CoNS</i>	29 (59.2%)	67 (67%)	19 (37.3%)	151 (52.3%)	48 (48.0%)	218 (56.0%)
<i>Acinetobacter</i> spp.	1 (2.0%)	4 (4.0%)	0 (0%)	43 (14.9%)	1 (1.0%)	47 (12.1%)
<i>Klebsiella</i> spp.	0 (0.0%)	4 (4.0%)	6 (11.8%)	32 (11.1%)	6 (6.0%)	36 (9.3%)
<i>S. aureus</i>	0 (0%)	1 (1.0%)	5 (9.8%)	9 (3.1%)	5 (5.0%)	10 (2.6%)
<i>E. coli</i>	0 (0.0%)	7 (7.0%)	4 (7.8%)	19 (6.6%)	4 (4.0%)	26 (6.7%)
<i>Streptococcus</i> spp.	4 (8.2%)	5 (5.0%)	3 (5.9%)	6 (2.1%)	7 (7.0%)	11 (2.8%)
Other <i>Staphylococcus</i> spp.	6 (12.2%)	9 (9.0%)	4 (7.8%)	16 (5.5%)	10 (10.0%)	25 (6.4%)
<i>Enterococcus</i> spp.	8 (16.3%)	1 (1.0%)	2 (3.9%)	2 (0.7%)	10 (10.0%)	3 (0.8%)
<i>Serratia</i> spp.	0 (0.0%)	2 (2.0%)	0 (0%)	10 (3.5%)	0 (0%)	12 (3.1%)
<i>S. maltophilia</i>	1 (2.0%)	0 (0%)	6 (11.8%)	0 (0.0%)	7 (7.0%)	0 (0.0%)
<i>P. aeruginosa</i>	0 (0.0%)	0 (0%)	1 (2.0%)	1 (0.4%)	1 (1.0%)	1 (0.3%)
<i>Enterobacter</i> spp.	0 (0.0%)	0 (0.0%)	1 (2.0%)	0 (0%)	1 (1.0%)	0 (0.0%)

MDR: Multidrug-resistant; SD: Standard deviation; CoNS: coagulase-negative *Staphylococci*.

**Table 2.** Antibiotic MDR patterns in newborns with LOS and EOS due *CoNS* infection.

Ranking	<i>CoNS</i> antibiotics MDR pattern	EOS N (%)	LOS N (%)
Top 1	CFX + CFP + CLD + GTM + LVF + SXT	8 (14.6)	42 (42.6)
Top 2	CFX + CFP + GTM + LVF + SXT	3 (5.5)	11 (10.9)
Top 3	CFX + CDM + GTM + LVF		7 (6.9)
Top 4	CFX + CPF + CDM + LVF + SXT		5 (5.0)
Top 5	CFX + CLD + GTM	4 (7.3)	
Top 6	CFX + CFP + CLD + GTM + LVF	4 (7.3)	
Top 7	CFX + GTM + SXT	3 (5.5)	
Top 8	Different combinations with frequencies < 5%	33 (60.0)	35 (34.7)

MDR: Multidrug-resistant; EOS: early-onset sepsis; LOS: late-onset sepsis; *CoNS*: Coagulase-negative *Staphylococci*; CFX: cefoxitin; CFP: cefepime; CLD: clindamycin; GTM: gentamicin; LVF: levofloxacin; SXT: trimethoprim/sulfamethoxazole.

*Antimicrobial susceptibility*

After analyzing the top five most frequent isolates among newborns with LOS (Table 2), we found the highest antibiotic resistance rates among *Acinetobacter* isolates (top 2), showing resistance rate greater than 90% (range: 94% to 97%) to all the antibiotics tested. Moreover, the most common enterobacteria among newborns with LOS were *Klebsiella* (top 3) and *E. coli* (top 4), presenting high resistance rates (> 50%) to conventional antibiotics including ampicillin, amoxicillin / clavulanic acid, and trimethoprim/sulfamethoxazole, monobactams

(aztreonam), and cephalosporins (cefepime, cefoxitin, and cefotaxime). On the other hand, both enterobacteria exhibited high susceptibility (100%) to carbapenems (imipenem and meropenem). Similarly, both *CoNS* (top 1) and *Staphylococcus* spp. (top 5) showed high resistance rates (over 50%) to ciprofloxacin, gentamicin and SXT, including 82% of resistance to cefoxitin, suggestive for methicillin resistant *CoNS*. Antibiotic resistance rates were lower for amikacin, rifampin, vancomycin, and tetracycline (all < 50%). (Table 2,3).

Contrary to LOS, the top five most frequent isolates from newborns with EOS exhibited a wide range of

**Table 3.** Antibiotic resistance among the five most frequent isolates among newborns with EOS and LOS.

Antibiotic	EOS: Resistant / (Resistant + Susceptible) (%)					LOS: Resistant / (Resistant + Susceptible) (%)				
	Top 1 <i>CoNS</i> N = 96	Top 2 <i>Staph.</i> N = 15	Top 3 <i>Enteroc.</i> N = 9	Top 4 <i>Strept.</i> N = 9	Top 5 <i>E. coli</i> N = 7	Top 1 <i>CoNS</i> N = 170	Top 2 <i>Acinetob.</i> N = 43	Top 3 <i>Klebsiella</i> N = 38	Top 4 <i>E. coli</i> N = 23	Top 5 <i>Staph.</i> N = 20
<b>β-Lactams</b>										
Ampicillin			0/7 (0)	0/1 (0)	5/5 (100)			27/27 (100)	16/16 (100)	3/7 (43)
Amox./Clav.					1/1 (100)			5/7 (71)	3/3 (100)	
Aztreonam					3/5 (60)				11/19 (58)	
Cefepime					6/6 (100)		34/34 (100)		11/19 (58)	
Cefotaxime				0/1 (0)	5/5 (100)		34/34 (100)	21/29 (72)	12/19 (63)	
Cefoxitin	64/94 (68)	6/10 (60)			0/6 (0)	133/163 (82)		0/29 (0)	0/19 (0)	
Ceftazidime					4/6 (67)		34/34 (100)	20/30 (67)	11/19 (58)	
Imipenem					0/6 (0)			0/25 (0)	0/17 (0)	
Meropenem					0/6 (0)		33/34 (97)	0/30 (0)	0/19 (0)	
Penicillin	10/10 (100)					16/16 (100)				
<b>Another antibiotic</b>										
Amikacin	18/76 (19)	1/11 (9)		3/8 (38)	1/6 (17)	48/164 (29)	33/33 (100)	11/29 (38)	9/19 (47)	
Ciprofloxacin	51/93 (55)	6/12 (50)	7/7 (100)		6/6 (100)	116/161 (72)	32/33 (97)	18/30 (60)	13/17 (76)	
Clindamycin	52/96 (54)			4/7 (57)		124/169 (73)				5/8 (63)
Erythromycin			3/7 (43)	5/8 (63)						6/8 (75)
Gentamicin	47/86 (55)	6/10 (60)		2/4 (50)	4/6 (67)	117/150 (78)	30/31 (97)	13/27 (48)	11/17 (65)	2/3 (67)
Levofloxacin	42/86 (49)	5/10 (50)	0/5 (0)	2/6 (33)	6/6 (100)	152/152 (100)	28/28 (100)	27/27 (100)	19/19 (33)	4/4 (100)
Nalidixic acid					2/2 (100)			11/12 (92)	2/3 (67)	
Rifampin	7/74 (9)	0/9 (0)	1/6 (17)			31/142 (22)				
Teicoplanin			0/5 (0)							
Tetracycline	4/11 (36)	1/3 (33)	0/2 (0)	1/3 (33)		7/31 (23)				1/2 (50)
TMP/SMX	38/71 (53)	5/10 (50)			6/6 (100)	111/140 (79)	32/33 (97)	23/27 (85)	17/19 (89)	

EOS: early-onset sepsis; LOS: late-onset sepsis; *CoNS*: Coagulase-negative *Staphylococci*; TMP/SMX: Trimetoprim/Sulfamethoxazol.

antibiotic resistance rates (range: 0% to 100%). The most common etiological agent (Top 1) among EOS was *CoNS*, which showed high resistance rates (> 50%) to penicillin, ciprofloxacin, gentamicin, clindamycin, levofloxacin, and trimethoprim/sulfamethoxazole, with values to ceftiofloxacin close to 60% of resistance. The second most frequent etiological agents (top 2) were other *Staphylococcus*, which was found to be highly resistant (> 50%) to ceftiofloxacin, gentamicin, ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole. The third and fourth most frequent etiological agents were *Enterococcus* (top 3) and *Streptococcus* (top 4), which were highly resistant (> 50%) to a few commonly used antibiotics. Finally, *E. coli* (top 5) also showed high resistance rates (67% to 100%) to standard antibiotics such as ampicillin, amoxicillin/clavulanic acid, ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole, and cephalosporins (cefepime and cefotaxime), but with high sensitivity (100%) to carbapenems (imipenem and meropenem). We also observed that *CoNS* (top 1) and *Staphylococcus* spp. (top 5) isolates showed resistance rates greater than 50% to most of the antibiotics tested, except for amikacin, rifampin, and tetracycline (all < 36%) (Table 2,3).

*Extended-Spectrum β-Lactamase (ESBL) phenotype*

Overall only 64 isolates were tested for ESBL production, most being from newborns with LOS (84%). Among the 10 ESBL isolates (six *E. coli* and four *K. pneumoniae*) from newborns with EOS, 90% (9/10) were ESBL positive, including 100% (6/6) of the *E. coli* and 75% (3/4) of the *K. pneumoniae* isolates. Among the 54 isolates analyzed from newborns with LOS, 93% (50/54) were ESBL positive, including 93% (13/14) of the *E. coli*, 89% (25/28) of the *K. pneumoniae*, 100% of the (8/8) *Serratia* spp. and 100% (4/4) of the *S. maltophilia* isolates.

*Carbapenemase screening*

Overall, 13 isolates were tested for the presence of carbapenemases, all of which were from newborns with LOS. Among these isolates, 62% (8/13) were

carbapenemase positive, all of which were *Acinetobacter* spp. strains (8/8). The five isolates that tested negative to carbapenemases included three *K. pneumoniae*, one *E. coli*, and one *P. aeruginosa*.

*MDR patterns*

We performed a sub-analysis among newborns infected with *CoNS* to compare the antibiotic MDR patterns between EOS and LOS (Table 3) and observed that a small subset of antibiotic MDR patterns characterized most of the EOS and LOS cases among newborns (40% and 65%, respectively). Specifically, we identified that the most frequent antibiotic MDR pattern was resistance to ceftiofloxacin, cefepime, clindamycin, gentamicin, levofloxacin, and trimethoprim/sulfamethoxazole. This pattern alone characterized 15% of EOS cases and 43% of LOS cases. Furthermore, overall, the *CoNS* isolated exhibited similar patterns of antibiotic MDR patterns, which were characteristically resistant to ceftiofloxacin, gentamicin, and clindamycin, or levofloxacin.

*MDR-associated factors*

In our study, we identified that MDR was significantly associated with LOS and *CoNS* infection. In our multivariable regression analysis, we observed that the prevalence of MDR was higher among cases with LOS compared to those with EOS (adjusted prevalence ratio [aPR] = 1.28; 95% CI: 1.14 -o 1.45), and among BSI due to *CoNS* compared to those by other bacteria (aPR = 1.10; 95% CI: 1.01 - 1.20). Bivariate analysis showed that prematurity might represent another risk factor for MDR among newborns. However, the multivariate regression analysis showed that both LOS and *CoNS* represented the most critical MDR risk factors in our study population (Table 4).

**Discussion**

In the present study, we observed that the MDR rate among newborns with sepsis was exceptionally high. Furthermore, it was of note that this rate was even higher among newborns with LOS compared to those with EOS, and among newborns infected with *CoNS*

**Table 4.** Regression analysis for MDR among newborns with BSI.

Associated Factor	cPR (95% CI)	p-value	aPR (95% CI)	p-value
LOS	1.27 (1.12, 1.43)	< 0.001	1.28 (1.14, 1.45)	< 0.001
<i>CoNS</i>	1.07 (0.97, 1.17)	0.156	1.10 (1.01, 1.20)	0.037
Preterm	1.11 (1.02, 1.21)	0.020		
Male gender	0.97 (0.88, 1.06)	0.519		
Outpatient	0.96 (0.75, 1.22)	0.717		

MDR: Multidrug-resistant; cPR: crude prevalence ratio; aPR: adjusted prevalence ratio; LOS: Late-onset sepsis; *CoNS*: *Coagulase-negative Staphylococci*.

compared to those infected with other bacteria. Additionally, we observed that *CoNS* was associated with a limited subset of MDR patterns, which could be useful to guide therapeutic decisions.

In Peru, neonatal sepsis represents a significant cause of neonatal morbidity and mortality, with an estimated incidence of 4.1 per 1000 live births (95% CI: 2.7 - 5.5) [18]. The etiological agents of sepsis vary significantly from country to country and even from institution to institution. However, the role of *Enterobacteriaceae* and *Acinetobacter* as the leading etiological agents worldwide is commonly accepted. In our study, we observed that the most common cause of neonatal sepsis was *CoNS*, followed by *Enterobacteriaceae* and Gram-negative non-fermenters, especially *Acinetobacter* spp.

We also observed isolates of *Klebsiella* with high levels of antibiotic resistance. This is consistent with a previous Peruvian study that reported high levels of resistance to trimethoprim/sulfamethoxazole and ciprofloxacin (73% and 65%, respectively) as well as high positivity to ESBL (75%) [9]. Recently, *Klebsiella* is in the spotlight due to the increase of the extremely antibiotic resistance associated with significant clinical outbreaks worldwide, including in Peru [19, 20].

In the present study *E. coli* isolates with MDR rates (~87%) were found, being higher than rates reported previously by Palma N *et al.* [21] in Peru (~80%). These researchers reported *E. coli* isolates with high levels of resistance to ampicillin (93%), nalidixic acid (66%), and trimethoprim/sulfamethoxazole (66%) [21]. However, in our study, we reported even higher levels of resistance to *E. coli* showing 100% resistance to ampicillin, amoxicillin plus clavulanic acid, levofloxacin, 92% resistance to trimethoprim/sulfamethoxazole and 65% to gentamicin. This is of particular concern, since ampicillin plus gentamicin is the most common empiric antibiotic combination used in neonates. Only last resort antibiotics such as colistin are available to treat extensively drug-resistant *Acinetobacter* (> 97% of resistance to all antimicrobials tested); it is important to highlight the elevated rates of antimicrobial resistance in these *Acinetobacter* found in these neonates with bacteremia that could be a clone installed in the NICU with similar antibiotic resistance pattern. Thus, *Acinetobacter* spp is usually related to intestinal carriage and is linked to neonatal outbreaks in Neonatal Intensive Care Units (NICU) [22]. These bacteria are associated with increased mortality in NICU, and active surveillance is recommended as a strategy to control neonatal colonization and avoid posterior infection or

dissemination for the hospital setting [22]. In general, the high antibiotic resistance rates of Gram-negative bacilli in bacteremia in neonates are in accordance with similar studies performed in other low- and middle-income countries such as southeast Asian [23].

In our study, three *S. aureus* isolates were identified in newborns with neonatal sepsis who were positive to specific test for MRSA (100%); we only have the data of 10 *S. aureus* tested for ceftazidime, being 60% resistant to this antibiotic indicating near 60% MRSA. Attending to *CoNS* the resistance to ceftazidime, was 68% in EOS and 82% in LOS. These rates were higher than recent Peruvian reports, at least regarding MRSA, which described 54% of MRSA positivity [24]. Moreover, a high proportions of nasal carriage of MRSA have been found among health care workers in previous studies made in Lima, which reported MRSA from nasal carriage to belong to similar lineages that were recovered from their patients with bacteremia [25].

It is essential to highlight that *CoNS* is not only the most frequent bacteria isolated from human skin and mucous, but it also represents an increasing cause of health care-associated bacteremia [26]. Moreover, what is more worrisome is that *CoNS* is strongly associated with MDR. In our study, *CoNS* was the most common cause of neonatal sepsis. It was associated with high resistance, historically to penicillin, but also to ceftazidime, gentamicin, clindamycin, ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole, all being higher among LOS than among EOS cases. Antibiotic resistance of skin isolates with *CoNS* has been reported to be increased during neonatal hospitalization (mainly during the first week) [27]. Although the levels of MDR in EOS with *CoNS* are considerable, they may be related to neonatal colonization with resistant *CoNS*, especially in the gut, immediately after birth [27].

We observed that the prevalence of MDR was higher among LOS compared to EOS cases (85% vs. 67%), in relation to the length of hospitalization, with the levels of resistance increasing with longer hospital stays. Usually in clinical settings, the most common sources of *CoNS* are related to the patient's microbiota and the hands of healthcare workers, thus, this could also mean that the bacteria related to this infection possibly come from these hospital environments compared to EOS, the origin of which is more linked to bacteria transmitted through the mother [28]. This finding is important because although MDR is multifactorial, the excessive use of broad-spectrum antibiotics in NICUs is a key MDR risk factor [29]. Apart from the use of antimicrobials themselves [30]

and the important role played by *CoNS* in MDR risk, medical care personnel could act as a reservoir for antibiotic-resistant bacteria [31]. Furthermore, the presence of MDR bacteria, including ESBL-producing microorganisms, has commonly been isolated in health worker devices, such as mobile phones and workwear [32].

Peru, as other low-middle income countries, presents a weak and fragmented health system, which some health institutions do not have semi-automatic systems to evaluate Minimum Inhibitory Concentration, for this reason we did not have information about vancomycin susceptibility levels, as an important limitation. Nonetheless, this study shown important data of antibiotic resistance levels in vulnerable population such as neonates, contributing to improve antibiotic resistance surveillance and control. Moreover it is recommended a antimicrobial use optimization programs (PROA), for a better antibiotic resources management, which reduces and controls the antimicrobials use in the NICU. The high relevance of *CoNS* in the present study, also, suggests a reinforcement in the handwashing and other hygiene measures of health workers, because they could become the reservoirs and / or dispersers of antimicrobial resistant bacteria.

## Conclusions

In conclusion, MDR is high among Peruvian newborns with neonatal sepsis and is associated with the presence of LOS and *CoNS*. These findings are important, mainly because of the lack of a robust health surveillance system in Peru. Furthermore, considering the strong association between MDR and LOS, we strongly recommended introducing antibiotic resistance surveillance systems (infection and colonization), at least in every NICU with a high MDR rate, as well as prioritizing increased hygiene measures and good practices for medical care personnel.

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### Corresponding author

Maria J. Pons, BS, MSc, PhD, Researcher,  
 Universidad Científica del Sur  
 Carr. Panamericana Sur 19, Villa EL Salvador, Lima, Peru, 15067  
 Tel: +51(1) 610-6400  
 Email: ma.pons.cas@gmail.com

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