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Dissemination of a multidrug resistant CTX-M-65 producer *Salmonella enterica* serovar Infantis clone between marketed chicken meat and children

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ABSTRACT

The objective of the present study was to characterize *Salmonella enterica* serovar Infantis isolated from chicken meat determining their clonal relationships with *S.* Infantis isolated from children with diarrhea. Fifteen meat-recovered *S.* Infantis were analyzed. Susceptibility levels to 14 antibacterial agents, the presence of ESBL and that of inducible plasmid-mediated AmpC (i-pAmpC) were determined by phenotypical methods. The presence of ESBL and pAmpC was confirmed by PCR, and detected ESBL-encoding genes were sequenced and their transferability tested by conjugation. The presence of *gyrA* mutations as well as Class 1 integrons was determined by PCR. Clonal relationships were established by REP-PCR and RAPD. In addition, 25 clinical isolates of *S.* Infantis were included in clonality studies. All meat-recovered *S.* Infantis were MDR, showing resistance to ampicillin, nitrofurans and quinolones, while none was resistant to azithromycin, ceftazidime or imipenem. ESBL ($bla_{CTX\text{-}M\text{-}65}$) and i-pAmpC ($bla_{DHA}$) were detected in 2 and 5 isolates respectively (in one case concomitantly), with $bla_{CTX\text{-}M\text{-}65}$ being transferable through conjugation. In addition, 1 isolate presented a bla_{SHV} gene. All isolates presented $D_{87}Y$ at GyrA, nalidixic acid active efflux pump and a Class 1 integron of ~1000 bp (*aadA1*). Clonal analysis showed that all isolates were related. Further they were identical to MDR $bla_{CTX\text{-}M\text{-}65}$-producing *S.* Infantis isolates causing children diarrhea in Lima. The dissemination of MDR $bla_{CTX\text{-}M\text{-}65}$-producing *S.* Infantis between marketed meat and children highlights a public health problem which needs be controlled at livestock level.

1. Introduction

Non-Typhi *Salmonella* (NTS) are common inhabitants of the gut of poultry and other animals for food consumption, including cattle and pig (Threlfall, 2002), being a common contaminant of marketed meat (Arnedo-Pena et al., 2016; Khan et al., 2018; Ruiz-Roldán et al., 2018). NTS are also present in other sources such as eggs, fish or contaminated irrigation water (Martínez et al., 2017; Ruiz et al., 1999; Silva et al., 2014). These findings result in the food-chain being the most common route of acquisition of NTS infections (Threlfall, 2002).

Thus, NTS are a common cause of gastrointestinal disturbances such as diarrhea in both adult and infants (Cabrera et al., 2004; Granda et al., 2019). In addition, NTS have also been involved in extraintestinal infections such as local abscesses, bacteremia or meningitis, among others (Alonso et al., 2007; Mandomando et al., 2015; Molyneux et al., 2009; Sugimoto et al., 2017). While NTS diarrhea are described in all geographical areas and age-segments, extraintestinal NTS infections are of special relevance in low- and middle-income areas and in specific at-risk populations (*e.g.*: HIV or immunocompromised patients) (Chou et al., 2016; Mandomando et al., 2015; Molyneux et al., 2009).

NTS diarrhea is often self-limiting and does not require the use of antimicrobial agents, except in long-standing diarrhea, the presence of

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severe symptoms, young children (< 3 months), the presence of diverse comorbidities or in immunocompromised patients (Lübbert, 2016; Threlfall, 2002). However, extraintestinal cases are life-threating and may result in fatal outcomes or severe sequelae (Alonso et al., 2007; Ao et al., 2015; Chou et al., 2016; Mandomando et al., 2015; Molyneux et al., 2009).

Classically, NTS had been treated with antibiotics such as ampicillin, chloramphenicol or cotrimoxazole (van Duijkeren and Houwers, 2000). Nonetheless, since the 1990's, a rise in the levels of NTS resistance to these antibiotics has been observed (Cabrera et al., 2004; Gallardo et al., 1999; Mandomando et al., 2015; Ruiz et al., 1999), and their role as first-line treatment has declined (Ao et al., 2015; Mandomando et al., 2015). Currently, when treatment of NTS causing diarrhea is needed, the antimicrobial agents most frequently used are fluoroquinolones or azithromycin (Cabrera et al., 2004; Lübbert, 2016), while NTS extra-intestinal infections are often treated with fluoroquinolones or cephalosporins (Crump et al., 2015). Nonetheless, in the present century the spread of NTS exhibiting multidrug resistance (MDR) patterns, including resistance to fluoroquinolones, macrolides, 3rd and 4th generation cephalosporins, carbapenems, fosfomycin, or even to polymyxins has been described (Carattoli et al., 2017; Granda et al., 2019; Hawkey et al., 2019; Quino et al., 2019).

The number of described NTS serotypes is higher than 2500, with a few being frequently described worldwide as a cause of human illness, and a high number being sporadically described as infecting patients. In this scenario, *Salmonella enterica* serovar Infantis ranks worldwide among the common NTS serotypes isolated as a cause of human disease, after those NTS belonging to serovars Typhimurium and Enteritidis (Lamas et al., 2018).

Since 2010, in Peru a series of diarrhea cases related to MDR *S.* Infantis have been detected both in Lima and other regions of the country (Gonzales-Escalante, 2015; Granda et al., 2019; Quino et al., 2019). Further, an increase of ESBL-carrying *S. enterica* isolates emerged in 2011 (Gonzales-Escalante, 2015).

In the present study a series of MDR *S.* Infantis isolated from meat samples were characterized and clonal relationships with *S.* Infantis isolated from children with diarrhea were established.

2. Material and methods

2.1. Microorganisms

Fifteen non-duplicate *S.* Infantis isolated in August 2012 from fresh meat samples were included in the present study. Of these, 13 were from chicken meat, and the other 2 isolates were from beef and pork samples (Martínez-Puchol et al., 2020; Ruiz-Roldán et al., 2018).

Briefly, the samples included in the above-mentioned study were randomly bought in 5 traditional markets of 3 different areas, South, Center and North, of Lima (Peru), in a study designed to determine the burden of *Enterobacteriaceae* in meat samples (Ruiz-Roldán et al., 2018). Regarding samples containing *S.* Infantis, these were bought in 4 out of 5 sampling markets representing the 3 above-mentioned areas (Martínez-Puchol et al., 2020). In the previously mentioned study *Salmonella* strains were ~3% of the total microorganisms isolated ($N = 830$) (Ruiz-Roldán et al., 2018).

In all cases the isolates were identified by biochemical tools and confirmed by *invA* gene amplification and sequencing (Barletta et al., 2013; Ruiz-Roldán et al., 2018). Furthermore, after recovery from frozen stock and prior to use, the microorganisms were reconfirmed as *S. enterica* by PCR amplification of the *16S rRNA* gene (Salazar de Vegas et al., 2006).

In addition, 27 *S.* Infantis from other sources were included in the analysis of clonal relationships (see Section 2.9 - Clonal determinations); when available, antibiotic resistance levels and molecular data of these isolates were also compared with the results obtained analyzing food-related *S.* Infantis.

2.2. Antibiotic susceptibility

The antibiotic susceptibility to ampicillin (β-lactam - βL), amoxicillin plus clavulanic acid (β-lactam plus β-lactamase inhibitor - βL-I), cefotaxime, ceftazidime (cephalosporins - CPH), chloramphenicol (phenicol - CHL), cotrimoxazole (antifolate - AF), gentamicin (aminoglycoside - AMG), imipenem (carbapenem - CBP), nalidixic acid, ciprofloxacin (quinolones - Q), azithromycin (macrolides - MC) and tetracycline (tetracyclines - TC) was established by disk diffusion in accordance with CLSI guidelines (CLSI, 2018).

The nalidixic acid breakpoint considered was that of *Enterobacteriaceae*. Regarding azithromycin, as no generic *S. enterica* clinical susceptibility cut-off has been established, the disk diameters were interpreted following the CLSI criteria for *Salmonella enterica* serovar Typhi (CLSI, 2018). Meanwhile, data on furazolidone and nitrofurantoin (nitrofurans - NF) resistance have previously been reported (Martínez-Puchol et al., 2020).

Multidrug resistance (MDR) was defined as a resistance to at least 1 antibacterial agent from 3 unrelated families. In all cases, the *Escherichia coli* ATCC 25922 was used as Minimal Inhibitory Concentration (MIC) quality control strain.

2.3. Phenotypic detection of ESBL

In the food-recovered isolates the presence of ESBL was phenotypically established in all isolates, irrespective of the pattern of susceptibility/resistance to CPH, by double disk methodology (Palma et al., 2017; Ruiz-Roldán et al., 2018). Briefly, cefotaxime and ceftazidime disks were placed at 20 mm (center-center distance) of an amoxicillin plus clavulanic acid disk. For a representative image see Lezameta et al. (2010).

2.4. Phenotypic detection of inducible plasmidic AmpC

The presence of inducible plasmidic AmpC (i-pAmpC) was determined in all isolates, irrespective of the susceptibility/resistance to CPH, using the double disk test induction with imipenem and ceftazidime as described previously (Ruiz-Roldán et al., 2018). Briefly, ceftazidime and amoxicillin plus clavulanic acid disks were placed at 20 mm (center-center distance). For a representative image see Del Valle Martínez Rojas (2009).

2.5. Molecular detection of β-lactamases

The presence of bla_{CARB}, bla_{TEM}, bla_{SHV}, bla_{OXA-1}-like, bla_{OXA-2}-like and bla_{OXA-5}-like was determined by Polymerase Chain Reaction (PCR) in all the isolates. Meanwhile, the presence of bla_{CTX-M} and bla_{DHA} was determined by PCR in those isolates displaying a phenotype compatible with the presence of ESBL and i-pAmpC, respectively.

The strategy to amplify bla_{CTX-M} was a preliminary screening with bla_{CTX-M} universal primers followed, in positive isolates, by the use of 5 pairs of primers able to amplify different bla_{CTX-M} groups ($bla_{CTX-M-1,\ 2,\ 8,\ 9,\ 10}$ - like) as described elsewhere (Palma et al., 2017). In all cases the primers and amplification conditions have been described previously (Palma et al., 2017) (Table 1).

All PCR products were resolved in a 2% agarose gel which was stained with Sybr Safe (Invitrogen, Carlsbad, USA). The amplified products were purified (Gel Extraction Kit, Omega Bio-tek Norcross, GA, United States) and sequenced (Beckman Coulter; Takeley, United Kingdom).

2.6. ESBL transferability

The transferability of detected ESBL was determined by conjugation in Luria-Bertani broth (Conda, Madrid, Spain) using the azide-resistant *E. coli* J53 and Mueller-Hinton supplemented with sodium azide (150

Table 1
Primers used in this study.

Gene	Sequence	Size (bp)	°C[a]	Cycles	Reference
bla_{CTX-M}	CGA TGT GCA GTA CCA GTA A TTA GTG ACC AGA ATC AGC GG	585	53	35	Palma et al., 2017
$bla_{CTX-M-1G}$	GTT ACA ATG TGT GAG AAG CAG CCG TTT CCG CTA TTA CAA	1041	50	35	Palma et al., 2017
$bla_{CTX-M-2G}$	ATG ATG ACT CAG AGC ATT CG TCA GAA ACC GTG GGT TAC	876	52	35	Palma et al., 2017
$bla_{CTX-M-8G}$	TGA TGA GAC ATC GCG TTA AG TAA CCG TCG GTG ACG ATT TT	875	52	35	Palma et al., 2017
$bla_{CTX-M-10G}$	CCG CGC TAC ACT TTG TGG C TTA CAA ACC GTT GGT GAC G	944	55	35	Palma et al., 2017
$bla_{CTX-9/14G}$	TGA CCG TAT TGG GAG TTT CAG GAT TTA TTC AAC AAA ACC AG	917	56	35	Palma et al., 2017
bla_{CARB}-like	AAT GGC AAT CAG CGC TTC CC GGG GCT TGA TGC TCA CTC CA	586	56	30	Palma et al., 2017
bla_{OXA-1}-like	ACC AGA TTC AAC TTT CAA TCT TGG CTT TTA TGC TTG	598	55	30	Palma et al., 2017
bla_{SHV}-like	ATG CGT TAT ATT CGC CTG TG TTA GCG TTG CCA GTG CTC G	841	55	30	Palma et al., 2017
$bla_{OXA-5/7}$-like	TAT ATT CCA GCA TCA ACA TT ATG ATG CCC TCA CTT GCC AT	605	55	30	Palma et al., 2017
$bla_{OXA-2/3}$-like	CGA TAG TTG TGG CAG ACG AA CCA CTC AAC CCA TCC TAC CC	550	55	30	Palma et al., 2017
bla_{DHA}	AAC TTT CAC AGG TGT GCT GGG T CCG TAC GCA TAC TGG CTT TGC	405	64	30	Palma et al., 2017
bla_{TEM}-like	ATT CTT GAA GAC GAA AGG GC ACG CTC AGT GGA ACG AAA AC	1150	55	30	Palma et al., 2017
gyrA	AAA TCT GCC CGT GTC GTT GGT GCC ATA CCT ACG GCG ATA CC	343	55	30	Vila et al., 1995
RAPD[b]	CCG AAG CTG C	–	35	39[c]	Lin et al., 1996
REP-PCR[b]	GCG CCG ICA TGC GGC ATT	–	40	30	Granda et al., 2019
Class 1 integron	GGC ATC CAA GCA GCA AG AAG CAG ACT TGA CCT GA	Variable	59	30	Levesque and Roy, 1993

bp: base pair.
[a] Annealing temperature.
[b] Both RAPD and REP-PCR are typing PCR, therefore do not amplify any specific gene and are expected to obtain a variable number of bands with different sizes.
[c] The program used was: 4 cycles of 94 °C for 4 min, 35 °C for 4 min, and 72 °C for 4 min; 30 cycles of 94 °C for 30 s, 35 °C for 1 min, and 72 °C for 2 min; and final cycle of 72 °C for 5 min was added for final extension (Lin et al., 1996).

μg/mL) and cefotaxime (10 μg/mL) following the methodology previously described (Pons et al., 2015).

2.7. Mechanisms of quinolone resistance

The presence of mutations in the *gyrA* gene was determined by PCR and sequencing as previously described (Vila et al., 1995) (Table 1). In addition, the MIC of nalidixic acid and ciprofloxacin was performed in the presence and absence of 20 μg/mL of Phenyl-Arginyl-β-Naphtylamide (PAβN). The PAβN effect on the MIC levels was considered when $MIC_I/MIC_{PA\beta N} > 2$ (Palma et al., 2017).

2.8. Class 1 Integron

The presence of Class 1 integrons was established by PCR following the procedures previously described (Levesque and Roy, 1993) (Table 1). The amplified products were recovered and sequenced as above.

2.9. Clonal determinations

The clonal relationships between the *S.* Infantis isolates were established by two different typing-PCR: Repetitive Extragenic Palindromic (REP)-PCR and Random Amplified Polymorphic DNA (RAPD). In both cases the techniques were performed following methodologies described previously (Granda et al., 2019; Lin et al., 1996).

As indicated above, in addition to the isolates recovered from food samples, 25 *S.* Infantis previously reported as a cause of children's diarrhea in Lima, from which data on antibiotic susceptibility and presence of extended-spectrum β-Lactamases (ESBL) were available, were included in the analysis. Two other *S.* Infantis, kindly provided by Dr. Mandomando (CISM, Manhiça, Mozambique) and by Dr. Garcia (UPCH, Lima, Peru), isolated as a cause of infantile diarrhea in Mozambique and from a bacteremia in Peru respectively, were also included in the clonal analysis. In addition, *Salmonella enterica* belonging to other serotypes were also used as controls.

RAPD and REP-PCR profiles were analyzed using the fingerprinting software GelJ (Heras et al., 2015). Phylogenetic trees were constructed using the Dice coefficient with clustering by the unweighted pair-group method with arithmetic mean (UPGMA) with a 1% tolerance in band position differences. The isolates were considered to belong to the same epidemiological group when the profiles showed ≥80% of homology.

2.10. Ethical issues

The study was approved by the Ethical Review Board of the Universidad Peruana Cayetano Heredia (UPCH).

3. Results

Food-recovered *Salmonella* were identified through phenotypic and genotypic methods, with a full concordance.

All food-recovered *S.* Infantis displayed high levels of antibiotic resistance irrespective of the origin of the sample (chicken, pork or beef). Furthermore, all isolates were MDR displaying resistance to at least 4 different antibacterial agent families. Eight different antibiotic resistance patterns were observed, with the most common antibacterial agent resistance profile being Q, NF, TC, βL (4 isolates) followed by Q, AF, NF, TC, βL (3 isolates) and Q, CHL, AF, NF, TC, βL and Q, NF, βL, βL-I (2 isolates each), with all the remaining antibiotic-resistant profiles accounting for 1 isolate each (Table 2). All 8 antibiotic resistance patterns were classified as MDR, showing resistance to 4 to 8 different antibiotic families.

The highest levels of resistance were those to ampicillin, furazolidone, nitrofurantoin, nalidixic acid and ciprofloxacin with 100% of the isolates exhibiting a resistance phenotype, followed by tetracycline (13

Table 2
Antibiotic resistance profiles of meat-recovered *S.* Infantis.

		Antibiotic resistance[a]											
Profile	N	βL	βL-I	CPH	NF	Q	AF	CHL	AMG	TC	MDR	ESBL	i-pAmpC
I	4	■			■	■				■	Yes	-	-
II	3	■			■	■	■			■	Yes	-	-
III	2	■			■	■	■	■		■	Yes	-	-
IV	2	■	■		■	■					Yes	-	Yes
V	1	■	■	■	■	■	■	■	■	■	Yes	Yes	Yes
VI	1	■		■	■	■	■	■	■	■	Yes	Yes	-
VII	1	■	■		■	■				■	Yes	-	Yes
VIII	1	■	■		■	■	■			■	Yes	-	Yes

N: Number of isolates; MDR: Multidrug resistance; ESBL: Extended-spectrum β-lactamase; i-pAmpC: inducible plasmid mediated AmpC.
βL: β-lactams; βL-I; β-lactams plus β-lactamase inhibitor; CPH: Cephalosporins; NF: Nitrofurans; Q: Quinolones; CHL: Phenicols; AF: Antifolate; AMG: Aminoglycosides; TC: Tetracyclines.
a = At least to one antibiotic of the antibiotic-families considered. No isolate was resistant to carbapenems or macrolides.

isolates, 86.7%). Meanwhile, no isolate was resistant to azithromycin, ceftazidime, or imipenem and only 2 showed gentamicin or cefotaxime resistance (Table 3).

The MIC to quinolones showed that all isolates displayed nalidixic acid MIC levels of >256 μg/mL with a ciprofloxacin MIC of 1 μg/mL. In all cases, resistance to nalidixic acid was associated with the presence of a $D_{87}Y$ amino acid change in GyrA plus an active efflux pump. In 3 and 11 nalidixic acid-resistant isolates the use of PAβN resulted in decreases from >256 μg/mL to 64 μg/mL and to 32 μg/mL respectively ($MIC_I/MIC_{PA\beta N}$ >4) remaining classified as nalidixic acid-resistant, while in the last isolate the nalidixic acid MIC decreased to 16 μg/mL ($MIC_I/MIC_{PA\beta N}$ > 16), just under the CLSI resistance breakpoint.

The search for ESBL and i-pAmpC showed the presence of 1 isolate carrying an ESBL, 4 carrying a i-pAmpC and 1 carrying both ESBL and i-pAmpC. In addition, in 1 case (concomitantly with a i-pAmpC) the presence of a bla_{SHV} was detected. No βL resistance mechanism was detected in 9 βL-resistant isolates. When the ESBL and i-pAmpC were determined the results showed the presence of $bla_{CTX\text{-}M\text{-}65}$ (ESBL) and bla_{DHA} (i-pAmpC) (Table 3). Of note, the presence of a positive result for a specific $bla_{CTX\text{-}M}$ group does not preclude the absence of another; the presence of all 5 $bla_{CTX\text{-}M}$ groups sought was performed in all isolates in which the presence of a $bla_{CTX\text{-}M}$ was detected irrespective of the detection of the presence of a group. Of note, the 2 $bla_{CTX\text{-}M\text{-}65}$-carrying isolates were those exhibiting resistance to cefotaxime. Regarding horizontal transferability, the conjugation assays showed the transferability of $bla_{CTX\text{-}M\text{-}65}$.

Finally, all isolates presented a Class 1 integron of c. 1000 bp, which contained an *aadA1* gene.

Both the REP-PCR and the RAPD analyses showed visual identical band-pattern in all food-related *S.* Infantis, which was confirmed by dendrogram construction (minimal RAPD identity degree: 100%, minimal REP-PCR identity degree >94%) being therefore considered as belonging to the same clone (data not shown). Furthermore, the incorporation of diarrhea-related *S.* Infantis to the analysis highlighted that these isolates belonged to the same clone. The bacteremic isolate incorporated in the analysis showed a distinctive band pattern, being then considered as belonging to another clone. In all cases the external controls showed different band-patterns, irrespective of the *Salmonella* serotype (data not shown).

4. Discussion

Antibiotic resistance is an emerging threat which affects most clinically relevant microorganisms. This problem is of special concern when food-transmissible pathogens are affected, because this may easily result in large difficult to treat outbreaks (Plumb et al., 2019). The presence of antibiotic-resistant microorganisms in marketed meat may be related to different factors, but the prophylactic, therapeutic or grown-promoter use of antibiotics during animal production is by far the most relevant cause acting as a powerful evolutionary force that selects antibiotic-resistant microorganisms (Angulo et al., 2000). In this context, a series of *S.* Infantis were recovered from meat samples in the area of Lima, mostly from chicken samples, but also from other meat sources.

Identification was done by both phenotypic and genotypic tools. Phenotypic approaches, such as biochemical tools, have the advantage to become accessible on low-income areas, providing of useful results. Nevertheless, these techniques need for an additional culture, and then final results are delayed. Genotypic tools, such as *invA* detection, while unaffordable in a series of low-income areas, provide of rapid and reliable results (Barletta et al., 2013). Of note, the amplification and sequence of the *16S rRNA* gene, while time consuming, may provide of data to resolve discrepancies between identification approaches (Wang et al., 2006), providing an accurate identification.

In *S. enterica*, the presence of the phenotype of nalidixic acid resistance of high level, and ciprofloxacin resistance of low level is a frequent event, being concordant with the presence of a single amino acid substitution in GyrA and a basal PAβN-inhibitible efflux pump activity (Kim et al., 2011; Merino et al., 2007). Furthermore, our results fully agree with the previously described more efficient extrusion of nalidixic acid than that of ciprofloxacin by *Enterobacteriales* PAβN-inhibitible efflux pump (Sáenz et al., 2004). None of the present isolates showed resistance of high level to ciprofloxacin.

Despite the description of successful *S. enterica* clones expressing resistance of high level to fluoroquinolones such as ciprofloxacin (Le Hello et al., 2013), it has been suggested that as a general rule the presence of additional quinolone-target alterations, be able to result in high MIC levels of fluoroquinolones, also have a strong impact on *Salmonella* fitness (Fàbrega et al., 2014). Regarding the amino acid substitution $D_{87}Y$, its presence in *Salmonella enterica* has been previously reported (Cabrera et al., 2004), including the detection in *S.* Infantis recovered in Peru from both human and meat samples (Quino et al., 2019; Vallejos-Sánchez et al., 2019). In fact, while in other *Enterobacteriales* as well as other Gram-negative bacteria the first point mutation usually arises on position 83 (*E. coli* or *Salmonella* numeration) (Ruiz, 2003; Vila et al., 1995), in *Salmonella* the presence of isolates presenting position 87 as first point mutation seems to be especially frequent (Cabrera et al., 2004; Quino et al., 2019; Ruiz et al., 1999). Of note, the use of the *S. enterica* CLSI MIC breakpoints for ciprofloxacin before 2012 would have resulted in discordant nalidixic acid and ciprofloxacin susceptibility patterns, as reported in a series of studies developed previous to this change (Bertrand et al., 2006; Cabrera et al., 2004; Girish et al., 2013; Pérez-Moreno et al., 2013; Ruiz et al., 1999).

The presence of isolates with and without $bla_{CTX\text{-}M\text{-}65}$ and/or bla_{DHA} may be interpreted as the presence of different plasmid profiles either related to the acquisition or the loss of plasmid content. In this line, previous studies have shown the presence of $bla_{CTX\text{-}M\text{-}65}$ within

Table 3
Antibiotic susceptibility and mechanisms of antibiotic resistance of meat-recovered *S.* Infantis.

Strain	Source	Area	NAL	CIP	C	SXT	FX	NIT	GM	AZM	IMP	TE	AMP	AMC	CAZ	CTX	*gyrA*	β-lactamases[a]					Class 1 integron	
																		bla_{SHV}	ESBL	$bla_{CTX\text{-}M}$	i-pAmpC	bla_{DHA}	Size (bp)	Cassette
S1	Chicken	South	R	R	R	R	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–	–	–	~1000	aadA1
S2	Chicken	North	R	R	R	R	R	R	R	S	S	R	R	R	S	R	$D_{87}Y$	–	+	***bla*$_{CTX\text{-}M\text{-}65}$**	+	***bla*$_{DHA}$**	~1000	aadA1
S3	Chicken	Center	R	R	S	S	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–	–	–	~1000	aadA1
S4	Chicken	Center	R	R	R	R	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–	–	–	~1000	aadA1
S5	Chicken	North	R	R	S	R	R	R	S	S	S	R	R	S	S	R	$D_{87}Y$	–	–	–	–	–	~1000	aadA1
S6	Chicken	North	R	R	S	S	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–	–	–	~1000	aadA1
S7	Chicken	North	R	R	R	R	R	R	R	S	S	R	R	S	S	S	$D_{87}Y$	–	+	***bla*$_{CTX\text{-}M\text{-}65}$**	–	–	~1000	aadA1
S8	Chicken	North	R	R	S	S	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–		–	~1000	aadA1
S9	Pork	South	R	R	S	S	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–		–	~1000	aadA1
S11	Chicken	Center	R	R	S	S	R	R	S	S	S	S	R	R	S	S	$D_{87}Y$	–	–	–	+	***bla*$_{DHA}$**	~1000	aadA1
S12	Beef	Center	R	R	S	S	R	R	S	S	S	R	R	R	S	S	$D_{87}Y$	–	–	–	+	***bla*$_{DHA}$**	~1000	aadA1
S16	Chicken	South	R	R	S	R	R	R	S	S	S	R	R	R	S	S	$D_{87}Y$	+	–	–	+	***bla*$_{DHA}$**	~1000	aadA1
S17	Chicken	Center	R	R	S	R	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–	–	–	~1000	aadA1
S18	Chicken	Center	R	R	S	R	R	R	S	S	S	R	R	S	S	S	$D_{87}Y$	–	–	–	–	–	~1000	aadA1
S19	Chicken	Center	R	R	S	S	R	R	S	S	S	S	R	R	S	S	$D_{87}Y$	–	–	–	+	***bla*$_{DHA}$**	~1000	aadA1

NAL: Nalidixic acid; CIP: Ciprofloxacin; C: Chloramphenicol; SXT: Cotrimoxazole; FX: Furazolidone; NIT: Nitrofurantoin; GM: Gentamicin; AZM: Azithromycin; IMP: Imipenem; TE: Tetracycline; AMP: Ampicillin; AMC: Amoxicillin plus clavulanic acid; CAZ: Ceftazidime; CTX: Cefotaxime; ESBL: Extended-spectrum β-lactamase; i-pAmpC: inducible plasmid mediated AmpC. bp: base pair.

[a] No bla_{CARB}, bla_{TEM}, $bla_{OXA\text{-}1\text{-}like}$, $bla_{OXA2/3\text{-}like}$ or $bla_{OXA5/7\text{-}like}$ was detected.

megaplasmids (125–>300 Kb) (Riccobono et al., 2015; Silva et al., 2017; Tate et al., 2017; Vallejos-Sánchez et al., 2019). Further, an in-silico comparison of plasmids p-F219 (~321 Kb; GenBank access CP038508) and pFSIS1502916 (~322 Kb; Genbank access: CP016409), from a Peruvian and USA *S.* Infantis isolates respectively, showed ~99% of identity, despite being reported as belonging to different Inc. groups (IncI - p-F219; IncFI1 - pFSIS1502916) (Tate et al., 2017; Vallejos-Sánchez et al., 2019). Interestingly, while in the studies of Granda et al. (2019) as well as Quino et al. (2019) almost all the isolates analyzed possessed the $bla_{CTX\text{-}M\text{-}65}$ gene, in the present meat samples this gene was present in the 2/15 (13.3%) of the isolates.

Previous data by Riccobono et al. (2015) showed that when the $bla_{CTX\text{-}M\text{-}65}$ plasmid spreading in Bolivia is transferred to *Salmonella enterica*, it tends to be lost in the absence of selective pressure. While the analyzed Bolivian plasmid is different to p-F219, reported in Peru, both plasmids have ~40% of identity (Riccobono et al., 2015; Vallejos-Sánchez et al., 2019). In the absence of further data, it can be proposed that a similar *Salmonella* instability may take place in $bla_{CTX\text{-}M\text{-}65}$-carrying plasmids spreading in Peru; the heavy level of use of CPH in both hospital and community settings likely underlie the differences in the prevalence of $bla_{CTX\text{-}M\text{-}65}$ in meat and humans. These findings suggest the presence of a widely spreading conjugative $bla_{CTX\text{-}M\text{-}65}$-borne megaplasmid among present isolates.

Although the presence of the most common β-lactamases was sought, the βL resistance mechanisms remained to be established in 9 out of 15 *S.* Infantis. This finding suggests the presence of an uncommon mechanism of βL resistance (either β-lactamase or not), and has also been observed in a βL-resistant *S.* Infantis isolated as a cause of children's diarrhea in the area of Lima in which no specific βL resistance mechanism was identified (Granda et al., 2019). Meanwhile the resistance to cefotaxime observed in the 2 CTX-M-65-producer isolates is in agreement with the high cefotaxime hydrolysis efficiency of CTX-M (Rossolini et al., 2008).

The presence of bla_{DHA} has been scarcely reported in *S. enterica* (Pérez-Moreno et al., 2013). While very few data on the prevalence of i-pAmpC in Peru are available, with descriptions in *E. coli* recovered from chicken and beef samples (Ruiz-Roldán et al., 2018), a high rate of pAmpC producing *Enterobacteriaceae* acquisition has been observed in travelers returning from Peru (Lorme et al., 2018). Meanwhile, $bla_{CTX\text{-}M\text{-}65}$ has been previously reported as being frequent in the area, including its detection in *S.* Infantis, being described in Peru and in neighboring countries, such as Bolivia, Chile or Ecuador (Cartelle Gestal et al., 2016; Fuentes-Castillo et al., 2019; Riccobono et al., 2015; Sánchez-Salazar et al., 2020). Regarding Peru, the $bla_{CTX\text{-}M\text{-}65}$ gene has been previously described in bacteremic *E. coli*, and also in *S.* Infantis recovered from human and meat samples (Granda et al., 2019; Palma et al., 2017; Quino et al., 2019; Tate et al., 2017; Vallejos-Sánchez et al., 2019). In fact, during the last years there has been continuous isolation of MDR and ESBL-producer *S.* Infantis in Lima, as well as in other Peruvian areas as a cause of diarrhea or other human infections, mostly affecting children (Gonzales-Escalante, 2015; Granda et al., 2019; Quino et al., 2019).

In this scenario the isolation of food-carried *S.* Infantis also displaying an MDR phenotype and some carrying $bla_{CTX\text{-}M\text{-}65}$ was suggestive of the presence of a possible clonal relationship. Thus, 25 *S.* Infantis clinical isolates from Granda et al. (2019) study were compared with present meat recovered *S.* Infantis. Twenty-four out of 25 clinical isolates presented the $bla_{CTX\text{-}M\text{-}65}$ gene, showing a resistance pattern (Q, CHL, AF, NF, TC, βL, CPH) compatible with present pattern VI, with 10 isolates also presenting resistance to azithromycin and without data of resistance to AMG (Granda et al., 2019). REP-PCR and RAPD analysis confirmed clonal relationship, highlighting the transference of *S.* Infantis between food samples and humans resulting in the development of infectious diarrhea. Furthermore, in addition to the above-mentioned frequent description of diarrhea cases related to *S.* Infantis in other Peruvian region, the present data open the door to the possible presence of at least a nationwide disseminated highly resistant clone. Furthermore, the presence of MDR ESBL-producer *S.* Infantis in marketed

bushmeat in Peruvian Amazonia has just been highlighted (Maguiña-Molina et al., 2021). In the absence of molecular comparisons, this finding strongly suggests the dissemination of this *S.* Infantis clone out of human and poultry borders. In this sense, the presence of reports showing the presence of *bla*$_{CTX-M-65}$-producer *S.* Infantis in neighboring countries such as Chile or Ecuador is of note. Thus, in Chile a *bla*$_{CTX-M-65}$-producer *S.* Infantis was recovered in a wild bird admitted to a wildlife rescue and rehabilitation center in Chile (Fuentes-Castillo et al., 2019), while in Ecuador *bla*$_{CTX-M-65}$-producer *S.* Infantis have been isolated from patients, farm-environment, farm animals or animal-feed (Cartelle Gestal et al., 2016; Sánchez-Salazar et al., 2020). In the absence of molecular determinations, it cannot be excluded that these isolates, together with those recovered in Peru, belong to a common *S.* Infantis clone disseminated throughout different countries.

The transference of this *S.* Infantis clone between food and humans may be related to different situations, including poor cooking, or indirect transmission related to food manipulation and/or poor hygienic practices. In this sense, the presence of *S.* Infantis from the same clone in beef and pork samples may be related to either the true presence of the microorganism in the animal environment, or cross-contamination from the slaughterhouse to retail stalls.

While most *Salmonella* infections are self-limiting and do not require the use of antibacterial agents, the presence of concomitant comorbidities, the severity, long duration, the presence of the infection in newborns or the elderly or some other specific circumstances may require the use of antibacterial agents (Lübbert, 2016; Threlfall, 2002). The present meat-recovered isolates displayed high levels of resistance to almost all antibiotics, usually administered in the treatment of gastrointestinal *Salmonella* infections, with the exception of azithromycin, highlighting the need to preserve the activity of this antibacterial agent, and strongly alerting about the need to restrict the use of macrolides outside of human health. Of concern, 40% of clinical isolates were resistant to azithromycin (Granda et al., 2019), with this finding probably being related to the antibiotic pressure exerted on clinical settings and community.

In the present study no data about the slaughterhouse origin of samples was recovered, and therefore being unknown whether the chickens were grown on the same or on different farms, thereby limiting the knowledge about the real dissemination of this *S.* Infantis clone in the farm environment. Furthermore, this lack of knowledge does not allow the risk of cross-contamination in the slaughterhouse to be established. Nonetheless, the wide distribution of samples analyzed in Lima, strongly suggests the presence of chicken meat from different origins and slaughterhouses, and subsequently the relevant presence of this *S.* Infantis clone on chicken farms. Of note, Sánchez-Salazar et al. (2020) described the presence of *S.* Infantis carrying the *bla*$_{CTX-M-65}$ gene in poultry feed from Ecuadorian farms. This possibility remains to be analyzed in Peruvian farms, but suggests the possible transmission of ESBL-producer *S.* Infantis from poultry feed through marketed chicken meat to humans.

The wide spreading of CTX-M-65-producer *S.* Infantis in Peru, which has been isolated in distant areas, from a variety of sources, including marketed chicken meat and human infections, and suggested in bushmeat, shows the need to develop further studies to expand present clonal studies, determine the relationships between these isolates and establish measures to break dissemination pathways.

In summary, the present study demonstrates the food-origin of the diarrhea-related *S.* Infantis clone which is spreading in Lima. On the other hand, the description of *bla*$_{CTX-M-65}$-possessing *S.* Infantis in both other Peruvian regions and neighboring countries suggests extensive spreading of this clone. Present data strongly suggest the *in vivo* farm-selection of MDR microorganisms and warms about on the imperious necessity to implement effective non-antibiotic human/animal-pathogen control pathways in order to reduce the use of antimicrobial agents in food-production.

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Maria J. Pons: Conceptualization, Investigation, Formal analysis, Writing - review & editing, Supervision.

Joaquim Ruiz: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alonso, D., Muñoz, J., Ruiz, J., Carmona, F., Nadal, A., Gascón, J., 2007. *Salmonella* ovarian abscess following travel diarrhoea episode. Arch. Gynecol. Obstet. 276, 551–553. https://doi.org/10.1007/s00404-007-0380-y.

Angulo, F.J., Johnson, K.R., Tauxe, R.V., Cohen, M.L., 2000. Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. Microb. Drug Resist. 6, 77–83. https://doi.org/10.1089/mdr.2000.6.77.

Ao, T.T., Feasey, N.A., Gordon, M.A., Keddy, K.H., Angulo, F.J., Crump, J.A., 2015. Global burden of invasive nontyphoidal *Salmonella* disease, 2010. Emerg. Infect. Dis. 21, 941–949. https://doi.org/10.3201/eid2106.140999.

Arnedo-Pena, A., Sabater-Vidal, S., Herrera-León, S., Bellido-Blasco, J.B., Silvestre-Silvestre, E., Meseguer-Ferrer, N., Yague-Muñoz, A., Gil-Fortuño, M., Romeu-García, A., Moreno-Muñoz, R., 2016. An outbreak of monophasic and biphasic *Salmonella* Typhimurium, and *Salmonella* Derby associated with the consumption of dried pork sausage in Castellon (Spain). Enferm. Infecc. Microbiol. Clin. 34, 544–550. https://doi.org/10.1016/j.eimc.2015.11.016.

Barletta, F., Mercado, E.H., Lluque, A., Ruiz, J., Cleary, T.G., Ochoa, T.J., 2013. Multiplex real-time PCR for detection of *Campylobacter*, *Salmonella*, and *Shigella*. J. Clin. Microbiol. 51, 2822–2829. https://doi.org/10.1128/JCM.01397-13.

Bertrand, S., Weill, F.X., Cloeckaert, A., Vrints, M., Mairiaux, E., Praud, K., Dierick, K., Wildemauve, C., Godard, C., Butaye, P., Imberechts, H., Grimont, P.A., Collard, J.M., 2006. Clonal emergence of extended-spectrum β-lactamase (CTX-M-2)-producing

Salmonella enterica serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). J. Clin. Microbiol. 44, 2897–2903. https://doi.org/10.1128/JCM.02549-05.

Cabrera, R., Ruiz, J., Marco, F., Oliveira, I., Arroyo, M., Aladueña, A., Usera, M.A., Jiménez de Anta, M.T., Gascón, J., Vila, J., 2004. Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing of traveler's diarrhea. Antimicrob. Agents Chemother. 48, 3934–3939. https://doi.org/10.1128/AAC.48.10.3934-3939.2004.

Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A., Pezzotti, G., Magistrali, C.F., 2017. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro. Surveill. 22, 30589. https://doi.org/10.2807/1560-7917.ES.2017.22.31.30589.

Cartelle Gestal, M., Zurita, J., Paz y Mino, A., Ortega-Paredes, D., Alcocer, I., 2016. Characterization of a small outbreak of *Salmonella enterica* serovar Infantis that harbour CTX-M-65 in Ecuador. Braz. J. Infect. Dis. 20, 406–407. https://doi.org/10.1016/j.bjid.2016.03.007.

Chou, Y.J., Lin, H.W., Yang, C.J., Chen, M.Y., Sheng, W.H., Liu, W.C., Chang, S.Y., Hung, C.C., Hsueh, P.R., Chang, S.C., 2016. Risk of recurrent nontyphoid *Salmonella* bacteremia in human immunodeficiency virus-infected patients with short-term secondary prophylaxis in the era of combination antiretroviral therapy. J. Microbiol. Immunol. Infect. 49, 760–767. https://doi.org/10.1016/j.jmii.2015.07.005.

Clinical Laboratory Standard Institute (CLSI), 2018. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-Eight Edition (M100-S28). CLSI, Wayne (PA).

Crump, J.A., Sjölund-Karlsson, M., Gordon, M.A., Parry, C.M., 2015. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. Clin. Microbiol. Rev. 28, 901–937. https://doi.org/10.1128/CMR.00002-15.

Del Valle Martínez Rojas, D., 2009. Betalactamasas tipo AmpC: generalidades y métodos para detección fenotípica. Rev. Soc. Venez. Microbiol. 29, 78–83.

van Duijkeren, E., Houwers, D.J., 2000. A critical assessment of antimicrobial treatment in uncomplicated *Salmonella enteritis*. Vet. Microbiol. 73, 61–73. https://doi.org/10.1016/s0378-1135(00)00156-5.

Fàbrega, A., Soto, S.M., Balleste-Delpierre, C., Fernández-Orth, D., Jiménez de Anta, M. T., Vila, J., 2014. Impact of quinolone-resistance acquisition on biofilm production and fitness in *Salmonella enterica*. J. Antimicrob. Chemother. 69, 1815–1824. https://doi.org/10.1093/jac/dku078.

Fuentes-Castillo, D., Farfán-López, M., Esposito, F., Moura, Q., Fernandes, M.R., Lopes, R., Cardoso, B., Muñoz, M.E., Cerdeira, L., Najle, I., Muñoz, P.M., Catão-Dias, J.L., González-Acuña, D., Lincopan, N., 2019. Wild owls colonized by international clones of extended-spectrum β-lactamase (CTX-M)-producing *Escherichia coli* and *Salmonella* Infantis in the Southern Cone of America. Sci. Total Environ. 674, 554–562. https://doi.org/10.1016/j.scitotenv.2019.04.149.

Gallardo, F., Ruiz, J., Marco, F., Towner, K.J., Vila, J., 1999. Increase in incidence of resistance to ampicillin, chloramphenicol and trimethoprim in clinical isolates of *Salmonella* serotype Typhimurium with investigation of molecular epidemiology and mechanisms of resistance. J. Med. Microbiol. 48, 367–374. https://doi.org/10.1099/00222615-48-4-367.

Girish, R., Kumar, A., Khan, S., Dinesh, K.R., Karim, S., 2013. Revised ciprofloxacin breakpoints for *Salmonella*: is it time to write an obituary? J. Clin. Diagn. Res. 7, 2467–2469. https://doi.org/10.7860/JCDR/2013/7312.3581.

Gonzales-Escalante, E., 2015. Incremento de aislamientos de *Salmonella* spp. productora de β-lactamasas de espectro extendido en pacientes pediátricos del Instituto Nacional de Salud del Niño. Rev. Peru. Med. Exp. Salud Publica. 32, 605–607.

Granda, A., Riveros, M., Martínez-Puchol, S., Ocampo, K., Laureano-Adame, L., Corujo, A., Reyes, I., Ruiz, J., Ochoa, T.J., 2019. Presence of extended-spectrum β-lactamase, CTX-M-65, in *Salmonella enterica* serovar Infantis isolated from children with diarrhea in Lima, Peru. J. Pediatr. Infect. Dis. 14, 194–200. https://doi.org/10.1055/s-0039-1685502.

Hawkey, J., Le Hello, S., Doublet, B., Granier, S.A., Hendriksen, R.S., Fricke, W.F., Ceyssens, P.J., Gomart, C., Billman-Jacobe, H., Holt, K.E., Weill, F.X., 2019. Global phylogenomics of multidrug-resistant *Salmonella enterica* serotype Kentucky ST198. Microb. Genom. 5, e.0.000269 https://doi.org/10.1099/mgen.0.000269.

Heras, J., Domínguez, C., Mata, E., Pascual, V., Lozano, C., Torres, C., Zarazaga, M., 2015. GelJ - a tool for analyzing DNA fingerprint gel images. BMC Bioinformatics. 16, 270. https://doi.org/10.1186/s12859-015-0703-0.

Khan, A.S., Georges, K., Rahaman, S., Abdela, W., Adesiyun, A.A., 2018. Antimicrobial resistance of *Salmonella* isolates recovered from chickens sold at retail outlets in Trinidad. J. Food Prot. 81, 1880–1889. https://doi.org/10.4315/0362-028X.JFP-18-223.

Kim, K.Y., Park, J.H., Kwak, H.S., Woo, G.J., 2011. Characterization of the quinolone resistance mechanism in foodborne *Salmonella* isolates with high nalidixic acid resistance. Int. J. Food Microbiol. 146, 52–56. https://doi.org/10.1016/j.ijfoodmicro.2011.01.037.

Lamas, A., Miranda, J.M., Regal, P., Vázquez, B., Franco, C.M., Cepeda, A., 2018. A comprehensive review of non-enterica subspecies of *Salmonella enterica*. Microbiol. Res. 206, 60–73. https://doi.org/10.1016/j.micres.2017.09.010.

Le Hello, S., Bekhit, A., Granier, S.A., Barua, H., Beutlich, J., Zając, M., Münch, S., Sintchenko, V., Bouchrif, B., Fashae, K., Pinsard, J.L., Sontag, L., Fabre, L., Garnier, M., Guibert, V., Howard, P., Hendriksen, R.S., Christensen, J.P., Biswas, P. K., Cloeckaert, A., Rabsch, W., Wasyl, D., Doublet, B., Weill, F.X., 2013. The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. Front. Microbiol. 4, 395. https://doi.org/10.3389/fmicb.2013.00395.

Levesque, C., Roy, P.H., 1993. PCR analysis of integrons. In: Persing, D.H., Smith, T.F., Tenover, F.C., White, T.J. (Eds.), Diagnostic Molecular Microbiology: Principles and Applications. American Society for Microbiology, Washington DC, pp. 590–594.

Lezameta, L., Gonzales-Escalante, E., Tamariz, J.H., 2010. Comparación de cuatro métodos fenotípicos para la detección de beta-lactamasas de espectro extendido. Rev. Peru. Med. Exp. Salud Publica. 27, 345–351. https://doi.org/10.1590/S1726-46342010000300006.

Lin, A.W., Usera, M.A., Barrett, T.J., Goldsby, R.A., 1996. Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella enteritidis*. J. Clin. Microbiol. 34, 870–876.

Lorme, F., Maataoui, N., Rondinaud, E., Esposito-Farèse, M., Clermont, O., Ruppe, E., Arlet, G., Genel, N., VOYAG-R study group, Matheron, S., Andremont, A., Armand-Lefevre, L., 2018. Acquisition of plasmid-mediated cephalosporinase producing *Enterobacteriaceae* after a travel to the tropics. PLoS One 13, e0206909. https://doi.org/10.1371/journal.pone.0206909.

Lübbert, C., 2016. Antimicrobial therapy of acute diarrhoea: a clinical review. Expert Rev. Anti-Infect. Ther. 14, 193–206. https://doi.org/10.1586/14787210.2016.1128824.

Maguiña-Molina, C., Pons, M.J., Beltran, M., Morales-Cauti, S., 2021. Multidrug-resistant *Salmonella enterica* isolated in paca (*Cuniculus paca*) carcasses from the Belen Market, Iquitos - Peru. Foodborne Pathog. Dis. 18, 131–138. https://doi.org/10.1089/fpd.2020.2836.

Mandomando, I.M., Bassat, Q., Sigauque, B., Masora, S., Quintó, L., Acacio, S., Nhampossa, T., Vubil, D., Garrine, M., Macete, E., Aide, P., Sacoor, C., Herrera-León, S., Ruiz, J., Tennant, S.M., Menéndez, C., Alonso, P., 2015. Invasive *Salmonella* infections among children from rural Mozambique, 2001-2014. Clin. Infect. Dis. 61, S339–S345. https://doi.org/10.1093/cid/civ712.

Martínez, M.C., Retamal, P., Rojas-Aedo, J.F., Fernández, J., Fernández, A., Lapierre, L., 2017. Multidrug-resistant outbreak-associated *Salmonella* strains in irrigation water from the Metropolitan Region, Chile. Zoonoses Public Health 64, 299–304. https://doi.org/10.1111/zph.12311.

Martínez-Puchol, S., Pons, M.J., Ruiz-Roldán, L., Laureano-Adame, L., Corujo, A., Ochoa, T.J., Ruiz, J., 2020. Análisis de los mecanismos de resistencia a nitrofuranos en *Salmonella* spp. aisladas de muestras cárnicas para consumo humano. Rev. Peru. Med. Exp. Salud. Pública. 37, 99–103.

Merino, L.A., Alonso, J.M., Ruiz, J., Vila, J., 2007. Resistance to quinolones in *Salmonella infantis* due to overexpression of an active efflux system and a mutation in the *gyrA* gene. Enferm. Infecc. Microbiol. Clin. 25, 357–358. https://doi.org/10.1157/13102275.

Molyneux, E.M., Mankhambo, L.A., Phiri, A., Graham, S.M., Forsyth, H., Phiri, A., Walsh, A.L., Wilson, L.K., Molyneux, M.E., 2009. The outcome of non-typhoidal *Salmonella* meningitis in Malawian children, 1997–2006. Ann. Trop. Paediatr. 29, 13–22. https://doi.org/10.1179/146532809X401980.

Palma, N., Pons, M.J., Gomes, C., Mateu, J., Riveros, M., García, W., Jacobs, J., García, C., Ochoa, T.J., Ruiz, J., 2017. Resistance to quinolones, cephalosporins and macrolides in *Escherichia coli* causing bacteraemia in Peruvian children. J. Global Antimicrob. Resist. 11, 28–33. https://doi.org/10.1016/j.jgar.2017.06.011.

Pérez-Moreno, M.O., Picó-Plana, E., de Toro, M., Grande-Armas, J., Quiles-Fortuny, V., Pons, M.J., Gomes, C., Sáenz, Y., Torres, C., Ruiz, J., 2013. β-Lactamases, transferable quinolone resistance determinants, and class 1 integron-mediated antimicrobial resistance in human clinical *Salmonella enterica* isolates of non-Typhimurium serotypes. Int. J. Med. Microbiol. 303, 25–31. https://doi.org/10.1016/j.ijmm.2012.11.003.

Plumb, I.D., Schwensohn, C.A., Gieraltowski, L., Tecle, S., Schneider, Z.D., Freiman, J., Cote, A., Noveroske, D, Kolsin, J, Brandenburg, J., Chen, J.C., Tagg, K.A., White, P. B., Shah, H.J., Francois Watkins, L.K., Wise, M.E., Friedman, C.R., 2019. Outbreak of *Salmonella* Newport infections with decreased susceptibility to azithromycin linked to beef obtained in the United States and soft cheese obtained in Mexico - United States, 2018-2019. MMWR Morb Mortal Wkly Rep. 68 (33), 713–717. https://doi.org/10.15585/mmwr.mm6833a1.

Pons, M.J., Vubil, D., Guiral, E., Jaintilal, D., Fraile, O., Soto, S.M., Sigaúque, B., Nhampossa, T., Aide, P., Alonso, P.L., Vila, J., Mandomando, I., Ruiz, J., 2015. Characterisation of extended-spectrum β-lactamases among *Klebsiella pneumoniae* isolates causing bacteraemia and urinary tract infection in Mozambique. J. Glob. Antimicrob. Resist. 3, 19–25. https://doi.org/10.1016/j.jgar.2015.01.004.

Quino, W., Hurtado, C.V., Escalante-Maldonado, O., Flores-León, D., Mestanza, O., Vences-Rosales, F., Zamudio, M.L., Gavilán, R.G., 2019. Multidrogorresistencia de *Salmonella infantis* en Perú: un estudio mediante secuenciamiento de nueva generación. Rev. Peru. Med. Exp. Salud Publica. 36, 37–45. https://doi.org/10.17843/rpmesp.2019.361.3934.

Riccobono, E., Di Pilato, V., Di Maggio, T., Revollo, C., Bartoloni, A., Pallecchi, L., Rossolini, G.M., 2015. Characterization of IncI1 sequence type 71 epidemic plasmid lineage responsible for the recent dissemination of CTX-M-65 extended-spectrum β-lactamase in the Bolivian Chaco region. Antimicrob. Agents Chemother. 59, 5340–5347. https://doi.org/10.1128/AAC.00589-15.

Rossolini, G.M., D'Andrea, M.M., Mugnaioli, C., 2008. The spread of CTX-M-type extended-spectrum β-lactamases. Clin. Microbiol. Infect. 14 (Suppl. 1), 33–41. https://doi.org/10.1111/j.1469-0691.2007.01867.x.

Ruiz, J., 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA Gyrase protection. J. Antimicrob. Chemother. 51, 1109–1117. https://doi.org/10.1093/jac/dkg222.

Ruiz, J., Capitano, L., Nuñez, L., Castro, D., Sierra, J.M., Hatha, M., Borrego, J.J., Vila, J., 1999. Mechanisms of resistance to ampicillin, chloramphenicol and quinolones in multiresistant *Salmonella typhimurium* strains isolated from fish. J. Antimicrob. Chemother. 43, 699–702. https://doi.org/10.1093/jac/43.5.699.

Ruiz-Roldán, L., Martínez-Puchol, S., Gomes, C., Palma, N., Riveros, M., Ocampo, K., Durand, D., Ochoa, T.J., Ruiz, J., Pons, M.J., 2018. Presencia de *Enterobacteriaceae* y *Escherichia coli* multirresistente a antimicrobianos en carne adquirida en mercados tradicionales en Lima. Rev. Peru. Med. Exp. Salud Publica. 35, 425–432. https://doi.org/10.17843/rpmesp.2018.353.3737.

Sáenz, Y., Ruiz, J., Zarazaga, M., Teixidó, M., Torres, C., Vila, J., 2004. Effect of the efflux pump inhibitor Phe-Arg-β-naphthylamide on the MIC values of the quinolones, tetracycline and chloramphenicol, in *Escherichia coli* isolates of different origin. J. Antimicrob. Chemother. 53, 544–545. https://doi.org/10.1093/jac/dkh117.

Salazar de Vegas, E.Z., Nieves, B., Araque, M., Velasco, E., Ruiz, J., Vila, J., 2006. Outbreak of infection with *Acinetobacter* strain RUH 1139 in an intensive care unit. Infect. Control Hosp. Epidemiol. 27, 397–403. https://doi.org/10.1086/503177.

Sánchez-Salazar, E., Gudiño, M.E., Sevillano, G., Zurita, J., Guerrero-López, R., Jaramillo, K., Calero-Cáceres, W., 2020. Antibiotic resistance of *Salmonella* strains from layer poultry farms in central Ecuador. J. Appl. Microbiol. 128, 1347–1354. https://doi.org/10.1111/jam.14562.

Silva, C., Calva, E., Maloy, S., 2014. One health and food-borne disease: *Salmonella* transmission between humans, animals, and plants. Microbiol. Spectr. 2, OH-0020-2013 https://doi.org/10.1128/microbiolspec.OH-0020-2013.

Silva, C., Betancor, L., García, C., Astocondor, L., Hinostroza, N., Bisio, J., Rivera, J., Perezgasga, L., Pérez Escanda, V., Yim, L., Jacobs, J., García-del Portillo, F., SalmoIber CYTED Network, Chabalgoity, J.A., Puente, J.L., 2017. Characterization of *Salmonella enterica* isolates causing bacteremia in Lima, Peru, using multiple typing methods. PLoS One 12, e0189946. https://doi.org/10.1371/journal.pone.0189946.

Sugimoto, R., Suzuki, H., Nei, T., Tashiro, A., Washio, Y., Sonobe, K., Nakamura, Y., Wakayama, N., Inai, S., Izumiya, H., 2017. Neck abscess due to *Salmonella choleraesuis*: case study and literature review. JMM Case Rep. 4, e005109 https://doi.org/10.1099/jmmcr.0.005109.

Tate, H., Folster, J.P., Hsu, C.H., Chen, J., Hoffmann, M., Li, C., Morales, C., Tyson, G.H., Mukherjee, S., Brown, A.C., Green, A., Wilson, W., Dessai, U., Abbott, J., Joseph, L., Haro, J., Ayers, S., McDermott, P.F., Zhao, S., 2017. Comparative analysis of extended-spectrum-β-lactamase CTX-M-65-producing *Salmonella enterica* serovar Infantis isolates from humans, food animals, and retail chickens in the United States. Antimicrob. Agents Chemother. 61, e00488-17 https://doi.org/10.1128/AAC.00488-17.

Threlfall, E.J., 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol. Rev. 26, 141–148. https://doi.org/10.1111/j.1574-6976.2002.tb00606.x.

Vallejos-Sánchez, K., Tataje-Lavanda, L., Villanueva-Pérez, D., Bendezú, J., Montalván, A., Zimic-Peralta, M., Fernández-Sánchez, M., Fernández-Díaz, M., 2019. Whole-genome sequencing of a *Salmonella enterica* subsp. *enterica* serovar Infantis strain isolated from broiler chicken in Peru. Microbiol. Resour. Announc. 8, e00826-19 https://doi.org/10.1128/MRA.00826-19.

Vila, J., Ruiz, J., Goñi, P., Marcos, M.A., Jiménez de Anta, M.T., 1995. Mutation in the *gyrA* gene of quinolone-resistant clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 39, 1201–1203.

Wang, T.K., Yam, W.C., Yuen, K.Y., Wong, S.S., 2006. Misidentification of a mucoid strain of *Salmonella enterica* serotype choleraesuis as *Hafnia alvei* by the Vitek GNI+ card system. J. Clin. Microbiol. 44, 4605–4608. https://doi.org/10.1128/JCM.01488-06.