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Genomic characterization of plasmids of *mcr-1*-positive *Escherichia coli* isolated from cohabiting rats, dairy cattle and pigs

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Abstract

Background Antimicrobial resistance has become a significant global issue impacting humans, animals, and the environment. Currently, the focus of concern has shifted to the environment, which can act as a reservoir and significantly contribute to the spread of resistance genes. This study aimed to elucidate the potential transmission of *mcr-1*, which confers colistin resistance, among *Escherichia coli* isolates from pigs, dairy cattle, and co-habiting rodents. In March 2018, 30 fecal samples were collected from three pig farms and one mixed cattle farm, and 31 cecal contents from rats (*Rattus norvegicus*) captured from the same four animal farms were analyzed.

Results Out of 26 *mcr-1* positive *E. coli* isolates, 16 came from six rats, 10 from four pigs, and none from dairy cattle. The *mcr-1*-positive isolates from cohabiting rats and pigs were genetically unrelated, based on different XbaI-PFGE profiles. The plasmid profiles of one isolate per animal from each farm were analyzed by S1-PFGE. *E. coli* isolates from cohabiting rats and pigs showed plasmid bands of similar sizes (33 or 65 kb). To investigate the horizontal transfer of these plasmids between the animals, two pairs of *E. coli* isolates from pig farms 1 and 3 were selected for WGS analysis. Three of the isolates (EcoP3-1, EcoC2-1 from pigs, and Eco1266-6 from a rat) belonged to clonal complex 10 (CC10), while the other rat isolate (Eco1284-6) belonged to CC398 (ST398). Eco1266-6 (rat) and EcoC2-1 (pig) from cohabiting animals in pig farm 1 carried IncX4 plasmids with the *mcr-1.1* variant. The plasmid sequences were almost identical (99.98% identity), both carrying the *mcr-1.1/pap2* segment. pEcoC2-1 had a complete *ISVsa5* insertion sequence upstream of the *mcr-1* gene. Eco1284-6 (rat) and EcoP3-1 (pig) from pig farm 3 carried IncI2 plasmids with different allelic variants of *mcr-1* (*mcr-1.5* and *mcr-1.1*).

Conclusions *E. coli* isolates from cohabiting rats and pigs were genetically distinct, but one pair of isolates had very similar IncX4 plasmids, suggesting the potential for horizontal spread of plasmids carrying *mcr* genes. These findings

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suggest a threat of resistant *E. coli* spreading between cohabiting animals and into the environment. This underscores the importance of conducting integrated One-Health studies.

Keywords Antimicrobial resistance, *Escherichia coli*, Spread of resistance genes, Colistin resistance, *mcr-1*, Horizontal transfer, Plasmid profiles and one-health studies

Introduction

In recent decades, antimicrobial resistance has become a growing public and animal health problem, altering the human-animal-environment interface. Within this framework, livestock production systems have multiple routes that allow the introduction and dissemination of resistant microorganisms. Among these pathways, wildlife can interact directly with livestock, serving as reservoirs of antimicrobial-resistant bacteria and allowing the exchange of genetic determinants of antimicrobial resistance. Wild animals act as mechanical vectors that spread bacteria into the food chain and across the environment [1]. One of the few last-line antimicrobials for the treatment of multidrug resistant *Enterobacterales* in human medicine is colistin. In 2015, a plasmid-encoded colistin resistance gene, known as mobilized colistin resistance gene (*mcr-1*), was described in China [2]. The *mcr-1* gene is transferred between bacteria, increasing the likelihood of acquiring colistin resistance by pathogens affecting humans and animals [3]. The main carrier of *mcr-1* is *Escherichia coli*, a microorganism that can be found in humans, animals, and the environment, making it an ideal indicator organism for *mcr-1* monitoring and surveillance [4]. Worldwide, bacteria harboring *mcr-1* have been found within the gastrointestinal tracts of wildlife, suggesting that this gene could have an important role in the development and dissemination of colistin resistance through fecal-mediated contamination. Rodents have long shared living environments with humans, playing a role as reservoirs of agents for various bacterial, viral, and parasitic zoonoses [5–7]. Additionally, rats have been previously reported to carry multidrug-resistant bacteria [8, 9]. The aim of the present study was to characterize plasmids carrying the *mcr-1* gene in *E. coli* isolates obtained from dairy cattle, pigs, and cohabiting rodents, with the aim to shed light on the potential of transmission of this determinant of resistance between *E. coli* isolates from these animal populations.

Results

Of the *E. coli* isolates grown on the MC-COL selective media, 48 were selected for molecular characterization and antimicrobial susceptibility testing of which 26 (54.2%) were positive for the *mcr-1* gene, with a minimum inhibitory concentration (MIC) to colistin ≥ 4 µg/mL. The remaining 22 *E. coli* isolates were negative for *mcr-1*, with a MIC to colistin < 4 µg/mL (Table 1). From the *mcr-1*-positive isolates, 16 were recovered from six rats, and 10 from four pigs. No isolates carrying the *mcr-1* gene were found in the daily cattle samples. The colistin MIC distribution among the *mcr-1*-positive isolates was: 21 with 4 µg/mL, four with 8 µg/mL, and one with 16 µg/mL (Table 1).

When analyzing the genetic relationship between the *mcr-1*-positive isolates obtained from pigs and rats from the same productive farm, they were not found to be genetically related among them, showing different XbaI-digested pulsed-field gel electrophoresis (XbaI-PFGE) profiles. To analyze the presence of shared plasmids among isolates from the same farm, plasmid profiles of one isolate per animal from each productive farm (pig farm 1: PF1, mixed farm: MF, pig farm 2: PF2 and pig farm 3: PF3) were analyzed by S1-PFGE (Supplementary Fig. 1). A diverse plasmid content was found by S1-PFGE, but some *E. coli* isolates from rats and pigs from the same farm (e.g., Eco1266-6 and EcoC2-1 from PF1, and Eco1284-6 and EcoP3-1 from PF3) showed plasmid bands with similar size (33 or 65 kb, respectively; Supplementary Fig. 1). Therefore, to analyze the possibility of horizontal plasmid transference, the two pairs of *E. coli* isolates from PF1 and PF3 were selected for whole-genome sequencing (WGS) analysis. All four isolates belonged to different sequence types (STs), showing a polyclonal expansion of *E. coli* bearing *mcr-1* plasmids. However, three of them, two of porcine origin, EcoP3-1 (ST10) and EcoC2-1 (ST34), and one of rat origin, Eco1266-6 (ST744), belonged to the clonal complex 10 (CC10). The other rat isolate, Eco1284-6, belonged to CC398 (ST398) (Table 2).

The Eco1266-6 (rat) and EcoC2-1 (pig) isolates were recovered from cohabiting animals in PF1 and harbored the IncX4 plasmids, pEco1266-6_33 (33,304 bp) and pEcoC2-1_34(34,643 bp), respectively, carrying the *mcr-1.1* variant (Table 2). Comparison of the plasmid sequence pEco1266-6_33 and pEcoC2-1_34 plasmid sequences showed that they were almost identical (99.98% identity), both containing the *mcr-1.1*/*pap2* segment. pEcoC2-1_34 also had a complete copy of the *IS*Vsa5 insertion sequence (1,329 bp), located 158 bp upstream of the *mcr-1* gene. The Eco1266-6 isolate harbored a truncated *IS*Vsa5 sequence but inserted into the chromosome (Supplementary Fig. 2). The other two isolates, Eco1284-6 (rat) and EcoP3-1 (pig), recovered from PF 3, harbored the IncI2 plasmids, pEco1284-6_62 (62,877 bp) and pEcoP3-1_63 (63,005 bp), carrying different allelic variants of *mcr-1* (*mcr-1.5* and *mcr-1.1*,

Table 1 Characteristics of the 48 *E. coli* recovered from different farms in Buenos Aires, Argentina, 2018

Farm	Sample	Origin	Location	Isolates	mcr-1*	MIC (μg/mL) colistin (int)**
Pig farm 1	Cecal content	*R. norvegicus*	Las Heras	**1266-6**	Positive	4 (R)
				1266-7	Positive	4 (R)
				1266-8	Positive	8 (R)
Pig farm 1	Cecal content	*R. norvegicus*	Las Heras	**1288-6**	Positive	8 (R)
				1288-7	Positive	4 (R)
				1288-8	Positive	16 (R)
Pig farm 1	fecal	pig	Las Heras	**C2-1**	Positive	4 (R)
				C2-2	Positive	4 (R)
				C2-3	Positive	4 (R)
Pig farm 1	fecal	pig	Las Heras	C5-2	Negative	2 (S)
				C5-3	Negative	2 (S)
				C5-4	Negative	2 (S)
Mixed farm	Cecal content	*R. norvegicus*	Marcos Paz	1275-7	Negative	2 (S)
				1275-8	Negative	2 (S)
				1275-9	Negative	2 (S)
Mixed farm	Cecal content	*R. norvegicus*	Marcos Paz	1286-7	Negative	1 (S)
				1286-8	Positive	8 (R)
				1286-10	Negative	2 (S)
Mixed farm	fecal	daily cattle	Marcos Paz	S 1–7	Negative	1 (S)
				S 1–8	Negative	2 (S)
				S 3 – 1	Negative	2 (S)
Mixed farm	fecal	daily cattle	Marcos Paz	S 5–6	Negative	1 (S)
				S 5–7	Negative	2 (S)
				S 5–8	Negative	1 (S)
Pig farm 2	Cecal content	*R. norvegicus*	Marcos Paz	**1278-6**	Positive	4 (R)
				1278-7	Positive	4 (R)
				1278-8	Positive	4 (R)
Pig farm 2	Cecal content	*R. norvegicus*	Marcos Paz	**1295-7**	Positive	4 (R)
				1295-8	Positive	8 (R)
				1295-9	Positive	4 (R)
Pig farm 2	fecal	pig	Marcos Paz	T2-2	Negative	1 (S)
				T2-3	Positive	4 (R)
				T2-4	Negative	2 (S)
Pig farm 2	fecal	pig	Marcos Paz	**T3-1**	Positive	4 (R)
				T3-2	Positive	4 (R)
				T3-3	Positive	4 (R)
Pig farm 3	Cecal content	*R. norvegicus*	Marcos Paz	1271-6	Negative	2 (S)
				1271-7	Negative	1 (S)
				1271-8	Negative	1 (S)
Pig farm 3	Cecal content	*R. norvegicus*	Marcos Paz	**1284-6**	Positive	4 (R)
				1284-7	Positive	4 (R)
				1284-8	Positive	4 (R)
Pig farm 3	fecal	pig	Marcos Paz	**P3-1**	Positive	4 (R)
				P3-2	Positive	4 (R)
				P3-3	Positive	4 (R)
Pig farm 3	fecal	pig	Marcos Paz	P4-1	Negative	2 (S)
				P4-2	Negative	2 (S)
				P4-3	Negative	2 (S)

Isolates in bold were positive for the *mcr-1* gene. * PCR to detect the *mcr-1* gene. **R, resistant; S, susceptible by the broth microdilution method to colistin

Table 2 Genomic features of *mcr-1*-positive *E. coli* isolates recovered from rodents and pigs

Isolate	Animal, Farm	*mcr-1* plasmid size (bp)	Sequence type (ST)	Clonal complex (CC)	Incompatibility group (Inc)	Genetic context of the *mcr-1* gene	Antimicrobial Resistance Gene Families					
							Aminoglycosides	ß-lactams	Phenicols	Tetracyclines	Sulfonamides	Others
Eco1266-6	rodent, farm 1	33,304	ST744	CC10	X4	ORF/ORF/***mcr-1.1***/*pap2* /ORF	*aph(3)-Ia, aph(3)-Ib, aph(6)-Id*	bla_{TEM-1B}	*floR.2, catA1*	*tet(B)*	sul2.3	*mdf(A)*
EcoC2-1	pig, farm 1	34,643	ST34	CC10	X4	ORF/ORF/ISVas5/***mcr-1.1***/*pap2*/ORF	*aph(3)-Ib, aph(6)-Id*	bla_{TEM-1B}	*floR.2*	*tet(B)*	-	*mdf(A)*
Eco1284-6	rodent, farm 3	62,877	ST398	CC398	I2	ISApl1/***mcr-1.5***/*pap2*/ISApl1	*aph(3)-Ib, aph(6)-Id*	bla_{TEM-1B}	-	*tet(B)*	-	*mdf(A)*
EcoP3-1	pig, farm 3	63,005	ST10	CC10	I2	ORF/***mcr-1.1***/*pap2*/ORF	*aph(3)-Ib, aph(6)-Id,*	$bla_{TEM-214}$	-	*tet(B)*	-	*mdf(A)*

respectively). Comparison between pEco1284-6_62 and pEcoP3-1_63 showed only 86% of the sequences with 99.98% of identity (Supplementary Fig. 3). The *mcr-1.5*/*pap2* fragment in these plasmids was flanked by two copies of IS*ApI1* (IS*ApI1-mcr-1.5-pap2*-IS*ApI1*, called Tn*6330*). The *mcr-1.1/pap2* fragment was located between the *nikB* (relaxase) and *top* (DNA topoisomerase III) genes, without the IS*ApI1* insertion sequences, described for the *mcr-1.5* variant.

Discussion

In the present study, we demonstrated the circulation of the *mcr-1* gene in a significant proportion of *E. coli* isolates from pigs and cohabiting rats, and found negative detection in isolates from cattle, which is consistent with previous reports [10, 11]. In the pig production chain, pigs are administered a high level of antibiotics, including colistin, mainly due to management practices. In contrast, the use of colistin in cattle production is uncommon. In other words, differences in the production practices applied to these animals could explain the variation in the acquisition of colistin resistance here observed [12].

Numerous *E. coli* isolates with different sequence types (STs) carrying *mcr-1* have been identified in animals, food products, and humans [13]. Among these, ST744 is an important member of the CC10 group and one of the most prevalent reported in *E. coli* isolates carrying the *mcr-1* gene in wildlife [14]. *mcr-1*-bearing CC10 *E. coli* has been described in different hosts and is widely disseminated in humans, food and animals, posing a potential threat to public health [2]. In this study, no dominant PFGE profile was found and, despite the diversity of STs detected, CC10 was the most prevalent, resulting as an important reservoir for the simultaneous spread of the *mcr-1* gene between pigs and rodents.

The diversity of plasmids observed using S1-PFGE in *mcr-1*-positive isolates, although not all were sequenced, suggests that there are several types of plasmids involved in the dissemination. In the present study, the comparison between the plasmid sequences of pEco1266-6_33 and pEcoC2-1_34 recovered from cohabiting animals, specifically rodents and pigs from farm 1, showed a remarkable similarity (99.98%). Further comparative analysis indicated that these plasmids were nearly identical, with 99.95% identity (100% coverage) to the IncX4 *mcr-1*-harboring *E. coli* plasmid pCSZ4 (GenBank KX711706.1) isolated from pigs in China [15], and to isolates (99.99% identity) obtained from human clinical samples in Argentina [16]. The differences described between pEco1284-6_62 and PEcoP3-1_63, recovered from pig farm 3, suggest that these plasmids were not closely related between them, and that their presence in both isolates would be via independent events. Additionally,

pEco1284-6_62 showed 99.9% genetic identity with the IncI2-type pMCR-M15049 (GenBank KY471308) recovered from a human clinical *E. coli* isolate [17], while pEcoP3-1_63 showed 100% identity to the fragment on plasmid pMCR-15,244 (GenBank KY471309) [17].

Although the *E. coli* isolates obtained from rats and pigs cohabiting the same farm were genetically different, one pair of isolates harbored highly similar IncX4 plasmids, suggesting a possible horizontal dissemination of plasmids containing *mcr* genes. These results imply that the transmission and dissemination of resistant *E. coli* between cohabiting animals and in the environment is not the only mechanism involved in the dissemination of *mcr-1* in farms. Thus, a deeper analysis about the mechanisms involved in the spread of the *mcr-1* resistance gene in animals and the environment is still necessary.

Conclusions

This work highlights the need to carry out integrated studies from a One Health perspective, as similar plasmids containing *mcr-1*, belonging to the IncX4 and IncI2 incompatibility groups, have been described in rats and pigs as well as in clinical isolates in our country. Further studies are needed to understand the evolution and possible dissemination of antimicrobial resistance through genetic platforms.

Materials and methods

In March 2018, a total of 30 fecal samples (25 from pigs and 5 from dairy cattle) were collected from three pig farms and one mixed cattle farm, along with 31 cecal contents from rats (*Rattus norvegicus*) captured in the same farms. These intensive farms were located in Marcos Paz (34°46′00″S, 58°50′00″W) and Las Heras (34°56′00″S, 58°57′00″W) departments in Buenos Aires province, Argentina (Table 1). These farms were part of a larger project that included sampling in 11 farms within an agricultural region representative of the livestock production sector in the region [9]. In these farms, multidrug-resistant *E. coli* isolates and one *E. coli mcr-1* strain were detected in rodents. Thus, these farms were subsequently selected for this study to collect samples from both rodents and livestock animals cohabiting within these production systems. Regarding the farms studied, the pig farms performed full-cycle operations (from breeding to finishing), while the mixed cattle farm was a dairy farm raising some pigs; however, cattle and pigs did not share the same area or have direct contact.

Rats were trapped with cage live traps (15 × 16 × 31 cm) baited with meat and carrot. On each farm, a total of 25 or 30 traps were set al.ong trap lines for three consecutive nights and checked for captures daily in the morning [18]. These traps were placed in representative environments within the farms, mainly in dairy or pig sheds, food storage sheds, and drainage channels, among others. Traps were set both inside and outside, except for those in drainage channels and pig sheds. Captured rats were anesthetized with 1:10 ketamine hydrochloride: xylazine sulphate injected intramuscularly, and humanely sacrificed to collect tissue samples. Rats were trapped, handled and euthanized according to the protocols and procedures approved by national and international guidelines for animal care, such as the Argentine Law 14,346 on the Animal Care, the Argentine Society for Mammalian Studies [19] the American Society of Mammalogists [20] and the Ethics Committee for Research on Laboratory, Farm, and Wild Animals of the National Council of Scientific and Technical Research of Argentina (CONICET; Resolution 1047, Sect. 2, Annex II). Samples were transported at 4 °C to the of General Bacteriology Laboratory of the National Institute of Agricultural Technology (INTA), Castelar, province of Buenos Aires, within 72 h of their collection.

For bacterial enrichment, 0.5 g of fecal samples and cecal contents were added with 4.5 mL of buffered peptone water and incubated for 18 h at 37 °C [9]. To select for colistin-resistant bacteria, MacConkey agar plates supplemented with 3 µg/mL of colistin (MC-COL) [21] were inoculated with 20 µL of the enriched cultures. For quality control of the MC-COL plates, previously characterized positive (colistin-resistant *E. coli*, Eco1082) and negative (colistin-susceptible *E. coli*, *E. coli* ATCC 25922) control strains were also included [9]. When lactose-fermenting colonies were obtained, five different colonies were randomly selected from each sample. Presumptive *E. coli* identifications were confirmed using standard biochemical tests (catalase, oxidase, indole, methyl red, Voges-Proskaeur, citrate, nitrates, TSI, hydrogen sulfide, and gas production) and PCR for the *ycjM* gene, to be able to identify enteric *E. coli* [22]. Of the 145 *E. coli* grown on the MC-COL selective agar, obtained from 29 (16 pigs, 11 rats and 2 dairy cattle) out of 61 animals (4 farms), 48 isolates were randomly selected for further testing. From pig farms 1, 2, and 3, six strains were isolated from pigs and six from rats. Meanwhile, from the mixed farm, six strains were isolated from dairy cattle and six from rats, resulting in a total of 12 isolates per farm. Antimicrobial susceptibility was evaluated in all selected isolates by the broth microdilution method, according to the guidelines of the Clinical and Laboratory Standards Institute [23]. PCR was performed to detect the *mcr-1* gene [2] (Table 1). The genetic relationship between *mcr-1*-positive *E. coli* isolates obtained from pigs and rats was evaluated by XbaI-digested pulsed-field gel electrophoresis (XbaI-PFGE). Plasmid content was assessed by S1 nuclease (Promega, Southampton, UK) digestion followed by PFGE [24], using a Chef-DR® III System (Bio-Rad™, Hercules, CA, United States). Briefly,

DNA fragments were resolved in 1% agarose gel applying a switch time of 2.4 to 54.2 s during 20 h at 14 °C. PFGE patterns showing > 6 bands of difference were considered to be non-genetically related. After analysis of the plasmid profiles, four *mcr-1*-positive *E. coli* (pigs and rats pairs) were selected for WGS based on band presence and size similarity (see Results section, and Supplementary Fig. 1). Whole bacterial DNA was extracted using the Master Pure Complete DNA & RNA Purification kit (Epicenter Illumina, Wisconsin, USA). Illumina and Oxford Nanopore Technologies (ONT) sequencing was performed as previously described [25]. Illumina-ONT hybrid assembly of short and long reads was done with Unicycler v0.4.8-beta [26]. The quality of the assembled genomes was assessed using the Quality Assessment Tool for Genome Assemblies (QUAST). Open reading frames were annotated using PROKKA [27] and manually curated. Sequence types (STs) and clonal complexes (CC) were determined by in silico analysis using the multilocus sequence typing (MLST) profile according to the Achtman scheme. Incompatibility groups, resistance genes and insertion sequences were identified using PlasmidFinder [28], ResFinder [29], and ISFinder [30], respectively, using the services of the CGE database (https://www.genomicepidemiology.org/) (accessed on 17 January 2024). Sequence comparisons were performed with nucleotide BLAST (BLASTn), using the NCBI Nucleotide Collection Database, and the Artemis Comparative Tool [31].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04665-4.

Supplementary Material 1: **Plasmid profile. S1 nuclease (S1-PFGE).** Gel order: Lane 2, Eco1266-6; 3, Eco1288-6; 4, EcoC2-1; 5, EcoC2-3; 6, Ecp1286-8; 7, Eco1278-6; 8, Eco1295-7; 9, EcoT2-3; 10, EcoT3-3; 11, EcoT3-3; 12, EcoT3-2; 13, Eco1284-6; 14, EcoP3-1; lanes 1 and 15, ***S***. Branderup. Pig farm 1 (PF1), mixed farm (MF), pig farm 2 (PF2) and pig farm 3 (PF3). Yellow arrows highlight plasmids containing the ***mcr-1*** gene.

Supplementary Material 2: **Analysis of insertion site of*ISVsa5* in pEcoC2-1_34.** In red and italics is a partial ***ISVsa5*** insertion sequence, only inverted repeat left (IRL) and right (IRR) sequences are shown. Target site duplication TA is in bold. Underlined sequence (TACTGA) is a possible recognition site for insertion of ***ISVsa5***. In purple, reverse and complement, is the ORF of ***mcr-1*** gene.

Supplementary Material 3: **Sequence comparison between IncI2 plasmids.** pEco1284-6_62 (upper sequence) and pEcoP3-1_63 (lower sequence) were compared using BLASTn and graphed by the Artemis Comparison Tool (ACT).

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Author contributions
JD, RC, RM, AC, DF and MFM participated in the design of the study. JD, FM, NC, CS and DF carried out the experiments. JD, FM, RL, NC, RM, DF and MFM analyzed the data. RL and RC collected fecal samples and captured the animals. JD and NC conducted the E. coli strains isolations. JD, NC, RL, RM and DF handled the preparation and editing of the manuscript. All authors contributed to the critical revision of the manuscript and have seen and approved the final draft. All authors read and approved of the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Trapping, handling, and euthanasia were performed according to procedures approved by the Ley 14346 de Protección Animal, the Argentine Society for the Study of Mammals (Giannoni et al. 2003), the American Society of Mammalogists (Sikes et al. 2011), and the Ethical Framework for Biomedical Research on laboratory, farm and wild-caught animals of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET; resolución 1047, sección 2, anexo II). Additionally, this project was reviewed by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL, its acronym in Spanish) of the Facultad de Ciencias Exactas y Naturales de la Universidad de Buenos Aires (Protocol No. 125).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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