



## Evaluation of heavy metal tolerance genes in plasmids harbored in multidrug-resistant *Salmonella enterica* and *Escherichia coli* isolated from poultry in Brazil

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### ABSTRACT

The emergence and spread of bacteria tolerant to heavy metal ions used in disinfectants have become a new threat to antimicrobial therapies. In this study, metal tolerance genes were analyzed in multidrug-resistant (MDR) *Enterobacteriaceae* isolated from chickens in Brazil. Different tolerance genes were found disseminated among the MDR isolates.

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## 1. Introduction

Infections caused by multidrug-resistant (MDR) nontyphoidal *Salmonella* and *Escherichia coli* have increased worldwide and are major public health concerns. In addition to antibiotic resistance, tolerance to other antimicrobials, such as heavy metal based disinfectants, has been investigated to understand the association and dissemination of different adaptive traits in bacterial species (Andrade et al. 2018). Silver compounds have also been incorporated in medical devices, and they are frequently used in a wide variety of household products as antibacterial treatments of materials. The zinc and copper salts are used for disinfection in animal husbandry and are also used as feed additives to improve health of livestock animals (Antunes et al. 2016).

In industrialized countries, the main reservoir of nontyphoidal *Salmonella* is the intestinal tract of food-producing animals, which may readily lead to contamination of diverse foodstuffs (Antunes et al. 2016). The intestinal tract of food-producing animals has also been characterized as an important reservoir of MDR *E. coli* (Liebana et al. 2013).

This study evaluated the occurrence of metal tolerance genes in 58 nontyphoidal *Salmonella enterica* and 200 *Escherichia coli* isolates, previously characterized as MDR (Ferreira et al. 2016, 2017, 2018), and

determined the location of the *silA* gene in *Salmonella enterica* isolates to track the dissemination of this gene in pathogenic bacteria. The bacteria were isolated from healthy chickens from 2 different regions (Sao Paulo state and Goias state) in Brazil between 2010 and 2015. The acquired metal tolerance genes were analyzed by PCR including genes involved in enzymatic detoxification or efflux of metals: *pcoA* (multicopper oxidase) (Mourao et al. 2015), *silA* (silver inner-membrane proton/cation antiporter) (Mourao et al. 2015), *arsB* (arsenite transmembrane pump) (Garcia Fernandez et al. 2007), *merA* (mercuric reductase) (Liebert et al. 1997), and *terF* (tellurite resistance protein) (Garcia Fernandez et al. 2007). The resistance genes screened were *bla*<sub>CTX-M</sub> (groups 1, 2, 9, 8, and 25), *bla*<sub>CMY</sub>, *bla*<sub>MOX</sub>, *bla*<sub>FOX</sub>, *bla*<sub>IAT</sub>, *bla*<sub>ACT</sub>, *bla*<sub>MIR</sub>, *bla*<sub>DHA</sub>, *bla*<sub>MOR</sub> and *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *acc(6')*-Ib-cr, *qepA*, and *oqxAB* by PCR as previously described (Cattoir et al. 2007; D'Andrea et al. 2006; Minarini et al. 2008; Saladin et al. 2002; Wang et al. 2009). The location of *silA* gene in *Salmonella* isolates was performed by PFGE after *S1*-nuclease digestion, Southern blot, and hybridization with specific probe.

Among all 200 *E. coli* isolates, only *terF* gene was found in 33 (16.5%), only *pcoA* gene in 20 (10%) isolates, only *silA* gene in 8 (4%) isolates, only *merA* gene in 4 (2%) isolates, and only *arsB* gene in 3 (1.5%) isolates. Among all *E. coli* isolates, 126 had 1 or more of the heavy metal tolerance genes, and 74 (34%) isolates had no tolerance gene. The MDR *E. coli* isolates were CMY-2, CTX-M2, CTX-M8, or CTX-M15 producers, and 54 MDR *S. enterica* isolates were CTX-M-2 producers (Table 1). Among

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**Table 1**

Heavy metal tolerance genes, *bla* genes and PMQR in *E. coli* and *Salmonella enterica* isolated from poultry from Brazil.

Species	Isolates (n)	Heavy metal tolerance genes	<i>bla</i> genes/PMQR <sup>a</sup>	
<i>E. coli</i>	8	<i>silA</i>	<i>cmy-2</i>	
	21	<i>pcoA</i>	<i>cmy-2</i> or <i>ctx-m-2</i> or <i>ctx-m-8/qnrB</i>	
	33	<i>terF</i>	<i>cmy-2</i> or <i>ctx-m-2/qnrB</i>	
	4	<i>merA</i>	<i>cmy-2</i> or <i>ctx-m-2/qnrB</i>	
	3	<i>asrB</i>	<i>cmy-2</i>	
	5	<i>silA</i> + <i>pcoA</i> + <i>terF</i>	<i>cmy-2</i> or <i>ctx-m-2/qnrS</i>	
	1	<i>silA</i> + <i>terF</i> + <i>asrB</i>	<i>ctx-m-15/qnrS</i>	
	19	<i>silA</i> + <i>pcoA</i>	<i>cmy-2</i> or <i>ctx-m-8</i>	
	1	<i>silA</i> + <i>terF</i>	<i>cmy-2</i>	
	1	<i>pcoA</i> + <i>merA</i>	<i>cmy-2</i>	
	7	<i>pcoA</i> + <i>terF</i>	<i>cmy-2/qnrB</i>	
	16	<i>terF</i> + <i>merA</i>	<i>cmy-2</i>	
	7	<i>terF</i> + <i>asrB</i>	<i>cmy-2</i>	
	<i>S. enterica</i>	4	<i>silA</i> + <i>pcoA</i> + <i>terF</i> + <i>merA</i> + <i>asrB</i>	<i>ctx-m-2/qnrB</i>
		34	<i>silA</i> + <i>pcoA</i> + <i>terF</i> + <i>merA</i>	<i>ctx-m-2/qnrB</i>
		9	<i>silA</i> + <i>pcoA</i> + <i>terF</i>	<i>ctx-m-2/qnrB</i>
3		<i>silA</i> + <i>pcoA</i> + <i>merA</i>	<i>ctx-m-2/qnrB</i>	
1		<i>silA</i> + <i>pcoA</i>	<i>ctx-m-2/qnrB</i>	
1		<i>silA</i> + <i>merA</i>	<i>ctx-m-2/qnrB</i>	
1		<i>terF</i> + <i>merA</i>	<i>ctx-m-2/qnrB</i>	
4	<i>merA</i>	<i>qnrB</i>		

<sup>a</sup> The resistance genes screened were *bla*<sub>CTX-M</sub> (groups 1, 2, 9, 8 and 25), *bla*<sub>CMY</sub>, *bla*<sub>MOX</sub>, *bla*<sub>FOX</sub>, *bla*<sub>ACT</sub>, *bla*<sub>ACT</sub>, *bla*<sub>MIR</sub>, *bla*<sub>DHA</sub>, *bla*<sub>MOR</sub> and *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *acc*(6')-Ib-cr, *qepA*, and *oqxAB*.

MDR *S. enterica*, 54 (93%) isolates showed more the 1 tolerance gene. Furthermore, 65% (38/58) co-harbored at least 4 heavy metal tolerance genes and 90% (52/58) showed *silA* gene. In *Salmonella enterica* the hybridization of the *silA* probe confirmed the presence of the gene in a ~280-kb plasmid, and it was characterized as IncHI2A by PCR-based Replicon Typing (PBRT). This plasmid also carried important antibiotic resistance genes, including *bla*<sub>CTX-M-2</sub> and *qnrB* (Table 1), a dangerous combination that may increase the co-selection rates of these pathogenic isolates. IncHI2A plasmids are frequently found in clinical enterobacterial strains associated with the dissemination of relevant antimicrobial resistance genes; these plasmids commonly show a very high molecular weight (>250 kb). In Portugal, plasmid IncHI2 carrying metal tolerance genes was already found in MDR *Salmonella* Typhimurium isolate. Furthermore, the IncHI2 has been described worldwide in *Salmonella* sp., *Klebsiella* sp., *Enterobacter* sp., and *E. coli* isolated from humans and food-producing animals (Campos et al. 2016; Mourao et al. 2015). Silver is the main nonantibiotic substance that has long been used as an antimicrobial agent, and it seems to be an important indicator of heavy metal tolerance. Heavy metals, such as silver, are currently used in medicine for the treatment of burned skin surfaces, open wounds, and specific eye infections and have also been incorporated in medical devices (McDonnell and Russell 1999; Silver 2003). The increased number of enterobacteria carrying heavy metals tolerance genes brings novel public health concerns. The intrinsic increased in the risk of co-selection of antimicrobial resistant bacteria could impact in the management of food safety as well as in the therapeutic options in food production animal and human infections. The findings of the present study bring new insights and data for discussion about the use of copper and zinc salts used as feed additives to improve animal health (Aarestrup and Hasman 2004), which may cause the maintenance and dissemination of these genes and other co-harbored antimicrobial resistant genes in the animal environment, although little is known currently. Moreover, this is the first

study showing the occurrence of genes encoding tolerance to metals, disseminated among commensal and pathogenic MDR *Enterobacteriaceae* isolates from poultry in Brazil.

### Conflict of interest statement

None to declare.

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