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Successful multidrug-resistant and copper-tolerant *Salmonella* clones are enriched in arsenic tolerance genes

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Recently our team highlighted tolerance to copper and silver in emergent and multidrug-resistant (MDR) *Salmonella* serotypes/clones, which constitute an advantage for survival and persistence in metal-contaminated environments of animal-production (PMID:25816978, 27118781). Arsenic contamination by anthropogenic activities (e.g. coccidiostatics/pesticides) is also frequent in these environments and may represent a long-term selective pressure driving the selection of MDR serotypes/clones. In Gram-negative bacteria, diverse arsenic tolerance (AsT) mechanisms have already been described. Nevertheless, dispersion and genetic location of AsT genes and their corresponding phenotypic behaviour are lacking in *Salmonella*. From collections of Portugal and Austria, 410 *Salmonella* isolates (2000-2016; humans/foods/animal/environment) from 59 serotypes (including the most frequent: *S*. Enteritidis/n=31; *S*. Typhimurium/n=63; *S*. 4,[5],12:i:-/n=82; *S*. Rissen/n=15) were selected based in PFGE-type and different profiles of antibiotic-resistance or metal tolerance (copper/silver/mercury/tellurite). Screening of AsT genes, *arsB* and *acr3* (both coding for arsenical pump membrane proteins) was performed by PCR/sequencing. Minimum inhibitory concentrations (MICs) to Na_2HAsO_4 (0.25-128 mM; pH=7.0) were determined in aerobic and anaerobic atmospheres by the agar dilution method. Genomic location (I-*Ceu*I/S1/*Xba*I-PFGE/hybridization) and plasmid analysis (PBRT/sequencing) were done in selected isolates. A high frequency of AsT genes was found (55%-n=226/410) involving diverse serotypes (44%-n=26/59): i) *arsB* (21%-n=88/410; 6 serotypes); ii) *acr3* (33%-n=135/410; 18 serotypes) or iii) *arsB*+*acr3* (1%-n=3/410; 3 serotypes). AsT isolates carried frequently copper+silver±mercury+tellurite tolerance genes (n=148/226-65%; "European clone" of *S*. 4,[5],12:i:- and *S*. Typhimurium, *S*. Rissen plus 17 serotypes). Different genetic contexts for AsT genes were detected. The emergent pig-associated MDR "European clone" (n=83/83-100%) carried only *arsB* gene in the chromosome, co-located with other metal tolerance (copper+silver±mercury) and antibiotic resistance genes. In other 3 less frequent serotypes/clones (n=3), the *arsB* gene was found associated with MDR IncHI2 (240-330 Kb) plasmids. The *acr3*, also chromosomal located, was highly dispersed including in two emerging pig-associated serotypes *S*. Rissen (n=15/15-100%) and *S*. Derby (n=19/19-100%). An absence of AsT genes (45%-n=184/410) was observed in *Salmonella* serotypes/clones commonly associated with poultry/eggs production such as *S*. Enteritidis (n=16) or *S*. Infantis (n=20). Phenotypic assays of all isolates showed higher $MIC_{Na2HAsO4}$ in isolates carrying *arsB* ($MIC_{50/90}$=>128/>128mM) and *acr3* ($MIC_{50/90}$=8/16mM) than without these genes ($MIC_{50/90}$=2/4mM) in aerobic/anaerobic conditions. These data suggest that tolerance to As along with to other metals, might have contributed to the adaptation and success of the emergent MDR pig-associated *Salmonella* clones to the selective food-animal farm environments.

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