Resistance to biocides in *Salmonella* from Portugal: a multilayered approach

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Background. Antibiotic resistant (ABR) *Salmonella* clones and the contributions of diverse environmental stressors (e.g. metals) widely used in food animal production (feed/disinfectants/antiseptics) for their selection/persistence are a current public health concern. We aimed to characterize metal tolerance and its associated genetic elements in clinical relevant ABR *Salmonella* clonal lineages. **Methods.** Portuguese *Salmonella* non-typhoid isolates (n=121/2000-2011) from human/non-human sources belonging to 16 serotypes were selected. The isolates included the 2 most frequent serotypes, *S*.Enteritidis (n=4) and *S*.Typhimurium (n=12), and the emergent *S*.4,[5],12:i:- (n=64) and *S*.Rissen (n=26). They are representative of different PFGE and Sequence Types (ST) and associated with particular ABR phenotypes/genotypes. Genes associated with ABR/integrons (Int), Cu^R (*pcoD*), Ag/Cu^R (*silA-silE*), Hg^R (*merA*), As^R (*arsB*) or Te^R (*terF*) were searched by PCR/sequencing. MICs to $CuSO_4$ and $AgNO_3$ were determined in aerobic/anaerobic atmospheres by agar dilution method and susceptibility to 10 antibiotics by disk diffusion methods. Conjugation assays, genomic location (I-*Ceu*I/S1-PFGE/hybridization) and plasmid (PL) analysis (PBRT/sequencing) were done. **Results.** Metal tolerance genes *silA-silE* (69%), *pcoD* (51%), *merA* (50%), *terF* (2%) or *arsB* (1%) were found in different serotypes/clones. *S*.Rissen (ST469; 62%-*bla*TEM-*aadA2-sul1*/*sul3-tetA-dfrA12)* carried *pcoD*+*silA-silE* in chromosome (Ch) as *S*.4,[5],12:i:- from European clone (n=22/ST34), which also have co-located ABR genes (*blaTEM-strA-strB-sul2-tetB*) and the majority *merA*. In contrast, *S*.Typhimurium monophasic variant of the Spanish (n=5/ST19) and Portuguese (n=5/ST19) MDR clone carried *merA* and/or *silA-silE* on large non-transferable IncA/C (130-170Kb) or IncR PL (110-140Kb) respectively. MDR *S*. Typhimurium (n=12; 5 clones; ST19/ST313) carried *silA-silE* (n=3/DT104 clone) with atypical type I-*sul3* integron on IncN PL (135Kb) or *merA* (n=4) with *intI1-oxa30-aadA1*/*intI1-aadA1* on transferable IncFII PL (120-140Kb). In *S*. Enteritidis (ST11) only *merA* (n=2/4) was detected on transferable IncP PL (80Kb) along with *intI1-dfrA1-aadA1*. In isolates of other MDR clones (n=15/12 serotypes), *merA* (n=13) and/or *silA-silE*+*pcoD* (n=6) were co-located with different Int on large plasmids (>120Kb; IncHI1/IncP/IncI1). The *arsB* (n=1) and/or *terF* (n=3) were located on transferable IncHI2 (220Kb) with $bla_{CTX\text{-}M\text{-}9}$ or IncP (265kb) (250kb) PL with *int1-aadA1* or *dfrA1-aadA1*/*pcoD*/*silA*/*merA*. Higher $CuSO_4$ MIC values were obtained in aerobiosis in contrast with anaerobiosis, where a correlation between MICs and Cu^R genes was found (MIC_{50}=28mM *pco*D/*sil*A-*sil*E^+ vs 1mM *pco*D/*sil*A-*silE*$^-$). MICs values for $AgNO_3$ were higher in anaerobic than aerobic conditions and a slightly difference between *sil*A-*sil*E^+ (MIC_{50}=0,32/0,25mM) and *sil*A-*sil*E^- (MIC_{50}=0,25/0,16mM) was observed in both atmospheres. **Conclusions.** High prevalence of metal tolerance genes associated with increased copper/silver tolerance, and the co-location with ABR genes in different plasmids, suggests that besides antibiotics, metals used in the animal production setting may have contributed for the selection/maintenance of *Salmonella* clinical relevant clonal lineages. **Acknowledgments:** This research was supported by UP/Santander Totta "Projectos Pluridisciplinares 2011" and FCT-PEst-C/EQB/LA0006/2011.