

DETECTION OF *SALMONELLA* HEIDELBERG RESISTANT TO COLISTIN IN THE INTESTINAL CONTENT OF PIGS AT SLAUGHTER

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Abstract

Salmonella Heidelberg has increasingly been reported as cause of human salmonellosis worldwide. In Brazil, *S. Heidelberg* has been reported in poultry but it is infrequently isolated from pigs. Here, we describe the isolation of *S. Heidelberg* resistant to colistin from slaughter pigs. Five pigs and their carcasses belonging to a same slaughter batch in ten consecutive days were sampled for fragment of intestine in the ileocecal region and sponges rubbed on the carcass surface (400 cm²) before chilling. *Salmonella* detection was performed according to the ISO 6579:2002. Intestinal content was also subjected to *Salmonella* enumeration by a miniaturized Most Probable Number (MPN) protocol. *Salmonella* isolates were characterized by antimicrobial resistance by the disk diffusion test, the minimum inhibitory concentration to colistin determination and to gene *mcr-1* investigation by PCR. *Salmonella* was isolated from the intestinal content of 64% (32/50) of the pigs, in amounts that varied from 2.7 to >1,400 MPN/g. *Salmonella* Heidelberg was the most frequent serovar identified in the intestinal content samples (20/50; 40%), and this serovar was present in eight of the ten pig batches sampled. At the prechill, *Salmonella* was isolated from 8% of carcasses, and *S. Heidelberg* was not detected. *Salmonella* Heidelberg strains were resistant against ampicillin (n=9), tetracycline (n=8), sulfonamide (n=8) and gentamicin (n=5). Nine multi-drug resistant strains were detected; among them four strains were positive for the gene *mcr-1*. In these strains the MIC value was 8 µg.mL⁻¹, while in the strains without the *mcr-1* gene it ranged from 2 µg.mL⁻¹ to 4 µg.mL⁻¹. Therefore, humans in contact with carrier pigs or their environment may be exposed to *S. Heidelberg*, including strains harboring the gene *mcr-1*.

Introduction

Salmonella enterica figures amongst the most important foodborne transmitted bacteria, and pork has been reported as a vehicle in outbreaks worldwide (EFSA, 2015). *Salmonella* strains isolated from pig carcasses are originated from the intestinal content of *Salmonella*-carrier pigs or are acquired by the contact with residual contamination of the slaughterhouse environment (BUNCIC et al., 2011). Therefore, the delivery of pig batches with a large number of animals excreting *Salmonella* represents a burden to the slaughtering process, in terms of bacterial load and serovar diversity. In Brazil, the high prevalence of *Salmonella* carrier pigs at slaughter has been reported, demonstrating that this may be a relevant factor in the *Salmonella* control in pork (KICH et al., 2011; SILVA et al., 2012).

The *Salmonella* serovars vary in prevalence overtime and according to the region. Recently, *Salmonella* Heidelberg has increasingly been reported in poultry and as cause of human salmonellosis (COLLA et al., 2012; CDC, 2016; GIERALTOWSLI et al., 2016). This serovar has also been pointed out as more invasive and carrying multi-drug resistance (MDR), which represents a further concern for human and animal health (CDC, 2016; GIERALTOWSLI et al., 2016). In swine, reports of *S. Heidelberg* are still rare, being *S. Typhimurium* and *S. Derby* among the most isolated serovars worldwide (KICH et al., 2011; DENIS et al., 2013). In this study, we describe the isolation of *Salmonella* Heidelberg in pig batches processed in a slaughter plant from Southern Brazil and their antimicrobial resistance profile.

Material and methods

Five slaughtered pigs and their carcasses belonging to a same slaughter batch in ten consecutive days were sampled for: *i.* blood collected at bleeding; *ii.* sponges (Nasco[®]) rubbed on the carcass surface (400 cm²) after bleeding and before chilling; *iii.* fragment from the ileocecal region of intestine.

Serum samples were tested by the ELISA-Typhimurium test as previously described (KICH et al., 2007). The cut-off point of the test was the OD 0.169 (KICH et al., 2016). Sponges were individually suspended in 30 mL of buffered peptone water 1%, and 5 mL of suspension was used for Total Aerobic Mesophilic (TAM) enumeration in Plate Count Agar (PCA) (DOWNES et al., 2011). The remaining suspension volume was used for *Salmonella* detection according to the ISO 6579:2002. Intestinal content (25 g) was suspended in 225 mL buffered peptone water 1% and subjected to *Salmonella* detection (ISO 6579:2002). It was also subjected to *Salmonella* enumeration by a miniaturized Most Probable Number (MPN) protocol (PAVIC et al., 2010). *Salmonella* isolates were serotyped at the Fundação Instituto Oswaldo Cruz (FIOCRUZ).

Antimicrobial resistance profiling was determined against twelve antimicrobials by disk-diffusion test method performed and interpreted by Clinical and Laboratory Standards Institute, document M100 (CLSI, 2016). The following antimicrobials disks were tested: ampicillin (10µg), azithromycin (15µg), ceftiofur (30µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), ceftiofur (30µg), ciprofloxacin (5µg), gentamicin (10µg), meropenem (10µg), sulfonamide (300µg) and tetracycline (30µg). Furthermore, the Minimum Inhibitory Concentration (MIC) to colistin was also determined (EUCAST, 2016). *Escherichia coli* ATCC[®] 25922 was used for quality control purpose. These isolates were screened for the presence of *mcr-1* gene by PCR with primers CLR5-F (5'-CGGTCAGTCCGTTTGTTC-3') and CLR5-R (5'-CTTGGTCGGTCTGTAGGG-3') (LIU et al., 2016).

The TAM results were calculated as CFU.cm⁻² and transformed into log₁₀ for the analysis. Means of log₁₀ CFU.cm⁻² TAM were compared by the Tukey test (p=0.05) of the SPSS software.

Results and discussion

All 50 sampled pigs were positive in the ELISA-Typhimurium test demonstrating that they had been exposed to *Salmonella* on farm. A positive result in the ELISA-test cannot be interpreted as an active infection at slaughter; however, seropositive slaughter batches have higher chances of including pigs excreting *Salmonella* at slaughter (KICH et al., 2007). In this study, *Salmonella* was isolated from the intestinal content of 64% (32/50) of the seropositive pigs, in amounts that varied from 2.7 to >1,400 MPN/g, indicating that the burden of *Salmonella*-carrier pigs to the slaughter processing may be very high. Thus, hygienic measures are of utmost importance to avoid the contact of the carcass with the intestinal content and the environment contaminated with feces.

The carcasses sampled after bleeding presented TAM average of $3.28 \pm 0.53 \log \text{cfu.cm}^{-2}$ and *Salmonella* was isolated from 16% (8/50) of them. At the pre-chill step, the same carcasses presented a TAM average of $1.84 \pm 0.49 \log \text{cfu.cm}^{-2}$, representing a logarithmic reduction ranging from 0.64 to 2.35 $\log \text{cfu.cm}^{-2}$. Moreover, the frequency of *Salmonella*-positive carcasses decreases to 8% (4/50). Slaughter steps, such as singeing, can decrease the superficial microbial contamination, while others represent a hazard of adding bacteria to the surface of the carcass (BUNCIC et al., 2011). Since the monitoring of TAM reflects the change on contamination throughout the processing, the logarithmic reduction achieved demonstrated that all batches were processed in accordance with hygiene standards. As a result, the number of *Salmonella*-positive carcasses detected after bleeding was decrease by the half, despite the high load of *Salmonella* present in the intestinal content.

Salmonella Heidelberg was the most frequent serovar identified in the intestinal content samples (20/50; 40%), and this serovar was present in eight of the ten batches. In spite of that, *S. Heidelberg* was detected neither in carcasses after bleeding nor at the prechilling, which indicate that the slaughter process might have been able to control the contamination by this serovar. *Salmonella* Heidelberg has infrequently been reported in pigs and pork (KICH et al., 2011; EFSA, 2015). On the other hand, this serovar has been increasingly prevalent in poultry and humans (COLLA et al., 2012; FOLSTER et al., 2012; GIERALTOWSKI et al., 2016; PALMEIRA et al., 2016). Noteworthy is that *S. Heidelberg* has been associated with invasive human infections and high mortality rates (CRUMP et al., 2011). Moreover, a *S. Heidelberg* outbreak in humans in close contact with cattle was reported (CDC, 2016), demonstrating that this is another possible transmission route to humans. Antimicrobial resistance in *S. Heidelberg* is of particular concern, since MDR strains have been implicated in human outbreaks (GIERALTOWSKI et al., 2016). In this sense, the 20 *Salmonella* strains tested were fully susceptible to azithromycin, cephalosporin (ceftiofur, cefotaxime, ceftazidime, ceftriaxone, and cefoxitin), ciprofloxacin and meropenem, antimicrobials considered as highly critical to human treatment (WHO, 2012). Resistance was detected against ampicillin (n=9), tetracycline (n=8), sulphonamide (n=8), gentamicin (n=5). A total of nine MDR strains (resistant to ≥ 3 antimicrobial classes) were identified. Four MDR strains presenting a profile including all the four aforementioned antimicrobials were also positive for the gene *mcr-1*, demonstrating that they were resistant to colistin. In all resistant strains the MIC value was $8 \mu\text{g.mL}^{-1}$, while in the susceptible strains it ranged from $2 \mu\text{g.mL}^{-1}$ to $4 \mu\text{g.mL}^{-1}$. The presence of the gene *mcr-1* in *Salmonella* is a concern, since colistin is considered the last resource for treatment of MDR bacteria in humans and the gene is usually located in transferable genetic elements (LIU et al., 2016).

Conclusion

Although not detected in carcasses in this study, the identification of *S. Heidelberg* in the intestinal content demonstrated that this serovar is circulating in pigs and might eventually also be found in pork. Therefore, humans in contact with carrier pigs or their environment may be exposed to *S. Heidelberg*, including to strains harboring the gene *mcr-1*.

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