Use of heavy metals in swine feed and its association with the co-selection of metal tolerant and multi-drug resistant Salmonella

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Abstract

This study was conducted to characterize the role of chemical interventions, biocides and heavy metal micronutrients, in particular, in swine production systems on the emergence of heavy metal and biocide tolerant Salmonella and its association with antimicrobial resistance. A total of 353 Salmonella isolates with different antimicrobial resistance profiles identified from 36 barns exposed to three different classes of biocides were analyzed. The sources of isolates included feed (n=30), fecal (n=226), and environment (n=97) samples that were systematically selected. The minimum inhibitory concentrations (MIC) of each isolate against heavy metals copper (Cu) and zinc (Zn) was determined on Mueller-Hinton-II (MH) agar plates containing serial dilutions of copper sulfate (1-32mM) and zinc chloride (0.25-16mM). A non-parametric Wilcoxon Rank Sum test for trends across ordered groups (Stata 10, College Station, TX) was used to determine association between concentration of metal in feed and MIC. The most common MDR patterns among the more heavy metal tolerant isolates were AmClStSuTe (n=81) and AmStTeKm (n=58), which are common multi-drug resistance patterns found in swine production systems. There was a significant association between the concentration of copper in feed and the MIC of isolates recovered from fecal samples for copper ($p<0.001$). Heavy metal tolerance was also significantly associated with distinct multi-drug resistance types. The odds of finding high Zinc MIC were 15 times higher for the AmClStSuTe R-type than AmStTeKm (Chi-square= 47.2; $p<0.05$). On the other hand, the odds ratio for association between copper tolerance and R-type AmStTeKm was 4.6 (Chi-square=17.9; $P<0.05$). No association between biocide use and heavy metal tolerance was detected in this study. Unique genes that encode for tolerance to copper and zinc and physical linkage to antibiotic resistance determinants are being investigated. The findings in this study suggest that the use of copper in swine feed results in higher tolerance of Salmonella strains to copper which in turn co-selects for antimicrobial resistance.

Introduction

Non-typhoidal Salmonella serovars are among the most important foodborne bacterial pathogens worldwide. Many strains of commonly occurring Salmonella serovars have been shown to exhibit multi-drug resistance (MDR), however, the role of non-antiotic chemical agents as a selective pressure for emergence and persistence of antimicrobial resistance is poorly understood (Aarestrup and Hasman, 2004). In commercial swine production systems, heavy metals such as copper , zinc, manganese, and others in feed are used to assist with normal growth of pigs and to provide cytotoxic effects on bacteria (NRC, 1998). The role of chemical interventions, biocides and heavy metal micronutrients, in particular, in swine production systems on the emergence of heavy metal and biocide tolerant Salmonella and its association with antimicrobial resistance is not well known. The present study investigates the occurrence and significance of tolerance to copper and zinc, its association with the concentration in swine feed and co-selection with antimicrobial resistance in Salmonella isolates recovered from swine production units.

Materials and Methods

Study design and sample collections: This was part of a study conducted to study the role of specific classes of biocides and heavy metals (copper and zinc) in the occurrence and persistence of multidrug resistant Salmonella in swine production units. Briefly, a longitudinal randomized control study was conducted on 36 swine barns that originated from three production systems and 12 farms of independent production pyramids. Feed (pooled one per barn) and fecal (48 per barn) samples collected from the 36 barns were tested for copper and zinc concentrations. Samples were collected in four replicates. The details of the study design and sample collections were previously described (Zewde et al., 2009).

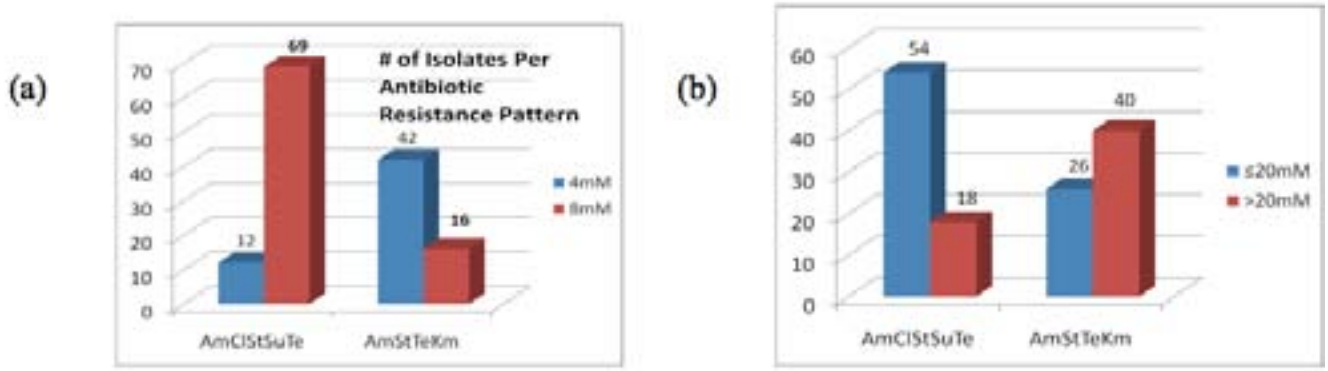
Salmonella isolation and antimicrobial susceptibility testing: Salmonella was isolated using conventional culture and identification systems (Gebreyes et al., 2004). Brief**f** y, buffered peptone water was added at a 1:9 W:V for each of the samples and incubated at 37C for 24h. A 100uL suspension was transferred to Rappaport Vassiliadis media and further incubated at 42C over night. A loopful of the suspension was streaked on to XLT-4 selective media and incubated at 37C for 24h. Presumptive isolated black colonies were tested for via triple sugar iron (TSI) and urease reactions. Further confirmation was done by serogrouping using uni- and polyvalent antisera. Antimicrobial susceptibility testing was done using kiby-Bauer disc diffusion testing. A total of 353 Salmonella isolates with different antimicrobial resistance profiles identified from 36 barns exposed to three different classes of biocides were analyzed. The sources of isolates included feed (n=30), fecal (n=226), and environment (n=97) samples that were systematically selected.

Minimum inhibitory concentrations (MIC): The minimum inhibitory concentrations (MIC) of each isolate against heavy metals copper (Cu) and zinc (Zn) was determined using a agar gel dilution system on Mueller-Hinton-II (MH) agar plates containing two fold serial dilutions of copper sulfate (1-32mM) and zinc chloride (0.25-16mM). The pH of the copper media was adjusted to 7.2 and the zinc to 5.5 to allow for solubility of the metal in the media. Zinc susceptibility was recorded at 4mM and 8mM and copper susceptibility at 2mM, 4mM, 16mM , 20mM, and 24mM. Two MH plates of each dilution were inoculated with the selected isolates at a uniform concentration using repeat inoculators and incubated overnight at 37°C. MIC was determined by the absence of growth at a given concentration. A non-parametric Wilcoxon Rank Sum test for trends across ordered groups (Stata 10, College Station, TX) was used to determine association between concentration of metal in feed and MIC.

Results and Discussion

The most common MDR patterns among the more heavy metal tolerant isolates were AmClStSuTe (n=81) and AmStTeKm (n=58), which are common multi-drug resistance patterns found in swine production systems. There was a significant association between the concentration of copper in feed and the MIC of isolates recovered from fecal samples for copper ($p<0.001$).

Heavy metal tolerance was also significantly associated with distinct multi-drug resistance types. The odds of finding high Zinc MIC were 15 times higher for the AmClStSuTe R-type than AmStTeKm (Chi-square= 47.2; $p<0.05$), Fig 1a. On the other hand, the odds ratio value for association between copper tolerance) and MDR AmStTeKm was 4.6 (Chi-square=17.9; $P<0.05$), Fig 1b.



No association between biocide use and heavy metal tolerance was detected in this study. Unique genes that encode for tolerance to copper and zinc and physical linkage to antibiotic resistance determinants are being investigated. The findings in this study suggest that the use of copper in swine feed results in higher tolerance of Salmonella strains to copper which in turn co-selects antimicrobial resistance. The findings imply that the use of heavy metals in swine feed may contribute to the persistence of multi-drug resistant Salmonella of pork safety significance.

References

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