Association between biocide use and antimicrobial resistance of *Salmonella* in swine production environment

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Abstract

Swine are important reservoirs of antimicrobial resistant *Salmonella*. The emergence and dissemination of multidrug resistant (MDR) *Salmonella enterica* has also become a major concern globally. Studies on the association between biocide use and emergence and persistence of MDR *Salmonella* in swine production environment are very limited. The aim of this study was to determine the role of specific classes of biocides (disinfectants) in the emergence and persistence of multidrug resistant *Salmonella* in swine production environments. Barn floor swab samples were aseptically collected pre-disinfection (n=1,360) and post-disinfection (n=1,360) from randomly selected pens (n=10 per barn) in 36 barns in four replicates. Samples were cultured for *Salmonella* isolation. Isolates recovered pre- and post-disinfection were tested for antimicrobial resistance to a panel of 12 antimicrobials. A significant reduction of *Salmonella* was detected in post-disinfected barn floors (6.2%) compared to pre-disinfection (14.4%). Of the total *Salmonella* isolates recovered from barn floors pre-disinfection (n=460) and post-disinfection (n=341), 87.4% and 96.5% of the isolates respectively were resistant to one or more of the antimicrobials tested. A significant reduction in pansusceptible isolates was detected in isolates recovered from barn floors post-disinfection (3.5%; 12/341) versus pre-disinfection (12.6%; 58/460) indicating the increase in resistant strains. *Salmonella* isolates recovered pre- and post-disinfection from control barns did not show a change in antimicrobial resistance profiles. A high proportion of multidrug resistance (≥3 antimicrobials) was detected in *Salmonella* isolates post-disinfection (60.1%) compared to those recovered pre-disinfection (52.6%). The increase in antimicrobial resistance in post-disinfection barns tends to be associated with specific classes of biocides, particularly the quaternary-ammonium glutaraldehyde combination (Synergize®). The highest increase in proportion of resistant isolates was to tetracycline, streptomycin, and sulfisoxazole, which might be associated with efflux systems induced by the biocides or persistence of class 1 integrons.

Introduction

Non-typhoidal *Salmonella* is one of the important foodborne pathogens causing gastroenteritis worldwide. It is one of the most common causes of foodborne infections in the United States. Swine are important reservoirs of antimicrobial resistant *Salmonella* and it is well known that *Salmonella* can persist in the swine production environment for long periods (1). The emergence and dissemination of multidrug resistant (MDR) *Salmonella enterica* has also become a major concern globally. Previous studies conducted on *Salmonella* in swine and swine production environments in the study areas indicated the epidemiology and antimicrobial resistance of *Salmonella* (2-4). Studies on the association between biocide use and emergence and persistence of MDR *Salmonella* in swine production environment are very limited. The aim of this study was to determine the role of specific classes of biocides in the emergence and persistence of multidrug resistant *Salmonella* in swine production environments.
Materials and Methods

Study design and sample collections: This study was part of a project to study the role of specific classes of biocides and heavy metal micronutrients in the occurrence and persistence of multidrug resistant Salmonella in swine production environments. In this longitudinal group randomized controlled study, in three vertically-integrated commercial swine production systems, three farms per system were selected conveniently. In each farm four barns were selected randomly and assigned to four different treatment groups: Biosentry® (0.4%), Virkon-S® (1%), Synergize® (33%) and control (water). Each type of the disinfectant was used to disinfect designated swine barns before swine were placed in each barn. Barn floor swabs were aseptically collected using conventional drag swabs pre-disinfection (n=1,360) and post-disinfection (n=1,360) in four replicates. Post-disinfection swab samples were collected within an hour after completion of disinfection of barns and before swine are placed in each of the disinfected barns. From each barn a total of 10 drag swab samples (Tyco Healthcare/Kendall, Mansfield, MA) and negative controls (pre- and post-disinfection) per barn were collected from randomly selected pens following previously described methods (5). Repeated measures after biocide intervention on finishing barns is being undertaken by prospective follow up of the selected barns under each farm.

Salmonella isolation and identification: Samples were cultured for Salmonella isolation using conventional methods as described previously (3-5).

Antimicrobial susceptibility testing: Salmonella isolates recovered from barn floor swabs pre- and post-disinfection were tested for antimicrobial susceptibility to a panel of 12 antimicrobials using the Kirby-Bauer disc diffusion method following the guidelines established by the Clinical Laboratory Standards Institute, CLSI, formerly National Clinical Laboratory Standards (6). The following antimicrobials with the respective disc concentrations used: ampicillin (Am; 10 µg), amoxicillin-clavulanic acid (Ax; 30 µg), amikacin (An; 30 µg), ceftriaxone (Ce; 30 µg), cephalothin (Ch; 30 µg), chloramphenicol (Cl; 30 µg), ciprofloxacin (CIP; 5 µg), gentamicin (Gm; 10 µg), kanamycin (Km; 30 µg), streptomycin (St; 10 µg), sulfisoxazole (Su; 250 µg) and tetracycline (Te; 30 µg). We used Escherichia coli ATCC 25922, E. faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, and Pseudomonas aeruginosa ATCC 27853 as reference strains.

Serogrouping: The Salmonella isolates were serogrouped using commercially available polyvalent O and group specific (B, C, D, E and G) antisera (Mira Vista, Copenhagen, Denmark).

Results and Discussion

A significant reduction of Salmonella was detected in post-disinfected barn floors (6.2%) compared to pre-disinfection (14.4%), however, many of the barns remained Salmonella positive after disinfection irrespective of the type of biocides used to disinfect the barns (Table 1). We noted that none of the disinfection procedures we applied to disinfect swine barn floors was able to completely eliminate Salmonella from the barns. The majority of the isolates recovered pre-disinfection and post-disinfection belonged to serogroup B and C.

Table 1: Proportion of Salmonella isolates from swine barn floors before and after disinfection by treatment group

<table>
<thead>
<tr>
<th>Treatment group (number of barns/replicate)</th>
<th>Pre-disinfection</th>
<th>Post-disinfection</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Biosentry (9)</td>
<td>20.3% (71/350)</td>
<td>8.6% (30/350)</td>
</tr>
<tr>
<td>Synergize (9)</td>
<td>13.1% (46/350)</td>
<td>6.3% (22/350)</td>
</tr>
<tr>
<td>Virkon-S (9)</td>
<td>10% (35/350)</td>
<td>5.1% (18/350)</td>
</tr>
<tr>
<td>Control (9)</td>
<td>13.7% (48/350)</td>
<td>22.6% (79/350)</td>
</tr>
</tbody>
</table>

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Of the total *Salmonella* isolates recovered from barn floors pre-disinfection (n=460) and post-disinfection (n=341), 87.4% and 96.5% of the isolates respectively were resistant to one or more of the antimicrobials tested. A significant reduction in pansusceptible isolates was detected in isolates recovered from barn floors post-disinfection (3.5%; 12/341) versus pre-disinfection (12.6%; 58/460) indicating the increase in resistant strains. *Salmonella* isolates recovered pre- and post-disinfection from control barns did not show a major change in antimicrobial resistance profiles (Figure 1D). None of the isolates from barn floor swabs collected before and after disinfection was resistant to ciprofloxacin, ceftriaxone and amikacin. A high proportion of multidrug resistance (≥3 antimicrobials) was detected in *Salmonella* isolates post-disinfection (60.1%) compared to those recovered pre-disinfection (52.6%). The increase in antimicrobial resistance post-disinfection was mainly to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline and cephalothin (Figure 1).

Figure 1: Antimicrobial resistance profiles of *Salmonella* isolates recovered from swine barn floors before and after disinfection

In summary, the increase in antimicrobial resistance in post-disinfection barns tends to be associated with specific classes of biocides, particularly the quaternary-ammonium glutaraldehyde

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combination (Synergize®), Figure 1 (B). The highest increase in proportion of resistant isolates was to tetracycline, streptomycin, and sulfoxizoxazole, which might be associated with efflux systems induced by the biocides or persistence of class 1 integrons.

References

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