Role of Biocides in Occurrence and Persistence of Biocide-tolerant and Multi-drug Resistant Salmonellae

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SUMMARY

The aim of this study was to characterize the role of biocide interventions in the emergence of biocide-tolerant *Salmonella*, its co-selective association with multidrug-resistant *Salmonella* and the association with carriage of specific efflux gene markers. *Salmonella* isolates were detected in 13.9% (208/1,497) and 6.7% (98/1,468) of swine barn drag swab samples at pre- and post-disinfection with biocide, whereas, *Salmonella* isolates were detected in 17.2% (1,180/6,842) and 7.1% (431/6,093) of the early and late finishing pigs, respectively. Barn-level prevalence of antimicrobial resistance among *Salmonella* isolates recovered from the floor swab samples was 92.3% (1,503 of 1,628), whereas, the barn-level prevalence of antimicrobial resistance among *Salmonella* isolates recovered from the fecal samples was 98.02% (4,415 of 4,504). A total of 348 *Salmonella* isolates from swine barns formerly disinfected with Biosentry and 428 from swine barns disinfected with Synergize were subjected to minimum inhibitory concentration (MIC) testing for Biosentry and Synergize, respectively. In addition, selected *Salmonella* isolates were also genotyped to identify the carriage of quaternary ammonium compound (*qac*) tolerance genes. Interestingly, 22.9% (98 of 428) of *Salmonella* isolates from swine barns disinfected with Synergize carried the *qac* gene and the 86% of isolates that carried the *qac* gene also carried attenuated *qacEΔ1*. Nearly 31% (109 of 348) of *Salmonella* isolates from swine barns disinfected with Biosentry carried the *qac* gene. Furthermore, out of 109 *Salmonella* isolates that carried *qac* genes, 94.5% of the isolates contained attenuated *qacEΔ1* gene, whereas 72.5% of the isolates carrying *qacEΔ1* gene also carried class 1 integrons which is associated with both antibiotic and quaternary ammonium compound resistance. Although biocides are effective in limiting the growth, load and the colonization of bacterial pathogens, this study underscores the contribution of biocides in selective pressure towards antibiotic resistant in *Salmonella*.

Keywords: *Antimicrobial resistance, Biocide, Biocide tolerance, efflux genes, Salmonella.*

INTRODUCTION

Salmonella are bacteria capable of infecting both human and animals to cause gastrointestinal disease commonly known as salmonellosis. The disease occurs globally and is more wide spread in countries where the use of biocides and antimicrobials in human and veterinary practices is not well regulated (Threlfall *et al*., 2000; Fraise *et al*., 2002). The main reservoir of *Salmonella* spp. is the gastro-intestinal tract of the food animals. The Salmonellae are frequently recovered from food and animal products such as meat, milk and eggs (Zhao *et al*., 2008; Hue *et. al*., 2010). Control measures in terms of disinfection of food animal contact surfaces such as barn floors, are very important for limiting the risk of contaminated animal products from reaching the consumers and public as a whole (Fraise, 2002; Lo Fo Wong *et al*., 2002; Gantzhorn *et al*., 2014). Reduction of *Salmonella* load and colonization in food animals is achieved

if proper and effective detection and control measures are put in place at all levels of food production and in particular the animal production farms as the primary production level. As such, hygienic practices have been a basis of efforts to control foodborne pathogens in farm environments.

At the farm level, the control programmes include proper cleaning and disinfection (Lo Fo Wong *et al*., 2002; Zewde *et al*., 2009). The participation of all sectors involved throughout the food production levels is critical in ensuring a safe food from farm to table. Various studies have clearly demonstrated that colonized food-producing animals are the main source of *Salmonella* at the slaughterhouse (Botteldoorn *et al*., 2003; Arguello *et al*., 2013).

Biocidal substances (disinfectants) have been used to assist improved hygiene (Morente *et al*., 2013; Barillo and Marx, 2014; Wales and Davies, 2015). Several biocidal substances such as glutaraldehydes and quaternary ammonium compounds (QACs) are commercially available for disinfection purposes at the farm level and other relevant stages of food production chain aimed at inhibiting bacterial growth, load and colonization (Russell, 2002a; Russell, 2002b; Maillard, 2007).

However, the use of disinfectants (biocides) is implicated in the emergence of disinfectant tolerant foodborne and environmental bacterial pathogens (Russell *et al*., 1999; Fraise, 2002; Chapman, 2003; Russell, 2004; Carson *et al*., 2008).

A number of studies on the use of different biocides including glutaraldehydes, quaternary ammonium compounds, intercalating dyes and diamidines have shown that the biocides are implicated in selection of bacteria with low-level of antimicrobial resistance (McDonnell and Russell, 1999; Russell, 1999; Gaze *et al*., 2005; Whitehead *et al*., 2011). Previous studies have also reported that bacterial resistance can be induced as a result of exposure to a low concentration of a biocide (Kolar *et al*., 2001; Braoudaki and Hilton, 2004a; Braoudaki and Hilton, 2004b; Karatzas *et al*., 2007; Randall *et al*., 2004; Randall *et al*., 2005).

In addition, previous studies have shown a close association between resistance to biocides and antimicrobial agents such as antibiotics, and the genetic determinants to these agents are commonly linked with each other. For instance, the *qac* genes such as $qacE_1$ and $qacE\Delta 1$ genes are often present on the plasmids together with other resistance genes (Weigel *et al*., 2003; Jaglic and Cervinkova, 2012). If antibiotic and *qac* resistance genes are both carried on class I integrons, selection for *qac* resistance may result as co-selection of antibiotic resistance in microbial population.

Co-existence of resistance determinants such as antibiotic and *qac* resistant genes, on mobile genetic elements, provides a potential reservoir of antibiotic-resistant bacteria in *qac*-polluted environments (Russell, 2000; Gaze *et al*., 2005). The present study was conducted to characterize the role of biocide (three types of disinfectants) interventions (such as those by Biosentry, Synergize and virkon-S) in the emergence of biocide-tolerant *Salmonella* and also its co-selective association with multidrug-resistant *Salmonella*.

We also further, investigated the association with carriage of specific efflux gene markers.

MATERIALS AND METHODS

Study design and sample collection

This study was conducted from October 2007 to November 2009 in Raleigh, North Carolina, United States.

It was a longitudinal group-randomized controlled study designed to investigate the association of different kinds of biocides with the occurrence and persistence of

biocide tolerant and multi-drug resistant (MDR) *Salmonella*.

Briefly, three vertically integrated commercial swine production systems (systems 1, 2 and 3) were selected based on history of occurrence of *Salmonella*. From each of the production system, three farms were selected and from each farm, four barns were randomly selected for further follow-up in this study, and all barns used standardized electrostatic disinfection systems to limit introduction of additional potential confounding effects.

We visited each farm at two stages (early finishing and late finishing) in four replicates (repeated visits to the same barns during the study period of October 2007 to November 2009). Each replicate visit consisted of sampling assigned barn floors before and after disinfection and pigs at early and late finishing stages. Sampling was done from all the 36 barns for twelve consecutive months for a period of 2 years.

Barn floor swab samples were aseptically collected (10 samples per barn in 5 replicates) pre-disinfection (n = 1,800) and post-disinfection (n = 1,795) from randomly selected pens (n = 10 samples per barn) in 36 barns. A total of 48 fresh fecal samples of about 25g were aseptically collected from each barn in four replicates at the early finishing (6 to 9 weeks of age) (n = 6,842) and at late finishing stages (26 to 28 weeks of age) of production (n = 6,093) from individual pigs. As a result of unanticipated sampling logistics, some samples were missed at different stages of the study (48 samples per barn x 36 barns x 4 replicates = 6,912 samples). About 100 g of pooled feed samples (1 sample per barn collected from all 36 barns at 2 stages and 4 replicates with 13 losses to follow-up) was aseptically collected from 36 barns (n = 275) over a period of 2 years. Each pooled feed sample per barn was aseptically collected from the feeder bin in sterile Whirl-Pak bags and shipped to the laboratory on the same day.

Isolation and identification of *Salmonella*

Salmonella isolates were recovered and identified following conventional methods as described previously (Gebreyes *et al*., 2000; Molla *et al*., 2010). Briefly, a 10g portion of each fecal and feed sample was pre-enriched in 90 ml of buffered peptone water (BPW; Becton Dickinson, Sparks, MD) and 90 ml of BPW was added to each Whirl-Pak bag containing individual drag swabs, and incubated at 37°C overnight.

The remaining portions of fecal and feed samples were stored at -20°C. After overnight incubation, 100 µl of the pre-enriched suspension was added into 9.9 ml of Rappaport-Vassilliadis (RV) enrichment broth (Becton Dickinson, Sparks, MD) and incubated at 42°C overnight. A 10 µl of the suspension was inoculated onto Xylose-lactose-Tergitol 4 (XLT-4) agar (Becton Dickinson, Sparks, MD) plates and incubated at 37°C for 24 h and incubation was extended to 48 h in cases where colonies were doubtful.

Three *Salmonella* colonies were selected from each positive plate for biochemical testing. Each of selected *Salmonella* colonies were then inoculated onto triple sugar iron (TSI) agar (Becton Dickinson, Sparks, MD) slants, lysine iron agar (LIA) slants (Becton Dickinson, Sparks, MD) and urea broth (Becton Dickinson, Sparks, MD) and incubated at 37°C overnight.
All biochemically confirmed *Salmonella* isolates were then stored at -80°C until further testing.

Phenotyping

Salmonella isolates recovered from swine feed (n = 30), swine barn floors (n = 1628) and swine feces (n = 4504) were serogrouped using commercially available polyvalent O and group-specific antisera

(Mira Vista, Copenhagen, Denmark) following the recommendations of the manufacturer. All (n = 6162) *Salmonella* isolates were biochemically confirmed.

Isolates were tested for antimicrobial susceptibility to a panel of 12 antimicrobials using the Kirby-Bauer disc diffusion method following the guidelines of the CLSI (2009). The antimicrobials used and their respective disc potencies were as follows: ampicillin (Am; 10 µg/ml), amoxicillin-clavulanic acid (Ax; 30 µg/ml), amikacin (An; 30 µg/ml), ceftriaxone (Ce; 30 µg/ml), cephalothin (Ch; 30 µg/ml), chloramphenicol (Cl; 30 µg/ml), ciprofloxacin (CIP; 5 µg/ml), gentamicin (Gm; 10 µg/ml), kanamycin (Km; 30 µg/ml), streptomycin (St; 10 µg/ml), sulfisoxazole (Su; 250 µg/ml), and tetracycline (Te; 30 µg/ml).

We used *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 as control strains. *Salmonella* isolates showing resistance to three or more classes of antimicrobials were identified as multidrug resistant (MDR) and for those isolates showing intermediate resistance were considered as susceptible (Wei *et al*., 2019).

Minimum Inhibitory Concentration (MIC) of the Synergize and Biosentry

For Synergize tolerance test, a total of four hundred twenty-eight *Salmonella* isolates were involved, including 222 isolates recovered from floor swabs pre-disinfection, 96 isolates post-disinfection, 72 isolates from swine at early finishing stage, 27 isolates from swine at late finishing stage and 11 isolates from swine feed, whereas, for Biosentry tolerance test, total of three hundred forty-eight *Salmonella* isolates, including 176 isolates from floor swabs pre-disinfection, 112 isolates post-disinfection, 42 isolates from swine at early finishing stage, and 18 isolates from swine at late finishing stage were involved.

All *Salmonella* isolates selected for Synergize and Biosentry tolerance tests contained different antimicrobial resistance patterns. They were recovered from swine barns disinfected with Synergize and Biosentry, respectively. The agar plate-dilution method was used to determine the MIC against *Salmonella* following the methods described before (Aarestrup and Hasman, 2004; Kawamura-Sato *et al*., 2010; Medardus *et al*., 2014). The susceptibilities were determined on Mueller-Hinton (MH)-II agar plates containing two fold serial dilutions of biocides (Synergize and Biosentry.

All *Salmonella* isolates were tested for the following dilution ranges of Synergize: 0, 20, 40, 80, 160, 320, 330, 640 and 1280 µg/ml. Biosentry solutions contained the following dilution ranges: 0, 20, 40, 80, 160, 320, 330 and 640 µg/ml. Briefly, 500 ml of 20,000 µg/ml of biocide stock solutions and the 2L of MH agar were autoclaved and cooled to 60-70^{0}C in a water bath.

The specific volumes of Synergize and Biosentry solutions to be added to each dilution tube of 100 ml of MH agar were calculated according to the desired concentrations. About 25ml of MH agar was aseptically dispensed and allowed to solidify. Bacterial suspensions were adjusted to 0.5 McFarland (equivalent to 1-2 x10^{8}CFU/ml) in 2 ml of sterile 0.85% NaCl solutions. The suspensions were diluted to approximately 10^{7}CFU/ml (100µl of each inoculum at 0.5 McFarland + 900µl of sterile 0.85% NaCl solutions) in 1.5 ml eppendorf tubes before adding to the inoculum replicator block. About 400 µl of each suspension was aseptically aliquoted to a corresponding well of the replicator inoculum block. All test *Salmonella* isolates and control strains were tested in triplicate. The inoculated plates were allowed to stand at room temperature for 15-20 min and incubated at 37^{0}C for 16 to 20 h.

After incubation, the plates were assessed for growth and determine the MIC. *E. faecium* A17 sv 1 HHA 210, *S. aureus* C10682, *S. aureus* ATCC 29213 and *S. aureus* SO385 were used as reference strains. The MIC was defined as the lowest concentration that inhibits the visible growth of *Salmonella* after an overnight incubation.

Identification of qactolerance genes, class 1 integrase gene (*intI1*) and gene cassettes

Seven hundred seventy-six *Salmonella* isolates were tested for the carriage of quaternary ammonium compound (*qac*) tolerance genes (*qacE1* for multi-drug efflux and *qacEΔ1* for attenuated variant *qacE1*) and the presence of class 1 integrase (*intI1*) and gene cassettes integrated between conserved segments (5'-3'CS) of class 1 integrons were detected using PCR.

Briefly, *Salmonella* isolates were inoculated onto Tryptic Soy agar (TSA) plates and incubated at 37°C for overnight. The genomic DNA was extracted using Qiagen DNeasy tissue kit following the manufacturer's instruction (Qiagen Ambion, Austin, Texas, USA).

The sets of primers used to screen for presence of *qac* [*qacE1* or *qacEΔ1*] were 5'-ATCGCAATAGTTGGCGAAGT-3' and 5'-CAAGCTTTTGCCCATGAAGC-3' as forward and reverse primers. Following screening for *qac* [*qacE1* or *qacEΔ1*], other sets of primers used for amplification of the *qacEΔ1* were: 5'-ATCGCAATAGTTGGCGAAGT-3' and 5'-TTAGTGGGCACTTGCTTTGG- 3' as forward and reverse primers, whereas, for *qacE1* the set of primers used were 5'-ATCGCAATAGTTGGCGAAGT-3' and 5'-AACACCGTCACCATGGCGTCG-3' as forward and reverse primers.

PCR thermocycling conditions included initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min and final extension was done at 72°C for 7 min and amplification was done in 30 cycles (Sandvang *et al*., 1997; Kazama *et al*., 1998).

Primers used for amplification of the *intI1* were 5'-GCCTTGCTGTTCTTCTACGG-3' and 5'-GATGCCTGCTTGTTCTACGG-3'as forward and reverse primers (Levesque *et al*., 1995) and those for conserved segments were 5'-GGCATCCAAGCAGCAAG-3' and 5'-AAGCAGACTTGACCTGA-3' as previously described (Ploy *et al*., 2000).

PCR conditions included initial denaturation at 94°C for 5 min, followed by 25 cycles of 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec, and the final extension was done at 72^0C for 7 min (Lindstedt *et al*., 2003). About 10 µl of the PCR product of each isolate tested were electrophoresed on 1% agarose gel stained with 5 µl of 10 mg/ml ethidium bromide for 1 hr at 120 volts using 0.5X Tris-borate EDTA (TBE) as running buffer. A 1-kb plus DNA ladder was used as molecular size marker.

Data analysis

Data were entered into the Microsoft excel 2007 (Ms Corp., Redmond, WA, USA) and the statistical analyses were conducted using MedCalc® software version 12.7.1.0 (Ostend, Belgium), and the construction of graphs were conducted with Microsoft excel 2007 (MsCorp, Redmond, WA, USA). Descriptive statistics summarized the levels of *Salmonella* prevalence in different samples, proportions of isolates that were resistant or were carrying *intI1*, and *qac* tolerance genes (*qacE1* and *qacEΔ1*). Change in environmental *Salmonella* prevalence was calculated as the difference between the number of *Salmonella* isolates recovered from the swine barns before and after disinfection of barns. A value of $P< 0.05$ was considered statistically significant

RESULTS

Prevalence and antimicrobial resistance of *Salmonella* isolates from the floor

Salmonella isolates were detected in 13.9% (208/1497) and 6.7% (98/1468) of swine barn drag swab samples at pre- and post-disinfection with biocide, whereas, from control swine barns treated with pressurized hot water, *Salmonella* isolates were detected in 16.7% (75/450) and 27.6% (124/450) of drag swab samples collected pre- and post-disinfection, respectively. *Salmonella* prevalence on swine barn floors varied from 0 to 100% in different replicate samplings, with 191 (53.1%) of the 360 barn-level estimates being 0 (no *Salmonella*-positive samples were found) and 0.8% (3 of 360) being 100 % (all collected floor swabs in the barn at sampling time were found to be positive for *Salmonella* (Figure 1). The change in environmental *Salmonella* prevalence between pre- and post-disinfection samplings varied from -0.7 to 0.7. Most frequently, in 43.3% (78 of 180) of the replicates there was no change in the prevalence observed, however, in 33.9% (61 of 180) of the replicates the prevalence increased and in only 22.8% (41 of 180) of cases prevalence decreased (Figure 2).

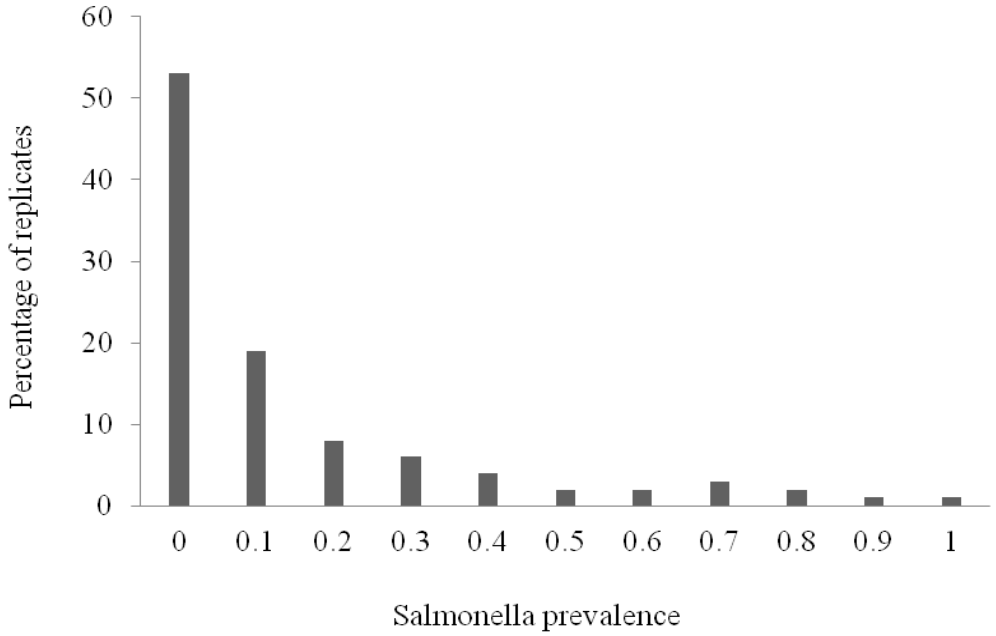

Figure 1. Environmental *Salmonella* prevalence at 360 samplings. Each prevalence estimate was based on 10 floor swab samples collected at the same time point in each swine barn.

Of the 1628 *Salmonella* isolates from the drag swab samples, the most common antimicrobial resistance was found with tetracycline (79.9%, 1300 of 1628), followed by streptomycin (64.2%, 1045 of 1628), sulfisoxazole (44.9%, 731 of 1628), ampicillin (27.9%, 455 of 1628), chloramphenicol (14.7%, 239 of 1628), kanamycin (12.3%, 201 of 1628), cephalothin (4.3%, 70 of 1628), amoxicillin-clavulanic acid (3.6%, 59 of 1628), gentamycin (1.9%, 31 of 1628), ceftiofur (1.3%, 21 of 1628), ceftriaxone (1%, 16 of 1628), amikacin (0.2%, 4 of 1628), and ciprofloxacin (0.1%, 2 of 1628).

Antimicrobial resistance was found to be common among the isolates, with 89.5% (401 of 448) of *Salmonella* isolates that originated from drag swabs from swine barns pre-disinfected with Biosentry, 87% (287 of 330) of isolates from drag swabs

from swine barns pre-disinfected with Synergize, 93.9% (229 of 244) of isolates from drag swabs from swine barns pre-disinfected with virkon-S, and 96.7% (586 of 606) of isolates from drag swabs from swine barns pre-treated with pressurized hot water showing resistance to one or more of the antimicrobials tested.

Almost 44% (195 of 448) of the *Salmonella* isolates recovered from drag swabs from swine barns pre-disinfected with Biosentry, 43% (142 of 330) of the isolates from drag swabs from swine barns pre-disinfected with Synergize, 69.3% (169 of 244) of the isolates from drag swabs from swine barns pre-disinfected with virkon-S, and 54.5% (330 of 606) of isolates from drag swabs from swine barns pre-treated with pressurized hot water were MDR.

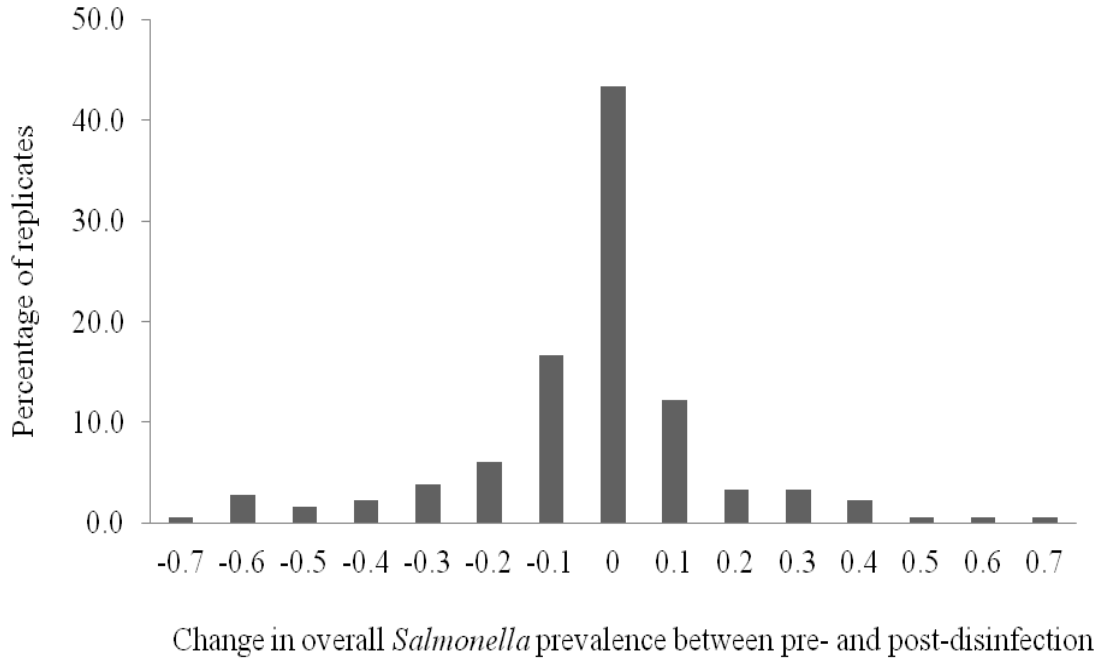

Figure 2. Overall *Salmonella* prevalence between pre-and post-disinfection at 360 samplings.

Barn-level prevalence of antimicrobial resistance (isolate resistant to at least one antimicrobial) among *Salmonella* isolates recovered from the floor swab samples was 92.3% (1503 of 1628) (ranging from 0 to 100%). The overall MDR *Salmonella* isolates originating from floor swab samples was 51.4% (836 of 1628 (Table 4). The barn-level MDR among the environmental isolates from floor swabs was 67.3%, with a median of 100% (range from 0 to 100%).

Results from the mixed model approach suggested that the cleaning method or compound had a significant impact on the change in *Salmonella* prevalence in the swine barn environment from pre-disinfection to post-disinfection, (P = 0.0003). The estimates for change in *Salmonella* prevalence for each biocide treatment are presented in Table 1. As a result of cleaning and disinfection of the swine barns with biocide, the percentage reduction in *Salmonella* prevalence between pre- and post-disinfection was 7.2%, significantly lower at post-disinfection than it was at pre-disinfection (% reduction in *Salmonella* prevalence = 7.2%, 95% CI, 4.99 to 9.42; P< 0.0001).

Besides the reduction in the prevalence of *Salmonella* between pre- and post-disinfection stages of swine barns, the proportions of MDR *Salmonella* isolates significantly increased by 8.17% from pre- to post-disinfection of swine barns (% increase in MDR = 8.17%, 95% CI, 3.18 to 13.11; P = 0.0013).On the other hand, the average prevalence of *Salmonella* isolates increased by 10.9% following cleaning of swine barns with pressurized hot water (% increase in *Salmonella* prevalence = 10.9%, 95% CI, 5.34 to 16.4; P< 0.0001).

The odds of recovering *Salmonella* isolates from swine barns before disinfection were 2.26 times higher than after disinfection of the barns (OR = 2.26; 95% CI; 1.75 to 2.90; P <0.0001). *Salmonella* prevalence change estimates after all biocide treatments were significantly different from those when cleaning with water only, Biosentry against water, P = 0.006; Synergize against water, P = 0.0018; virkon-S against water, P = 0.0356).

The largest decrease in *Salmonella* prevalence between pre- and post-disinfection was observed for Biosentry with 10.4% decrease, followed by Synergize (8.9% decrease) and virkon-S (3.8% decrease). However, there were no statistically significant differences between the three biocide treatments in terms of the change in *Salmonella* prevalence (P > 0.6).

Salmonella fecal prevalence and antimicrobial resistance

Barn-level fecal *Salmonella* prevalence varied between 0 and 100%, with an average prevalence of 12.4% (Table 2). Prevalence of *Salmonella* at late finishing stage (7.1%) was significantly lower than at early finishing stage (17.2%) at the time of pig placement in the barns (% reduction in *Salmonella* prevalence = 10.1%, 95% CI, 8.99 to 11.21; P< 0.0001). The odds of recovering *Salmonella* isolates from early finishing stage of placement of pigs to disinfected barns were 2.74 times higher than at late finishing stage of pigs (OR = 2.74; 95% CI; 2.44 to 3.08; P <0.0001).

The change in fecal *Salmonella* prevalence between early and late finishing stages among growing pigs was not significantly associated with biocide treatment in the barns (P = 0.9119). Of the 4504 *Salmonella* isolates from the swine fecal samples, the most common antimicrobial resistance was found with tetracycline (96.6%, 4349 of 4504), followed by streptomycin (75.2%, 3389 of 4504), ampicillin (57.5%, 2588 of 4504), sulfisoxazole (50.7%, 2284 of 4504), kanamycin (39.1%, 1760 of 4504), chloramphenicol (19.9%, 897 of 4504), amoxicillin-clavulanic acid (8.8%, 395 of 4504), cephalothin (8.2%, 369 of 4504), ceftiofur (4.2%, 187 of 4504), gentamycin (3.6%, 162 of 4504), ceftriaxone (1.4%, 61 of 4504), ciprofloxacin (0.1%, 4 of 4504), and amikacin (0.04%, 2 of 4504).

Antimicrobial resistance was observed among *Salmonella* isolates, with 98.6% (1260 of 1278) of isolates that originated from fecal samples of swine placed in barns pre-disinfected with Biosentry, 99.2% (970 of 978) of isolates from fecal samples of swine placed in barns pre-disinfected with Synergize, 97.4% (1066 of 1095) of isolates from fecal samples of swine placed in barns pre-disinfected with virkon-S, and 97.1% (1119 of 1153) of isolates from fecal samples of swine placed in barns pre-treated with pressurized hot water showing resistance to one or more of the antimicrobials tested.

Approximately 77% (984 of 1278) of isolates recovered from fecal samples of swine placed in barns pre-disinfected with Biosentry, 77.1% (754 of 978) of isolates from fecal samples of swine placed in barns pre-disinfected with Synergize, and 80.5% (882 of 1095) of isolatesfrom fecal samples of swine placed in barns pre-disinfected with virkon-S, and 70.1% (808 of 1153) of isolates from fecal samples of swine placed in barns pre-treated with pressurized hot water were MDR Salmonella isolates. The verall, barn-level prevalence of

antimicrobial resistance whereby isolates recovered from faecal samples are resistant to at least one antimicrobial was 98% (4,415 of 4,504) (ranging from 0 to 100%). The overall fecal MDR Salmonella isolates were recorded to be as high to over a half of isolates, with 76.1% (3,428 of 4,504) of the isolates originating from swine fecal samples (Table 4). Multi-drug resistance was found in fecal Salmonella from 163 (90.6%) of the 180 replicate samplings. The barn-level MDR varied from 0 to 100%, with median of 100% and average MDR prevalence being 79%. Fecal MDR prevalence was not associated with biocide treatment used in the barn (P = 0.9201).

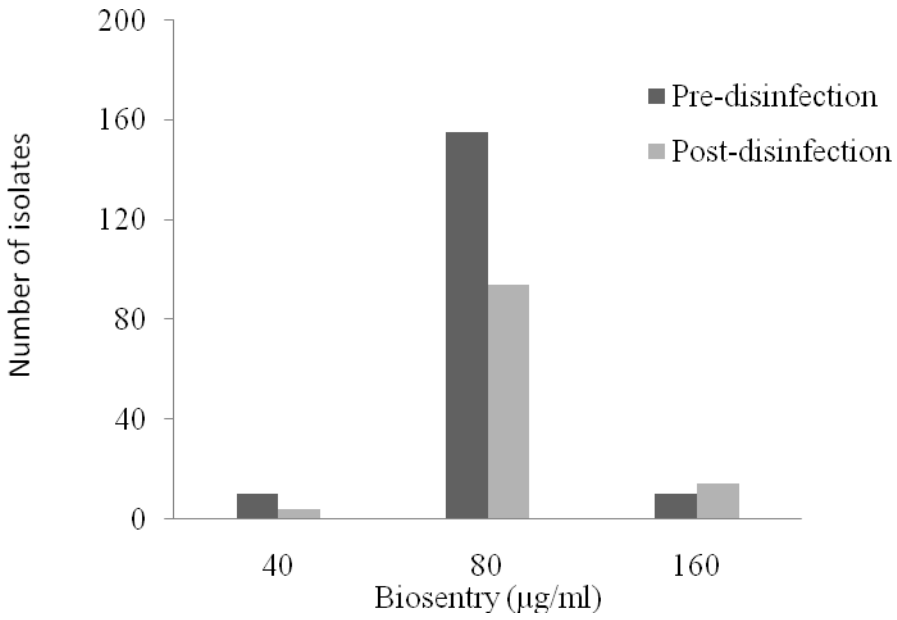

Figure 3. MIC of Biosentryfor isolates obtained before and after disinfection of swine barns

Analysis of Biosentry tolerance

A total of 348 Salmonella isolates were systematically selected, in which 328 isolates were obtained from the Biosentry pre-disinfected swine barn floors and feces from swine placed in the respective swine barns, and the rest 20 isolates were obtained from the control swine barns which were previously pre-treated with pressurized hot water. About half (50.6%; 176 of 348) of the isolates selected for Biosentry tolerance test originated from drag swabs from Biosentry pre-disinfected swine floors (Figure 3).

Of the 176 Salmonella isolates, a total of 156 (88.6%) were resistant to at least one antimicrobial, and 47.2% (83) were MDR Salmonella isolates. Also, a total of 112 (32.2%, 112 of 348) Salmonella isolates selected for Biosentry tolerance test, originated from floor drag swabs post-disinfection, in which 89.3% were resistant to at least one antimicrobial, and 50% were MDR Salmonella isolates. Overall, of the 348 Salmonella isolates, nearly ninety-one percent (315 of 348) tested for tolerance to Biosentry were resistant to at least one antimicrobial and 9% (33 of 348) of the isolates were pansusceptible.

On tolerance test, almost eighty-eight percent (305 of 348) of the isolates showed tolerance to Biosentry at a breakthrough point of 80µg/ml, whereas, the remaining 4.9% (17 of 348) and 7.5% (26 of 348) showed tolerance at MICs of < 80 µg/ml and > 80 µg/ml, respectively.

Of the 331 *Samonella* isolates with Biosentry MIC > 40 µg/ml, 90.3% were resistant to at least one antimicrobial, whereas 9.7% were pansusceptible. Generally, the prevalence of pansusceptible*Salmonella* isolates was 3.5% (217 of 6162).

Of the 33 *Salmonella* isolates with pansusceptible phenotypes selected for Biosentry MIC testing, approximately 97% (32 of 33) of the isolates showed high resistance to Biosentry at the MIC breakthrough point of ≥ 80 µg/ml.

The mean difference of Biosentry MICs of *Salmonella* isolates obtained from floor drag swabs post-disinfecion was 21.0 times higher than for *Salmonella* isolates obtained from floor drag swabs pre-disinfection ($\bar{x}$ difference = 6.64; 95% CI, 0.83 to 12.45; *P* = 0.0253).

Of the 348 *Salmonella* isolates screened for MDR efflux (e.g. *qacE1, qacEΔ1*) and *intI1* genes, about 31.3% carried the *qac* gene, of which 89% (97 of 109) showed a MIC of 80 µg/ml. The *qac* tolerance genes were also detected from 2% (7 of 348) and 1.4% (5 of 348) of *Salmonella* isolates at MICs of <80 µg/ml and > 80 µg/ml, respectively.

Of the 109 *Salmonella* isolates that carried *qac* genes, 94.5% contained attenuated *qacEΔ1* gene, whereas 68.8% (75 of 109) of the isolates carrying *qacEΔ1* gene were found to be associated with class 1 integrons and the resistance gene cassettes of different sizes and patterns. The carriage of the *qac* gene versus Biosentry MIC in *Salmonella* isolates recovered from fecal matter, feed, and barn floors is shown in Table 5.

The odds of isolating resistant *Salmonella* isolates from isolates with high MIC of Biosentry (≥ 80 µg/ml) were 1.71 times higher than for isolates with low MIC (<80 µg/ml) of Biosentry, (OR = 1.71; 95% CI; 0. 22 to 13.34; *P* = 0.6076). Assessment of association between tolerance and gene carriage showed that isolates with a high Biosentry MIC were more likely to carry the *qac* gene (Table 3).

The odds of *qac* gene carriage were 1.57 times higher for isolates with high Biosentry MICs than for those with low Biosentry MICs (OR = 1.57; 95% CI, 0.58 to 4.25; *P* = 0.3726), whereas, the odds of *qac* gene carriage were 85.22 times higher for MDR *Salmonella* isolates with high Biosentry MICs than for those none MDR *Salmonella* isolates with low Biosentry MICs (OR = 85.22; 95% CI, 26.41 to 274.98; *P*< 0.0001).

We also found out that the odds of *intI1* gene carriage were 184.01 times higher for MDR *Salmonella* isolates with high Biosentry MICs than for none MDR *Salmonella* isolates with low Biosentry MICs (OR = 184.01; 95% CI, 11.29 to 2999.46; *P* = 0.0003). The correlation analysis showed only weak correlation between MDR *Salmonella* isolates and *qac* gene carriage (*r* = 0.55; 95% CI for *r,* 0.47 to 0.62; *P*< 0. 0001) and MDR *Salmonella* and *intI1* gene carriage (*r* = 0.46; 95% CI for *r,* 0.37 to 0.54; *P*< 0. 0001).

Analysis of Synergize tolerance

A total of 428 *Salmonella* isolates were systematically selected for Synergize tolerance test. About 94% (403 of 428) of the *Salmonella* isolates tested for tolerance to Synergize were resistant to at least one antimicrobial and 6% (25 of 428) of the isolates were pansusceptible.

Of 222 *Salmonella* isolates selected for Synergize tolerance test, originating from floor drag swabs pre-disinfection, 89.2% were resistant to at least one antimicrobial, of which 44.4% (88 of 198) of the isolates were MDR *Salmonella* isolates.

Out of a total of 96 *Salmonella* isolates selected for Synergize tolerance test, originating from floor drag swabs post-disinfection, 96.9% were resistant to at least one antimicrobial, whereas, of which 72% (67 of 93) were MDR *Salmonella* isolates.

Despite the reduced *Salmonella* prevalence between pre- and post-disinfection stages of swine barns, the proportions of MDR *Salmonella* isolates increased by 27.6% between pre- and post-disinfection stages of swine barns (Figure 4).

Eighty-percent (341 of 428) of the isolates showed tolerance to Synergize at a breakthrough point of 160 µg/ml, whereas, the remaining 5.8% (25 of 428) and 17.8% (76 of 428) showed tolerance at MICs of <160 µg/ml and >1600 µg/ml, respectively.

Of the 417 *Samonella* isolates with Synergize MIC > 80 µg/ml, 90.9% were resistant to at least one antimicrobial. A small proportion (6.7%) of the isolates was pansusceptible to antimicrobials used.

On the other hand, a total of 29 *Salmonella* isolates with pansusceptible phenotypes were selected for Synergize MIC testing of which 96.6% showed high resistance to Synergize at the MIC breakthrough point of ≥ 160 µg/ml. The MICs mean difference of *Salmonella* isolates obtained from floor drag swabs post-disinfecion was 21.0 times lower than for *Salmonella* isolates obtained from floor drag swabs ($\bar{x}$, 21.0; 95% CI of difference, 3.38 to 38.61; $P = 0.0197$).

Of the 428 isolates screened for *qac* tolerance gene, 22.9% carried the *qac* gene, of which 77.6% (76 of 98) showed the MIC of ≥160 µg/ml. Almost 86% (84 of 98) of isolates that carried the *qac* gene also carried attenuated *qacEΔ1* and 14% (14 of 98) did not carry *qacEΔ1* gene which is part of the 3' of the conserved segment (CS) of class 1 integrons.

Of the 84 *Salmonella* isolates that carried attenuated *qacEΔ1* gene, 48.8% were found to be associated with class 1 integrons and the resistance gene cassettes of different sizes and patterns (Table 3).

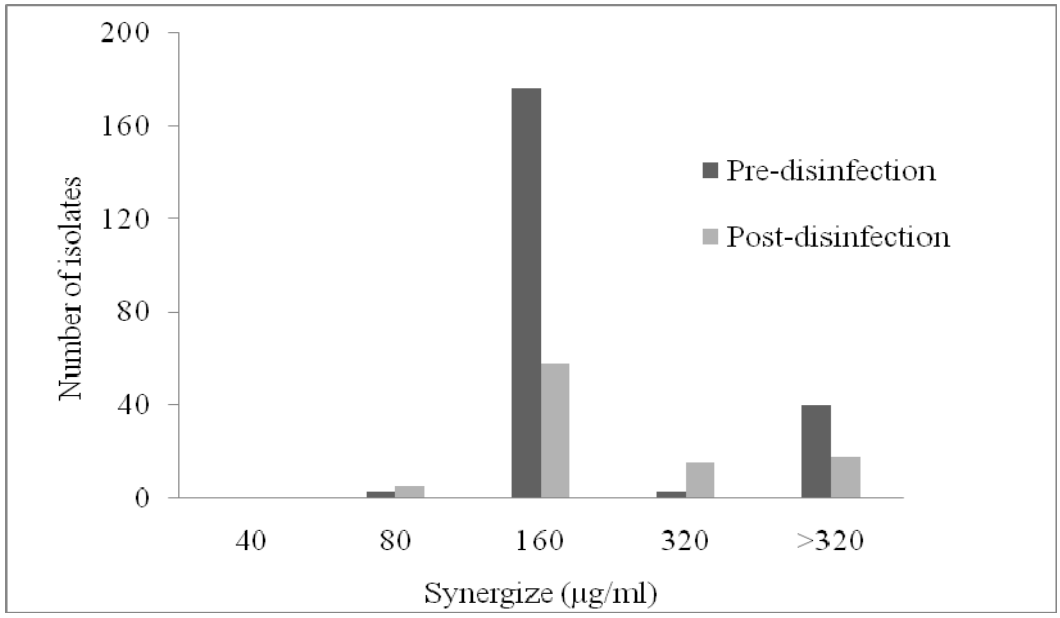

Figure 4. MIC of Synergize for isolates obtained before and after disinfection of swine barns

Genes such as *qac*, *qacEΔ1* and *intI1* were found among the *Salmonella* isolates that originated from drag swabs and feces from swine placed in the barns pre-disinfected with Biosentry, with 29.6% (97 of 328), 27.7% (91 of 328), and 20.1% (66 of 328) of the isolates carrying the genes, respectively.

Almost 21% (83 of 398), 19.6% (78 of 398), 10.1% (40 of 398) of *Salmonella* isolates originating from drag swabs and feces from swine placed in the barns pre-disinfected with Synergize, were found to carry *qac*, *qacEΔ1* and *intI1* genes, respectively, whereas, 54% (27 of 50), 36% (18 of 50), and 20% (10 of 50), of *Salmonella* isolates originating from floor drag swabs and feces from swine placed in the barns pre-treated with pressurized hot water were also found to carry *qac*, *qacEΔ1* and *intI1* genes, respectively.

Overall, genes such as *qac*, *qacEΔ1* and *intI1* were detected from nearly 27% (207 of 776), 24.1% (187 of 776), and 14.9% (116 of 776), of *Salmonella* isolates systematically selected for Biosentry and Synergize MICs testing, respectively (Table 4). None of the *Salmonella* isolates harbored $qacE_1$. The *intI1* genes were observed in 62% (116 of 187) of the *qacEΔ1*-positive strains. Thirty eight-percent (71 of 187) of the *qacEΔ1*-positive strains did not contain the *intI1* genes.

The odds of isolating resistant *Salmonella* isolates from isolates with high Synergize MIC (≥160 µg/ml) was 1.39 times higher than for isolates with low MIC (< 160 µg/ml), (OR = 1.39; 95% CI; 0. 17 to 11.25; P = 0.7580). Assessment of an association between tolerance and gene carriage showed that isolates with a high Synergize MIC were more likely to carry the *qac* gene (Table 3).

The odds of *qac* gene carriage were 7.09 times higher for isolates with high Synergize MICs than for those with low Biosentry MICs (OR = 7.09; 95% CI, 0.41 to 121.42; P = 0.1765), whereas, the odds of *qac* gene carriage were 10.38 times higher for MDR *Salmonella* isolates with high Synergize MICs than for those none MDR *Salmonella* isolates with low Synergize MICs (OR = 10.38; 95% CI, 5.06 to 21.30; P< 0.0001).

The odds of *intI1* gene carriage were 5.82 times higher for MDR *Salmonella* isolates with high Synergize MICs than for none MDR *Salmonella* isolates with low Synergize MICs (OR = 5.82; 95% CI, 2.24 to 15.15; P = 0.0003). Analysis of correlation showed weak association between MDR *Salmonella* isolates and *qac* gene carriage (r = 0.36; 95% CI for r, 0.27 to 0.44; P< 0. 0001) and MDR *Salmonella* and *intI1* gene carriage (r = 0.19; 95% CI for r, 0.10 to 0.28; P< 0. 0001).

DISCUSSION

Disinfection is regarded as a crucial step in achieving a desired hygiene in food handling environment. Lack of a thorough disinfection may pose a risk of transfer of foodborne pathogens to animal products.

In the current study, the prevalence of *Salmonella* isolates detected from swine barn floors before disinfection was two times higher (13.9%) than after disinfection (6.7%) and the odds of recovering *Salmonella* isolates from swine barns before disinfection were two times higher than after disinfection of the barns, suggesting the importance of disinfecting barns and in a way reducing *Salmonella* load and colonization in pigs or food animals.

This observation is in line with the finding from another study conducted in Denmark, where *Salmonella* spp. before chemical disinfection was found to be 17.7% and was then reduced to 5.2% after disinfection of abattoir (Gantzhorn *et al*., 2014).

In the current study, the prevalence of *Salmonella* isolates observed in the swine barns pre-treated with pressurized hot water

(control group) increased from 16.7% to 27.6% before and after disinfection, suggesting that the cleaning of the swine barn floors with pressurized hot water is the least efficient method of clearing *Salmonella* isolates over the chemical disinfection.

The prevalence of *Salmonella* at late finishing stage was significantly lower (7.1%) than at early finishing stage (17.2%) at the time of placement of the barns.The long term effect of biocide use for disinfection of the swine barnfloors is perhaps responsible for the reduced *Salmonella* prevalence by 10.1%. The observed reduction in the prevalence of *Salmonella* isolates between the two stages of production is further supported by the observed higher odds of recovering *Salmonella* isolates from early finishing stage than at late finishing stage of pigs, further supports the the significance of the long term effect of the disinfectant in the biocide treated swine barns. Observations on the significance of biocides in reducing the surface load of bacteria in production environments are in line with other previous studies (Møretrø *et al*., 2009; Soumet *et al*., 2016).

Environmental *Salmonella* prevalence before and after disinfection samplings indicates that there was no change in 43% of the replicates. However, in nearly 34% of the replicates the prevalence increased; and decreased in only 23%. This reduction in the prevalence of environmental *Salmonella* is perhaps due to chemical disinfection, however, there might be other reasons that are currently not known for increased *Salmonella* prevalence. For instance, efficacy of a biocide is impended by factors such as the pH, temperature, humidity, surface organic matters and the active ingreadients of the disinfectants.

In this study, the prevalence of MDR *Salmonella* was observed to increase by 8.2%, despite a significant reduction by 7.2% of the *Salmonella* prevalence. Also, the current study shows that almost all *Salmonella* isolates with MDR resistance phenotypes were also biocide-tolerant isolates. Though biocides play important role in limiting the potential sources of infection, there is a rising concern over the decreased susceptibilities of foodborne pathogens to biocides (McDonnell and Russell 1999; Russell 1999; Russell *et al*., 1999; Russel, 2002; Chapman, 2003; Braoudaki and Hilton, 2004a; Braoudaki and Hilton, 2004b; Randall *et al*., 2004; Gradel *et al*., 2005; Randall *et al*., 2005).

Quaternary ammonium compounds (QACs) can induce leakage of intracellular components, which is an indication of membrane damage (Takasaki *et al*., 1994; Tattawasart *et al*., 1999; Morente *et al*., 2013). As a resultof the membrane leakage, cross-resistance can potentially occur to allow different antimicrobials (antibiotics and biocides) to escape out of the bacterial cells and thus no cell death is initiated as the threshold required to kill the microorganisms is not attained (Guimaraes *et al*., 2000; Chapman, 2003; Futoma-Koloch *et al*., 2013; Morente *et al*., 2013).

In the current study, nearly 25% of *Salmonella* isolates carried the *qacEΔ1* and about 15% of the *qacEΔ1* was disseminated among the *Salmonella* isolates by means of the class 1 integrons. Therefore, it is concluded that all of the *intI1*-positive isolates carried *qacE*Δ1 in their 3′ conserved segments, confirming that the *qacE*Δ1 gene is linked to the integrons. As a whole, in the current study, no correlation between increased MIC values to Biosentry and Synergize to carriage of *qacE*Δ1 and *intI1* was observed. This finding is similar to the previous reports in clinical isolates of Gram-negative bacteria (Kucken *et al*., 2000) and *Salmonella* isolates from poultry and swine (Chuanchuen *et al*., 2007).

In the current study, the comparisons of abundances of class 1 integrons and disinfectant resistance genes (*qacEΔ1*) in

Salmonella isolates showed a significant correlation, suggesting that the MDR efflux pumps, such as the *qacEΔ1* in Gram-negative bacteria such as *Salmonella* spp., *E. coli*, *Acinetobacter* spp. *Campylobacter* spp., *Pseudomonas* spp. and also in Gram-positive such as *Staphylococcus aureus*, are common transporters known as membrane-associated proteins that extrude a range of structurally dissimilar toxic compounds from the cytoplasm of the bacterial cells (Piddock *et al*., 2006; Kawamura-Sato *et al*., 2010; Quinn *et al*., 2011; Wan and Chou, 2015).

In addition, *Salmonella* serovars also contain a 43-kb *Salmonella* Genomic Islands 1 [SGI-1, wherein a *qacEΔ1*-encoding gene is linked to class 1 integrons located on the chromosomes. The SGI-1 in *Salmonella* serovars was reported to be characterized by resistance to five or six drugs such as ampicillin, chloramphenicol, streptomycin, spectinomycin, sulfonamides, and tetracycline (Ridley and Threlfall, 1998; Threlfall *et al*., 1998; Boyd *et al*., 2002; Carattoli *et al*., 2002; Doublet *et al*., 2003); associated with penta-resistance transmission in *Salmonella* serovars. Besides the *qacEΔ1* MDR efflux pumps, *Salmonella* spp. and *E.coli* also possess well characterised membrane transport proteins called *AcrAB-TolC*, *AcrEF-TolC* and *EmrE* (Piddock, 2006), and the over-expression of *AcrAB-TolC* efflux pumps in *Salmonella* and *E. coli* was reported to confer resistance to triclosan and pine oils (Moken *et al*., 1997).

In view of the results obtained, it is noted that detection of class 1 integrons in the biocide-tolerant and MDR *Salmonella* raise more concern on the public health and multidrug resistance dissemination among important foodborne pathogens such as *Salmonella* spp. Interestingly, all of the *Salmonella intl1*-positive isolates were resistant to at least two antibiotics, and almost over a half (51%) of the *intl1*-positive isolates, showed a wide range of resistance patterns, mainly characterized byresistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracycline, and additional resistance patterns such as resistance to amoxicillin/clavulanic acid, amikacin, ceftriaxone, ceftiofur, cephalothin, gentamycin and kanamycin were observed.
These extremely MDR *Salmonella intl1*-positive isolates, whose class 1 integrons are responsible for multiple resistance phenotypes are often contained in 43-kb *Salmonella* Genomic Islands (SGI-1) located on the chromosomes (Ridley and Threlfall, 1998; Threlfall *et al*., 1998; Carattoli *et al*., 2002).

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CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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TABLES

Table 1. *Salmonella* prevalence estimates in pre- and post-disinfection barn samples

Treatment	Estimate (post-pre prevalence)
Biosentry	-0.1044[a]
Synergize	-0.0889[a]
Virkon-S	-0.0378[a]
Water	0.1089[b]

[a]Estimates with the same superscript did not differ significantly. [b]Estimates with different superscript differed significantly.

Table 2. Barn-level fecal *Salmonella* prevalence among growing pigs in different production systems and at early and late finishing stages of the production cycle

	Salmonella prevalence [%] Mean, median (range)	F2-F1[1], % Mean, median (range)
Overall	12.4, 4.2 (0, 100)	-10.1, -5.6 (-83.3, 50.0)
Production system 1	9.5, 4.2 (0, 61.7)	-10.9, - 8.3 (-61.7, 37.5)
Production system 2	23.5, 10.3 (0, 100)	-15.6, -6.2 (-83.3, 50.0)
Production system 3	4.3, 2.0 (0, 37.5)	-4.0, -2.1 (-37.5, 10.4)
Early finishing stage (F1)	17.3 8,3 (0, 100)	N/A
Late finishing stage (F2)	7.0, 2.1 (0, 93.7)	N/A

[1]Difference in *Salmonella* prevalence between late finishing (F2) and early finishing (F1) of production stage, calculated as [F2-F1]

Table 3. MIC of biocides, multidrug resistance and the presence of *qac*, *qacEΔ1* and *intI1* genes in *Salmonella* isolates

Salmonella	Biosentry MIC (µg/ml)						
	[n (%)]						
[n = 348)]	320	160	80	40	20	0	Total
Number		26 (7.5)	305 (87.6)	16 (4.6)	1 (0.3)		348 (100)
qac		5 (1.4)	97 (27.9)	7 (2)	0		109 (31.3)
qacEΔ1		5 (1.4)	92 (26.4)	6 (1.7)	0		103 (29.6)
intI1		4 (1.1)	70 (20.1)	1 (0.3)	0		75 (21.6)
MDR		13 (3.7)	177 (50.9)	7 (2)	1 (0.3)		198 (56.9)

Salmonella	Synergize MIC (µg/ml)						
	[n (%)]						
[n = 428)]	640	330	320	160	80	40	Total
Number		58 (13.6)	18 (4.2)	341 (79.7)	11 (2.6)		428 (100)
qac		10 (2.3)	12 (2.8)	76 (17.8)	0		98 (22.9)
qacEΔ1		9 (2.1)	12 (2.8)	63 (14.7)	0		84 (19.6)
intI1		8 (1.9)	8 (1.9)	25 (5.8)	0		41 (9.6)
MDR		30 (7)	14 (3.3)	199 (46.5)	7 (1.6)		250 (58.4)

Table 3. Resistance profile, efflux genes and *intI1*of *Salmonella* isolates isolated when three types of biocides were used.

Type of sample	*Salmonella* resistance pattern	Classes of biocides used for disinfection of swine barns, [n (%)]					Detection of MDR efflux genes and class 1 integrons from selected *Salmonella* isolates								
		Biosentry	Synergize	Virkon-S	Hot water	Total	Biosentry [n = 348]			Synergize [n = 428]			Total [n = 776]		
							qac	*qacEΔ1*	*intI1*	*qac*	*qacEΔ1*	*intI1*	*qac*	*qacEΔ1*	*intI1*
Swine feces	Pansusceptible	18 (0.4)	8 (0.2)	29 (0.6)	34 (0.8)	89 (2)									
	Resistant	1260 (28)	970 (21.5)	1066 (23.7)	1119 (24.8)	4415 (98)	30 (8.6)	26 (7.5)	15 (4.3)	35 (8.2)	31 (7.2)	9 (2.3)	65 (8.4)	57 (7.3)	24 (3.1)
	MDR	984 (21.8)	754 (16.7)	882 (19.6)	808 (17.9)	3428 (76.1)	30 (8.6)	26 (7.5)	15 (4.3)	31 (7.2)	29 (6.8)	7 (1.8)	61 (7.9)	55 (7.1)	22 (2.8)
Swine barns	Pansusceptible	47 (2.9)	43 (2.6)	15 (0.9)	20 (1.2)	125 (7.7)									
	Resistant	401 (24.6)	287 (17.6)	229 (14.1)	586 (36)	1503 (92.3)	79 (22.7)	77 (22.1)	60 (17.2)	63 (14.7)	53 (12.4)	32 (7.5)	142 (18.3)	130 (16.8)	92 (11.9)
	MDR	195 (12)	142 (8.7)	169 (10.4)	330 (20.3)	836 (51.4)	76 (21.8)	74 (21.3)	60 (17.2)	58 (13.6)	48 (11.2)	29 (6.8)	134 (17.3)	122 (15.7)	89 (11.5)
Swine feed	Pansusceptible				3 (10)	3 (10)									
	Resistant		9 (30)	6 (20)	12 (40)	27 (90)									
	MDR		6 (20)		6 (20)	12 (40)									

Table 4. Characterization of the patterns of the associated gene cassettes from swine barns pre and post-disinfection

Biocide	MIC (ug/ml)	[n (%)] *Salmonella*	*qac*[a]	*qacE1*[a]	*qacEΔ1*[a]	*intI1*[a]	Cassette pattern [n]	R-type
Biosentry	0 - 20							
	40	1 (0.3)	+	−	+	+	1,1.2 kb [1]	AmStTeAxChKm
	80	39 (11.2)	+	−	+	+	1 kb [21]; 1,1.2 kb [7]; 1, 1.5 kb [1]; 1,1.2, 1.5 kb [10]	AmClStSuTe/Ax/An/Ch/Gm/Km
		3 (0.9)	+	−	+	+	1 kb [2]; 1,1.2 kb [1]	AmStTe/Ax/Ch/Gm/Km/Su
		28 (8)	+	−	+	+	1 kb [27]; 2 kb [1]	StSuTe/Gm/Km
	160	1 (0.3)	+	−	+	+	1 kb [1]	AmClStSuTe
		3 (0.9)	+	−	+	+	1 kb [3]	AmStSuTeKm/Ax/Gm
	320							
Synergize	0 - 80							
	160	14 (3.3)	+	−	+	+	1 kb [6]; 1.2 kb [1]; 1.5 kb [2]; 1,1.2 kb [2]; 1,1.2, 1.5 kb [3]	AmClStSuTe/Ax/Ce/Ch/Gm/Km/XNL
		3 (0.7)	+	−	+	+	1 kb [3]	AmStSuTe/Ax/Ce/Ch/Gm/Km/XNL
		1 (0.2)	+	−	+	+	1 kb [1]	AmStSuTeKm
		7 (1.6)	+	−	+	+	1 kb [7]	StSu/Te
	320	6 (1.4)	+	−	+	+	1 kb [6]	StSuTe/Ax/Am/Ch
		2 (0.5)	+	−	+	+	1 kb [2]	StSu
	330	5 (1.2)	+	−	+	+	1 kb [2]; 1,1.2,1.5 kb [3]	AmClStSuTe
		3 (0.7)	+	−	+	+	1 kb [3]	StSu/Am/Su/Te

+

(plus), positive result; - (minus), negative result; Ax, amoxacillin-clavulanic acid; Am, ampicillin; Cl, chloramphenicol; CIP, Ciprofloxacin; An, amikacin; Gm, gentamycin; Km, kanamycin; S, streptomycin; Su, sulfisoxasole; TMP, trimethoprim; Te, tetracycline; XLN, ceftiofur; Ce, ceftriaxone; Cf, cephalothin