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Complete List of Authors:	Espinosa-Gongora, Carmen; University of Copenhagen, Department of Veterinary Disease Biology Damborg, P; University of Copenhagen, Department of Veterinary Disease Biology Nielsen, Soren; University of Copenhagen, Department of Large Animal Sciences; Gibbs, Shawn; University of Nebraska Medical Center, Department of Environmental, Agricultural & Occupational Health Guardabassi, Luca; University of Copenhagen, Department of Veterinary Disease Biology
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Effect of a disinfectant powder on methicillin-resistant *Staphylococcus aureus* in pigs, bedding and air samples under simulated farm conditions

Carmen Espinosa-Gongora[1*], Peter Damborg[1], Søren Saxmose Nielsen[2], Shawn Gibbs[3], Luca Guardabassi[1]

[1]*Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.*

[2]*Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.*

[3]*Department of Environmental, Agricultural & Occupational Health, College of Public Health, University of Nebraska Medical Center, USA.*

***Corresponding author:** Mailing address: Department of Veterinary Disease Biology, University of Copenhagen, Stigbøjlen 4, Frederiksberg C, 1870, Denmark. Phone +45 35333742. E-mail ceg@life.ku.dk

Summary

Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) is an emerging zoonotic agent that can be transmitted to people exposed to contaminated farms. This study was performed to evaluate the efficacy of a commercial farm disinfectant in reducing LA-MRSA contamination under controlled experimental conditions. Treatment and control group were both composed by four pigs naturally colonized with LA-MRSA clonal complex CC398. The animals were housed for 37 days (day -7 to day 30) into two separate farm-style chambers (Danbox Danmark ApS) designed for evaluation of farm decontamination technologies. The treatment group received one daily application of the disinfectant on days 1, 2, 3, 7, 10, 13 and 16. MRSA load was measured in samples from pigs, bedding material and air on days 1, 6, 9, 12, 15, 18, 23 and 30. MRSA concentrations were determined by selective culture and analysed statistically. While pig samples yielded variable MRSA counts and remained positive throughout the study, the amount of MRSA in the air and bedding material increased significantly during the first week and then was gradually reduced during the following weeks in both groups. A significant difference in the MRSA air load was observed between the two groups after six applications of the product (day 15), and MRSA couldn't be isolated from air and bedding material after seven applications (day 18). The load of MRSA increased immediately after discontinuation of treatment (day 23 and 30). This experimental study suggests that this type of disinfectant is not able to eradicate LA-MRSA from animals but continued application might be able to reduce the load of LA-MRSA in the farm environment and ultimately to minimize the risk of zoonotic transmission. This hypothesis shall be further evaluated by farm trials where the product is applied for longer periods, alone or in combination with other control measures.

Keywords

Swine, MRSA, decolonization, decontamination, eradication.

Introduction

Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) belonging to clonal complex (CC) 398 have emerged and spread recently in Europe, North America and Asia (Huijsdens et al., 2006; Smith et al., 2008; Yu et al., 2008). Within pig farms, LA-MRSA can be isolated from animals as well as dust, surfaces, feed and air (Friese et al., 2012). Various studies have shown that people exposed to livestock have an increased risk of becoming colonized and infected with LA-MRSA (van Loo et al., 2007; Lewis et al., 2008; Graveland et al., 2011). The frequency of human cases of LA-MRSA infection and carriage has increased over the last years (Van Cleef et al., 2011). Thus, effective intervention strategies are urgently needed to prevent further spread of LA-MRSA in the human population. In order to minimize human-to-human spread, pig farmers in certain countries are screened for MRSA upon admittance to hospitals and positive individuals are quarantined and decolonized (Dutch Working Group on Infection Prevention [WIP], 2007). Very little is known about how to reduce the burden of LA-MRSA at the farm level. Recently, one experimental cohort study showed that the combined use of UVA-activated photocatalytic paint, air purification and charged electrochemical solutions reduced levels of MRSA in pigs and their environment significantly (Giotis et al., 2011a).

The present experiment was undertaken to investigate whether a commercial farm disinfectant product (Stalosan®F, Stormøllen A/S, 4682 Tureby, Denmark) may be used to reduce the burden of MRSA in the farm environment. Stalosan®F is a disinfectant powder containing phosphates, clay, and iron compounds, and has been used in farms for many years against bacteria, fungi, viruses and parasites. The objective was to evaluate the short-term effect of Stalosan®F against LA-MRSA under simulated farm conditions.

Material and methods

Pigs and facilities

Eight pigs of six weeks of age were purchased from a Danish pig farm known to be contaminated with LA-MRSA (Espinosa-Gongora et al., 2011; Broens et al., 2012). Pigs were divided into two groups of four pigs (treatment and control group) and each group was housed in a farm-like chamber (Danbox Danmark ApS) designed for evaluation of farm decontamination technologies. Each Danbox was equipped with independent ventilation and heating systems and was designed as a biosecurity class II facility (Giotis et al., 2011b). The floors of the pig rooms had a surface area of 5.7 m^2 and were periodically covered by straw and wood bedding. The pigs were fed twice a day (*Grisette*, DLG Service A/S, Copenhagen, Denmark) and had *ad libitum* access to water. Relative humidity was measured daily at the same time in the pig rooms.

Study design

Upon arrival, pigs were individually ear-tagged and acclimatized for one week (day -7 to day 0). In the treatment group, Stalosan®F was applied on days 1, 2, 3, 7, 10, 13 and 16 using the dosage recommended by the manufacturer (50g/m^2). At first application, 90g of the product were spread on the floor of the pig room and 90g in the air with the aid of a bellow; in the second and third applications, 45g of Stalosan®F were spread on the floor and the rest in the air; and in the remaining applications 280g of product were spread in the air..

Samples were taken from air, bedding material and pigs on days 1 (just before the first treatment), 6, 9, 12, 15, 18 (end of treatment), 23 and 30 at the same time of the day (9:00 a.m.). An air sampler (Sampl'air Pro, AES Chemunex, France) was used in combination with MRSA-selective agar plates (Brilliance MRSA2, Oxoid, UK) for duplicate sampling of 100, 200 and 500 L of air from each box. The sampler was placed 150 cm above floor level. As a control of the inflowing air, two additional samples of 1000 L were taken outside at the air entry point. Bedding samples

representing four different spots on the floor were taken in duplicate from each box. Pigs were sampled by rubbing the mucosa of the outer area of both nostrils with a dry cotton swab. Selected samples were confirmed to be CC398 MRSA at the beginning and at the end of the study as previously described (Stegger et al., 2010).

Sample processing

Air samples

The MRSA selective agar plates from air samples were incubated at 37°C and presumptive MRSA colonies were counted 24h later. Counts of colonies were adjusted by the positive-hole correction table provided by the manufacturer, and a final average colony forming units per cubic meter (CFU/m^3) was calculated for each sampling moment.

Bedding samples

The weight of each bedding sample was adjusted to 1g, followed by addition of 50ml of saline, mixing and filtering through sterile gauze: 1ml was centrifuged (10,000 rpm for 5 min), the supernatant was removed and remaining 100µl were plated onto Brilliance MRSA2 Agar plates. After overnight incubation at 37°C, colonies were counted to calculate the concentrations (CFU/mg) of presumptive MRSA.

Nasal swabs

Individual nasal swabs were vortexed for 30 seconds in 1ml of saline. After removal of the swab, the solution was centrifuged (10,000 rpm for 5 min), supernatant was removed, and the remaining 100µl were plated onto Brilliance MRSA2 Agar. MRSA counts (CFU/swab) were performed as described for other sample types.

Statistical analyses

Mean CFU/m^3 of air within treatment group at each treatment day was compared using the Mixed procedure in SAS (SAS Institute Inc., Cary, NC, USA). The model took the repeated measurements

into account through inclusion of an autoregressive type 1 correlation structure. Mean counts for each treatment group at each day of the treatment period were then compared by least square means, and p-values <0.05 were considered significant. The assumption of independent identically distributed Normal residuals was assessed by visual inspection of heteroscadiscity and qq-plots.

Results

During the first two weeks, a significant difference in the MRSA air load was observed between the two groups after six applications of the product (day 15) and MRSA could not be isolated from air and bedding material after seven applications (day 18). However, the load of MRSA increased immediately after treatment was discontinued (day 23 and 30), and the overall MRSA load was not significantly different between the two groups. Figure 2 shows the difference in least square means between the two groups. A similar pattern was observed in the amount of MRSA in air and bedding samples collected from the two groups, irrespective of treatment. The numbers of MRSA were low on day 1, increased significantly during the first week of the experiment, and decreased to the initial levels in the following weeks (Table 1, Figure 1).

The air samples taken outside the facilities were always negative for MRSA. Mean relative humidity was 73.5% (58-85%) in the treatment group and 75.9% (60-86%) in the control group with no statistical difference between the two groups. Pig samples remained positive throughout the study with variable MRSA counts.

Discussion

The difference observed in the MRSA counts of environmental samples between the treatment and the control group was not statistically significant, indicating a limited efficacy of Stalosan®F following a treatment period of 16 days. However, the lack of statistical significance could be

influenced by the limited number of pigs and observations or by the low levels of environmental contamination resulting from the presence of four animals in the chambers. The fact that MRSA was not isolated from the air and the bedding material in the treatment group on day 18 suggests that Stalosan®F may be able to reduce the load of MRSA in the farm environment. The later increase of CFU during the two post-treatment weeks illustrates that continuous treatment may be needed to obtain a long-term reduction in the numbers of MRSA.

Higher MRSA levels (over 600 CFU/m^3) were detected in the air of the farm supplying pigs to our study compared to those measured in the Danboxes during the experiment. Thus, our experimental setup never reached the same colonization pressure as in the supplier farm. This is probably due to multiple factors including low pig density, high hygienic standards and absence of risk factors known to be associated with presence and transmission of LA-MRSA in pig farms such as use of risk antimicrobials (i.e. β-lactams and tetracycline), mingling of animals or introduction of new positive pigs in the pen (Broens et al., 2011; Broens et al., 2012). Based on these considerations, future experiments involving decontamination of MRSA CC398 should take place on farms and have longer treatment periods. Other possible shortcoming of the study was the use of straw and wood bedding, which is not used in pig pens in several countries but was required for this study due to local regulations. It is likely that MRSA have survived better in this bedding material compared to on a raw surface, which is easier to clean. Our results may therefore underestimate the effect of Stalosan®F in farm environments with less or even no bedding material for pigs.

Counts of MRSA in piglets, and to some extent in bedding material, fluctuated throughout the study and did not decline as observed in the air samples. This could indicate that Stalosan®F has the most predominant effect on air. However, it may also reflect the lower reproducibility of MRSA counts in samples other than air. Nasal swabs are not easy to quantify as the numbers of bacteria present on the swab may be influenced by the sampling technique and by the presence of other material (e.g.

faeces) in the nostrils of pigs during sampling. The MRSA concentration in bedding material may also vary substantially depending on where this material is taken from. Thus, despite our attempts to standardize all sampling techniques, it appears that MRSA counts from air samples are the most reliable for this type of study. Moreover, air contamination is likely to play an important role in the zoonotic transmission of LA-MRSA to humans, since humans are expected to be colonized by airborne particles from heavily contaminated pig environments (Gibbs et al., 2004; Friese et al., 2012). Therefore, the level of air contamination may be regarded as an important parameter to evaluate the efficacy of control measures to prevent LA-MRSA transmission to farm workers and other people exposed to contaminated farms.

The worldwide spread of LA-MRSA in livestock has resulted in a public health concern, especially in large-scale pig-producing countries with a low prevalence of MRSA in the human population, such as Denmark and the Netherlands. These and other countries have experienced a recent increase in LA-MRSA infections amongst humans (Grisold et al., 2010; Hartmeyer et al., 2010; Schijffelen et al., 2010; Lozano et al., 2011). There are some recommendations that may be adopted to keep a low prevalence in the farm, such as prudent antimicrobial and zinc use (Broens et al., 2011; Cavaco et al., 2011), purchasing animals from MRSA-negative suppliers (Espinosa-Gongora et al., 2012), isolating and decolonizing pigs before entering the farm, and minimizing mingling of animals that would expose non-carriers to carriers (Broens et al., 2012). Stalosan®F is intended for continuous use in farms, thus the efficiency of this and similar environmental disinfectants against MRSA should be further studied by farm trials over long time periods. Since none of the currently available strategies against MRSA can completely eradicate this pathogen from pig farms, a combination of disinfectant and other measures to combat MRSA may be tried to reduce the load of bacteria to which humans are exposed. Such studies are needed to develop and implement effective programs for control of LA-MRSA in livestock production.

At the conditions tested, application of Stalosan®F did not eradicate LA-MRSA from pigs but could reduce the bacterial loads in the farm environment, contributing to minimize the transmission from the environment to farm workers. This product and other disinfectants with similar efficacy represent a possible inexpensive approach to be considered in the development of control programs of this zoonotic MRSA clone in pig farming.

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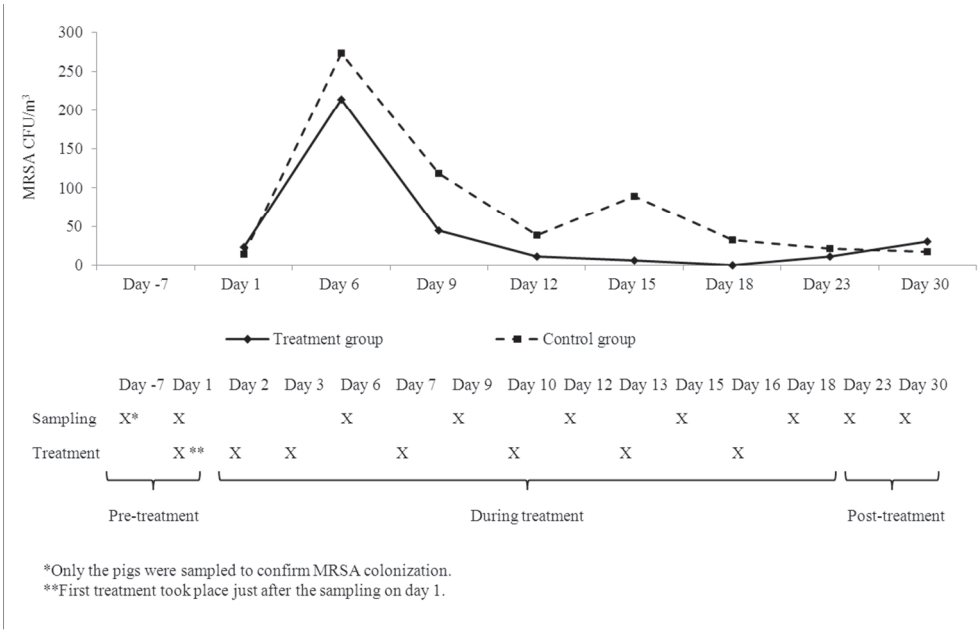
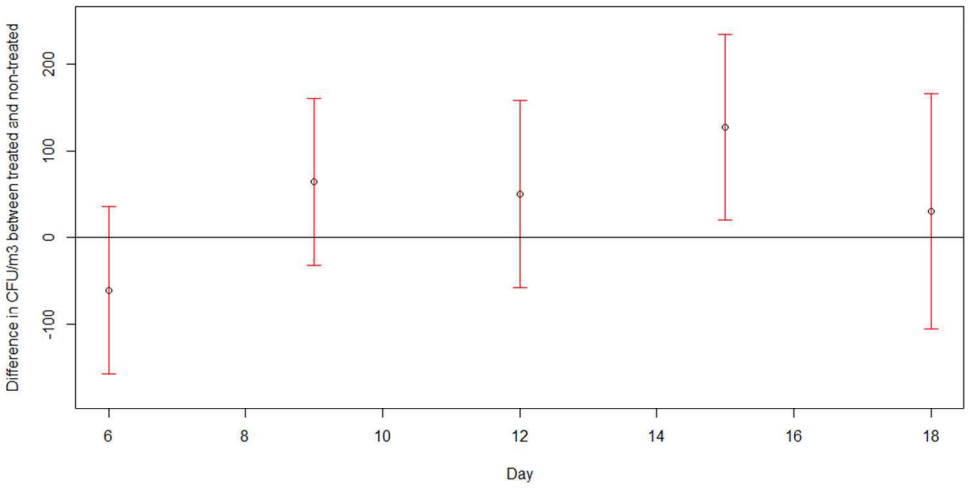
Table 1. Mean MRSA counts in Treatment and Control groups indicated as colony forming units (CFU) in nasal swabs of pigs (CFU/swab), in the air (CFU/m^3) and in the bedding material (CFU/mg) before, during and after treatment with Stalosan®F.

	Pre-treatment	During treatment					Post-treatment	
Pigs (CFU/swab)	Day 1	Day 6	Day 9	Day 12	Day 15	Day 18	Day 23	Day 30
Treatment group (n=4)	22	308	55	19	83	2	22	69
Control group (n=4)	13	48	84	50	25	44	20	1
%Reduction from day 1 in treatment group		-	82	94	94	99	93	78
Air (CFU/m^3)								
Treatment group (n=2-5)	23	215	44	11	6	0	11	30
Control group (n=2-6)	14	274	119	38	89	32	21	17
Reduction from day 1 in treatment group		-	80	96	98	100	96	90
Bedding (CFU/mg)								
Treatment group (n=2)	0.18	3.3	3.4	1.8	0.15	0	0.25	1.9
Control group (n=2)	0.70	1.5	2.9	2.1	1.35	5.6	2.8	1.0
Reduction from day 1 in treatment group		-	-3	45	95	100	92	42



Densities of LA-MRSA (CFU/m3) in the air of the farm-like chambers before, during and after application of Stalosan®F.
214x133mm (150 x 150 DPI)



Differences in least square means between Treatment and Control group at each day. Error bars indicates the 95% confidence interval at each time point.
277x158mm (96 x 96 DPI)