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EFFECT OF A DISINFECTANT POWDER ON METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN PIGS, BEDDING AND AIR SAMPLES UNDER SIMULATED FARM CONDITIONS

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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 conditions. Treatment and control groups were both composed of pigs naturally colonised with LA-MRSA. The animals were housed for 37 days in two separate farm-style chambers (Danbox Danmark ApS) designed for evaluation of farm decontamination technologies. The treatment group received seven applications of the disinfectant. Methicillin-resistant Staphylococcus aureus (MRSA) load was measured in samples from pigs, bedding material and air and analysed statistically. While pigs remained positive with variable MRSA counts, the amount of MRSA in the air and bedding material increased significantly during the first week and then was gradually reduced in both groups. MRSA could not be isolated from air and bedding material in the treatment group after seven applications and the load of MRSA increased immediately after discontinuation of treatment. This study suggests that this type of disinfectant is not able to eradicate LA-MRSA from animals but continued application might reduce the load of LA-MRSA in the farm environment and ultimately to minimise the risk of zoonotic transmission.

INTRODUCTION

Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) belonging to clonal complex (CC) 398 has emerged and spread recently in Europe, North America and Asia (Huijsdens *et al.*, 2006; Smith *et al.*, 2009; 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 colonised and infected with LA-MRSA (Graveland *et al.*, 2011; Lewis *et al.*, 2008; van Loo *et al.*, 2007). The frequency of human cases of LA-MRSA infection and carriage has increased over the last few years (van Cleef, 2011). Thus, effective intervention strategies are urgently needed to prevent further spread of LA-MRSA in the human population. In order to minimise human-to-human spread, pig farmers in certain countries are screened for methicillin resistant *Staphylococcus aureus* (MRSA) upon admittance to hospitals and positive individuals are quarantined and decolonised (Dutch Working Group on Infection Prevention, 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 Ultraviolet A (UVA)-activated photo-catalytic 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) could 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 on 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 (Broens *et al.*, 2012; Espinosa-Gongora *et al.*, 2011). 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 acclimatised for one week (day minus seven to day zero). In the treatment group, Stalosan®F was applied on days one, two, three, seven, 10, 13 and 16 using the dosage recommended by the manufacturer (50g/m^2). At first application, 90g of the product was spread on the floor of the pig room and 90g in the air with the aid of bellows; in the second and third applications, 45g of Stalosan®F was spread on the floor and the rest in the air; and in the remaining applications 280g of product was spread in the air.

Samples were taken from air, bedding material and pigs on days one (just before the first treatment), six, nine, 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 500L of air from each box. The sampler was placed 150cm above floor level. As a control of the inflowing air, two additional samples of 1,000L 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.*, 2011).

Sample processing

Air samples

The MRSA selective agar plates from air samples were incubated at 37°C and presumptive MRSA colonies were counted 24 hours 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 five minutes), 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 five minutes), 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 one, increased significantly

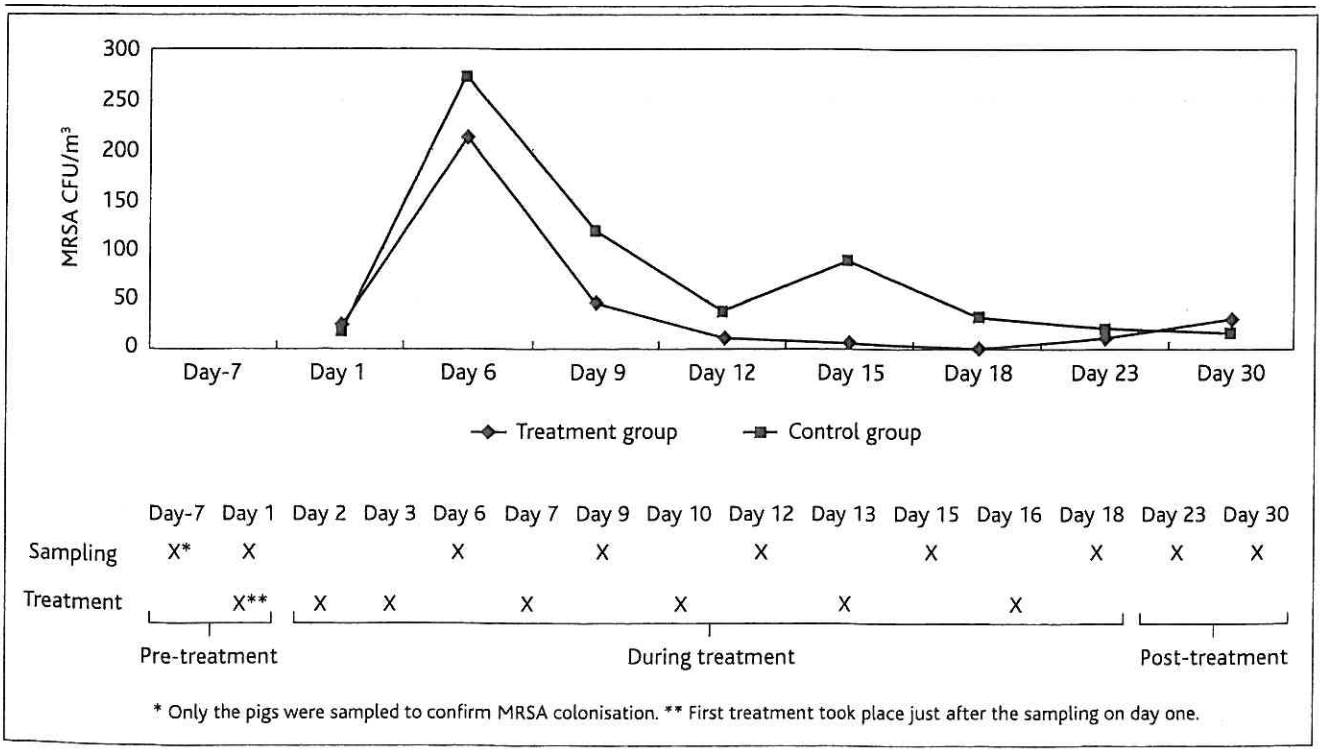
during the first week of the experiment, and decreased to the initial levels in the following weeks (Table 1, Figure 1).

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 (Figure 2).

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/m³) and in the bedding material (CFU/mg) before, during and after treatment with Stalosan®F*

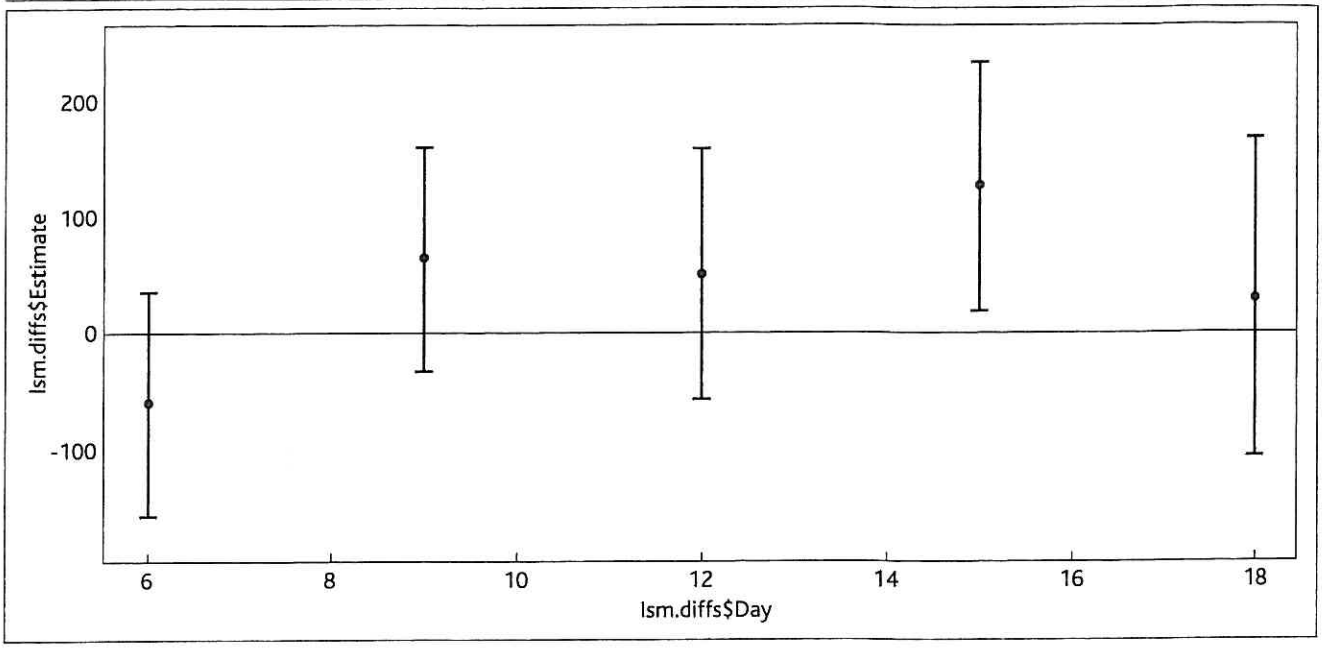
	Pre-treatment	During treatment					Post-treatment	
Pigs (CFU/swab)	**Day 1**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 23**	**Day 30**
Treatment group (n=4)	22	308	55	19	83	2	22	69
Control group (n=4)	13	48	84	50	25	44	20	1
% Reduction from day one in treatment group		–	82	94	94	99	93	78
Air (CFU/m³)								
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 one 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	9.5	100	92	42

Figure 1 – *Densities of LA-MRSA (CFU/m³) in the air of the farm-like chambers before, during and after application of Stalosan®F*



* Only the pigs were sampled to confirm MRSA colonisation. ** First treatment took place just after the sampling on day one.

Figure 2 – *Differences in least square means between Treatment and Control group at each day. Error bars indicate the 95% confidence interval at each time point*



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 colonisation pressure as on 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 the 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. Another 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 could have survived better in this bedding material compared with 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 standardise 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 colonised by airborne particles from heavily contaminated pig environments (Friese *et al.*, 2012; Gibbs *et al.*, 2004). 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 (Grishold *et al.*, 2010; Hartmeyer *et al.*, 2010; Lozano *et al.*, 2011; Schijffelen *et al.*, 2010). 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 decolonising pigs before entering the farm, and minimising 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 periods of time. 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.

Under the conditions tested, application of Stalosan®F did not eradicate LA-MRSA from pigs but could reduce the bacterial loads in the farm environment, minimising 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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