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Short communication

Comparison of sampling methods used for MRSA-classification of herds with breeding pigs

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ABSTRACT

Since the first report on methicillin resistant *Staphylococcus aureus* (MRSA) CC398 in pigs, several countries have determined the prevalence of MRSA-positive pig herds using different sampling and laboratory techniques. The objective of the study was to compare three sampling methods for MRSA-classification of herds. Therefore, nasal swabs of pigs and environmental wipes were collected from 147 herds with breeding pigs. Per herd, laboratory examination was done on 10 pools of 6 nasal swabs (NASAL), 5 single environmental wipes (ENVSINGLE) and one pool of 5 environmental wipes (ENVPOOL). Large differences in apparent prevalence of MRSA-positive herds between methods were found: 19.1% for ENVPOOL, 53.1% for ENVSINGLE, and 70.8% for NASAL. Pairwise comparisons of methods resulted in relative sensitivities of 26.9% (ENVPOOL vs. NASAL), 34.6% (ENVPOOL vs. ENVSINGLE), and 72.1% (ENVSINGLE vs. NASAL) with relative specificities of respectively 100%, 98.6% and 93.0%. Cohen's kappa was respectively 0.18, 0.32 and 0.55, thus varying between very poor and moderate agreement. Examination of environmental wipes is an easy and non-invasive method to classify herds for MRSA. The number of environmental wipes needed depends on e.g. required detection limits and within-herd prevalence. In low prevalent herds (e.g. herds with <3 positive pools of nasal swabs), 25 single environmental wipes are required to be 90% sure that MRSA is detected at a detection limit similar to analyzing 10 pools of nasal swabs. Individual analysis of environmental wipes is highly recommended, as pooling 5 environmental samples resulted in a substantial reduction of the apparent prevalence.

1. Introduction

A distinct clone of methicillin resistant *Staphylococcus aureus* (MRSA CC398) has emerged among pigs, veal calves, poultry and people in contact with livestock since 2005 (Voss et al., 2005; Graveland et al., 2008; Mulders et al., 2010). Several countries have determined national prevalences of MRSA-positive pig herds and a EU-wide baseline survey on MRSA-prevalence in herds with breeding pigs was performed in 2008 (Broens et al., 2008; Dewaele et al., 2008; Khanna et al., 2008; Smith et al., 2008; EFSA, 2009).

To determine the MRSA-status of pig herds, different sampling methods and laboratory techniques are used. A

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procedure using pre-enrichment in combination with selective enrichment for MRSA detection in nasal swabs from pigs was evaluated as good by Graveland et al. (2009). Several studies have also detected MRSA in dust from inside stables (Broens et al., 2008; Dewaele et al., 2008; Van den Broek et al., 2009). In a prior study, Cohen's kappa was 0.68, indicating a good agreement between MRSA-classification of 50 pig herds based on the results of either 10 pools of nasal swabs or 5 single environmental wipes (Cohen, 1960; Broens et al., 2008). Taking environmental wipes to determine herd status, might therefore be a feasible option to minimize animal handling. To reduce expenses further, environmental wipes could be pooled, but this might have an effect on the performance of the test method; especially if within-herd prevalence is low (Munoz-Zanzi et al., 2006).

The objective of this study was to compare three sampling methods for MRSA-classification of herds with breeding pigs. Herd classification was based on either 10 pools of 6 nasal swabs, or 5 single environmental wipes, or 1 pooled sample of 5 environmental wipes. To determine the feasibility of taking environmental wipes instead of nasal swabs for MRSA-classification of herds, the number of environmental wipes required to detect MRSA in a herd at a detection limit similar to taking nasal swabs, was calculated.

2. Materials and methods

2.1. Sampling and laboratory analysis

From January to December 2008, 147 herds were randomly selected out of the national database of herds with breeding pigs. Per herd, 60 pigs were sampled using nasal swabs (Medical Wire and Equipment, MW102, United Kingdom); pigs of each age group present (sows, gilts, suckling piglets, weaned piglets and finishing pigs) were randomly sampled. Additionally, 10 moist environmental wipes (Sodibox, s1 kit ringer solution, France) were taken from surfaces in farm sections, where also pig samples were taken. These environmental wipes were taken in pairs from adjacent surfaces to enable proper comparison between results of single and pooled analyzed environmental wipes.

All samples were immediately transported to the laboratory of the Dutch Animal Health Service. Samples were stored at 4 °C until processing, which occurred within 7 days after sampling. Laboratory examination took place on 10 pools of 6 nasal swabs, each pool containing swabs from only one age group and section (NASAL), 5 single environmental wipes (ENVSINGLE) and one pool of 5 environmental wipes (ENVPOOL).

Microbiological analysis was done as described in procedure 2 by Graveland et al. (2009). In brief, selective enrichment using Phenol Red Mannitol broth with 75 mg/l aztreonam and 4 mg/l ceftizoxime (PMB+; BioMérieux, NL020, France) was preceded by pre-enrichment using Mueller–Hinton broth with 6.5% NaCl (MHB+). A chromogenic MRSA screen agar (Oxoid, PO5196A, United Kingdom) was used for culture and confirmation of one suspected colony per sample was done using two PCR-tests for the *S. aureus* specific DNA-fragment (Martineau et al., 1998) and the *mecA* gene (De Neeling et al., 1998) respectively.

To ensure that all samples (swabs or wipes) were totally immersed in MHB+, different volumes of MHB+ were used for each method. Pooled nasal swabs were put into 10 ml MHB+, each single environmental wipe into 100 ml MHB+ and 5 environmental wipes were pooled into 600 ml MHB+; samples were stirred and shaken by hand before and after incubation to ensure proper homogenization.

2.2. Statistical analysis

For all methods (NASAL, ENVSINGLE and ENVPOOL) a herd was classified positive if at least one sample tested positive. Pairwise comparison of methods was performed; relative sensitivity and specificity, and Cohen's kappa (Cohen, 1960) were calculated. The association between the number of positive single environmental wipes or the number of positive pools of nasal swabs per herd and the percentage of herds with an MRSA-positive pool of environmental wipes was calculated using logistic regression. The probability of one (out of five) environmental wipe to be positive (=Prob) was calculated based on the number of positive pools of nasal swabs per herd; logistic regression with a random herd effect (PROC GLIMMIX; SAS Institute Inc., 2004) was performed. The probability to find at least 1 positive wipe (out of five) equals to $1-(1-\text{Prob})^5$. The number of wipes (n) needed to be e.g. 90% sure to find at least one positive environmental wipe, could then be solved from: $1-(1-\text{Prob})^n \geq 0.9$, yielding $n = \log(0.1)/\log(1-\text{Prob})$.

3. Results

3.1. Test evaluation

Apparent prevalences of MRSA-positive herds ranged from 19.1% for one pool of environmental wipes (ENVPOOL), and 53.1% based on 5 single environmental wipes (ENVSINGLE) to 70.8% for 10 pooled nasal swabs (NASAL) (Table 1). The combination of NASAL and ENVSINGLE showed the highest prevalence and resulted in three extra positive herds, i.e. 72.8%. By adding ENVPOOL, no extra herds were classified positive.

The relative sensitivity was 26.9% comparing ENVPOOL with NASAL, 34.6% comparing ENVPOOL with ENVSINGLE, and 72.1% comparing ENVSINGLE with NASAL. Relative specificity was respectively 100%, 98.6% and 93.0%. Cohen's kappa was respectively 0.18, 0.32 and 0.55 (Table 2), thus varying between very poor and moderate agreement.

3.2. Herd classification

On 107 farms, classified MRSA-positive by either NASAL and/or ENVSINGLE, on average, 31.2% (median = 20; Q1–Q3 = 0–40) of the 5 single environmental wipes and 62.4% (median = 70; Q1–Q3 = 50–80) of the 10 pools of nasal swabs tested positive.

The percentage of herds classified positive based on ENVPOOL increased with the number of ENVSINGLE wipes per herd ($P < 0.01$), with a probability of classifying a herd MRSA-positive based on the pool of environmental wipes equal to $1/(1+\exp^{(2.65-0.7847 \times \text{number of positive single wipes per}}$