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Livestock-associated methicillin-resistant *Staphylococcus aureus* in slaughtered pigs in England

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Abstract

This study was performed to investigate the occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in batches of pigs at slaughter and at different stages along the slaughter line. Nasal and ear skin swabs were collected from 105 batches of 10 pigs at six abattoirs. Cultures (pooled or individual) were performed for MRSA using selective media; presumptive MRSA were confirmed by *mecA* and *nuc* gene detection and a selection was *spa*-typed. MRSA was detected in 46 batches. All *spa*-types detected were those associated with LA-MRSA clonal complex 398. The proportion of positive batches varied among abattoirs (0–100%). Two abattoirs were subsequently further investigated, with samples taken at post-stunning, chiller and either at lairage or post-singe. Results suggested cross-contamination occurred between the lairage and point of post-stunning, but the slaughter processes appeared effective at reducing contamination before carcases entered the chiller. One abattoir provided only negative samples in the initial study and in the subsequent study along the slaughter line (26 batches in total), suggesting differences possibly in the MRSA status of pigs on arrival from supply farms or in its abattoir practices affecting the MRSA status of pigs at the sampling points. This study highlights that in the investigated abattoirs, MRSA was detected in 43.8% of batches of pigs at slaughter using sensitive selective culture methods.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious human pathogen, resistant to most beta-lactams and frequently resistant to multiple other antibiotics. The bacteria can cause skin and wound infections, abscesses or joint infections, endocarditis, pneumonia and bacteraemia [1]. Strains of MRSA can be divided into three broad categories based on their epidemiological and molecular characteristics, namely healthcare-associated MRSA, community-associated MRSA and livestock-associated MRSA (LA-MRSA) [2]. LA-MRSA has been detected in pigs, poultry and other farm animals in many countries globally, and persons in prolonged close contact with colonised animals have an increased risk of being infected or colonised with LA-MRSA [2, 3].

LA-MRSA belonging to multi-locus sequence types within clonal complex (CC) 398, was first described in pigs, farm workers and veterinarians in the Netherlands in 2005 [4]. It has been reported from pigs or pig farms from a number of countries in Europe, the Americas and Asia [2, 5–7], as well as from a wide range of food-producing and other animal species from studies in Europe [8, 9]. A European baseline study was performed in 2008 to investigate the occurrence and diversity of MRSA in breeding pigs on breeding and production holdings, through the examination of environmental samples [5]. The reported prevalence at the herd level of MRSA belonging to CC398 varied between the different European countries, ranging from 0% to 46% for breeding holdings and 0% to 50% for production holdings; MRSA was not detected on any UK pig holdings in the 2008 baseline study.

Numerous European and North American surveillance and research studies have described the occurrence of MRSA at slaughter [9, 10], from pigs on arrival [11, 12], after stunning [13–15] and on carcases during chilling [16, 17]. The majority of the studies performed in European countries reported data on the occurrence of MRSA from nasal swabs along the slaughter process, where the prevalence ranged from 0% in Ireland to 49–71% in Germany and 99.5% in Netherlands [14, 18, 19].

The MRSA prevalence in pigs at slaughter in the Netherlands has been found to be higher than that detected when pigs are sampled on farms [11] and cross-contamination between animals during transport to the abattoir or in the slaughterhouse lairage may account for the higher holding level prevalence observed in pigs at slaughter. However, a comparison between prevalence in another European country (Italy) did not find any significant difference between

holding prevalence estimated from slaughtered pigs and that estimated from environmental samples from production holdings [5, 20].

The relative sensitivity of different sampling sites in pigs and on farms has been investigated [21–23]. These investigations found that sampling both nasal swabs and ear skin swabs had a higher relative sensitivity (98.2%) than sampling either site alone, or sampling perineum, or other combinations of these three sites. A relative sensitivity for ear skin swabs of 90% was detected when compared against sampling air filters (78%), dust (43%) and nasal swabs (78%) [22].

In the UK, LA-MRSA CC398 was first identified from a food-producing animal from a poultry holding in 2013 through scanning surveillance of clinical diagnostic material and additional cases were detected through scanning surveillance after 2013 [24, 25]. LA-MRSA was first detected in the UK in pigs in 2014, in Northern Ireland and also in England [26, 27]. Up to October 2017, scanning surveillance had detected 16 occurrences of MRSA CC398 across the UK, with the majority (11) from pigs, and single occurrences in turkeys, poultry, pheasants, beef cattle and dairy cattle. However, these results are a likely underestimate of the true occurrence of LA-MRSA as UK scanning surveillance focuses on clinical disease incidents in livestock and LA-MRSA rarely causes animal disease [2]. The prevalence of MRSA in UK pigs has not been determined since the European Food Safety Authority (EFSA) baseline survey of 2008 [5], either through the investigation of the farms of origin or through investigation of pigs at slaughter. This study was performed to investigate the occurrence of MRSA in batches of pigs at selected English abattoirs. The study also investigated the occurrence of MRSA at different stages along the slaughter line.

Methods

Sample collection

As the MRSA batch level prevalence was unknown in England, a sample size of 90 was selected which would detect a 5% prevalence with 4.5% precision and 95% confidence but could also detect a 30% prevalence with at least a 10% precision. The sample size was increased by 15–105 batches, to allow for individual samples to be collected from additional batches, to provide a limited investigation of within-batch prevalence.

A convenience sample of six abattoirs (designated A–F) was selected for inclusion in the prevalence study. Each was sampled between January and February 2016, with between 15 and 20 batches to be sampled per day. All were standard electric stunning plants, apart from abattoir D which used gas stunning. The 105 sampled batches came from 100 unique farms. These batches consisted of 89 batches of finisher pigs, 15 sow batches and a single batch which contained a mix of boars and sows. Sow and mixed sow/boar batches were only sampled at abattoirs A and E.

On each sampling day, nasal and ear skin swabs were collected, with swabs from 2–3 batches of pigs per day cultured individually; swabs from the remaining sampled batches from that day were pooled to create a single pooled sample for ear skin swabs and one for nasal swabs per batch. For each sampled batch, 10 pigs from the same batch were sampled post-stunning by collecting both nasal and ear skin swabs (swabs of the skin fold caudal to the base of the pig's ear) from each pig. Ear skin swabs were collected to optimise sensitivity of detecting MRSA on each carcase and to allay concerns about any potential adverse effect on the recovery of MRSA of blood present on the nose after slaughter. The method used to sample the skin behind the ear was as previously described [22], with a single dry swab used to swab the skin behind both ears over a width of approximately 2 cm over the entire length of the skin where the ear joins the body. Both nostrils of the selected pig were swabbed using a single dry swab. The edges of the nostrils were swabbed and the swab was inserted into each nostril to a depth of 2.5–5 cm and then rotated. Charcoal transport swabs were used to ensure optimal conditions for bacterial survival and samples were transported at ambient temperature and were processed on the day of receipt (which was the day after sampling).

For the investigation of the occurrence of MRSA at different stages of the slaughter line, two abattoirs were visited: a new gas stunning plant (abattoir G) in December 2016 and abattoir D in June 2018. A different sampling procedure was used than in the prevalence study. On each sampling visit, samples were to be taken from three different points along the slaughter line. From the two abattoirs, pigs originating from 10 and 11 different batches were identified, with 10 pigs selected from each of these batches for sampling. Pigs were swabbed at the following locations in the abattoir: lairage – ear swab only; immediately post-stun – ear and nasal swab; and in the chiller – ear and nasal swab. However, at abattoir D, samples could not be collected as planned from the lairage and samples were collected at post-singe instead. Both a pooled ear and a pooled nasal sample were collected from this location, meaning that six pooled samples were collected per batch rather than five. The swabs collected from each anatomical site and abattoir location were pooled for testing for each batch of pigs and no individual swabs were cultured for MRSA. Although 10 pigs from each batch were sampled at each sampling site in the abattoir, these were not necessarily the same individual pigs at each point.

Sample testing

Culture of swabs was performed in accordance with the EU Reference Laboratory for Antimicrobial Resistance protocol for MRSA isolation recommended at the time of the study. This comprised of culture of 10 swabs into 100 ml of Mueller–Hinton broth (BPU Media) with 6.5% NaCl (16–20 h incubation at 37 °C) for pooled samples and one swab into 10 ml for individual swabs. Then 1 ml of enriched broth was inoculated into 9 ml of tryptone soya broth (BPU Media) plus 3.5 mg/l cefoxitin and 75 mg/l aztreonam (16–20 h incubation at 37 °C), followed by plating onto Oxoid Brilliance™ MRSA agar (24–48 h incubation at 37 °C). The media would have inhibited the growth of sensitive *S. aureus*. The media was subject to quality control before use to indicate that all stages could support the growth of MRSA for each batch of samples tested. Controls included an MSSA strain, MRSA strain and a negative control (reference strains – MSSA (ATCC 2913) and MRSA (NCTC 13142)).

Suspect MRSA isolates (up to three per culture plate) were examined by a multiplex polymerase chain reaction [28] to confirm bacterial identification (*S. aureus*) and confirm methicillin resistance by the detection of the *mecA* gene. A selection of confirmed MRSA isolates was then typed by *spa*-typing [29]. DNA sequences were analysed and *spa* types assigned using BioNumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium).

The numbers of isolates from pooled or individual samples that were subject to confirmatory molecular testing was agreed prior to the commencement of the study. The plan allowed for

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definitive confirmation of MRSA presence on at least one randomly selected sample (either pooled or individual) from each batch of pigs, which had been sampled and yielded suspect MRSA colonies on selective culture. Where multiple suspect MRSA isolates were detected in a batch, then not all isolates were subjected to confirmatory molecular testing. However, in the study of samples along the slaughter line at the two abattoirs, all suspect samples were sent for confirmatory testing.

Data analysis

Descriptive statistics provided the estimates of the prevalence of MRSA in the sampled pig batches. Summaries were produced for each of the sample types for confirmed MRSA presence and for the *spa*-types detected. A descriptive analysis was also completed to assess differences in the presence of MRSA by the hour in which samples were collected at each abattoir, to explore possible cross-contamination at slaughter, in particular related to contamination from those pigs which had been previously slaughtered. Chi-squared tests were used to compare results between selected categories of samples and batches or a McNemar's test if comparing sample results from the same carcase.

Prevalence was determined by two methods: (1) a summary of all results at the batch level, from both the pooled and individual samples and taken from both nasal and ear sample types, where any positive result indicated a positive batch; (2) prevalence indicated by pooled samples consisting of only nasal swabs and made from 10 swabs per batch. Analysis was conducted in Microsoft Excel 2013 and 95% confidence intervals (95% CI) were produced using the confidence command. A kappa test to determine agreement between the ear and nasal results from the same carcase was conducted in Minitab 16.

Results

In total, 479 samples were cultured from the 105 batches, of which 300 were cultured as individual samples (from 15 batches) and 179 were cultured as pooled samples (from 90 batches) (Table 1). One pooled sample of nasal swabs was missing, giving a total of 240 ear samples and 239 nasal samples for investigation. Confirmatory testing indicated that 67 (14.0%) individual or pooled swab samples were MRSA positive (Table 1). Overall, the ear samples (both pooled and individual) had a lower prevalence (31/240, 12.9%) detected than the nasal swabs (36/239, 15.1%) but the result was not significant when comparing results from the same carcase ($P_{\text{McNemar's}} > 0.05$) and indicated a fair-to-moderate agreement (0.39 kappa statistic). No difference in MRSA detection was detected from the sampled batches that were identified as being from the same herd (five batches were from herds already sampled within the population). Of the 15 batches that were investigated by individual sampling, four had negative MRSA results, whereas the others had between 1 and 14 (mean 5.8, standard deviation 5.1) of their 20 individual samples presumptive positive, with at least one per batch confirmed as MRSA.

Spa-typing was performed on 46 MRSA isolates, with the remaining 21 confirmed positives not *spa*-typed. The most frequent *spa*-type detected was t011 (32 isolates; 69.6% of typed isolates). Four other *spa*-types were identified: t034 (six isolates; 13.0%) and t4838 (six isolates; 13.0%), with single isolates of t108 and t2346 (2.2% each). These *spa*-types are all associated with LA-MRSA CC398.

Table 1. Batch-level results of selective culture for MRSA by sample type collected from slaughtered pigs

Sample type	Number of batches	Number of batches from which MRSA confirmed (% of those tested)
Individual nasal swabs	15	11 (73.3)
Pools of nasal swabs	89	25 (28.1)
Total for nasal swabs[a]	104	36 (34.6)
Individual ear skin swabs	15	5 (33.3)
Pools of ear skin swabs	90	26 (28.9)
Total for ear skin swabs[a]	105	31 (29.5)
Total for all nasal and ear skin swabs[a]	105	46 (43.8)

[a]Result for all samples, including both pooled and individual samples.

Batch prevalence results from sampled abattoirs

The overall batch-level prevalence of MRSA, utilising results from all sample types and from all abattoirs sampled, was 43.8% (95% CI 33.1–54.5). The proportion of MRSA positive batches varied among abattoirs. Abattoir D had no positive batches, whereas abattoir F had all batches positive, although only four batches could be collected on this sampling day (Table 2). Abattoir E was sampled on two separate days for convenience, with 37.5% of the 16 batches positive on the first day, and 60% of 10 batches on the second, giving an overall proportion of 46.2% MRSA positive batches of pigs at this abattoir.

Batches of sows or sows and boars (3/16 positive batches) were less likely to be MRSA positive than batches of finisher pigs (43/89 tested) which was statistically significant ($P_{\text{chisq}} = 0.03$). If these sow/sow and boar batches were removed from the population then the overall batch prevalence was 48.3%.

The available population for calculating prevalence based only upon pooled nasal swabs, consisting of 10 swabs per pool, was 83 batch samples. The prevalence of MRSA estimated from these samples, including all of the abattoirs, was 26.5% (95% CI 17.0–36.1) of batches of pigs MRSA positive. If only results from batches of finisher pigs were assessed then the prevalence was 31.0% (22/71 batches). Six pooled nasal samples were discounted from this analysis due to having less than 10 swabs within the pool. However, this had a limited effect on the overall estimate when compared to the total pooled nasal results, which had an overall prevalence of 28.1% (95% CI 25.6–33.4).

Results by hour of sampling

The samples were collected between hours 06:00 and 17:00. The proportion of MRSA-positive batches was highest (58.3–62.5%) from samples collected during the middle of the day (between 11:00 and 13:59), with 36% of all samples being taken in this 3 h period (Table 3).

The results of *spa*-typing from each abattoir by the hour of sampling are presented in Table 4. On each sampling day, a predominant *spa*-type was most frequently detected in each abattoir, although there was a little difference in the occurrence of two *spa*-types at abattoir B. *Spa*-type t011 appeared across the six sampling days in which MRSA was detected. *Spa*-types t011, t4838 and t034 were detected subsequently through the day after they were first detected in a batch of pigs, whereas t2346 and t108 did not

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Table 2. Confirmed MRSA batch-level results from all samples, by abattoir

Abattoir	No. of positive	No. of batches	% positive	95% CI	
A	8	20	40.0	18.0	62.0
B	5	20	25.0	5.5	44.5
C	17	19	89.5	75.3	100.0
D	0	16	0.0	–	
E	12	26	46.2	26.6	65.7
F	4	4	100.0	–	
Overall	46	105	43.8	34.3	53.3

Table 3. Confirmed MRSA batch-level results from all samples, by hour of sampling day

Time	No. of positive	No. of batches	% positive	% of total sampled batches
06:00	3	7	42.9	6.7
07:00	4	10	40.0	9.5
08:00	2	6	33.3	5.7
09:00	4	10	40.0	9.5
10:00	4	7	57.1	6.7
11:00	7	12	58.3	11.4
12:00	10	16	62.5	15.2
13:00	6	10	60.0	9.5
14:00	3	12	25.0	11.4
15:00	3	12	25.0	11.4
16:00	0	2	0.0	1.9
17:00	0	1	0.0	1.0

reappear. In abattoir E, which was sampled over 2 days, t011 was detected on both days.

Sampling along the slaughter line

In the follow-up investigation of samples collected along the slaughter line, MRSA was confirmed from abattoir G in all 20 suspect MRSA samples out of the 55 samples collected (36.3%), and the positive samples came from 10/11 batches (90.9%). Only 2/11 pooled (ear skin) swabs taken in the lairage were positive, and these batches also had positive results for both samples taken at post-stun but none of their chiller samples were positive. The proportion of positive samples was substantially higher at post-stun (17/22 samples, 77.3% positive (95% CI 59.3–95.2) than in the chiller (1/22 samples, 4.5% (0.0–13.5)) or lairage (2/11 samples, 18.2% (0.0–42.1)). Only one batch had both post-stun samples found to be negative and this batch also had negative lairage and chiller samples. Only one pooled sample (nasal swabs) was positive in the chiller and from that batch of pigs a nasal swab taken at post-stun was also positive.

At abattoir D, 10 batches were sampled. Eight additional individual swab samples were collected from three of the batches sampled in the chiller. All 68 samples (60 pooled and 8 individual swabs) tested MRSA negative.

Discussion

In this study, an overall MRSA prevalence at batch-level of 43.8% was detected from samples from batches of pigs at slaughter, combining all ear/nasal and pooled/individual swab results for each batch.

Samples from sow, or sow and boar, batches were significantly less likely to be MRSA positive when compared to finisher pigs. This may affect comparison with other studies which only sampled finisher pigs, although these only represented 15% of the batch population in this study. This difference may also have resulted in lower prevalence estimates from the abattoirs (A and E) from which sows or sows/boars were sampled. However, other studies have detected no substantial difference in the prevalence of MRSA in finisher pigs and sows, especially when from comparable farm types [2, 11]. The individual sample results, suggest a wide variation in the within-batch prevalence of MRSA, but this analysis used only presumptive positive results (where at least one confirmed MRSA was present in the batch) and a relatively small number of batches, and so was limited and requires further study to validate these findings.

When the results were compared to the relative estimates from a 2013 study in the Netherlands (testing pooled nasal swabs) [11], the results from the presented study indicated a much lower prevalence (26.5%) of MRSA, whereas the Netherlands study detected that 67% of breeding and 71% of finishing herds were positive. Although the same laboratory methods were used, the Dutch study used sampling on farm whereas this study sampled at the abattoir. However, Dutch nasal swab results from slaughtered fattening pigs, also from 2013, indicated that 91/93 (97.8%) were positive for MRSA [8]. Comparison to batch-level prevalence, where nasal samples were collected at stunning, from a slaughterhouse survey in Germany also indicated a greater prevalence than this study, with 71% (56 out of 79 herd batches) detected in Germany [30].

Previous reviews have suggested that the public health risk from consumption, handling or preparation of foodstuffs contaminated with MRSA is very low [31]. People working in abattoirs are occupationally exposed to MRSA, when it is present in the livestock being slaughtered [15], and have been shown to have a greater prevalence than the general population, although those working with live pigs have been shown to be at greater risk [32]. The degree of exposure is likely to be dependent on numerous factors including food processing procedures and slaughterhouse hygiene practices, as well as the level of contamination of MRSA on carcases and the susceptibility of workers [33, 34].

No MRSA was detected in the UK pig population from the EFSA baseline study of 2008 [5] and so the results here imply that there was subsequent introduction of MRSA and spread within livestock. However, the baseline study differed greatly from the presented study and because of the limited available data it is difficult to draw formal conclusions. The baseline survey investigated environmental samples from pig herds selected according to the survey criteria, was performed a considerable time before this current study, was a farm-based (rather than abattoir based) study and used a laboratory method which had slight variation from procedures adopted later. National data on MRSA bacteraemia cases in people suggests that the case rate reduced from 2008 to the period of this study (5.6 cases per 100 000

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Table 4. *Spa*-type of confirmed MRSA samples by abattoir and time of sampling

Abattoir	*Spa*-type	6:00–6:59	7:00–7:59	8:00–8:59	9:00–9:59	10:00–10:59	11:00–11:59	12:00–12:59	13:00–13:59	14:00–14:59	15:00–15:59	16:00–16:59	17:00–17:59	Total
A	ND	1	2			5			1					9
A	t011	1	2		1				2	1				7
A	t2346					1								1
B	ND								1	2				3
B	t011								2					2
B	t034									2	1			3
C	ND	1	13	1	2	2	13	3			1			36
C	t011	3	2		1	1		2	1		1			11
C	t108						1							1
C	t4838			1	1	1	2	1						6
E day 1	ND			1	10		1	3	1					16
E day 1	t011				1			1	1					3
E day 1	t034			1			1	1						3
E day 2	ND							1						1
E day 2	t011					1	1	4						6
F	ND						14	1			3			18
F	t011						2				1			3

ND, not determined.

population to 1.5 [35]), which may indicate that any increase in MRSA in pigs was having little effect on the prevalence of symptomatic human cases reporting to health care agencies. However, the detection of MRSA in this study suggests that a national monitoring scheme would be useful in order to provide a standardised methodology from which to detect changes in this prevalence estimate over time.

The analysis of results obtained for batches of pigs slaughtered at different times at each abattoir showed that the predominant occurrence of positive batches was at midday, where most of the batches were sampled and which may have been a busy period for the abattoirs. No accompanying management information was collected from the abattoirs (e.g. cleaning or breaks in throughput) and so little could be inferred on the potential cause of variation in results. A predominant *spa*-type was detected in each abattoir (apart from abattoir B); the predominant *spa*-type was generally either t011 or t034, types which are both relatively frequently detected in herd level studies of pigs in Europe [10]. The detection of MRSA in batches of pigs may represent contamination from between batches, from other sources within the abattoir, during transport to the abattoir, or persistence of MRSA in the abattoir after initial introduction, rather than accurately reflecting the status of the farm of origin [11, 12, 15, 36]. In abattoir E, which was sampled over 2 days, t011 was present on both days possibly suggesting that batches may have been contaminated at the abattoir, which was acting as a reservoir, although t011 tends to be a frequently detected *spa*-type of MRSA CC398 in pigs in Europe and repeated introduction is equally possible. However, in abattoir C, there were consistent detections of a less common *spa*-type (t4838) from batches every hour over a 5-h period. *Spa*-typing alone does not conclusively resolve whether the occurrence was related to cross-contamination, persistence in the abattoir or reintroduction of a *spa*-type in different batches of pigs. This highlights a need to further explore this and to assess the effectiveness of cleaning strategies for reducing the cross-contamination of MRSA, potentially using whole-genome sequencing methods to provide further discrimination of isolates of MRSA.

All *spa*-types detected were those associated with LA-MRSA CC398. A previous study of LA-MRSA CC398 isolates from animals in the UK concluded that there have been multiple independent incursions of LA-MRSA CC398 into the UK, with isolates most closely resembling isolates from Europe [37]. The routes of dissemination of LA-MRSA to the UK are not clear but it could be speculated from the results of this study that other MRSA clones besides CC398 have either not yet reached the UK pig population or were not detected in our study. This study was not designed to be representative of all English pig herds or abattoirs, and was not designed to investigate the national diversity and occurrence of MRSA.

The main source of variation of MRSA results was found at the abattoir level, with wide variation in the prevalence results shown between the six sampled abattoirs. A German study of five abattoirs also showed variability of results between the plants, with prevalence ranging from 59 to 80% [18]. Other studies have shown that slaughter capacity and hygiene practices may affect MRSA occurrence at slaughter [36, 38]. Some of this variance in the results at post-stunning may be related to variability on the source farms or in transport and lairage, but in the study of abattoir G a large difference in detection occurred between lairage and post-stunning within the same batches, which supports an abattoir effect. Of specific interest, was that the samples from abattoir D were negative, which was also negative when followed up with an additional sampling visit collecting samples at three points on the line. The prevalence estimated in this study using pooled nasal samples was 28.1%, which indicates that the samples collected from the 26 batches from abattoir D would have been sufficient to detect at least a single MRSA-positive sample with at least 95% confidence.

In the prevalence part of the study, abattoir D was the only gas stunning plant sampled in the prevalence study but the results from the other gas stunning abattoir (abattoir G) showed similar MRSA results to the sampled electric stunning plants. Given the potential for cross-contamination at abattoirs, the results from abattoir D suggests that all contributing batches were MRSA negative on that day of sampling. At the follow-up visit, samples could not be collected in the lairage at abattoir D which meant it was not possible to confirm the status of the pigs entering the abattoir. Additional samples were collected post-singeing, and these may have been more likely to be negative due to the scalding/singeing process.

The results from the three different sampling locations collected at abattoir G suggest that slaughter processes were effective at reducing contamination before carcases entered the chiller. As few lairage samples were positive but almost all post-stunning samples were positive, this suggests that cross-contamination occurred between these stages. However, the same pigs from each batch were not sampled at each location and it was identified that sampling in the lairage was difficult and may have affected the sensitivity of detection and accuracy of these comparisons. There was also a lack of consistent sampling of each batch, with some variation in the number of samples collected at each point and numbers of swabs per pool. Reducing the number of swabs per pooled sample may have reduced the sensitivity of MRSA detection, although not enough data were available to quantify any effect.

The method of isolation used was that recommended by the EU reference laboratory for antimicrobial resistance at the time the study was instigated. Of the suspect colonies from primary cultures undergoing confirmation, 10.3% were found to be negative. Subsequent modifications to the method have been suggested [39] to reduce the proportion of false-negative samples and these were recently adopted by the EU reference laboratory [40]. Not all positive cultures were confirmed by molecular testing, which may have introduced bias, although at least one positive sample per batch was confirmed and used for batch prevalence estimation. A higher proportion of nasal sample isolates were subject to confirmatory testing. However, the moderate agreement between nasal and ear skin samples may indicate that any bias would have had little effect. A greater level of agreement (0.7; good agreement) had been indicated between these sample types from a comparative farm study [22]. However, a similar level of agreement (0.4; moderate) had been shown by a previous abattoir study which assessed individual pigs [23], and this may indicate the importance of collecting both sample types from pigs to maximise the ability to detect MRSA at slaughter.

This study indicated that in those abattoirs which were investigated, MRSA was overall detected in 43.8% of batches of pigs at slaughter using sensitive selective culture methods. However, it has highlighted that the occurrence of MRSA was variable, specifically between abattoirs, suggesting that a number of factors may influence the occurrence of MRSA in pigs at slaughter. Detailed further investigation of the factors related to cross-contamination at slaughter and examination of different abattoir management practices may hold the key to managing and controlling the

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occurrence of MRSA in pigs at slaughter and thereby assist in reducing any associated human occupational exposure.

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Conflict of interest. None.

Data availability statement. Anonymised data from this study are available on request from the authors.

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