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Communication

Detecting *mecA* in Faecal Samples: A Tool for Assessing Carriage of Meticillin-Resistant Staphylococci in Pets and Owners in the Microbiological 'Fast Age'?

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Abstract: Sampling animals for carriage of meticillin-resistant, coagulase-positive staphylococci (MRCoPS), considered zoonotic pathogens, can be challenging and time-consuming. Developing methods to identify *mecA* from non-invasive samples, e.g., faeces, would benefit AMR surveillance and management of MRS carrier animals. This study aimed to distinguish MRS carriers from non-carriers from faecal samples using quantitative polymerase chain reaction (qPCR) for *mecA*. Paired faecal and nasal swab samples (n = 86) were obtained from 13 dogs and 20 humans as part of a longitudinal study. Nasal MRCoPS carriage (either MR-*Staphylococcus aureus* or MR-*Staphylococcus pseudintermedius* was confirmed by identification of species (*nuc*) and meticillin resistance (*mecA*) (PCR). Faecal DNA (n = 69) was extracted and a qPCR method was optimised to provide a robust detection method. The presence of faecal *mecA* was compared between MRS carriers and non-carriers (Kruskal–Wallis test). Nasal swabbing identified seven canine and four human MRCoPS carriers. *mecA* was detected in 13/69 faecal samples, including four MRCoPS carriers and nine non-carriers. For dogs, there was no significant association ($p = 1.000$) between carrier status and *mecA* detection; for humans, *mecA* was more commonly detected in MRCoPS carriers ($p = 0.047$). *mecA* was detected in faeces of MRCoPS carriers and non-carriers by qPCR, but larger sample sizes are required to determine assay sensitivity. This rapid method enables passive surveillance of *mecA* in individuals and the environment.

Keywords: MRSP; MRSA; surveillance

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1. Introduction

A pivotal part of veterinary antimicrobial stewardship is the use of infection prevention and control measures [1]. Detecting asymptomatic carriers of multidrug-resistant (MDR) nosocomial pathogens, including meticillin-resistant, coagulase-positive staphylococci (MRCoPS) such as MR-*Staphylococcus aureus* (MRSA) and MR-*S. pseudintermedius* (MRSP), quickly and reliably is a cornerstone for infection control [2–4]. MRCoPS have acquired *mecA*, which confers resistance to all β-lactam antibiotics and is a marker for MDR, incorporating most other antimicrobial classes [5]. Subsequent infection relating to MRCoPS has both morbidity and economic costs [6,7], further driving the need to detect sources of infection, such as carrier individuals.

Although staphylococci are normally carried on the skin and mucosae of mammals, MRSA has been found in the faeces of both humans [8,9] and dogs [10]. No study has

yet described faecal recovery of MRSP, but the perineal mucosa has been described as a common carriage site of *S. pseudintermedius* in dogs [11,12]. Asymptomatic carriage of MRCoPS has been shown to last many months [13–15], presenting a transmission risk within both the household and hospital environments [16,17]. Human MRSA carriage is most commonly screened for by nasal swabbing, alongside variable other sites. This has been extrapolated into veterinary medicine for canine MRSP carriers but is not always well-tolerated and must be performed by a veterinary professional. Subsequent processing should include enrichment culture due to the low numbers of MRCoPS present in carriage sites [18,19], which extends standard identification by up to 48 h. The development of a rapid method to detect MRCoPS carriers, using samples that can be easily collected by owners, would be beneficial. This would allow veterinary clinics to more easily request pre-admission screening for carriage of these staphylococci, especially in the case of high-risk elective or non-emergency procedures, without delaying for multiple days. The ability for owners to take samples themselves further reduces the potential costs of sampling that are associated with veterinarian time and would open the study of MRCoPS carriage to researchers who are not closely linked with suitably equipped veterinary premises for sampling of large numbers of animals. Furthermore, a reliable method to quantify *mecA* in faecal samples could allow for passive surveillance of antimicrobial resistance (AMR) abundance outside of the veterinary clinic.

This study used quantitative PCR (qPCR) to identify *mecA* in faecal samples from a population of dogs and their owners as a proof-of-concept study to investigate a possible alternative to traditional detection methods from nasal swabs.

2. Materials and Methods

Owners and their dogs were recruited from February 2018 to April 2019 as part of a larger, multi-centre longitudinal project investigating the transmission of multidrug-resistant bacterial pathogens within households. Recruitment, sampling and handling of results were approved by the RVC's Clinical Research Ethical Review Board (CRERB; URN 2017 1750-3) and the University of Lisbon's Comissão de Ética a Bem-Estar Animal (CEBEA; 027/2018), and owners gave written consent at enrolment.

2.1. Study Design

For this study, 35 canine and 51 human faecal samples and their corresponding nasal swab results were included from 13 dogs and 20 humans (one sample n = 1 dog, n = 2 humans; two samples n = 5 dogs, n = 8 humans; three sample n = 4 dogs, n = 7 humans; four samples, n = 3 dogs, n = 3 humans). Repeat samples were obtained with at least one month between sample dates. Faecal samples were grouped into those from MRCoPS nasal carriers or non-nasal carriers based on the detection of MRCoPS on nasal swabs. Bacterial culture was used to isolate MRCoPS from faeces, and a qPCR method was optimised to detect *mecA* in DNA extracted from faecal samples.

2.2. Isolation of Staphylococci

For nasal carriage sampling, a single dry, sterile swab (charcoal transport swabs, SLS, Nottingham, U.K.) was rolled on the inside of each nostril for 3–5 s. Dogs were swabbed by a veterinary surgeon; humans swabbed their own nostrils. Nasal swabs were enriched (37 °C/48 h) in 3 mL tryptone soya broth (CM0129; Thermo Fisher Scientific, Loughborough, U.K.) + 6.5 mg/L sodium chloride (Sigma-Aldrich Ltd., Gillingham, Dorset, U.K.).

A faecal sample was collected within 24 h of nasal swabbing and processed within 72 h of collection (transported at room temperature). Aliquots (0.5 g) of faeces were immediately frozen on receipt at the laboratory (maximum temperature −20 °C) for subsequent DNA extraction. A further 1 g was enriched (37 °C/24 h) in 10 mL peptone water (CM0009, Thermo Fisher Scientific) for bacterial culture.

Nasal swab and faeces enrichment broths were subsequently plated (100 µL) onto either mannitol salt agar (CM0085; Thermo Fisher Scientific) containing 6 mg/L oxacillin

(Sigma-Aldrich) (MS+) [20,21] or Oxoid Brilliance MRSA agar (PO5310, Thermo Fisher) at 37 °C for 48 h for selective growth of MR-staphylococci.

A minimum of four colonies of each distinct morphology (considering size, shape, colour and mannitol fermenting ability) were subcultured onto blood agar (CM0271; Thermo Fisher Scientific) containing 5% sheep blood (TCS BioScience, Buckingham, U.K.), and phenotypic identification of CoPS was made through a range of tests, including observation of characteristic colony morphology and haemolysis, slide coagulase testing, DNAse production and Voges–Proskauer test [22]. Species (thermonuclease *nuc*) and meticillin resistance (*mecA*) were confirmed by conventional PCR [23,24].

2.3. DNA Extraction from Faeces

DNA was extracted from frozen faecal aliquots ('faecal DNA') and subsequently confirmed for the presence of bacterial ribosomal DNA by PCR. Faecal DNA was extracted using a commercial kit (DNeasy PowerSoil Kit 100, Qiagen, Hilden, Germany) following the manufacturer's protocol with the exception of undertaking the final elution step in two 50 µL (cf., one 100 µL) volumes to thoroughly elute DNA from the column. DNA quantity (ng/µL) and purity (ratio of absorbance at 260 nm and 280 nm wavelength) were determined (Qubit 4 Fluorometer, Thermo Fisher Scientific; DeNovix DS-11, DeNovix Inc., Wilmington, DE, USA). Samples were excluded from analysis if <1 ng of DNA would be available for qPCR. The presence of bacterial DNA within the faecal extractions was confirmed through 16S PCR [25]; the absence of a positive band on 16S PCR excluded the sample from further analysis.

2.4. qPCR Method

A qPCR method [26] to detect *mecA* was optimised for its use for faecal DNA. Three changes were made to the method: (1) the primer concentration was reduced to 0.25 M (cf., 0.5 M) to reduce primer dimers (primer manufacturer Eurofins Genomics, Wolverhampton, U.K.), (2) 45 cycles (cf., 40) were undertaken to facilitate increased detection with lower DNA template concentrations and (3) the addition of a single step melt curve (65–95 °C, 0.5 °C every 5secimage q5sec) for confirmation of correct gene. A commercial mastermix (PowerUp™ SYBR™ Green Master Mix, Thermo Fisher Scientific) was used in a 20 µL reaction volume, analysed by the CFX96 Real-Time System C1000 Touch Thermal Cycler (Bio-Rad, Watford, U.K.). Correct melt curve temperature (75 °C) and subsequent 213 bp band on agarose gel electrophoresis (AGE) confirmed *mecA* detection.

To confirm that method optimisation resulted in the correct *mecA* PCR product, a previously characterised canine-derived MRSA (A16) [27] underwent the same amplification steps as for qPCR, but with a standard *Taq* PCR mastermix (*Taq* PCR core kit, Qiagen). After DNA clean-up (Monarch PCR & DNA Cleanup Kit, New England BioLabs, Ipswich, MA, USA), the product was sequenced (Sanger sequencing, Source BioScience, Nottingham, U.K.) and aligned to *mecA* from MRSA COL [28].

The concentration of extracted DNA from MRSA A16 (PurElute™ Bacterial Genomic Kit, Promega Southampton, U.K.) was measured (DeNovix DS-11) and dilutions were made ranging from 75.2 to 7.52×10^7 *mecA* copy numbers per reaction volume (cn), based on a single copy of *mecA* per bacterial genome. Triplicate assessment established a limit of detection (LOD) of 75.2 cn (Cq 34.67 ± 0.86), with the other remaining standards providing a quantifiable range from 188 to 7.52×10^7 cn. Extracted MRSP DNA (1726) [27] was quantifiable by this standard curve.

The blank faecal DNA extraction kit (using both PCR grade water and elution buffer), and extracted bacterial DNA, pure bacterial culture and a faecal sample spiked with *mecA*-positive DNA (all at the top and bottom of the standard range) were analysed to investigate extraction efficiency and potential for qPCR interference from faecal proteins.

For the faecal DNA, the detection of *mecA* above LOD—and quantification, where appropriate—was investigated in triplicate using 6 µL of DNA in each reaction to maximise the volume of DNA evaluated, alongside a standard curve and duplicate no template

controls (NTC) on every 96-well plate. Data were analysed using Bio-Rad CFX Manager (Bio-Rad) and Microsoft Excel 2016 (Microsoft, Reading, U.K.). All qPCR products underwent agarose gel electrophoresis (AGE) to confirm the presence/absence of correct size fragment.

2.5. Statistical Analysis

SPSS version 26 (IBM, Portsmouth, U.K.) was used to compare DNA quantity and quality (Kruskal–Wallis) and *mecA* detection (Fisher's Exact Test) between MRCoPS carriers and non-carriers.

3. Results

From the available 86 paired nasal and faecal samples, MRCoPS were isolated from 11 nasal samples (7 dog, 4 human) and 1 faecal sample (dog); this faecal carriage coincided with nasal carriage (Table 1). All MRCoPS isolated from dogs were confirmed as MRSP and all MRCoPS isolated from humans as MRSA.

Table 1. Results from faecal assessment through culture and qPCR detection of *mecA* with respect to meticillin-resistant, coagulase-positive *Staphylococcus* species (MRCoPS) nasal carrier status of 31 canine and 38 human samples.

Origin of Sample		Faecal Assessment	
Nasal Carrier Status	**Host Species**	**Number of MRCoPS-Culture-Positive Faecal Samples per Available Samples**	**Number of Samples with *mecA* Detected in Faecal DNA by qPCR per Available Samples (%)**
MRCoPS carrier	Dog	1/7	2/7 (28.6)
	Human	0/4	2/4 (50.0)
	Overall	1/11	4/11 (36.4)
MRCoPS non-carrier	Dog	0/24	7/24 (29.2)
	Human	0/34	2/34 (5.9)
	Overall	0/58	9/58 (15.6)

The DNA yield from 86 faecal samples ranged from 0.06 to 247.03 ng/µL. Of those, 17 were excluded from further analysis because of low levels of DNA (12/86 samples, 14.0%) or inability to detect 16S PCR product (5/86, 5.8%).

Faecal DNA subsequently used for qPCR represented 11 instances of MRCoPS carriage (7 canine, 4 human) and 58 instances of non-carriage (24 canine, 34 human) (Table 1). DNA extractions contained 25.23 ± 5.46 ng/µL DNA (mean ± SEM; range 0.18–247.03 ng/µL). All 69 extractions produced 16S PCR product indicating the presence of bacterial ribosomal subunits in the DNA. There was no difference in the quantity or purity of faecal DNA between MRCoPS nasal carriers and non-carriers (quantity $p = 0.611$; purity $p = 0.367$). Overall, 151.4 ± 16.5 ng (mean ± standard error) faecal DNA with purity of 1.62 ± 0.54 was used in each qPCR reaction.

The blank faecal extraction kit did not produce a signal on qPCR; however, the kit extraction resulted in up to a 3.71 $\log_{10}$ copy number reduction of *mecA* when used for known quantities of bacterial DNA, pure bacterial culture and *mecA*-spiked faeces. As a result, *mecA* detection above LOD is compared in all stated results, but *mecA* was not quantified. Primer efficiency was 97.1 ± 3.2% ($y = -3.305x + 40.84$, $r^2 = 0.9971$). NTCs showed late amplification curves (Cq 36.1 ± 1.9) with no detectable melt curve in the range for *mecA*.

Overall, *mecA* was detected in 13/69 faecal samples (n = 4 human, n = 9 canine), of which four were from MRCoPS carriers and nine from non-carriers (Table 1). All *mecA*-positive samples had correct melt curve temperature (qPCR) and band size (AGE). Overall, and for dogs alone, there was no association ($p = 1.000$ dog, $p = 0.199$ combined) between nasal carrier status and faecal *mecA* detection. For humans, *mecA* was more commonly detected in MRCoPS nasal carriers than non-carriers ($p = 0.047$).

4. Discussion

Faecal samples yielded detectable *mecA* using this optimised qPCR method. The association between *mecA* detection and MRCoPS carriage found in humans was encouraging for this novel method for carriage detection. Although not corroborated by the findings in dogs, or when dog and human results were combined, this may have related to low numbers within this study. This association should be investigated within a broader range of carriers in future. The detection of *mecA* in the faeces of non-carriers may be due to an increased sensitivity of the qPCR for detecting their presence, although one would expect it to have in this case detected all known MRCoPS carriers. This may be due to the need for sampling of multiple body sites to identify all potential MRCoPS carriers, as has been shown for both MRSA and MRSP [21,29]. It may also be due to the presence of meticillin-resistant, coagulase-negative staphylococci (MRCoNS) in these samples, which may have lower oxacillin breakpoints and therefore not have been detected on the 6 mg/L oxacillin-impregnated mannitol salt. However, this concentration of oxacillin has been shown reliable for previously detecting MRCoPS [20,21]. In fact, the lower concentrations of beta-lactam antimicrobial in the Oxoid Brilliance agar resulted in a number of non-*mecA* oxacillin-susceptible staphylococcal isolates being recovered (data not shown), which reinforces the importance of confirmatory testing of isolates through *mecA* PCR when using screening agars.

As for MRCoPS, MRCoNS have been described in faeces as well as the wider environment [30,31], and it is known that these were likely the precursor to acquisition of SCC*mec* in coagulase-positive species [32]. The method optimised in this study does not distinguish the origin of *mecA*, and confirmation of exactly which species was carrying it is beyond the capability of qPCR. However, the creation of a multiplex method, including the addition of a staphylococcal species-specific gene (e.g., thermonuclease, *nuc*) which could determine relative abundance of *S. aureus*, *S. pseudintermedius* and MRCoNS compared to *mecA* in the same samples would add evidence to support the presence or absence of MRCoPS. Nevertheless, the presence of *mecA* in faeces originating from any staphylococcal species is of concern due to the potential for horizontal gene transfer between species [33].

The presence of *mecA* in faecal samples indicates the utility of this method for surveillance of the spread of this significant resistance gene. The One Health importance of antimicrobial resistance, extending across human and veterinary healthcare into food production systems and the wildlife ecosystem, indicates the need for global monitoring of antimicrobial resistance [34]. In the case of meticillin resistance, more specifically, the presence of MRCoPS in other animal hosts may be linked to dissemination of this pathogen more widely. The risk of MRSA transmission from pigs to humans is widely known [35], but the more recent discovery of emergence of MRSA within wildlife hosts in the pre-antibiotic era [36] indicates the potential value of passive surveillance. These techniques can be used to track the emergence and spread of critically important resistance genes without the need for individual animal sampling which can pose many challenges.

This specific DNA extraction kit requires a small mass of faeces, which may not be representative of the wider bacterial population of the gastrointestinal tract and perianal mucosa. However, it did result in the extraction of a sufficient quantity of DNA, demonstrated by the presence of bacterial ribosomal subunits (16S). The quality of the faecal DNA was lower than ideal, likely due to the presence of faecal protein contamination, but was sufficient for successful qPCR amplification.

Overall, the detection of *mecA* in faeces indicates that there is a reservoir of this important resistance gene which is currently unassessed. Further analysis of human MRCoPS carrier faeces is needed to establish whether there is diagnostic merit in this approach for rapid identification of asymptomatic carriers. Regardless, this qPCR method provides a novel technique for the surveillance of the *mecA* reservoir in both individuals and the environment.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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