



Methicillin-resistant staphylococci amongst veterinary personnel, personnel-owned pets, patients and the hospital environment of two small animal veterinary hospitals

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ABSTRACT

This study investigated the transmission cycle of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) in small companion animal veterinary practice. Sampling was undertaken at two small animal veterinary hospitals in Sydney, Australia. Samples were collected from 46 veterinary personnel, 79 personnel-owned dogs and cats, 151 clinically normal canine hospital admissions and 25 environmental sites. Nasal swabs were collected from veterinary personnel. Nasal, oral and perineal swabs were collected from animals. Methicillin resistance was detected by growth on BrillianceTM MRSA 2 Agar and confirmed by ceftiofur and oxacillin broth microdilution for *S. aureus* and *S. pseudintermedius*, respectively. MRSA and MRSP isolates were characterised using whole genome sequencing including *mecA* gene screening and multilocus sequence typing. MRSA was isolated from four (8%) veterinary personnel but no animals. MRSP was isolated from 11/151 (7%) of canine hospital admissions and 4/53 (8%) of personnel-owned dogs but no veterinary personnel or cats. No MRSA or MRSP was isolated from the environment. MRSP isolates were resistant to significantly more antimicrobial classes than MRSA. The main MRSP clone carried by canine patients (ST496) was distinct to that carried by personnel-owned dogs (ST64). One veterinary nurse, who carried Pantone leucocidin-positive ST338 MRSA, also owned a ST749 MRSP-positive dog. Besides MRSP-positive dogs from the same household sharing the same clone of MRSP, MRSA and MRSP were not shared between humans, animals or environment. Therefore, in the non-outbreak setting of this study, there was limited MRSP transmission between veterinary personnel, their pets, patients or the veterinary environment.

1. Introduction

Staphylococcus aureus in humans and *Staphylococcus pseudintermedius* in dogs commonly cause opportunistic infections that are generally treatable with topical or systemic antimicrobials. However, the rise of methicillin-resistant *S. aureus* (MRSA) and *S. pseudintermedius* (MRSP) makes these bacterial infections difficult to treat with commonly available antimicrobials. Although MRSA is a significant pathogen for humans, it can cause disease in animals and has been isolated from a number of skin and soft tissue infections in Australian cats

and dogs (Worthing et al., 2018a). The lineages of MRSA found in Australian animal species are similar to those found in Australian veterinarians treating these animal species (Groves et al., 2016; Worthing et al., 2018a). The majority of MRSA found in companion animals originate from human healthcare-associated lineages (Harrison et al., 2014; Worthing et al., 2018a). Human physicians occasionally attribute pets as the source of MRSA infections in their owners (Manian, 2003), yet it appears that MRSA can move between humans and animals in a bi-directional manner. Veterinarians who treat companion animals are at higher risk of MRSA carriage than pet owners (Loeffler et al., 2010)

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or veterinarians who do not treat animals (Jordan et al., 2011). Veterinarians can also occasionally be the source of MRSA infections in their patients (Walther et al., 2008; Ishihara et al., 2010).

The epidemiology of *S. pseudintermedius* transmission between animals and humans is not well characterized. MRSP can certainly be carried by healthy pets (Bean and Wigmore, 2016), their owners (Gomez-Sanz et al., 2013) and veterinarians (Paul et al., 2011), and it can occasionally cause infections in immune-compromised people (Starlander et al., 2014). Although studies have documented concurrent carriage of MRSP and MRSA in veterinary dermatologists and their own pets (Morris et al., 2010), no studies have assessed carriage by non-dermatologist veterinarians and their pets. Veterinary personnel often bring their pets to their place of work thereby potentially exposing them to environmental and patient-associated organisms. Veterinary hospital visits and having an owner who works in healthcare are respective risk factors for MRSP and *S. aureus* carriage in dogs (Boost et al., 2008; Nienhoff et al., 2011), so it follows that pets owned by veterinary personnel may also be at increased risk of MRSA and MRSP carriage.

The prevalence of methicillin-resistant *Staphylococcus* (MRS) carriage in veterinary patients is variably reported, with MRSA and MRSP carriage in dogs sampled in their homes in the USA reported as 8% and 1% respectively (Iverson et al., 2015), while carriage of MRSP in canine hospital admissions in Portugal was 6.2% (Couto et al., 2011). MRSP carriage rate is much higher in dogs with clinical pyoderma, with reports ranging from 33% in the USA (Detwiler et al., 2013) to over 60% in reports from Japan (Kawakami et al., 2010). No MRSP was found in two Australian studies conducted between 2004 and 2007 (Malik et al., 2006; Gottlieb et al., 2008) while a 2016 study found two of 117 canine obedience school attendees were MRSP carriers (Bean and Wigmore, 2016). Although the methodology and results of veterinary MRS carriage studies vary, most studies show a steady upward trend of antimicrobial resistance in *S. pseudintermedius* as time progresses (Moodley et al., 2014), and global expansion of a relatively small group of MRSP lineages (Perreten et al., 2010). A recent Australian study revealed that the most common clone of MRSP amongst clinical samples from animals was ST71, which is similar to the rest of the world (Perreten et al., 2010; Worthing et al., 2018b), but the lineages of MRSP found amongst clinically normal dogs in Australia is as-yet unknown.

Molecular typing has shown that MRSA and MRSP found in the environment can be closely related to that carried by veterinary personnel and patients (Loeffler et al., 2005; Feßler et al., 2018). There is a growing body of literature investigating the transmission cycle of MRS in veterinary practice but no studies have concurrently examined MRSA and MRSP carriage in veterinary personnel, personnel-owned animals, patients and the veterinary hospital environment. This study therefore aimed to determine the prevalence of methicillin-resistant staphylococci amongst veterinary personnel, personnel-owned pets, hospital patients and environment of two small animal hospitals and to use molecular methods to determine the relatedness of MRS isolated from these groups.

2. Materials and methods

2.1. Sampling

Sample collection involved two veterinary hospitals in Sydney, Australia: a primary accession small animal hospital (Hospital A) and a multi-disciplined small animal referral hospital (Hospital B). Samples were collected from veterinary personnel, personnel-owned pets, canine hospital admissions and environmental sites within the veterinary hospital. All aspects of this study were approved by the Human and Animal Ethics Committees at the University of Sydney (Project numbers 2016/837, 2016/1072 and 2015/866, respectively).

2.2. Veterinary personnel and their pets

Sampling of veterinary personnel and their pets (dogs and/or cats) was undertaken over a two-week period in February 2017 (Hospital A) and April 2017 (Hospital B). Veterinary personnel included veterinarians and support staff (veterinary nurses, kennel hands and administrative staff). Personnel were invited to participate whether or not they had pets at home. Personnel were given written and verbal instructions on how to take samples, then took samples from themselves and their pets, undertaking sampling in their own home. For veterinary personnel, a single sterile swab was used to sample one nostril. For pet dogs and cats, personnel took three separate swabs: one each from the anterior nares (large dogs) or nasal planum (small dogs and cats), oral cavity and perineum. For multipet households, sampling was limited to three dogs and/or three cats. Personnel wore gloves during the procedure and refrigerated samples immediately after sampling. All swabs were collected using Amies™ Agar Gel swabs (Copan Diagnostics, USA).

2.3. Canine hospital admissions

Swabs were taken from the nares/nasal planum, oral cavity and perineum of canine hospital admissions from both veterinary hospitals. Convenience sampling of canine hospital admissions occurred in two sampling periods in August 2015 and August 2016 for Hospital A while sampling was sporadic from April 2016 to April 2017 for Hospital B. To minimise sampling from dogs with known MRSP carriage risk factors such as recent hospitalisation or antimicrobial treatment (Nienhoff et al., 2011), dogs had to meet the following selection criteria: a) they had been in the hospital for less than 10 min at the time of sampling (not including time spent in the waiting room); b) they were admitted for an elective procedure (for example: non-emergency surgery, routine blood tests, imaging, dentistry, boarding); c) they were deemed systemically well by the attending clinician; and d) they had no visible skin lesions suggestive of pyoderma.

2.4. Hospital environment

Environmental sampling was undertaken at Hospital A only, on the same day in February 2017 that samples were collected from personnel and their pets. Twenty-five swabs were taken from the following areas: cage floors and walls, waiting room chair legs and seats, door handles into the consultation, treatment, pharmacy, bathroom, radiology and boarding rooms, and computer keyboards and computer mice in the consulting, treatment and radiology rooms. Samples were taken by pre-moistening Amies™Gel agar swabs with sterile saline, then rolling the swab across the environmental surface for 10 s. Although MRSP-positive carriers and patients with MRSP-infected wounds were known to have been in the hospital within the last month, no known MRSP carriers or patients were present at the time environmental sampling. Therefore, it was assumed that environmental sampling was indicative of a non-outbreak setting for the hospital.

2.5. Phenotypic methicillin resistance screening

Human, animal and environmental swabs were all processed as follows. Samples were initially enriched by inoculation onto 2% Columbia sheep blood agar (SBA) (Oxoid, UK) and overnight incubation at 37 °C. If swarming colonies (presumed to be *Proteus* spp.) were noted after initial incubation, the swab was replated onto 4% SBA to allow easier subculturing of colonies with the morphologic appearance of staphylococci. After the enrichment step, a sterile microbiological loop was streaked across the blood agar plate to collect many colonies which were then subcultured onto the selective medium, Brilliance™ MRSA 2 Agar (Oxoid, UK) and incubated overnight at 37 °C (Horstmann et al., 2012). Samples that grew as blue colonies on the Brilliance™

agar underwent catalase and tube coagulase testing. Coagulase-positive BrillianceTM-positive isolates underwent species confirmatory testing with MALDI-TOF mass spectrometry (BDTM Bruker MALDI BiotyperTM) as previously described (Worthing et al., 2018b). Phenotypic methicillin resistance was confirmed using the Vitek 2TM automated antimicrobial susceptibility testing (AST) system (bioMérieux, USA). Methicillin resistance in *S. aureus* was identified by a cefoxitin MIC of ≥ 8 mg/L and in *S. pseudintermedius* by an oxacillin MIC of ≥ 0.5 mg/L (Saputra et al., 2017). Isolates also underwent testing by Vitek 2TM to the following antimicrobials: benzylpenicillin, enrofloxacin, erythromycin, gentamicin, clindamycin, tetracycline, chloramphenicol, rifampicin and trimethoprim-sulfamethoxazole. Clindamycin testing included screening for inducible clindamycin resistance and measurement of MIC by Vitek 2TM. Clinical breakpoints were used as described by the Clinical and Laboratory Standards Institute (CLSI, 2013a, b). Isolates with intermediate resistance were defined as resistant.

2.6. Molecular characterisation of methicillin-resistant isolates

Phenotypic MRSA and MRSP underwent whole genome sequencing (WGS) using the MiSeq system (Illumina, USA) as previously described (Worthing et al., 2018a, b). *De novo* assembly was performed CLC Genomics Workbench (Qiagen, USA). Multilocus sequence typing (MLST) was undertaken by uploading *de novo* contigs onto the respective MRSA and MRSP online MLST platforms hosted by the Center for Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/MLST/>) (Larsen et al., 2012). New MRSP sequence types were assigned by the *S. pseudintermedius* database curator (vincent.perreten@vetsuisse.uni-be.ch). All isolates were screened for the *mecA* gene, its homologues and the Pantone-Valentine leucocidin gene (*pvl*) using the bioinformatics websites, ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>) (Zankari et al., 2012) and (<https://cge.cbs.dtu.dk/services/VirulenceFinder/>) (Joensen et al., 2014), respectively. SCC_{mec}, *dru* and *spa* typing was undertaken using sequence data as previously described (Worthing et al., 2018a, b). A phylogenetic tree of MRSP isolates was generated using CSI Phylogeny 1.4 (<https://cge.cbs.dtu.dk/services/CSIPhylogeny/>) (Kaas et al., 2014). This program used a Maximum Likelihood algorithm to depict inferred phylogeny based on concatenated alignment of high quality single nucleotide polymorphisms. To construct the tree, fasta files of all MRSP genomes were uploaded to the online platform which aligned them to a reference genome (ST71 MRSP 081661, Accession number: CP16073.1 (Riley et al., 2016)). FigTree 1.4.3 (Rambaut, 2012) and interactive tree of life (iTOL) (Letunic and Bork, 2016) were used to optimize visualization of the phylogenetic tree.

2.7. Statistical analysis

Based on previously reported MRS prevalence rates amongst Australian dogs of between 1% (Malik et al., 2006; Bean and Wigmore, 2016) and 11.5% (Worthing et al., 2018b), power analysis suggested a sample size of at least 164 would be required to predict MRS carriage frequency with 95% confidence (<http://clincalc.com/stats/Sample-Size.aspx>). Categorical comparisons were undertaken by constructing contingency tables and performing Fishers exact test (GraphPad Prism 7, USA). Results were considered significant if $p < 0.05$.

3. Results

3.1. MRSA and MRSP in humans, animals and environment

Samples were collected from 46 veterinary personnel (19 veterinarians, 22 nurses, 2 receptionists, 3 kennel hands), 79 personnel-owned pets, 151 canine hospital admissions and 25 environmental sites. This resulted in 191 samples from Hospital A (118 canine hospital admissions, 21 veterinary personnel, 13 personnel-owned pet dogs, 14

Table 1

Methicillin-resistant coagulase-positive staphylococci isolated from veterinary personnel, canine hospital admissions, personnel-owned pets and the veterinary hospital environment.

Group	Number sampled	Number of carriers (%)	
		MRSA	MRSP
Veterinary personnel			
Veterinarians	19	3 (16%)	0
Support staff	27	1 (4%)	0
Total personnel	46	4 (8%)	0
Dogs			
Hospital admissions	151	0	11 (7%)
Personnel-owned	53	0	4 (8%)
Total dogs	204	0	15 (8%)
Cats			
Personnel-owned	26	0	0
Hospital environment			
Waiting room chairs	2	0	0
Door handles	8	0	0
Computer keyboards	3	0	0
Cage door handles	3	0	0
Cage interiors	9	0	0
Total environment	25	0	0
Total samples	301	4 (1%)	15 (5%)

personnel-owned pet cats and 25 environmental sites) and 110 samples from Hospital B (33 canine hospital admissions, 25 veterinary personnel, 40 personnel-owned pet dogs and 12 personnel-owned pet cats). The proportions of methicillin-resistant staphylococci (MRS) isolated from humans, animals and the environment are shown in Table 1. MRS were isolated from 4/46 veterinary personnel (8%), 11/151 (7%) canine hospital admissions, and 4/53 personnel-owned pet dogs (8%) but not from any cats or the veterinary environment. No MRSA was isolated from animals nor MRSP from humans. The MRS carriage rate in personnel-owned dogs was not significantly different to personnel-owned cats ($p = 0.3$). MRS frequency was proportional to sampling intensity from each hospital and was not significantly different between Hospital A and B ($p = 0.91$). Consequently, results from both hospitals were combined for analyses. The phenotypic antimicrobial resistance profile of all isolates is shown in Table 2. MRSP isolates were resistant to significantly more antimicrobial classes than MRSA ($p < 0.001$). All personnel- and animal-derived MRS samples underwent WGS and *in silico* molecular characterisation (Table 3). Two Brilliance-positive isolates (one *S. aureus* (KW8) and one *S. pseudintermedius* (KWBH2)) were positive for the *mecA* gene but methicillin-susceptible on Vitek 2 screening. It was assumed that a methicillin-susceptible subpopulation had been inadvertently subcultured for AST testing and thus these two isolates were still included in further analyses. The genomes obtained from whole genome sequencing have been deposited at the National Center for Biotechnology Information (NCBI) database, under Bioproject accession number PRJNA482500.

Table 2

Frequency (%) of antimicrobial resistance in MRSA and MRSP from veterinary hospitals.

Group	PEN	ENR	ERY	CLI	TET	CHLOR	RIF	TMS
MRSP (n = 15)	100	37	32	16	58	42	0	37
MRSA (n = 4)	100	0	0	0*	25	0	0	0

PEN = benzylpenicillin, ENR = enrofloxacin, ERY = erythromycin, CLI = clindamycin, TET = tetracycline, CHLOR = chloramphenicol, RIF = rifampicin, TMS = trimethoprim-sulfamethoxazole. *Susceptible to clindamycin by MIC testing and by screening for inducible clindamycin resistance using Vitek 2TM.

Table 3
Molecular epidemiology MRSA and MRSP isolated from veterinary personnel, personnel-owned dogs and canine hospital admissions in Sydney, Australia.

Isolate	Year isolated	Source	Species	Isolation site	Phenotypic methicillin resistance §	MLST	SCCmec	<i>Spa</i> type	<i>Dru</i> type
HOSPITAL A									
KW12	2017	Veterinarian	MRSA	Nares	+	ST59	IV	t316	None
KW1606 ^a	2015	Dog (H)	MRSP	Nares and perineum	+	ST496	Vt	t05	dt10a
KW1610 ^a	2015	Dog (H)	MRSP	Nares, oral cavity and perineum	+	ST496	Vt	t05	dt10a
KW1608	2015	Dog (H)	MRSP	Perineum	+	ST496	Vt	t05	dt10a
KW1614 ±	2015	Dog (H)	MRSP	Perineum	+	ST525	Vt	t09	dt9bd
KW1613 ±	2015	Dog (H)	MRSP	Oral cavity	+	ST68	Vt	t23	New
KW1607	2015	Dog (H)	MRSP	Nares	+	ST64	C1/ccrC6	None	dt7ae
KWAH3	2016	Dog (H)	MRSP	Oral cavity	+	ST496	Vt	t05	New
KWAH4	2016	Dog (H)	MRSP	Oral cavity and perineum	+	ST496	Vt	t02	New
KWAH1	2016	Dog (H)	MRSP	Oral cavity	+	ST64	C1/ccrC6	None	dt11af
KWAH2	2016	Dog (H)	MRSP	Oral cavity	+	ST64	C1/ccrC6	None	dt10cj
KW5	2017	Dog (P)	MRSP	Nares	–	ST749	IV	None	NT
HOSPITAL B									
KW7 ^c	2017	Veterinary nurse	MRSA	Nares	+	ST338	V	t441	NT
KW8	2017	Veterinarian	MRSA	Nares	–	ST81	IV	t177	dt7f
KW3	2017	Veterinarian	MRSA	Nares	+	ST59	IV	t976	dt7f
KWBH2 ‡	2016	Dog (H)	MRSP	Oral cavity and perineum	+	ST751	NT	None	NT
KW1	2017	Dog (H)	MRSP	Nares and oral cavity	+	ST496	Vt	t02	dt7f
KW10 ^b	2017	Dog (P)	MRSP	Nares	+	ST64	C1/ccrC6	None	New
KW11 ^b	2017	Dog (P)	MRSP	Oral cavity	+	ST64	C1/ccrC6	None	dt7f
KW6 ^c	2017	Dog (P)	MRSP	Nares, oral cavity and perineum	+	ST64	C1/ccrC6	None	New

§ = Phenotypic methicillin resistance as determined by Vitek² MIC testing: cefoxitin MIC for MRSA isolates; oxacillin MIC for MRSP isolates. ± = Isolates 1613 and 1614 originated from the same dog but were different MLST types. ‡ = Both isolates from dog KWBH2 underwent phenotypic and genotypic testing and both isolates were *mecA* positive but one isolate was resistant to oxacillin (MIC = 0.5mg/L) while the other was susceptible (MIC < 0.25mg/L). ^{a, b, c} = Isolates with the same superscript letters originated from the same household. Dog (H) = canine hospital admission; Dog (P) = personnel-owned dog. NT = not typable. Unless indicated, dogs that were positive at multiple sites carried the same MLST type at all sites.

3.2. Characterisation of MRSA from veterinary personnel

Of the four MRSA isolates from veterinary personnel, three were from veterinarians and one was from a veterinary nurse. MRSA carriage amongst veterinarians (16%) was not significantly different to support staff (4%; $p = 0.29$). Three of the four MRSA isolates were susceptible to all non- β -lactam antimicrobials. The fourth isolate, ST338 from a veterinary nurse (Table 3), was additionally resistant to tetracycline. One veterinarian from each of the hospitals carried ST59-IV MRSA. The two isolates were different *spa* types (t316 and t976). One MRSA isolate, ST338 SCCmec type V isolate from a veterinary nurse, harboured the Panton-Valentine leucocidin gene (*pvl*); the remaining isolates were *pvl*-negative. The final MRSA isolate, ST81 from a veterinarian, was phenotypically susceptible to oxacillin but harboured the *mecA* gene. *Post-hoc* power analysis found that with a sample size of 46, previously reported Australia-wide veterinarian MRSA carriage rate of 4.8% (Jordan et al., 2011) and observed MRS frequency rate of 8%, the personnel-sampling component of the study was underpowered (power = 23%). A sample size of 409 would have been required to obtain 80% power in the veterinary personnel component of the study.

3.3. Characterisation of MRSP from personnel-owned pets and canine hospital admissions

The MRSP carriage rate between personnel-owned pet dogs and canine hospital admissions was not significantly different ($p = 0.77$). Two main MRSP lineages were isolated: ST496 ($n = 6$) and ST64 ($n = 6$). ST496 was the most common clone amongst canine hospital admissions (6/12 dogs; 50%) but was not carried by any personnel-owned dogs. ST64 was the most common clone amongst personnel-owned dogs and was carried by all three personnel-owned dogs at Hospital B. Within each of the hospitals, none of the personnel-owned dogs carried the same clone as hospital admissions to that hospital. The three ST496 collected from Hospital A in 2015 were all the same *spa* and *dru* type (t05 and dt10a respectively) but subsequent isolates from later years and from Hospital B showed different *dru* and *spa* types (Table 2). Fig. 1 shows the phylogeny of MRSP isolated from canine

hospital admissions and personnel-owned dogs at both hospitals. Isolates clustered within their respective MLST lineages and isolates within MLST tended to cluster according to hospital, suggesting a degree of geographic clustering.

3.4. Shared MRS carriage in veterinary personnel and their pets

Of the 46 veterinary personnel that were sampled, 38 also had their pets sampled. All MRSA carriers were pet owners. Of the 38 personnel-pet groupings, concurrent MRS carriage was identified in one veterinary nurse and one of the two dogs owned by that nurse. The isolate from the nurse was ST338 MRSA with SCCmec type V while the nurse's pet dog carried ST64 MRSP with a *mec* complex type C1/ccrC6 SCCmec element. Besides both SCCmec elements carrying a type C1 *mec* gene complex, the nurse- and pet- derived MRS samples did not appear related. Two pairs of dogs from the same household were both MRSP carriers. The first pair were personnel-owned dogs from Hospital B. Both dogs carried ST64 which clustered closely in the phylogenetic tree. The dog's owner, a veterinarian, was not a MRS carrier. The second MRSP-positive pair were hospital admissions at Hospital A, admitted on the same day for routine dental care. The ST496 MRSP isolated from this pair clustered closer to each other than other ST496 isolates.

4. Discussion

This study detected MRSA amongst veterinary personnel and MRSP in dogs but did not find MRSP carriage in humans, MRSA carriage in animals, or any MRS in the environment. The absence of MRSA in animals and MRSP in humans supports the notion that *S. aureus* is generally more host-adapted to humans and *S. pseudintermedius* to dogs (Simou et al., 2005). Four of 46 veterinary personnel (8%) were MRSA carriers. Our results add to evidence that the rate of MRSA carriage in veterinary personnel, like human healthcare workers, is higher than what is reported in the general population (Moodley et al., 2008; Loeffler et al., 2010; Jordan et al., 2011; Eveillard et al., 2015). The three MRSA lineages isolated in our study (ST338-V, ST59-IV and ST81-IV) were not amongst the commonly reported MRSA lineages isolated

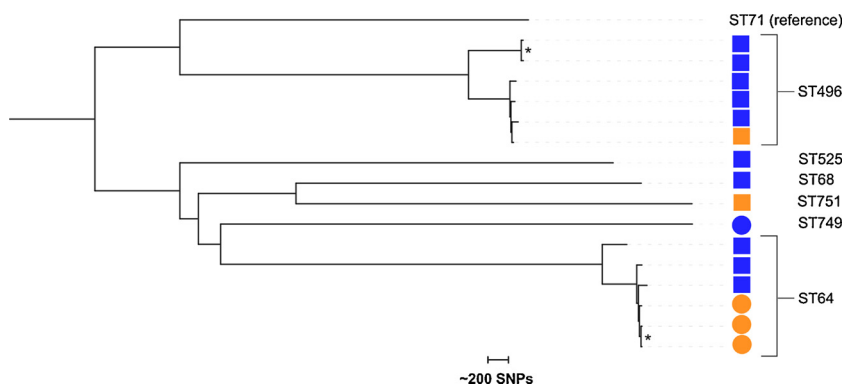


Fig. 1. Phylogenetic tree of methicillin-resistant *Staphylococcus pseudintermedius* from canine veterinary hospital admissions and personnel-owned dogs, generated using a Maximum Likelihood algorithm based on a concatenated alignment of 16,209 high-quality SNPs. The tree was constructed using CSIPhylogeny 1.4 and optimized using FigTree v1.4.3 and Interactive Tree of Life v3 (iTOL). ST71 MRSP 081661 (Accession: CP16073.1) was used as the reference genome. Circles indicate isolates from personnel-owned dogs. Squares indicate isolates from canine hospital admissions. Blue shapes are from Hospital A, orange shapes are from Hospital B. The MLST of each isolate is shown on the right-hand margin of the tree. * = indicates a pair of isolates from two dogs in the same household (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

from human hospitals in Sydney in the same time period, and the level of antimicrobial resistance to non- β -lactam antimicrobials was lower than healthcare associated MRSA clones in Sydney (Australian Commission on Safety and Quality in Health Care, 2017). The level of antimicrobial resistance amongst MRSA in this study was also lower than the resistance seen in ST22 MRSA, which is the lineage most commonly isolated from small animal veterinarians (Groves et al., 2016; Loeffler et al., 2005). The veterinary-specific risk factors for MRSA carriage are not yet known, but it is possible that veterinary personnel have similar occupational risk factors to workers in healthcare such as caring for patients with MRSA-infected wounds (Cox and Conquest, 1997). However, MRSA appears to be much more prevalent amongst human hospital patients than veterinary hospital patients, so it is likely that additional risk factors exist for veterinarians such as the handling of antimicrobial drugs (Moodley et al., 2008; Morris et al., 2010), or environmental exposure to biocides and patient-excreted antimicrobials. It is clear that an extensive prospective cross-sectional study is warranted to better define the occupational risk factors for MRSA carriage in veterinary personnel.

The rate of MRSP carriage amongst personnel-owned dogs (8%) was not significantly different to canine hospital admissions (7%), indicating that dogs of veterinary personnel are not at increased risk of MRSP or MRSA carriage compared to canine hospital admissions from the same geographic area. Previous studies have found that pets owned by human healthcare workers are at increased risk of carrying methicillin-susceptible *S. aureus* (MSSA) (Boost et al., 2008), but there are conflicting reports as to whether or not dogs owned by healthcare workers are at increased risk of MRSA carriage (Boost et al., 2008; Kottler et al., 2010). Future studies that directly compare the relative risk of MRSA and MRSP carriage amongst pets owned by veterinary personnel, human healthcare workers and non-healthcare workers are now warranted. All three MRSP-positive personnel-owned dogs at Hospital B carried ST64 which closely clustered on the phylogenetic tree and differed by less than 100 SNPs, suggesting that ST64 had circulated amongst personnel-owned pets in this hospital. Despite this, it was not isolated from any dogs admitted to the same hospital. Sampling of dogs upon discharge would have helped to determine whether canine hospital admissions subsequently acquired MRSP lineages carried by personnel-owned pets in the same hospital.

The rate of 7% MRSP carriage we found amongst canine hospital admissions is similar to some previous studies (Nienhoff et al., 2011), but higher than the 1% MRSP carriage rate in another Australian study that examined healthy dogs at an obedience school in regional Victoria (Bean and Wigmore, 2016). The difference could reflect increased local prevalence in Sydney compared to regional Victoria or increased prevalence amongst dogs attending veterinary hospitals compared to obedience school. ST496, a multidrug resistant MRSP strain, was the most common clone carried by canine hospital admissions in this study and was also the most common clone amongst clinical MRSP from dogs in greater Sydney in 2013 (Worthing et al., 2018b). ST496 has not yet

been reported outside Australia, but ST496 has become a common clone in Sydney that has evolved and diversified, evidenced by a greater diversity of *dru* types in this study compared to the 2013 surveillance study (Worthing et al., 2018b). Concurrent carriage of ST496 MRSP by two canine hospital admissions from the same household suggested intra-household transmission had occurred. Although neither patient showed signs of skin disease at the time of sampling, inspection of both patients' records revealed that one of the dogs had a long history of intermittent antimicrobial use to treat pyoderma secondary to flea allergy dermatitis. It is likely that the dog with a history of skin disease acted as a source of MRSP for the asymptomatic dog in the same household (Duijkeren et al., 2011; Laarhoven et al., 2011). While clear risk factors for MRSP carriage and infection such as previous antimicrobial use, frequent veterinary visits and a history of hospitalisation have already been identified (Nienhoff et al., 2011; Lehner et al., 2014), veterinarians should be aware that apparently healthy dogs can also carry MRSP, particularly if they live with an MRSP carrier.

It is noteworthy that the MRSP isolates in this study displayed a significantly higher level of antimicrobial resistance than the MRSA isolates, with 37% resistance to fluoroquinolones and trimethoprim-sulfamethoxazole and 32% resistance to erythromycin amongst MRSP compared to no resistance to these classes amongst MRSA isolates. While MRSP is not a major zoonotic pathogen, the expansion of multidrug-resistant MRSP lineages such as ST496 still presents a potential public health concern because such lineages may act as a reservoir for genetic resistance determinants. Animal-derived staphylococci can be the source of resistance determinants in human *S. aureus* (Rolo et al., 2017), but the extent to which *S. pseudintermedius* contributes to the resistance gene pool in human pathogens such as *S. aureus* is not fully determined (Frank et al., 2009) and could thus be examined in future genomic studies.

Besides concurrent MRSA carriage in a veterinary nurse and MRSP carriage in the nurse's pet dog, this study found no shared MRS carriage in veterinary personnel and their pets. The lack of MRSP isolation from veterinary personnel in this study likely reflects the general lack of human host tropism by MRSP but could also reflect the small sample size and the fact that animals with overt skin disease were intentionally omitted from this study. Additionally, MRSP has recently been isolated from the hands of veterinary personnel (Feßler et al., 2018), so the sensitivity of our study may have been improved had we included hand as well as nasal sampling of humans. Dogs with skin disease are more likely to carry MRSP than dogs with healthy skin (Griffeth et al., 2008), and transmission of MRSP from animals to owners is more commonly reported when the animal has clinical disease (Duijkeren et al., 2011). Certain MRSP lineages such as ST71 appear better able to colonize human corneocytes than MSSP or other MRSP lineages and thus may have better zoonotic potential (Latronico et al., 2014). It is therefore possible that the lack of human MRSP carriage in this study reflects the lack of ST71 in the animal population sampled. Although ST71 is a dominant MRSP clone in Europe (Perreten et al., 2010) and represents

34% of clinical MRSP in Australia overall (Worthing et al., 2018b), it was not isolated from any dogs in this study and was isolated from only 1/24 (4%) clinical MRSP cases around Sydney in 2013 (Worthing et al., 2018b). Overall, it appears that MRS carriage by veterinary personnel is influenced by their role within the veterinary hospital and the local prevalence and clonal distribution of MRS in their respective patient population.

MRSP was not isolated from the veterinary hospital environment. This could be attributed to the low sample size, the lack of longitudinal sampling, or the fact that equipment with high animal contact from which MRSP has been isolated in other studies, such as clippers (Feßler et al., 2018) or feeding bowls (Duijkeren et al., 2011), were not sampled. Alternatively, Hospital A had developed a hospital protocol for the management of known MRS-infected patients which was initiated six months prior to this study which may have effectively reduced the MRS load in the environment. Successful reduction of environmental MRSA was reported in a human hospital that revised its hospital infection control protocols to address an increase in MRSA cases (Rampling et al., 2001), but lack of longitudinal sampling pre- and post-cleaning protocol prevents us from assessing whether the absence of MRSP was truly due to a successful infection control program.

This study provides valuable insights into the molecular epidemiology of MRS within two veterinary hospitals. The clonal types of MRSP and MRSA found in veterinary personnel, personnel-owned pets and hospital admissions were distinct from each other. This suggests that limited MRS transmission occurs between these groups, at least in a non-outbreak setting as was examined in this study. The rate of MRS isolation was not significantly different between the tertiary referral hospital and a primary accession hospital, nor between personnel-owned dogs and hospital admissions. MRSP carriage was not detected amongst veterinary personnel. However, it is apparent that clonal types of MRSP vary with geography, so sampling of dogs and veterinary personnel in areas where ST71 MRSP is common would provide valuable comparative results for this study. Contrasting with the lack of MRSP carriage in our study, MRSP carriage has been twice documented in veterinary dermatologists (Morris et al., 2010; Paul et al., 2011). A study that simultaneously compares MRSP carriage amongst dermatologist and non-dermatologist veterinarians is warranted to evaluate whether specialty-specific risk for MRSP carriage exists amongst veterinarians. Lastly, our study adds to existing literature in reporting that veterinarians have a higher rate of MRSA carriage than the general population. A large-scale case-control study is thus warranted to further investigate the occupational risk factors for MRSA carriage in veterinarians.

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Conflict of interest statement

At the time of writing, KW was an employee of Hospitals A and B, and JB is an employee of Hospital B. All other authors have no conflict of interest to declare.

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