**SUPPORTING INFORMATION**

**Risk factors for meticillin-resistant *Staphylococcus aureus* (MRSA) carriage in MRSA-exposed household pets**

Meticillin-resistant *Staphylococcus aureus* (MRSA), a drug-resistant Gram-positive bacterium, causes clinical disease in humans and animals.1,2 In the early 2010s, the MRSA strain USA300 [typically staphylococcal protein A (*spa*-) type t008] was the major cause of human community-associated MRSA (CA-MRSA) infections in the USA.3

**METHODS S1**

**Human and animal subject protections**

Participants and pets were enrolled in the primary study or nested sub-study detailed here as described previously.4,5 This secondary data analysis was reviewed and approved by the Johns Hopkins School of Public Health Institutional Review Board (IRB00006259).

**Household recruitment and questionnaire administration**

PETS was a sub-study of a randomised controlled trial (RCT; Epidemiology and Transmission of MRSA in the Community, NCT00966446) which evaluated the effectiveness of MRSA decontamination through sampling household members for MRSA at bi-weekly home visits for six months.

Verbal questionnaires about medications, pet-related characteristics, and environmental factors were conducted at each PETS visit using an iFormBuilder (iFormBuilder; Herndon, VA, USA) survey application for iPad (Apple, Cupertino, CA, USA).

Human participants provided written informed consent for their participation in the study. Pet sampling was approved by the Johns Hopkins School of Public Health Animal Care and Use Committee (ACUC), and pet owners provided written informed consent. Human index patients diagnosed around one month previously with CA-MRSA SSTI, their cohabitating family members, and their domestic animals were enrolled.

**Pet sampling**

One hundred and eighty-four pets were enrolled, of which 179 (97.3%) were sampled at baseline for bacterial culture. See Table S1 for animal population description. Four individual (nonpooled) swabs were taken from each pet as described previously.5

At each household visit, all pets were sampled using dry culture swabs with transport media (BBL Culture Swabs, BD; Franklin Lakes, NJ, USA). Pet samples were taken at the nares or nasal planum, the tongue, gingiva or hard palate, and inguinal and perineal regions.

**Environmental sampling**

Sites of environmental surface dust sampling included: (1) the top of the refrigerator, (2) the handle of the refrigerator, (3) the top of the television, (4) the television remote, (5) a kitchen towel, (6) the bathroom faucet handle, (7) the index patient’s pillow, (8) the dusty surface of the headboard of the bed, and (9) any pet bedding, crates or cages.

**Human index case and family member sampling**

Eswabs (Copan Diagnostics; Murrieta, CA, USA) were used to sample from up to three sites – nares, axillae/groin, and the healed lesion site (index patients only) – to test for human carriage of MRSA by culture as described previously.4,6

**Bacterial culture and enrichment**

Human, pet and environmental bacterial samples were processed and identified using a previously described protocol including parallel arms for nonselective and meticillin-resistance-selective culture.6,7 MRSA positivity was determined by positive CHROMagar staphylococcal culture and susceptibility testing as described previously.4

One bacterial isolate sampled from each animal per visit was selected by one member of the study team (MFD) for subculture and speciation by PCR as described previously.8

**Risk factors of interest**

Relationships between pet carriage of MRSA and potential household, human and animal risk factors at the baseline visit were explored. Using a One-Health approach as defined in the Checklist for One Health Epidemiological Reporting of Evidence (COHERE) guidelines,9 previously known factors for human MRSA carriage were considered first. Age, sex and species of pet were determined *a priori* to be variables of interest. Due to low MRSA prevalence in pet reptiles, birds and fish in this study, pet species analysis was limited to dogs versus cats. Based on distribution of the data, dog breed was split into Labrador/Labrador mixes and non-Labradors, as well as small versus large breeds. Likewise we divided cat breed into domestic short hair (DSH) and non-DSH breeds. Age was calculated to the closest month and dichotomised above and below the median value of 24 months. Sex was coded as male or female, without regard to neuter status.

Biologically plausible mechanisms for pet MRSA carriage included household environmental MRSA contamination, pet bed MRSA contamination (including pet bedding and crates/cages), pet antimicrobial use, veterinary hospital contact, presence of fleas on the pet (visual observation of the pets only), kennel exposure and human–animal contact. Household income quartile, rural versus nonrural (i.e. urban and periurban) environments and meteorological season were evaluated as possible risk factors. Standard home environmental questions based on prior studies of household environmental persistence of MRSA10 were included, and any observed or reported presence of pests (i.e. cockroaches, mice) or visible mould/mildew in the home was recorded.

Veterinary hospital visit was defined as veterinary contact for any reason over the past year. Pet antibiotic use was defined as treatment of the pet with a systemic (oral or injectable) antimicrobial within the past 12 months. No pets received antibiotics that did not also have exposure to a veterinary clinic setting (Table S2); 100% of pets treated with antimicrobials in the past year also had veterinary clinic exposure in the past year, as a result of its requirement for a veterinary prescription. Because of this dependency, pet antimicrobial use was combined with veterinary clinic exposure (vet visit) and the combined variable was defined as pets receiving antibiotics within the past 12 months. A sensitivity analysis including veterinary clinic visit in the multivariable model instead of pet antimicrobial use was performed.

Of 63 cats and 71 dogs sampled at baseline, 10 (7.5%) pets (five dogs, five cats) received antibiotics within the past year. These antibiotics included cephalosporins (cephalexin or cefpodoxime, three dogs), amoxicillin and azithromycin (one cat), amoxicillin-clavulanic acid (three cats, one dog), clindamycin (one cat), and one dog was treated with an unknown antibiotic type. At the three month visit, five additional dogs had been treated with antibiotics: two were treated with cephalexin, two with clindamycin and one with doxycycline in the past three months.

Pet kennel exposure was defined as experience at a dog kennel or boarding facility (without distinction of vet hospital boarding or elsewhere) within the past 12 months. Presence or absence of household pests or mould was determined by survey reporting and home inspection. Season (meteorological) was defined as winter, spring, summer or autumn, with season end date cut-offs of 20March, 21June, 22September, and 21December. A previously developed scale defining human–animal contact within the home was extrapolated from human MRSA transmission research, with score points assigned for provision of care (feeding, bathing, medicating, grooming, exercising), close sleeping quarters (on the same bed), letting animals lick the human participant’s hands or face, and housing pets indoors, with a maximum point total of nine.11 Based on the distribution of data in this study (median survey response score of two), human–animal contact scores of two or above were characterised as “close human contact”, and scores less than two were labelled “casual human contact.”

**Statistical methods**

Variables were explored and aggregated if needed. Survey-weighting for unadjusted and adjusted logistic regressions accounted for clustering of pets within their respective households. Those variables showing unadjusted associations with MRSA detection with *p*-values <0.20 were further explored in adjusted logistic regression models. Species-stratified analysis was conducted to explore the potential for effect modification by species; limitations of sample size restricted this exploratory analysis to unadjusted logistic regression models only.

Variables with strong *a priori* evidence of epidemiological significance were retained in the final multiple logistic regression model of risk factors for pet carriage of MRSA at baseline. Forward and backward stepwise variable selection (with a retention α level of 0.05) and likelihood ratio tests were conducted to determine best model fit. Only one variable remained significant at α = 0.05 after using the Benjamini and Hochberg false discovery rate (FDR) ranking to consider the impact of multiple comparisons; *p*-values presented are not FDR-adjusted.

**RESULTS S1**

**Baseline analysis**

Because no pets carrying MRSA were unexposed to household environmental MRSA, this variable could not be included in multivariate models. A number of factors were of marginal significance (*p* > 0.05 and *p* < 0.20) in unadjusted analysis at baseline and were considered for multivariate evaluation. These included flea infestation at household examination, neutered status and exposure to a boarding kennel within the past year (Table 1b). False discovery rate analysis suggested that only pet bed MRSA contamination would remain significant after consideration of multiple comparisons. After adjusting for all other variables, pet neuter status and kennel exposure were no longer significant (*p* = 0.729 and *p* = 0.354, respectively).

On sensitivity analysis for an adjusted model including veterinary clinic visits instead of pet antibiotic use, the associations between MRSA isolation from pet bedding and fleas on pet exam were unchanged, and an association between vet clinic visit association and pet MRSA carriage was not significant (*p* = 0.118).

Stepwise forward and backward logistic regression selection resulted in retention of pet antimicrobial use, presence of fleas on the pet and pet bed isolation of MRSA in a multiple logistic regression model. Inclusion of the human–animal contact variable did not improve model fit.

Within a subset unadjusted analysis of dogs only, the odds of Labrador or Labrador-mix breeds to have detectable MRSA was 15-fold higher than other dog breeds (OR = 15.0; 95% CI 2.26, 99.68; *p* = 0.006; Table 1). As a consequence of the limited within-stratum sample size and the attendant power constraints, these results should be interpreted with caution. Nonetheless, the average human–pet closeness score of Labradors was 1.7, while the average human–pet closeness score of non-Labradors was 2.0, and no other measured variables were identified that changed the measure of association between Labrador breed and MRSA. Variables regarding pet owner ethnicity and household income were highly collinear in this population. Dog breed was not found to be collinear with owner’s ethnicity, household income or community location (rural versus nonrural).

**Risk factor analysis at three months and longitudinal implications**

At the three month visit for this pet population (after the RCT trial conducted a randomisation protocol for human treatment with one week nasal mupirocin and two chlorhexidine-based body washes), MRSA carriage was detected in seven of 86 dogs and cats, and only one of the seven MRSA-positive pets lived in a MRSA-negative environment based on swab samples (tables S3 and S4).

Of 74 pets tested both at baseline and three months, 13 (17.6%) were MRSA-positive at either or both time points. Of the nine longitudinally-sampled pets that were MRSA-positive at the first visit, five (55.5%) pets cleared MRSA carriage during this time and four (44.4%) pets had persistent carriage. Four (5.4%) pets had incident MRSA carriage (i.e. MRSA not present at baseline and then isolated at three month sampling). All pets with persistent carriage carried the same MRSA *spa*-type on both visits. Longitudinal comparisons of *spa*-types recovered from animal isolates at baseline and three months are provided in Table S5. See Results S1 and Table S4 for final risk factor model variable analysis at three months.

**DISCUSSION S1**

Use of antimicrobials in pets was 100% collinear with veterinary hospital exposure, making these two variables indistinguishable. However, because all enrolled pets were exposed to a CA-MRSA-positive human index patient and a community-associated *spa*-type was shared between them in most cases, it is more likely that MRSA transmission occurred in the home.

Notably, our group previously has identified that rates of multidrug resistance were higher among *S. aureus* than *S. pseudintermedius* or other staphyloccal strains recovered from pets in this study,12 which has clinical implications for treatment in the event of animal disease, such as the surgical site infection identified between baseline and follow-up visits in a single dog in this study.13

The presence of fleas on exam appeared to be associated with higher risk of MRSA carriage in our pet population. Flea bites may cause skin lesions vulnerable to infection; fleas also could perhaps serve as a mechanical vector carrying MRSA between humans and animals; or the presence of fleas could be associated with other measured or unmeasured variables. Flea allergy dermatitis (FAD) is a common precursor to skin disruption, excoriation and increased immunoglobulin (Ig)E titres in pets.14,15 We could hypothesise that fleas disrupt the skin’s barrier function as a consequence of pet self-trauma from pruritus and that this may increase the risk of MRSA carriage in pets exposed to a known MRSA case. Further studies should consider fleas/ectoparasites as a potential factor for MRSA carriage in pets.

No categorisation or dichotomisation of our person–pet contact score variable had a statistically significant association with pet carriage of MRSA. When the person–pet contact score variable was dichotomised at its median in this population (median contact score was 2 of 9, which is subjectively quite low), the odds ratio indicated the potential for person–pet contact to be a risk factor (OR 1.58; 95% CI 0.29, 8.67; *p* = 0.596), and this estimate was not statistically significant. Conversely, when the score was dichotomised with scores of 4, 5 or 6 of 9 as “close” contact and scores under 3 of 9 as “casual” contact, the odds ratio suggested person–pet contact as a protective factor for pet carriage of MRSA (OR 0.46; 95% CI 0.10, 2.08; *p* = 0.309), and again the estimate was nonsignificant. However, owing to small sample size neither of these effect sizes are reliable and the effect of person–pet contact on pet or human MRSA carriage remains a research question of interest.

Recall and ascertainment biases were limited in this study because the same staff member collected specimens simultaneously, and microbiological comparisons of isolates were performed for a large number of survey respondents. Data were evaluated through two separate cross-sectional analyses, preventing identification of causal association between risk factors and outcome.

Labradors and Labrador-mix dog breeds were associated with increased risk of MRSA carriage in our population, while cat breed had no association with MRSA carriage. In a previous analysis of this study population, rural households were 100% collinear with environmental multidrug-resistant (MDR) *Staphylococcus* contamination.7 In the present analysis, no relationship was found between dog breed and rural (versus suburban) residence, household income quartile or human–pet closeness score. There was no association between rural residence and any pet carriage of MRSA. While it is possible that the association we see between Labrador breed and MRSA carriage can be explained by other unmeasured variables, future studies should continue to evaluate the potential for this breed to be at higher risk for MRSA carriage in this context.

Another author has addressed cleaning/disinfection in relation to identification of MDR among MRSA detected from this population in a separate manuscript.2 The manuscript found that *not* using EPA-listed cleaning products as effective against MRSA was significantly protective against home contamination with MDR MRSA, suggesting the potential for use of MRSA-cidal cleaning products in the home to exert selective pressure and select for MDR strains. This has potential consequences for pet MRSA carriage, given that households in this study were randomised to household-wide human decolonisation treatment (nasal mupirocin, a topical antibiotic, and chlorhexidine body wash, a topical antiseptic) between the baseline and follow-up home visits. Supporting this, prior work on environmental MRSA isolates in this population suggested the potential for human decolonisation treatment with chlorhexidine to be associated with *qac*-gene positivity (a marker of potential chlorhexidine resistance) in the environmental reservoir.16 Human decolonisation treatment could reduce MRSA shedding into the home environment.7 In our current analysis, human MRSA decolonisation efforts, whether supervised or unsupervised, had no significant association with MRSA carriage in household pets at the follow-up home visit, although sample size was limited to identify small effects.

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**TABLE S1.**

|  |  |  |
| --- | --- | --- |
| **Pet enrollment and sampling** | **Baseline** | **Three month follow-up** |
| Households enrolled, no. | 95 | 65 |
| Households with pets, no. (%) | 67 (70.5%) | 44 (67.7%) |
| Pets enrolled, no. | 184 | 130 |
| Pets sampled, no. | 1791 (97.3%) | 1254 (96.2%) |
| Dogs, no. (%) | 71 (39.7%) | 38 (30.4%) |
| Cats, no. (%) | 63 (35.2%) | 48 (38.4%) |
| Small mammals2, no. (%) | 11 (6.1%) | 9 (7.2%) |
| Reptiles and birds3, no. (%) | 23 (12.8%) | 20 (16.0%) |
| Freshwater fish tank, no. (%) | 11 (6.1%) | 11 (8.8%) |

|  |
| --- |
| 1179 of 184 enrolled pets were sampled. Reasons for not sampling were (i) pet was outside or in an unattainable location, or (ii) pet was too aggressive to be sampled |
| 2Chinchillas, hamsters, rat, sugar glider, ferret, rabbit |
| 3Turtles (primarily aquatic), lizards, snake, parrots |
| 4108 continuing pets, 17 new pets at follow-up (10 cats, three reptiles, three fish, one hamster), 125 total sampled |

**TABLE S2. Pet antibiotic use and veterinary clinic visits at baseline**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **No vet visit or antibiotics** | **Vet visit without antibiotics** | **Vet visit with antibiotics** | **Antibiotics without vet visit** |
| Cats (N = 63) |  |  |  |  |
| MRSA-positive,  no. (%) | 3 (4.8%) | 1 (1.6%) | 3 (4.8%) | 0 |
| MRSA-negative,  no. (%) | 39 (61.9%) | 15 (23.8%) | 2 (3.2%) | 0 |
| Dogs (N = 71) |  |  |  |  |
| MRSA-positive,  no. (%) | 2 (2.8%) | 3 (4.2%) | 0 | 0 |
| MRSA-negative,  no. (%) | 37 (52.1%) | 24 (33.8%) | 5 (7.0%) | 0 |
|  |  |  |  |  |
|  | **No antibiotics**  **in one year** | **Last received antibiotics in past year** | **Last received antibiotics in past six months** | **Last received antibiotics in past month** |
| Cats (N = 63) |  |  |  |  |
| MRSA-positive,  no. (%) | 4 (6.3%) | 1 (1.6%) | 1 (1.6%) | 1 (1.6%) |
| MRSA-negative,  no. (%) | 54 (85.7%) | 0 | 1 (1.6%) | 1 (1.6%) |
| Dogs (N = 71) |  |  |  |  |
| MRSA-positive,  no. (%) | 5 (7.0%) | 0 | 0 | 0 |
| MRSA-negative,  no. (%) | 61 (85.9%) | 3 (4.2%) | 1 (1.4%) | 1 (1.4%) |

**TABLE S3. *spa*-typing at three months**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| House ID | Human | Pet(s)\* | Environment | No. cats positive | Total no. cats | No. dogs positive | Total no. dogs |
|  |  |  |  |  |  |  |  |
| A | NT | No MRSA | No MRSA | 0 | 2 | 0 | 3 |
| C | t008 | No MRSA | t008 | 0 | 2 | 0 | 0 |
| D | t008 | t008 | t008 | 1 | 9 | 0 | 0 |
| E | t12500 | t12500 | t12500 | 0 | 0 | 3 | 3 |
| F | t216 | t216 | t216 & t2008 | 0 | 0 | 1 | 2 |
| H | t121 | t121 | t121 | 1 | 3 | 0 | 0 |
| I | NT | UTBT | No MRSA | 0 | 0 | 1 | 3 |
| J | t008 | t008 | t008 & t179 | 0 | 6 | 1 | 1 |

\*No positive housemate pets were discordant with each other

UTBT, unable to be typed; NT, not tested

**TABLE S4. Dog and Cat 3-month Risk Factors, Unadjusted and Adjusted**

|  |  |  |
| --- | --- | --- |
| **Risk Factors** | **Unadjusted Logistic Regression** | **Adjusted, Multiple Logistic Regression** |
| **n=134** | **OR (95% CI)** | **OR (95% CI)** |
| **Species (cat v. dog)** | 0.57 (0.07, 4.53) | 0.55 (0.60, 5.08) |
| **Pet Age** | 0.69 (0.11, 4.35) | 0.45 (0.98, 2.09) |
| **Pet Sex** | 6.15 (0.71, 53.58) | 21.72 (3.24, 145.32) |
| **Environmental MRSA positivity** | 4.77 (0.48, 47.76) | 12.92 (0.57, 290.89) |
| **Pet bed MRSA positivity** | 2.03 (0.21, 19.77) | 2.45 (0.23, 26.67) |
| **Pet antibiotic use** | 3.13 (0.21, 45.73) | 10.08 (0.38, 265.01) |
| **Fleas on pet exam** | 1.03 (0.17, 6.14) | 0.47 (0.00, 0.09) |

\* p<0.05   
\*\* p<0.01   
\*\*\* p<0.001

**TABLE S5. Longitudinal Comparisons of Pet MRSA Isolates**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **All Pets, 74\*** | **Dogs, 38** | **Cats, 36** |
| **Negative - Negative** | 61(82.4%) | 30 | 31 |
| **Negative - Positive**  **(Incident)** | 4 (5.4%)† | 3 | 1 |
| **Positive - Negative (Cleared)** | 5 (6.8%)‡ | 3 | 2 |
| **Positive – Positive, *Spa*-type concordant**  **(Persistent carriage)** | 4 (5.4%)§ | 2 | 2 |
| **Positive – Positive, *Spa­*-type discordant** | 0 (0.0%) | 0 | 0 |

\*All pets represented in this table were sampled longitudinally, at both baseline and 3-month visits.

† *spa*-types t12500 and t008

‡ *spa*-types t008, t216 and t334

§ spa-types t12500, t008, t216 and t121