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The Common House Spider *Tegenaria saeva* is an unlikely vector or reservoir of Community-Associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA)

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Abstract

Aim: To identify whether the common house spider *Tegenaria saeva* can act as a potential vector and reservoir of Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA).
Methods and Results: *Tegenaria saeva* were screened for the presence of *S. aureus* and CA-MRSA on the fangs, legs, outer body and internal microflora. None of the spiders processed in this investigation carried MRSA. However, 37.5% did carry *S. aureus*. Overall, low levels of microbes were isolated from the spiders processed.
Conclusion: *T. saeva* is an unlikely vector of CA-MRSA. A large percentage of 'spider bite' lesions are normally misdiagnosed bacterial infections, with limited evidence linking them to spider bites.
Significance and Impact of Study: The consequence of misdiagnosed 'spider bite' lesions could lead to untreated bacterial infections. If this was to occur with a community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection, the outcome could be life threatening.

Keywords: Methicillin Resistant *Staphylococcus aureus*• MRSA • community-associated MRSA•CA-MRSA• *Tegenaria saeva*• vector• spider bite• spider bite lesions

Introduction

The burden of Methicillin-resistant *Staphylococcus aureus* (MRSA) is no longer simply confined to the hospital environment. With the emergence of community-associated MRSA (CA-MRSA), in the USA and the UK, infections have now been reported in seemingly healthy members of the public, with no compromised immune system. This is radically different from hospital-acquired MRSA (HA-MRSA) which usually only causes disease in individuals with a compromised immune system (Millar *et al.* 2007). All known MRSA stains carry a genetic transposable element known as the *Staphylococcal Cassette Chromosome* (SCC*mec*), this is a pathogenicity island containing the *MecA* gene. This gene codes for a penicillin-binding protein (PBP) known as 2a (Berger-Bachi & Rohrer 2002) which provides resistance against all licensed β-lactam antibiotics (Van Bambeke *et al.* 2008). There are currently five know SCC*mec* (I-V) found in MRSA isolates (Chongtrakool 2006). CA-MRSA is genotypically and phenotypically distinct from HA-MRSA, which allows it to be easily identified with the use of molecular techniques. The majority of CA-MRSA strains harbour the SCC*mec* types *IV* and *V*. These are smaller genetic units that only provide resistance against β-lactam antibiotics, and unlike their hospital-associated counterpart, are not resistant to a range of antibiotics (Elston & Barlow 2009). The clinical spectrum of CA-MRSA is clearly distinct from that caused by HA-MRSA. In a large majority of cases HA-MRSA causes blood stream infections and infections of the urinary and respiratory tracts (Kluytmans-VandenBergh & Kluytmans 2006). However, CA-MRSA has mainly been isolated from skin and soft tissue infections (SSTIs) (Fridkin *et al.* 2005). Although the large percentage of CA-MRSA infections are mild, they can be severe and result in death (Centres for Disease Control and Prevention 1999). Therefore, CA-MRSA is clinically important and the increase in the frequency of CA-MRSA infections has led to a need to identify potential vectors and reservoirs of CA-MRSA.

In many cases in the USA and the UK, where CA-MRSA has caused skin and soft tissue infections, it is often perceived as being preceded by a spider bite (Suchard 2009). There are several explanations to this; firstly, spiders introduce MRSA into a bite wound and thus act as vector for the spread of MRSA; secondly, MRSA colonization is a secondary event to a spider bite; and thirdly, spider bites are a misguided explanation for why patients have skin and soft tissue infections (Baxtrom *et al.* 2006). Therefore, if common household spiders do act as a vector for MRSA then they should be consequently colonized by MRSA.

The *Tegenaria saeva* (Family: *Agelenidae*) population is mainly concentrated in the South West of England and Southern Wales and individuals are commonly found in the household environment. *T. saeva* is regularly mistaken for other species in the *Tegenaria* genus, such as *Tegenaria duellica,* another common large house spider (Jones 1983). The female of the species is the larger at <18mm, where as the males are usually <14mm. Mature females can be found all year but the males tend to mature in the late summer and early autumn. They are distinguishable from their reproductive partner due to longer legs and their epigynes and male palp (Roberts 1995). *T. saeva* is a close relative to *Tegenaria agrestis*, the notorious "hobo spider". *T. agrestis* has been the centre of much debate in the USA regarding its medical importance, and many medical professionals have blamed necrotic lesions on bites from this organism (Vetter & Isbister 2004). If an infection occurs around an area of a suspected bite, the infection is termed *necrotic arachnidism*.

The aim of this study was to determine if the common household spider *T. saeva* could act as a potential vector and reservoir of CA-MRSA. Different parts of the

organism's body were investigated in order to determine the presence and distribution of microorganisms across *T. saeva*. As well as the external microflora, the internal microflora was investigated. For obvious medical reason, the fangs will be the main focus of this study. As *T. saeva* has been associated with human households for many years, it could be hypothesised that they will harbour similar organisms among their microflora to humans.

Materials and Methods

Spider Collection
Fellow students and members of staff assisted in collecting common house spiders in and around Plymouth. This study was focused on determining the presence of MRSA solely on *T. saeva*. Therefore, any other species handed in were not used and promptly released. Identification of species was achieved using Robert's (1995). Spiders were also collected in houses around Plymouth and the University of Plymouth's horticultural gardens. All spiders collected were stored in sterile specimen containers and processed within two days to prevent a misrepresentative change in microbial flora. Any spiders kept in captivity for over three days without being processed were not used in the investigation and promptly released. An email was circulated to students and staff at the University of Plymouth asking for any spiders found in the household environment to be collected and handed in for identification. Attempts were made during this study to obtain air time on a local radio station to promote the collection of household spiders from the general public. Unfortunately, attempts were unsuccessful.

Each spider collected was given a number and the location where the spider was found was also recorded.

Microbial Culture
Mannitol-Salt Agar (MSA: Oxoid, Basingstoke) was used in order to select for *S. aureus*. Colonies of *S. aureus* are easily identified by the fermentation of mannitol, as indicated by a change in the phenol red indicator, from red to yellow. Positive identification of *S. aureus* was achieved with the use of both the Gram-Stain and the catalase test. In order to select for CA-MRSA strains, oxacillin-mannitol salt agar (OMSA) was produced. Oxacillin is a β-lactam antibiotic and according to Gorak *et al.* 1999 this means that the strain can be classified as CA-MRSA. This was achieved by adding 8µg/µl of oxacillin to MSA. In order to determine the usability of the cultures, they were positively controlled with known MRSA strains and negatively controlled with methicillin-sensitive *Staphylococcus aureus* (MSSA). Growth of MRSA and inhibition of MSSA strains allowed the cultures to be used in the investigation. The MSA and OMSA cultures were incubated at 37ºC for 48 hours. A random sample of *S. aureus* that was isolated from the MSA was transferred onto OMSA plates, to ensure no MRSA was present.

Tryptone Glucose Agar (TGY: Oxoid, Basingstoke) was also produced in order to gain an understanding of the overall microflora of the organisms. The TGY cultures were incubated at 30ºC for 48 hours. Identification of bacterial species was achieved using Cowan and Steel (1993), Gram staining and microscope identification. Fungal species were identified using Gilman & Joseph (1998).

Spider Processing
All aspects of spider processing were completed under aseptic conditions. Gloves were always worn when dealing with the spiders and all dissecting tools (surgical

scalpel and surgical tweezers) were sterilised between each stage of the spider dissection, ensuring that limited cross contamination occurred between each body part. The spiders were terminated by removing the fangs with a sterile scalpel and then mechanically destroying the main ganglia in the head of the individual, instantly killing the organism. All of this work was carried out on a sterilised glass plate, which was sterilised between each spider. The following body parts were investigated:

Fangs: The fangs were instantly transferred into a sterile bijoux tube containing 200µl of sterile phosphate buffer saline (PBS) pH 7.2. This was vortexed for 30 seconds to suspend the microbes in the PBS, and 100µl of the original solution was plated out onto an OMSA plate using standard spread plate techniques. As little is known about the abundance of microorganisms on *T. saeva,* this first spread plate was carried out to ensure that the microbes were not 'diluted out' in the preceding dilution series. OMSA was the plate chosen to use for the 'pure culture' as the main aim of this investigation was to determine if *T. saeva* carries MRSA in its normal microflora. A serial dilution series was then carried out using the remaining 100µl of suspension. Each dilution was then spread out onto the OMSA plates, the MSA plates and the TGY plates, and incubated as described in the microbial culture section. It should be pointed out that each suspension was vortexed for 30 seconds between each stage of the dilution series and each sample was left no longer than 20 minutes before being spread out onto the plates.

Legs: Four of the organism's legs were removed and added to a bijoux tube containing 200µl of PBS. The same procedures described above were used in order to produce the spread plates.

External Microflora (Thorax and abdomen): In order to access the external microflora of the organism, the remaining legs were removed   and the body was dropped into 200µl of PBS and then vortexed for 30 second before being plated out using the procedure described.

Internal microflora: In order to culture microbes from the internal chambers of the organism, the following procedure was carried out. The body of the specimen was dipped into 98% alcohol in order to remove organisms still persisting on the outside on the body, which would contaminate the internal microflora. The body was then placed on a sterile Petri dish until the alcohol had visibly evaporated. The body was then placed into 200µl of PBS and homogenised thoroughly with a sterile glass rod. This was then vortexed for 30 seconds before being plated out using the procedure described above.

Results

In total 16 individual *T. saeva* were collected and processed in this investigation (table 1).

Table 1 Origin of Spiders Processed

Spider Number (s)	Location Found (% of total collected)
10,13,14,15,1,9	House (37.5%)
5,7,11,8	University Greenhouse (25%)
2,6,16	Garden Shed (18.5%)
4	Garden (6.25%)
3	Conservatory (6.25%)
12	University Building (6.25%)

OMSA Cultures
No MRSA was isolated from any of the body parts of the 16 *T. saeva* processed in this investigation.

MSA Cultures
S. aureus was isolated from 37.5% of the spiders processed and the colony counts are shown below in table 2. A much larger percentage of cultures actually showed little or no growth, with 62.5% of the cultures showing no microbial growth, even on the TGY plates.

Table 2 Methicillin-Sensitive *Staphylococcus aureus* colony counts isolated from different body parts of the *T. saeva* processed (body parts showing different subscripts are significantly different). Key to body parts: F= Fang, E= Exterior Flora, I= Internal Flora, L= Leg

Spider	Bodypart	CFU Per Body Part
2	F_a	460
	L_b	3960
	I_a	200
	E_a	700
5	F_a	1100
	L_b	4080
	I_a	480
	E_a	1160
6	F_a	2100
	L_b	14040
	I_a	1160
	E_a	4080
10	F_a	6000
	L_b	11600
	I_a	2020
	E_a	4140
13	F_a	1740
	L_b	4420
	I_a	960
	E_a	1320
16	F_a	1340
	L_b	3340
	I_a	1360
	E_a	1800

The results showed that the colony numbers from the external flora and legs were significantly different, the colony numbers from the fangs were significantly different from the legs and the numbers from the interior chambers were significantly different to the legs (*P value= 0.005*).

None of these isolates grew on the OMSA plates indicating that no MRSA was present on any of the 16 spiders investigated in this study. The mean colony numbers are shown in table 3.

Table 3 Mean colony counts of Methicillin-Sensitive *Staphylococcus aureus* found on spiders 2, 5, 6,10,13,16

Body Part	Mean CFU *S. aureus*	Standard Deviation
Fang	2123	1980
Legs	6907	4658
Internal	1030	648
External	2200	1521

TGY Cultures

Table 4 shows the microorganisms isolated from *T. saeva*. Individual spiders not shown had no growth anywhere on the body. *S. aureus* was not included in this table as its presence has already been show in table 2. It should be noted that the *S. epidermis* was isolated on the MSA cultures, it was identified by the fact no mannitol was fermented on the MSA cultures and using techniques described in the methods. It should be noted that all other TGY plates from the other ten spiders showed no growth.

Table 4 Identification of other microbes isolated from the TGY and MSA cultures. Key to body parts: F= Fang, E= Exterior Flora, I= Internal Flora, L= Legs

Spider	Body Part	Microbes identified
2	F	*Bacillus* sap, *Staphylococcus epidermidis*
	L	*S. epidermidis*
	I	*Corynebacterium*
	E	*Bacillus ssp*
5	F	No growth
	L	*S. epidermidis*
	I	*Bacillus ssp*
	E	*Bacillus ssp,*
6	F	No growth
	L	*S. epidermidis*
	I	No growth
	E	*Aspergillus,*
10	F	No growth
	L	*S. epidermidis*
	I	*Bacillus ssp*
	E	*S. epidermidis*
13	F	*S. epidermidis*
	L	*S. epidermidis*
	I	No growth
	E	*S. epidermidis*
16	F	No growth
	L	*Stachybotrys*
	I	*Bacillus ssp*
	E	*Aspergillus,*

Discussion

From the 16 spiders processed in this investigation, no MRSA was isolated from any of the organisms. The fact no MRSA isolates were found on the 'pure' cultures also provides evidence that no organisms were diluted out during the serial dilution. This could indicate that a low percentage of *T. saeva* in Plymouth carry MRSA strains among their natural microflora. However, it would be difficult to make any general assumptions seeing as the overall sample size was actually quite small (16 individuals). In order to be able to make a more reliable assessment of the prevalence of MRSA on *T. saeva,* a much larger sample size would be needed to be processed. One of the major limiting factors restricting the sample size in this investigation was the timing of the study. Work began on this project in October, 2009 and the majority of the spider collecting occurred in this period. The majority of *T. saeva* individuals mature in late summer and early autumn, and are normally found in houses in high numbers during this time. Therefore, in order to increase sample numbers, collecting spiders earlier in the year would be a huge advantage. In order to increase the number of specimens collected, it would have been beneficial to inform the general public about the investigation in the hope that samples could have been collected in homes and handed into the university. This could be achieved by creating a 'sample pack' which could be handed out to the general public in and around Plymouth. The pack could include items such as sterile sample pots, and identification keys.

The results obtained in this study do, however, coincide with a large scale study in the USA. Baxtrom *et al.* (2006) screened over 100 common house spiders and isolated no MRSA from any of the specimens sampled, including species in the *Agelenidae* family. However, one big difference between the studies did occur. They did not isolate any *S. aureus* from any of their specimens, which was markedly different from the results obtained in this study in which 37.5% of the organisms investigated carried *S. aureus* among their microflora. One reason for this observation could be due to the locations where the spiders were collected. Of the six spiders found carrying S*. aureus,* 66.6% were found outside the household environment, either in the university greenhouses or in garden sheds. *S. aureus* is a very widely dispersed organism and is frequently found in soils and in the outdoor environment. The majority of the organisms screened in Baxtrom's *et al.* investigation were found in the household environment. This could explain the difference between the investigations. The high standard deviations imply that there is a wide scope for *S. aureus* colonisation on *T. saeva.* Interestingly, if an organism was colonised with *S. aureus* it seemed to also be colonised with other microorganisms.

S*. aureus* is an opportunistic pathogen that can cause various diseases. The most common include minor infections of the skin, post-operative wound infections and necrotising pneumonia. It can also cause life threatening sepsis and toxic shock syndrome (Madigan *et al.* 2000). However, MSSA infections are easily treated with the use of modern antibiotics, and infections rarely cause mortalities.

Roberts (2010) and Staddon (2008 unpublished data) both isolated *S. aureus* from the *T. saeva* processed in their investigations. The distribution of *S. aureus* across the organism was strikingly different from the results obtained in this study. In their investigation *S. aureus* was predominantly found on the fangs and very low levels were isolated from any other body part. One of the main reasons for this

outcome could be due to a difference in methods used. In their experiments, only the fangs were placed in PBS and subsequently plated out. In comparison, the rest of the body parts were swabbed using sterile cotton swabs. This method would not have isolated as many microorganisms as the methods carried out in this investigation, where each body part was placed in PBS. Not only because the agitating of the body parts within the PBS would have transferred more microorganisms, but also because of the physical properties of the spider's skin. The surface of the spider's body is not a smooth flat surface, the chitin exoskeleton of many arthropods is rough and highly heterogeneous (Roberts 1995). This means that many microorganisms may not have been removed from the organism with the use of a crude swab. Therefore, it is plausible that the methods used in this investigation give a much more reliable representation of the diversity and distribution of microorganisms across the surface of the spider. In this investigation the legs of the organism harboured the highest number of isolates. This is not surprising as the spiders legs readily come into contact with many different environments and overall, they would have had a larger surface area.

From the results it can be seen that a large percentage of the spiders were associated with very low levels of microbialflora; 62.5% of the individuals processed showed no growth, even on the TGY cultures. There could be a number of reasons for this outcome. Firstly, the incubation temperature of the TGY cultures was 30°C. This may not have been a suitable incubation temperature for many microorganisms found on the spiders. In order to access this point, lower and more environmentally realistic incubation temperatures could be used.

The spiders are also known to clean themselves on a regular basis. This could have had an impact on the number of bacterial species isolated. In order to test this hypothesis a further test could be carried out. A number of individual spiders could be deliberately inoculated with a variety of known bacterial species and kept in clear containers. Their behaviour could then be monitored, either in person or with the use of a video camera, to observe for cleaning behaviour. Microbial numbers could then be assessed after a period of time and compared with the total numbers of 'cleans' performed. This may be able to determine if cleaning reduces microbialflora on the organism.

The cleaning performed by the spiders was one of the main reasons why the internal flora of the organism was investigated. This is because the spiders clean themselves by moving their legs through their chelicerates (Roberts, 1995) and microorganisms could have been transferred to the internal compartments of the individual. However, overall low levels of microbialflora were isolated from the internal compartments.

From the microbes isolated there were no medically important species present, and the majority of species identified are ubiquitous in the environment (i.e. *Bacillus spp, Corynebacterium*). The prevalence of *S. epidermidis* could be due to a number of reasons; it is part of the human skin flora and its presence could be due to contamination. However, as it was isolated from spiders 10 and 13, they could have acquired the microorganims from the household environment, supporting the hypothesis described in the introduction. The fungal species identified were of low medical importance and are found ubiquitously in soils, which is probably the reason why they were isolated from *T. saeva* found outdoors (Klich 2002).

There is a wide scope for further work in this field, not only based on the presence of medically important microbes on common spiders, but more generally on the microflora of spiders in general. There is a real lack of research in this field and little

is know about the common relationships found between microorganisms and spiders. A next logical step in this area of research would be to determine if MRSA strains can survive on the surfaces of *T. saeva*. This could be achieved by deliberately incubating spiders with known stains of MRSA and observing survival rates of MRSA on the spiders over a period of time. This would determine whether MRSA can be sustained on *T. saeva*, and it would also give an approximate time of survival of the isolates on the body. A similar experiment was carried out by Gaver-Wainwright *et al.* (2009, *pers. comm*) and the work presented at the 2009 annual meeting of the Entomology Society of America. Their investigation was based on the ability of *T. agrestis to* act as a vector of MRSA, and their results indicated that MRSA could not survive on *T. agrestis* for more than an hour. These results provide further evidence that common house spiders are unlikely vectors of MRSA.

As well as the evidence already discussed implying that common house spiders are unlikely vectors of MRSA, recent publications have also investigated the medical reports of 'spider bite' lesions. Suchard (2009) aimed to determine the percentage of patients reporting a 'spider bite' who received clinical diagnosis of spider bite by their physician vs. other causes, such as CA-MRSA infections (Suchard 2009). This study was conducted in an academic, suburban emergency department in the USA (ED). There were 194 patients reporting a 'spider bite' documented between January and November 2007. The great majority (83.5%) of these patients were diagnosed with SSTIs only. Only 3.8% of the patients had their complaint of a spider bite confirmed by their treating physicians and additional arachnid specialists. CA-MRSA is a common cause of SSTIs and it was isolated from 70% of all positive wound cultures obtained in the study. The misattribution of lesions caused by CA-MRSA seems to result from the frequent finding of central dermonecrosis. This is likely to be linked to the production of the Panton-Valentine toxin by CA-MRSA stains, which is known to cause dermonecrosis (Boyle-Vavra & Daum 2007). Numerous other case studies have provided evidence that would suggest that spiders do not act as vectors of CA-MRSA (Dominguez 2004). Therefore, it has been shown that a high percentage of lesions linked to 'spider bites' are actually bacterial infections, normally due to CA-MRSA, and unlikely to be caused by spider bites. It should be pointed out that 50% of the diagnosed spider bites were identified to be caused by *Latrodectus hesperus*, the clinically important 'black widow spider' and not members of the *Tegenaria* genus.

The clinical importance of *T. saeva* is negligible and is a non-aggressive, benign organism (Roberts, 1995). In numerous searches of the literature, no recorded bites from *T. saeva* were found, and more importantly, no CA-MRSA infections were associated with bites from this organism. Therefore, it could be concluded that *T. saeva* is an unlikely reservoir of MRSA strains, and an unlikely vector due to the fact inadequate evidence is available to support the notion they could transmit CA-MRSA to human hosts. The medical importance of *T. agrestis* is also up for much debate in the USA (Vetter & Isbister 2004).

It should also be acknowledged that the ecology of CA-MRSA in the environment is poorly understood. Further research needs to be carried out in order to address the survival and distribution of CA-MRSA in the environment in order to improve predictions of potential epidemics.

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