The European spider *Tegenaria agrestis* (Walckenaer) (hobo spider) has been implicated as a spider of medical importance in the Pacific Northwest since its introduction in the late 1980s. Studies have indicated that the hobo spider causes necrotic tissue lesions through hemolytic venom or through the transfer of pathogenic bacteria introduced by its bite. Bacterial infections are often diagnosed as spider bites, in particular the pathogenic bacteria methicillin-resistant *Staphylococcus aureus* (MRSA). This study examines three aspects of the potential medical importance of hobo spiders in part of its introduced range, Washington State. First, the bacterial diversity of the spider was surveyed using a polymerase chain reaction-based assay to determine whether the spider carries any pathogenic bacteria. Second, an experiment was conducted to determine the ability of the spiders to transfer MRSA. Third, the venom was evaluated to assess the hemolytic activity. We found 10 genera of ubiquitous bacteria on the exterior surface of the spiders. In addition, none of the spiders exposed to MRSA transferred this pathogen. Finally, the hemolytic venom assay corroborates previous studies that found hobo spider venom was not deleterious to vertebrate red blood cells.

**KEY WORDS** *Tegenaria agrestis*, hobo spider, methicillin-resistant *Staphylococcus aureus*, bacteria, spider bite
native Europe (Binford 2001). In fact, a venom analysis did not find any components that would induce tissue necrosis in mammals (Binford 2001; Gomez and Binford, personal communication). Therefore, an important step in verifying the potential toxicity of the hobo spider bite is to identify the microbial fauna associated with this spider, which may be vectored during a bite or accidental injury.

We identified the microbial fauna associated with *T. agrestis* as a first step toward establishing whether hobo spiders can carry and transfer pathogenic bacteria. To estimate bacterial biodiversity, universal primers were used in a polymerase chain reaction (PCR)-based assay to amplify the prokaryotic 16S rRNA gene. This technique has been used in a variety of natural environments, including marine sediments (Marchesi et al. 1998), cerebrospinal fluid (Lu et al. 2000), the termite gut (Hongoh et al. 2003), and the human gut (Ley et al. 2005). We amplified a conserved region of the bacterial 16S rRNA gene that contains enough sequence divergence to accurately distinguish between hundreds of bacterial species within a sample (Marchesi et al. 1998, Osborn et al. 2000). In addition to the bacterial diversity survey, we also used a more direct approach to assess the possibility that hobo spiders can mechanically acquire and transfer a highly pathogenic bacterium such as methicillin-resistant *Staphylococcus aureus* (MRSA). Finally, we conducted standard hemolytic assays to determine toxicity of *T. agrestis* venom to mammals to corroborate the results of Binford (2001) and Binford and Gomez (unpublished) venom analysis.

**Materials and Methods**

**Spider Collection.** To examine the microbial fauna associated with *T. agrestis*, spiders were collected into sterile 50-ml Falcon tubes from around the outside of homes in urban environments in the cities of Spokane, Pullman, Bellevue, and Puyallup, Washington State. Spiders were located by searching under rocks, concrete blocks, and landscape material using webbing as an indicator for potential habitat. Positive identification of adult spiders was accomplished by examination of sclerotized genitalia according to Vetter and Antonelli (2002). Immature spiders were identified to genus using Ubick et al. (2005), followed by an examination of seminal patterns as delineated in Vetter and Antonelli (2002). Spiders were individually held in sterile containers and tested upon arrival to the laboratory or within 48 h depending upon collecting location. No food or water was administered before the experiments described in the following paragraphs.

**Bacterial Diversity Assay.** To identify external and internal bacterial species associated with *T. agrestis*, we isolated microbes from spiders using the following sampling methods. First, whole spiders were individually ground with a mortar and pestle in Tris-ethylenediaminetetraacetic acid in a salt-buffered solution. Second, whole spiders were individually ground with mortar and pestle in Luria-Bertoni (LB) broth in case the bacteria were sensitive to the medium in which the spiders were ground. We chose a generalized, bacterial medium as the grinding solution. We dissected abdomens and ground them in fresh LB broth to isolate the internal gut-associated microbial fauna. Finally, multiple spiders were individually washed in 5 ml of LB medium for 1 min each to pool bacterial species associated with the exterior of the spiders. Multiple culture methods were used, including plating different concentrations of spider wash or ground spider materials onto LB agar or Baird-Parker agar, as well as growing different concentrations of spider wash or ground spider materials in LB broth at different temperatures for different amounts of time. Of these methods, only a dilution of 20 μl of LB spider wash in 5 ml of LB broth incubated at 37°C with agitation for 3 h produced positive results. A positive control with *Escherichia coli* and negative control with sterile double-distilled water were run with all treatments.

**Amplification and Sequencing of Bacteria.** To identify microbes associated externally and internally from *T. agrestis*, we amplified a conserved region of the 16S rRNA gene with universal bacterial primers. Primers used were as follows: forward primer, 63 F (5'-CAG GCC TAA CAC ATG CAA GTC-3') (Marchesi et al. 1998); reverse primer, 1387R (5'-GGG CGG WGT GTA CAA CAA GCC-3') (Ley et al. 2005), and reverse primer, 1389R (5'-ACG GCC GGT GTG TAC AAG-3') (Osborn et al. 2000). The predicted sequence length for the PCR product amplified with 63 F with 1387R and 63 F with 1389R was ~1,300 bp.

Samples from all of the spider treatments (whole ground Tris-ethylenediaminetetraacetic acid, whole ground LB, ground abdomen LB, whole wash LB, and multiple wash LB) were used as templates for PCRs with the bacterial universal primer pairs. In addition to using ground and washed spider media exactly as described as the template for PCR, we also used individual colonies from the cultured samples. The following materials were included in the PCRs: 1 μl of template (treatment solution, cultured medium, or colony), 1 μl each of 10 μM universal bacterial primers 63 F with 1387R and 63 F with 1389R, 12.4 μl of double-distilled water, 2 μl of 10X PCR buffer, 2 μl of 25 mM magnesium chloride (MgCl2), 0.4 μl of 10 mM dNTPs, 0.1 μl of dimethylsulfoxide, and 0.1 μl of *Taq* polymerase (Invitegen, Carlsbad, CA) run on a PTC-100 thermal cycler (MJ Research, Cambridge, MA) with the following program: 95°C for 10 min, 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, repeat (95°C for 30 s, 55°C for 30 s, 72°C for 1 min) 35 times, 72°C for 10 min, and 4°C hold. PCR products were visualized on a 1.5% agarose and Tris-acetate-EDTA buffer gel and cut out for sequencing. PCR products were isolated from the agarose gel fragment with a Bio-Rad Freeze and Squeeze kit (Bio-Rad, Hercules, CA). The resulting isolated PCR products were ligated into a pGEM Teasy kit (Promega, Madison, WI) for amplification. To isolate the amplified plasmids, we used a Qiagen mini-prep kit (Qiagen, Valencia, CA). Big Dye cycle sequencing was conducted at the Washington State...
University Molecular Biology Core Laboratory. Bacterial species were identified (95% similar or higher) by comparing the sequences to GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) with the tblastx algorithm and confirmed with the Ribosomal Database Project (http://rdp.cme.msu.edu/index.jsp).

### Potential of the Hobo Spider to Transfer MRSA.
To conduct a direct test of the ability of the hobo spider to acquire and transfer pathogenic bacteria from surfaces and to vector the pathogen during an accidental bite, we exposed hobo spiders to MRSA. We exposed living adult hobo spiders to MRSA-saturated substrates for a total of 5 min. Lyophilized MRSA cultures from American Type Culture Collection were reconstituted with tryptic soy broth and incubated overnight in a 37°C shaker bath, following American Type Culture Collection recommendations. Adult spiders were collected and identified, as described previously. A polyethylene splash apron (Fisher, Pittsburgh, PA) was chosen as the substrate for MRSA because it has been shown to persist on this specific material for up to 51 d (Neely and Maley 2000). The apron was cut into disks to fit 15 × 95-mm Fisher brand petri dishes, and then glued to the inside of the bottom dish with an Avery permanent acid-free glue stick. The polyethylene disks were then cleaned with 70% ethanol and allowed to air dry before treatment. The treatment included two separate MRSA cultures and a negative control of LB medium, which were swabbed onto the cleaned polyethylene disks and allowed to air dry. Spiders collected in sterile containers (n = 30) were manipulated to walk or stand only on the treated and control polyethylene disks in the petri dishes for 5 min. Treated spiders were removed and housed in individual sterile petri dishes. Spiders were then tested at 1, 2, 3, 4, and 24 h post-MRSA exposure for the presence of bacteria. To determine their ability to transfer MRSA, spiders were forced to walk on a clean polyethylene disk glued to a petri dish, and then washed with 5 ml of LB broth. Polyethylene disks that were exposed to spiders only and MRSA only (positive control) were then swabbed with a LB-moistened sterile cotton-tipped stick, which was then turned swabbed onto a blood agar plate. Blood agar plates and LB broth were incubated overnight at 37°C (with shaking for the liquid medium), and then tested for the presence of MRSA by use of a MRSA agglutination screening kit (Denka Seiken, Tokyo, Japan).

### Hobo Spider Venom Hemolysis Assay.
Hemolytic activity of spider venom was determined using the spectrophotometric hemolysis assay of Foradori et al. (1978) modified by Foradori et al. (2005). One venom gland from each of an adult male and female spider was individually homogenized in 50 µl of a 20 mM Tris-buffered saline with 8 mM CaCl₂, pH 7.4 (TBSC; after Babcock et al. 1981). The entire venom gland homogenate (VGH) was added to a 1.0-ml suspension of 2% sheep red blood cells in TBSC and incubated at 37°C for 1.5 h with gentle agitation. The suspension was then diluted with 3 ml of TBSC and centrifuged at 760 × g for 5 min. Optical densities of the supernatants were measured at 540 nm with a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA). Within each assay, a 1-ml aliquot of the sheep red blood cell suspension was incubated, as stated above, then lysed with 3 ml of distilled water, and used as the standard for 100% hemolysis. Values for percentage of hemolysis were determined against total hemolysis (Foradori et al. 2005).

### Results

**Spider Collection Notes.** Hobo spiders were observed in abundance around the urban homes where they were collected. Landscape blocks, bricks, rock beds, long grass, and stacked wood or boards were found to be preferred habitat for both adult and juvenile spiders. To allow for seasonal and temperature variations in microbial fauna, collections took place from June to October 2007 and February to October 2008. During the collection period, 102 T. agrestis adults were examined for bacteria.

**Bacterial Diversity Assay.** Seventy-three bacterial samples were isolated from the spiders, providing 45 bacterial sequences for a total of six Gram-positive and four Gram-negative bacteria genera identified (Table 1). The genera identified, followed by the percentage of sequence identity, include: *Bacillus* spp. (100%), *Paenibacillus* sp. (100%), *Aeromonas* sp. (99%), *Arthrobacter* sp. (98%), *Pseudomonas* spp. (98%), *Pantoea* sp. (97%), *Staphylococcus* sp. (97%), *Rahnella* sp. (97%), a bacterium in the order Actinomycete (95%),

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**Table 1.** Natural or cultured environmental locations of identified microbes isolated from the hobo spider, *Tegenaria agrestis*, using the *Manual of Clinical Microbiology*, 7th, 8th, and 9th editions

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gram-positive or negative</th>
<th>Environment</th>
<th>Pathogenic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycete sp.</td>
<td>+</td>
<td>Isolated from soil and organic matter</td>
<td>Forty genera relevant to human and animal health</td>
</tr>
<tr>
<td>Arthrobacter sp.</td>
<td>+</td>
<td>Present on human skin and in soil</td>
<td>Occasionally opportunistic in wounds</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>+</td>
<td>Isolated from soil</td>
<td>Some species opportunistic or obligate pathogens</td>
</tr>
<tr>
<td>Exiguobacterium sp.</td>
<td>+</td>
<td>Isolated from human skin</td>
<td>Not opportunistic pathogen</td>
</tr>
<tr>
<td>Paenibacillus sp.</td>
<td>+</td>
<td>Isolated from soil</td>
<td>Not opportunistic pathogen</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>+</td>
<td>Widespread in nature, including human skin</td>
<td>Some infection or disease-causing species</td>
</tr>
<tr>
<td>Aeromonas sp.</td>
<td>–</td>
<td>Isolated from moist environments</td>
<td>Potential intestinal pathogen</td>
</tr>
<tr>
<td>Pantoeca sp.</td>
<td>–</td>
<td>Isolated from plants</td>
<td>Occasionally opportunistic in wounds</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>–</td>
<td>Worldwide in moist environments</td>
<td>Occasionally opportunistic in wounds</td>
</tr>
<tr>
<td>Rahnella sp.</td>
<td>–</td>
<td>Isolated from water samples</td>
<td>Opportunistic pathogen</td>
</tr>
</tbody>
</table>
and *Exiguobacterium* sp., (93%). Identification to species level was confirmed, followed by the percentage of sequence identity, for *Bacillus megaterium* (100%), *Pseudomonas fluorescens* (100%), *Bacillus circulans* (99%), *Pseudomonas veronii* (99%), *Bacillus thuringiensis* (97%), *Pseudomonas poae* (97%), *Staphylococcus saprophyticus* (97%), and *Bacillus simplex* (95%). Descriptions of natural bacterial occurrences and pathogenic capabilities of isolates were found using *The Manual of Clinical Microbiology*, 7th, 8th, and 9th editions (Murray et al. 1999, 2003, 2007) (Table 1). All of the 10 genera identified with *T. agrestis* are ubiquitous bacterial fauna found in natural environments, with several occurring on human and animal skin.

**Potential of the Hobo Spider to Transfer MRSA.** Spiders were exposed to MRSA to determine their ability to acquire the pathogen from a MRSA-treated surface. This pathogen was chosen because the tissue lesions that causes are often misdiagnosed as resulting from spider bites (Dominguez 2004, Baxtrom et al. 2006, Vetter et al. 2006b, Cohen 2007). We used a commercially available MRSA screening kit to detect the acquisition and transfer of MRSA by the hobo spider from polyethylene disks. No MRSA was found either on the spiders or on the clean surfaces to which the MRSA-exposed spiders were subjected. There was no MRSA pathogen carried or transferred to another surface by the MRSA-exposed spiders, although MRSA was found to persist on the polyethylene disks.

**Hobo Spider Venom Hemolysis Assay.** A simple test that can help determine whether or not a spider may cause medically significant bites is a hemolysis assay. Compared with the known hemolytic activity (>37%) associated with *Loxosceles reclusa* Gertsch and Mulaik VGH, the potential of *T. agrestis* VGH to cause hemolysis was negligible at 0.62 and 0.93% for male (*n* = 5) and female (*n* = 7) spiders, respectively.

**Discussion**

We identified the sequences of seven Gram-positive and four Gram-negative bacteria genera associated with the hobo spider, *T. agrestis*, in Washington State (Table 1). The microbial fauna identified from our 102 *T. agrestis* specimens collected around the outside of homes in the PNW are all species previously cultured from natural environments. These include outdoor environments as well as the external surfaces of animal and human skin. Because the hobo spider builds its web near the ground, it is not surprising that they harbor the nonpathogenic bacteria that we identified, including species of *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Arthrobacter*, and *Actinomyces*, and species in the family Micrococaceae. Several of the bacteria we isolated from the spiders are also considered normal human fauna, such as species of *Staphylococcus* and *Arthrobacter* and the two species of *Exiguobacterium*, which have little or no pathogenic potential. Yet some of these naturally occurring bacteria include species that can be opportunistic or have pathogenic potential, such as species of *Bacillus*, *Rhahella*, *Aeromonas*, *Pseudomonas*, *Staphylococcus*, *Arthrobacter*, and *Actinomyces*. However, none of the eight bacterial species that we cultured from the hobo spider were recognized as a disease-producing agent, although some of the species have been isolated from human wounds or infections, including *P. fluorescens*, *P. poae*, *P. veronii*, *B. circulans*, *B. thuringiensis*, and *S. saprophyticus*. Our results are comparable to those of Baxstrom et al. (2006), who found that the majority of the 99 common North American house spiders they analyzed carried *Bacillus* spp. and *Staphylococcus epi-dermidis* among other diplheroid bacteria. However, by using the 16S rRNA gene and universal primers, we were able to culture higher numbers and, subsequently, a larger diversity for identification with more confidence than could be obtained with phenotypic identifications.

A primary goal of this study was to determine whether the hobo spider carried the necrotic lesion-inducing bacteria MRSA. Whereas we did not find MRSA on hobo spiders, that might be the result of sampling, as the MRSA strain is a community or nosocomial spread infection that has not been isolated from natural environments. Based on our ability to detect bacterial diversity and because of the number of MRSA infections initially reported as spider bites, we conducted a more direct experiment in which we exposed hobo spiders to a MRSA-saturated substrate to determine whether hobo spiders were capable of acquiring and transferring the bacteria from a contaminated surface. In our experiments, the hobo spider was unable to acquire MRSA after forced contact with a MRSA-saturated substrate for a full 5 min. This experiment was designed to mimic a natural setting in which the spider was subjected to an environment with a pathogenic bacteria such as MRSA (e.g., hospitals, nursing homes, prisons, athletic departments, and child care centers) (Moran et al. 2006, Klevenus et al. 2007). In this experiment, because the spider was not able to acquire MRSA, it also did not transfer the pathogen.

We also conducted a hemolytic assay of the venom of Washington State-collected spiders. The venom from United States hobo spider populations has been compared with populations from the native and introduced regions in Europe, with no significant variables found to explain the perceived envenomation concern in the United States (Binford 2001). The European populations are not known to cause necrotic arachnidism (Bettini and Brignnoli 1978, Binford 2001). Nevertheless, we believed that it was important to conduct a standard venom hemolytic assay on hobo spiders collected for this study. Foradori et al. (2005) examined the venom of 45 spider species for the ability to cause hemolysis or breakdown of red blood cell membranes. Of the spiders included in the assay, only two possessed venom enzymes that caused hemolysis. The venom of the brown recluse spider, *L. reclusa*, which contains the enzyme sphingomyelinase D, has been shown to produce necrotic lesions (Kurpiewski et al. 1981). The venom of the yellow sac spider, *C. mildei*, contains phospholipase A₂, an enzyme that may be capable of hemolytic activity (Fo-
The hobo spider has rapidly expanded its range since its introduction into the Seattle, WA area in the 1950s (Edline 1953, 1951), and has moved into neighboring states (Baird and Akre 1993, Baird and Stoltz 2002, Vetter et al. 2003). In one studied case that reported a necrotic lesion resulting from a putative T. agrestis envenomation, many of the details are unclear (Binford 2001, Vetter and Isbister 2004, 2008). The case described a 42-yr-old woman with a history of phlebitis who found a crushed brown spider on her ankle beneath her pant leg. The spider was reported as a hobo spider, but there is no explanation of how it was identified or by whom. In addition, the woman did not seek medical attention for 79 d after the incident, and therefore, questions surround the actual cause of the necrosis (Case 2, Vest 1996). Our study focused on identifying putative factors that could correlate T. agrestis bites with necrotic arachnidism based on widespread concern and some previous studies that have attributed necrotic lesions to the hobo spider. In the late 1980s, Vest (1987a) described the probable involvement of the hobo spider, T. agrestis, as the cause of necrotic lesions. Vest (1987b) further implicated T. agrestis in a preliminary study in which New Zealand White (NZW) rabbits and one California giant rabbit were subjected to envenomation by forced bite. Vest (1987b) observed that the four NZW rabbits bitten by male T. agrestis developed significant dermal lesions. This experiment was repeated by other researchers using =40 NZW rabbits over a 2-yr period (Gomez and Binford, unpublished). They injected 20 µl of venom intradermally at concentrations of 1:25, 1:75, and 1:150, which resulted in mild reactions, but no developing necrosis. They also injected pure undiluted hobo spider venom, which produced a modest dermal inflammation, but still not the significant necrosis that Vest described (Gomez and Binford, personal communication).

One possibility for the discrepancy in results could be that Vest forced the spider onto the skin of the rabbit, allowing for possible bacterial introduction and/or digestive fluids that may have been expelled in response to the forced pressure. We have ruled out bacteria as a cause of necrosis in the T. agrestis spiders that we surveyed, which is also supported by Binford (2001). Whereas researchers have focused on spider venom as the tissue-necrotizing agent, other factors besides bacteria could also have medical significance. Spider digestive fluids have been examined for potential necrotizing agents. The garden spider, Argiope aurantia Lucas, was found to possess collagenase in the gut, which cleaved connective tissue proteins, but did not produce necrotic lesions on rabbit test subjects (Foradori et al. 2001). Peptidases in the digestive fluid of A. aurantia were suggested to help inactivate the peptidases produced by its prey, whereas inhibitors in the gut of the spider may protect the spider from the serine peptidases of the prey during digestion (Foradori et al. 2006). Medical literature is reporting an increasing number of bacterial infections as a result of overuse of antibiotics and antibacterial cleansers depleting beneficial bacterial flora (Levy 2001). The microbial fauna on human skin can be beneficial to our health, but can also be the cause of infection or further injury to an existing wound (Cogen et al. 2008, Dethelefsen et al. 2007).

This study confirms previous results and provides further evidence that the hobo spider, T. agrestis, is not a spider of medical concern. We have identified the bacteria associated with T. agrestis as ubiquitous environmental fauna and displayed the spider’s inability to transfer a pathogenic bacteria. There is little evidence to support the claims that the hobo spider harbors or vectors microbial pathogens. The hemolytic venom assay demonstrates that the spider is incapable of causing severe cellular damage. Finally, the results from the mammalian assay strengthen the hypothesis that the hobo spider does not cause necrotic lesions. Although this introduced spider is common around homes in urban environments in the PNW and is expanding its range, we cannot substantiate its involvement in human necrotic tissue lesions as once suspected (Bennett 2002, 2004; Vetter and Isbister 2004, 2008).

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