Case Report

Characterization of a CC1153 PVL-producing community-acquired methicillin-resistant Staphylococcus aureus from a dog bite wound

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Abstract
The isolation of a rare community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) strain from an infected dog bite wound is here reported. A 27-year-old man presented with a deep open wound on his right hand caused by his dog’s bite at the outpatient clinic of Infectious Disease Hospital (IDH), Kuwait. A wound swab was obtained for bacteriological culture and susceptibility testing. The wound culture yielded pure heavy growth of an MRSA isolate, designated IDH70, which was susceptible to vancomycin, teicoplanin, erythromycin, clindamycin, trimethoprim, fusidic acid and rifampicin. The patient was successfully treated with a combination of rifampicin and cotrimoxazole twice daily for 10 days. Molecular characterization revealed that IDH70 was positive for genes encoding Panton-Valentine leucocidin. IDH70 also carried the SCCmec V genetic element, belonged to coagulase type XIIIa, spa type t903, and was assigned to clonal complex 1153 and sequence type ST1153 (ST1153-V-t903). The report highlights the increasing burden of CA-MRSA in the community and the risk of its acquisition from bites of companion animals.

Key words: MRSA; MLST; CC1153; PVL.

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Introduction
Methicillin-resistant S. aureus (MRSA) is a growing global problem in human and veterinary medicine [1-3]. Colonization of human or animal hosts by S. aureus may result in transmission between humans and animals [4]. Close contact between household pets and humans through petting, licking or biting, offers favorable conditions for the transmission of pathogenic bacteria [5]. Companion animals such as cats and dogs are frequently colonized by S. aureus including MRSA and can become infected [5-7]. Colonization or infection of dogs by MRSA exposes dog handlers and owners to MRSA infection through injuries from bites, minor lesions, traumatic injuries, or infections with severe complications such as septicemia [5,7]. Molecular characterization with SCCmec typing, spa typing, PFGE and multi locus sequence typing have identified differences in the genotypes of S. aureus found in companion animals and those associated with livestock. MRSA strains distributed in pet animals are mostly associated with clonal lineages that are also prevalent in human healthcare facilities. For example, the widely distributed epidemic MRSA-15 (EMRSA-15) clone has been isolated from humans as well as from companion animals [8,9]. In contrast, MRSA distributed among livestock predominantly belong to clonal complexes CC398, CC9 and CC97 [7].

MRSA infections of companion animals are of particular concern due to limited treatment options and their zoonotic potential [9,10]. Antibiotic resistance is a growing problem among S. aureus isolates obtained from healthy or infected companion animals since they may act as reservoirs of antimicrobial-resistant bacteria that may be transferred to humans when they interact with the animals [10,11].

This paper reports the characteristics of a rare strain of community-acquired MRSA (CA-MRSA) isolated from infected soft tissue of a patient bitten by his dog.

Case report
A 27-year-old man presented to the outpatient clinic of the Infectious Disease hospital (IDH) in Kuwait on February 5, 2009 with a deep open wound on his right hand caused by a bite from his dog which had occurred on January 6, 2009. Following the bite on January 6, the
wound was dressed without any antibiotics and the patient received a full course of rabies vaccination at his local health clinic. As the wound did not heal, the patient was referred to the IDH outpatient clinic on February 5, 2009. On examination, the wound was purulent and discharging. A wound swab was obtained for bacteriological culture and sensitivity testing while the patient was started empirically on oral amoxicillin-clavulanate, twice daily, for 7 days. Wound culture yielded pure growth of MRSA isolate, designated IDH70, which was susceptible to vancomycin, teicoplanin, erythromycin, clindamycin, trimethoprim, fusidic acid and rifampicin. Following the laboratory report, amoxicillin-clavulanate was discontinued, and the patient was placed on clindamycin thrice daily. No physical improvement was observed after 7 days on clindamycin. On February 17, 2009, the treatment was changed to a combination of rifampicin and cotrimoxazole, twice daily for 10 days. The patient did not report to the IDH again for treatment. When the patient was reached via telephone for follow up after 30 days, he stated that the wound had healed and he was well.

Isolate characterization

Wound swabs were inoculated onto sheep blood agar and mannitol salt agar (Oxoid, Basingstoke, UK) plates and incubated at 35°C for 48 hours. Colonies were identified as S. aureus by cultural characteristics, Gram-stain reaction (Gram-positive cocci in clusters), and positive catalase and tube coagulase tests. Susceptibility testing to antibacterial agents was done by the disc diffusion method [12]. Methicillin-resistance was confirmed by detecting PBP 2a in culture supernatant using latex agglutination test (Oxoid, Basingstoke, UK) and performed as described by the manufacturer. Minimum inhibitory concentration (MIC) was determined for cefoxitin, vancomycin, teicoplanin and mupirocin using Etest strips (BioMerieux, Marcy-Le Etoile, France).

Molecular characterization

The IDH70 isolate was investigated for Panton-Valentine leukocidin (PVL) genes carriage, using primers and protocols as described by Lina et al. [13]. Molecular typing was performed using staphylococcal cassette chromosome mec (SCCmec) typing as previously described [14]. Spa typing was performed as described by Harmsen et al. [15]. Multilocus sequence typing (MLST) was performed as previously described [16]. Coagulase typing was performed by multiplex PCR (I-X) as described by Hirose et al. [17]. The 3′ end of the variable region of the coagulase gene was amplified and sequenced using primers and conditions published previously [18]. PCR products were purified using the MicroElute cycle pure kit (Omega BioTekInc, USA) and sequenced using the BigDye Terminator v 3.1 Cycle Sequencing kit (Applied Biosystem, Foster City, CA) and a 3130xl Genetic Analyzer (Applied Biosystem, Foster City, CA) according to the manufacturer’s protocol. Coa sequences of the MRSA strain were analyzed and compared by BLAST (http://www.ncbi.nlm.nih.gov/BLAST.cgi) with coagulase gene sequences deposited in GenBank and by the pairwise sequence alignment of DNA sequences from EMBOSS (http://www.ebi.ac.uk/Tools/psa).

DNA microarray using IdentiBac S. aureus genotyping kit 2 (Alere Technology, Jena, Germany) was employed to investigate virulence and antibiotic resistance genes according to the manufacturer’s instructions.

Results

The S. aureus isolate IDH70 was positive for PBP 2a and was resistant to cefoxitin (MIC: 32 µg/ml), penicillin G and tetracycline. It was positive for genes encoding PVL.

The isolate carried the SCCmec type V genetic element which is a characteristic feature of CA-MRSA. It belonged to spa type t903 and sequence type 1153 (ST1153), and was deemed a CA-MRSA. Coagulase typing yielded negative results for types I–X. Subsequently, the coagulase gene was amplified and sequenced and its DNA sequence showed 99% similarity with the coagulase type XIIIa allele (accession number KT599478) [19]. Consequently, the MRSA isolate IDH70 was assigned to coagulase type XIIIa.

Results of DNA microarray analysis confirmed that IDH70 was positive for mecA and tetK, which encode methicillin and tetracycline resistance, respectively, but was negative for ermA, ermB,ermC, InuA, msrA, mefA and mphC, which encode resistance to macrolides and lincosamides. It was also negative for genes encoding aminoglycoside modifying enzymes.

The isolate was positive for accessory gene regulator type II (agr II), staphylococcal accessory regulator A (SarA), capsular polysaccharide type 5 (cap5), component A of haemolysin gamma (hlgA), haemolysin alpha (hla), putative membrane protein (hil), staphylokinase (sakA), Staphylococcal complement inhibitor (Scn), clumping factor A (clfA), clumping factor B (clfB), and biofilm-associated genes, icaA.
icaC and icaD. It was negative for genes encoding staphylococcal enterotoxins, toxic shock syndrome toxin, exfoliative toxins, epidermal cell differentiation inhibitors and arginine catabolic mobile element.

Discussion
This report documents the isolation of a PVL-positive CA-MRSA belonging to CC1153 (ST1153-V-t903) from an infected wound caused by a dog bite. Reports of ST1153 MRSA strains are rare in the literature and the current study is adding to the growing list of CA-MRSA clones reported previously in Kuwait hospitals [20]. Prior to this report, ST1153 MRSA has been only encountered in Thailand [21] However, since then sporadic cases of ST1153 MSSA have been reported for patients in Myanmar [19], Egypt [22], South Korea [23] and Malaysia [24,25].

Besides resistance to β-lactams, the ST1153-V-t903 in this report was only resistant to tetracycline. Similarly, the ST1153-MSSA strain from Egypt [22] was only resistant to sulphamethoxazole while the MSSA strain from Myanmar [19] was susceptible to non-β-lactam antibiotics suggesting that infections caused by these strains would be treatable with commonly available non-β-lactam agents. It is surprising that the patient failed to respond positively to treatment with clindamycin although the organism was susceptible to erythromycin and clindamycin, and was negative for genes encoding macrolide and lincosamide resistance. However, IDH70 carried genes for biofilm formation. It is not known whether biofilm formation could explain treatment failure with clindamycin or whether the poor outcome was related to lack of compliance. Nevertheless, treatment with a combination of rifampicin and cotrimoxazole, to which the CA-MRSA isolate was susceptible, yielded a positive outcome highlighting the importance of susceptibility testing of the associated organisms in the effective treatment of CA-MRSA infections.

Although the isolate was obtained from a wound caused by a dog bite, it is uncertain whether the dog was infected with the strain which infected the patient during the bite or whether the bite merely facilitated the contamination of the wound by an organism already present on the patient’s skin. Unfortunately, it was not possible to obtain swabs from the dog which would have definitely established transmission of the strain from dog to the patient. However, PVL-producing MRSA clones have been isolated from companion animals [2].

Conclusion
This study reports the isolation of a rare PVL-producing strain of CA-MRSA from a dog bite wound successfully treated with a combination of rifampicin and cotrimoxazole. The report highlights the increasing burden of CA-MRSA of diverse genetic backgrounds in the community and the risk of its acquisition from bites of companion animals.

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