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Case Report

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

Edet Ekpenyong Udo[1], Samar Saed Boswihi[1], Tsonyu Ivanov Dimitrov[2], Bobby Noronha[1], Bindu Mathew[1], Tina Verghese[1]

[1] *Department of Microbiology, Health Sciences Centre, Kuwait University, Kuwait, Kuwait*
[2] *Microbiology Laboratory, Infectious Disease Hospital, Kuwait, Kuwait*

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 SCC*mec* 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 SCC*mec* 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 35ºC 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 leucocidin (PVL) genes carriage, using primers and protocols as described by Lina *et al*. [13]. Molecular typing was performed using staphylococcal cassette chromosome *mec* (SCC*mec)* 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 Bio-TekInc, 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 SCC*mec* 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 (*hl*), 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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References

1. Baptiste KE, Williams K, Williams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T, Hart CA (2005) Methicillin-resistant staphylococci in companion animals. Emerg Infect Dis 11:1942-1944.
2. Faires MC, Travers M, Tater KC, Pearl DL, Weese JS (2010) Methicillin-resistant and – susceptible *Staphylococcus aureus* infections in dogs. Emerg Infect Dis 16: 69-75.
3. Rankin S, Roberts S, O'Shea K, Maloney D, Lorenzo M, Benson CE (2005) Panton-Valentine Leucocidin (PVL) toxin positive MRSA strains isolated from companion animals. Vet Microbiol 108:145-148.
4. Guardabassi L, Schwarz S, Lioyd DH (2004) Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother 54:321-332.
5. Oehler RL, Velez AP, Mizrachi M, Lamarche J, Gompf S (2009) Bite-related and septic syndromes caused by cats and dogs. Lancet Infect Dis 9: 439-447.
6. Goldstein EJ, Citron DM, Finegold SM (1980) Dog bite wounds and infection: a prospective clinical study. Ann Emerg Med 9: 508-512.
7. Jang Y, Bae DH, Cho JK, Bahk GJ, Lim SK, Lee YJ (2014) Characterization of methicillin- resistant *Staphylococcus* Spp isolated from dogs in Korea. Jpn J Vet Res62:163-170
8. Harrison EM, Weinert LA, Holden MT, Welch JJ, Wilson K, Morgan FJ, Harris SR, Loeffler A, Boag AK, Peacock SJ, Paterson GK, Waller AS, Parkhill J, Holmes MA (2014) A shared population of epidemic methicillin-resistant *Staphylococcus aureus* 15 circulates in humans and companion animals. MBio 5: 1-10.
9. Davis JA, Jackson CR, Fedorka-Cray PJ, Barret JB, Brousse JH, Gustafson J, Kucher M (2014) Carriage of methicillin-resistant Staphylococci by healthy companion animals in the US. Lett Appl Microbiol 59: 1-8.
10. Vincze S, Stamm I, Kopp PA, Hermes J, Adlhoch C, Semmler T, Wieler LH, Lubke-Becker A, Walther B (2014) Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010-2012. PLoS One 9: 1-6.
11. Kock R, Ballhausen B, Bischoff M, Cuny C, Eckmanns T, Fetsch A, Harmsen D, Goerge T, Oberheitmann B, Schwarz S, Selhorst T, Tenhagen BA, Walther B, Witte W, Ziebuhr W, Becker K (2014) The impact of zoonotic MRSA colonization and infection in Germany. Bert Munch Tierarztl Wochenschr 127:384 - 398.

12. Clinical and Laboratory Standard Institute (CLSI) Performance standards for antimicrobial susceptibility testing; Twenty-Second Informational Supplement. CLSI document, 2012; M100-S22, 32 (3), Wayne, PA.
13. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J (1999) Involvement of Panton–Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29: 1128–1132.
14. Zhang K, McClure J-A, Elsayed S, Louie T, Conly JM (2005) Novel multiplex PCR assay for characterization and concomitant subtyping of Staphylococcal cassette chromosome *mec*types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 43: 5026-5033.
15. Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U (2003) Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 41:5442-5448.
16. Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38:1008-1015.
17. Hirose M, Kobayashi N, Ghosh S, Paul SK, Shen T, Urushibara N, Kawaguchiya M, Shinagawa M, Watanabe N (2010) Identification of *Staphylococcus*co agulase genotypes I-X and discrimination of type IV and V subtypes by multiplex PCR assay for clinical isolates of *Staphylococcus aureus*. Jpn J Infect Dis 63: 257-263.
18. Goh S, Byrne SK, Zhang JL, Chow AW (1992) Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphism. J Clin Microbiol 30:1642-1645.
19. Aung MS, Zi H, Nwe KM, Maw WW, Aung MT, Min WW, Nyein N, Kawaguchiya M, Urushibara N, Sumi A, Kobayashi N (2016) Drug resistance and genetic characteristics of clinical isolates staphylococci in Myanmar: high prevalence of PVL among methicillin-susceptible *Staphylococcus aureus* belonging to various sequence types. New Microbes and New Infect 10:58-65.
20. Udo EE, Sarkhoo E (2010) The dissemination of ST80-SCCmec-IV community-associated methicillin-resistant *Staphylococcus aureus* clone in Kuwait hospitals. Ann Clin Microbiol Antimicrob 9:31.
21. Multilocus sequence typing for *Staphylococcus aureus.* Available at: http://Saureus.mlst.net [Accessed on 10 July 2015].
22. Enany S, Yaoita E, Yoshida Y, Enany M, Yamamoto T (2010) Molecular characterization of Panton-Valentine leucocidin–positive community-acquired methicillin-resistant *Staphylococcus aureus* isolates in Egypt. Microbiol Research 165:152-162.
23. Chong YP, Kim ES, Park S-J, Park K-H, Kim T, Kim MN, Kim SH, Lee SO, Choi SH, Woo JH, Jeong JY, Kim YS (2013) Accessory gene regulator (*agr*) dysfunction in *Staphylococcus aureus* bloodstream isolates from South Korean patients. Antimicrob Agents Chemother 57:1509-1512
24. Atshan SS, Shamsudin MN, Sekawi Z, Lung LTT, Hama RA, Karunanidhi A, Mateg Ali A, Ghaznavi-Rad E, Ghasemzadeh-Moghaddam H, Chong Seng JS, Nathan JJ, Pei CP (2012) Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus*. J Biomed Biotechnol 2012: 1-10.
25. Ghasemzadeh-Moghaddam H, Ghaznavi-Rad E, Sekawi Z, Yun-Khoon L, Aziz MN, Hamat RA, Melles DC, van Belkum A, Shamsudin MN, Neela V (2011) Methicillin-susceptible *Staphylococcus aureus* from clinical and community sources are genetically diverse. Int J Med Microbiol 301:347-353.

Corresponding author

Edet E Udo PhD
Department of Microbiology
Health Sciences Centre
Kuwait University
P. O. Box 24923, Safat
13110. Kuwait
Tel: +965 24636773.
Fax: +965 25332719
Email: EDET@hsc.edu.kw

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