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False Positives and Negatives Obtained with PCR-Based Identification of *Staphylococcus aureus* Clonal Complex 398

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Staphylococcus aureus belonging to multilocus sequence type (MLST) 398 (ST398) and their relatives in clonal complex 398 (CC398) have emerged recently as important zoonotic agents worldwide (1). Livestock, especially swine, are implicated in carriage and transmission of these organisms, as are individuals who work closely with livestock. However, ST398 strains have also been found in people without livestock exposure (2, 3) and have caused infections within hospitals and health care facilities (4). Since ST398 isolates are pulsed-field gel electrophoresis (PFGE) nontypeable with SmaI, belong to a wide variety of staphylococcal protein A (*spa*) types, and show a number of different antimicrobial resistance patterns, detection of ST398 is not a quick process. van Wamel et al. created two specific PCRs for detection of ST398 using two ST398-specific DNA sequences previously obtained from amplified fragment length polymorphism (AFLP) analyses (5). A07 and CO1 primer sets were reported to show 100% accuracy when tested against 133 isolates. Noting that the van Wamel et al. sets were targeted to a transposon, Stegger et al. developed a different PCR assay using the highly conserved *sau1-hsdS1* sequence in the CC398 lineage (6). PCR validation of this target showed 100% specificity (235/235 samples) and 100% sensitivity (1,072/1,072 samples).

We screened a total of 444 human colonization and 14 human infection isolates using the A07 or CO1 primer set representing the gene SAPIG2194 or SAPIG2195, respectively. All isolates were also *spa* typed, and a subset, including all potential false-positive and false-negative isolates, were typed by MLST. Sensitivity was 96.5% and specificity was 98.3% (Table 1). There were three false negatives that were all t571/ST398. The six false positives included two t922/ST1 isolates, one t045/ST1 isolate, one t337/ST9 isolate, one t505/ST45 isolate, and one t002/ST105 isolate (Table 2).

We screened the nine false positives and false negatives from the previous assay with the *sau1-hsdS1* assay. Two of the false positives were negative by this assay, while the other four were still positive. All three false negatives were positive by the *sau1-hsdS1* PCR assay (Table 2).

Molecular typing of *Staphylococcus aureus* is an incredibly important tool for surveillance and control. Both of these PCR assays show extremely high sensitivity and specificity yet still yielded false positives with our data set. Multiple tools should be used when screening for livestock-associated *Staphylococcus aureus* (LA-SA) to ensure diagnostic accuracy in the lab. Various *spa* types have been found in livestock (7–11), and it is possible that horizontal transfer of these gene fragments may be transmitted between co-colonized hosts or between host and environment (12). Therefore, while it is important to develop and use new molecular tools, gold standards, such as MLST, should still be used to identify CC398 and to understand the epidemiology of LA-SA.

TABLE 1 Summary of the numbers of true-positive, false-positive, false-negative, and true-negative results based on the A07/C01 ST98 PCR assay results, categorized by MLST results

	MLST result (no. of isolates) for:	
A07/CO1 PCR result	ST398 isolates	Non-ST398 isolates
Positive	True positive (82)	False positive (6)
Negative	False negative (3)	True negative (352)

TABLE 2 Summary of molecular characteristics of the false-negative and false positive isolates, with results from the A07/C01 PCR assay and *sau1-hsdS1* PCR assay[a]

Isolate	Presence of *mecA*	*spa* type	Presence of PVL gene	Antimicrobial agent(s) to which isolate was sensitive	MLST	A07/C01 PCR result	*sau1-hsdS1* PCR result
83	+	t571	−	OXA, TET	ST398	−	+
142	+	t002	+	OXA, ERY, LVX, CLI (intermed.)	ST105	+	+
303	−	t505	−	None	ST45	+	+
309	−	t337	−	TET, CLI, ERY	ST9	+	+
389	−	t571	−	GEN, ERY, CLI, TET, SXT, LVX	ST398	−	+
390	−	t571	−	GEN, ERY, CLI, TET, LVX	ST398	−	+
392	−	t922	−	None	ST1	+	−
393	−	t922	−	None	ST1	+	−
437	−	t045	−	TET, SXT	ST5	+	+

[a] PVL, Panton-Valentine leukocidin; OXA, oxacillin; TET, tetracycline; ERY, erythromycin; LVX, levofloxacin; CLI (intermed.), intermediate clindamycin; CLI, clindamycin; GEN, gentamycin; SXT, trimethoprim-sulfamethoxazole.

ACKNOWLEDGMENT

This work was funded in part by AHRQ award R18 HS019966 (T.C.S.).

REFERENCES

1. **Fluit AC.** 2012. Livestock-associated *Staphylococcus aureus*. Clin. Microbiol. Infect. **18:**735–744. http://dx.doi.org/10.1111/j.1469-0691.2012.03846.x.
2. **Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W.** 2009. Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. PLoS One **4:**e6800. http://dx.doi.org/10.1371/journal.pone.0006800.

Published ahead of print 13 November 2013
Editor: W. M. Dunne, Jr.
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doi:10.1128/JCM.03090-13

3. **Moritz ED, Smith TC.** 2011. Livestock-associated *Staphylococcus aureus* in childcare worker. Emerg. Infect. Dis. **17:**742–744. http://dx.doi.org/10.3201/eid1704.101852.
4. **Fitzgerald JR.** 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends Microbiol. **20:**192–198. http://dx.doi.org/10.1016/j.tim.2012.01.006.
5. **van Wamel WJ, Hansenova Manaskova S, Fluit AC, Verbrugh H, de Neeling AJ, van Duijkeren E, van Belkum A.** 2010. Short term micro-evolution and PCR-detection of methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398. Eur. J. Clin. Microbiol. Infect. Dis. **29:**119–122. http://dx.doi.org/10.1007/s10096-009-0816-3.
6. **Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L.** 2011. Rapid PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-modification system carrying *sau1-hsdS1*. J. Clin. Microbiol. **49:**732–734. http://dx.doi.org/10.1128/JCM.01970-10.
7. **Dressler AE, Scheibel RP, Wardyn S, Harper AL, Hanson BM, Kroeger JS, Diekema DJ, Bender JB, Gray GC, Smith TC.** 2012. Prevalence, antibiotic resistance and molecular characterisation of *Staphylococcus aureus* in pigs at agricultural fairs in the USA. Vet. Rec. **170:**495. http://dx.doi.org/10.1136/vr.100570.
8. **Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC.** 2013. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. PLoS One **8:**e53738. http://dx.doi.org/10.1371/journal.pone.0053738.
9. **Osadebe LU, Hanson B, Smith TC, Heimer R.** 2013. Prevalence and characteristics of *Staphylococcus aureus* in Connecticut swine and swine farmers. Zoonoses Public Health **60:**234–243. http://dx.doi.org/10.1111/j.1863-2378.2012.01527.x.
10. **Smith TC, Gebreyes WA, Abley MJ, Harper AL, Forshey BM, Male MJ, Martin HW, Molla BZ, Sreevatsan S, Thakur S, Thiruvengadam M, Davies PR.** 2013. Methicillin-resistant *Staphylococcus aureus* in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. PLoS One **8:**e63704. http://dx.doi.org/10.1371/journal.pone.0063704.
11. **Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, Capuano AW, Herwaldt LA, Diekema DJ.** 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One **4:**e4258. http://dx.doi.org/10.1371/journal.pone.0004258.
12. **Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nubel U, Fitzgerald JR.** 2009. Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U. S. A. **106:**19545–19550. http://dx.doi.org/10.1073/pnas.0909285106.