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Analysis of Morphologically Similar *Staphylococcus aureus* Colonies for Assessment of Phenotypic and Genotypic Correlation

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In an effort to track and control the transmission of *Staphylococcus aureus*, patient isolates are saved for epidemiological studies (1–3). Study investigators often assume that colonies with the same morphology on the original culture plate represent the same clone. However, there is very limited literature to support this assumption. Many studies do not address how isolates are selected from a culture (3–5) and state that one colony is chosen as a representative sample (6). Currently, the Treating Parents to Reduce NICU Transmission of *Staphylococcus aureus* (TREAT PARENTS) trial (registration no. NCT02223520) is assessing the concordance of *S. aureus* strains colonizing parents and their neonates (7). To test the aforementioned assumption, multiple *S. aureus* colonies were saved from a single culture plate and tested to determine their genotypes and susceptibility profiles.

Once parents or guardians consented to the TREAT PARENTS trial, swab samples were collected from the nares, throat, groin, and perianal region to screen for the presence of *S. aureus*. The samples were collected with the Copan Eswab transport system (Copan, Murrieta, CA). For each sample, 10 μl was aliquoted onto a quarter of one *S. aureus* selective chromogenic agar (SASelect; Bio-Rad, Hercules, CA) plate and one 5% sheep blood agar (SBA; Remel, Lenexa, KS) plate and incubated at 37°C for 16 to 24 h. At the same time, 100 μl of each sample was aliquoted into tryptic soy broth containing 6.5% sodium chloride (Bio-Rad, Hercules, CA) and incubated at 37°C for 16 to 24 h. After incubation, 10 μl of each broth was plated on SASelect medium and 5% SBA, streaked for isolation, and incubated at 37°C for 16 to 24 h. The colonies had to have the same color, size, consistency, and entirety to be called identical. Two medical technologists independently read the plates, and there were no inconsistencies between them in the determination of identical versus different morphologies on the basis of the criteria described. For each *S. aureus* morphology on every positive plate, five separate colonies were individually subcultured on 5% SBA and then frozen. These isolates were analyzed by pulsed-field gel electrophoresis (PFGE) and antimicrobial susceptibility testing (AST) with standard antistaphylococcal agents.

PFGE was performed in accordance with standard methods by using SmaI as the restriction enzyme (8). Restriction digestion patterns were analyzed with the Fingerprinting 2 software (Bio-Rad). PFGE results were interpreted by using modified Tenover criteria, and isolates were considered related if their patterns had three or fewer band differences (9).

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Isolate susceptibility was determined with the BD Phoenix 100 instrument (BD Diagnostics Inc., Sparks, MD) with Phoenix PMIC/ID-105 panels in accordance with the manufacturer's instructions.

A combination of 14 neonates and adult participants had positive *S. aureus* cultures that yielded 205 isolates (5 isolates from 41 morphologies observed). Of the isolates tested, 99.5% (204 of 205) had PFGE patterns that were identical to those of the other isolates of a particular morphology. One isolate in one group had a one-band difference from the other four isolates, and these are considered epidemiologically related.

AST indicated that 95.1% (195 of 205) of the isolates tested had the same profile of susceptibility to all 16 antibiotics tested (data not shown). All isolates showed categorical agreement (CA; agreement of susceptible/intermediate/resistant results) for all antibiotics except erythromycin (90% CA). When it could be determined, essential agreement (EA; MICs that match exactly or are within ±1 2-fold dilution) ranged from 90 to 100%. The 95% confidence interval for the proportion of morphologies with exact agreement is 0.91 to 1 when using an exact binomial confidence interval (10).

Because of its high colonization frequency and its role in health care-associated infections (1, 6, 8, 11), *S. aureus* will remain a commonly archived pathogen for epidemiological studies. A lack of literature specifying *S. aureus* colony selection seems to suggest that a fundamental assumption in these studies is that a single colony of the same morphotype is representative of the same strain. The results of our study show that this assumption is appropriate for morphologically similar colonies from a single culture. An interesting, but not fully unexpected, result was differing susceptibility profiles for 5% of the isolates that had identical banding patterns.

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REFERENCES

1. David MZ, Daum RS. 2010. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23:616–687. https://doi.org/10.1128/CMR.00081-09.
2. Loomba PS, Taneja J, Mishra B. 2010. Methicillin and vancomycin resistant *S. aureus* in hospitalized patients. J Glob Infect Dis 2:275–283. https://doi.org/10.4103/0974-777X.68535.
3. Fridkin SK, Hageman J, McDougal LK, Mohammed J, Jarvis WR, Perl TM, Tenover FC. 2003. Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, Unites States 1997–2001. Clin Infect Dis 36:429–439. https://doi.org/10.1086/346207.
4. Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, Korvick JA, Kauffman CA, Yu VL. 1993. Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. Am J Med 94:313–328. https://doi.org/10.1016/0002-9343(93)90063-U.
5. King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. 2006. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med 144:309–317. https://doi.org/10.7326/0003-4819-144-5-200603070-00005.
6. Klevens RM, Edwards JR, Richards CL. 2002. Estimating health care-associated infections and deaths in US hospitals. Public Health Rep 122:160–166. https://doi.org/10.1177/003335490712200205.
7. Milstone AM, Koontz DW, Voskertchian A, Popoola VO, Harrelson K, Ross T, Aucott SW, Gilmore MM, Carroll KC, Colantuoni E. 2015. Treating Parents to Reduce NICU Transmission of *Staphylococcus aureus* (TREAT PARENTS) trial: protocol of a multisite randomized, double-blind, placebo-controlled trial. BMJ Open 5:e009274. https://doi.org/10.1136/bmjopen-2015-009274.
8. Milstone AM, Goldner BW, Ross T, Shephard JW, Carroll KC, Perl TM. 2011. Methicillin-resistant *Staphylococcus aureus* colonization and risk of subsequent infection in critically ill children: importance of preventing nosocomial methicillin-resistant *Staphylococcus aureus* transmission. Clin Infect Dis 53:853–859. https://doi.org/10.1093/cid/cir547.
9. Tenover FR, Arbeit R, Goering R. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233–2239.
10. Brown LD, Cai TT, Das Gupta A. 2001. Interval estimation for a binomial proportion. Stat Sci 16:101–133. http://projecteuclid.org/download/pdf_1/euclid.ss/1009213286.
11. Xie X, Bao Y, Ouyang N, Dai X, Pan K, Chen B, Deng Y, Wu X, Xu F, Li H, Huang S. 2016. Molecular epidemiology and characteristic of virulence gene of community-acquired and hospital-acquired methicillin-resistant *Staphylococcus aureus* isolates in Sun Yat-sen Memorial Hospital, Guangzhou, Southern China. BMC Infect Dis 16:339–349. https://doi.org/10.1186/s12879-016-1684-y.