Cephid Xpert MRSA Cycle Threshold in Discordant Colonization Results and as a Quantitative Measure of Nasal Colonization Burden

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Running Title: MRSA Cycle Threshold and Burden of Nasal Colonization
We analyzed the cycle threshold ($C_t$) of PCR surveillance MRSA swabs in veterans. Lower $C_t$ on admission was associated with a positive discharge nasal culture. When compared to PCR, direct plating of nasal swabs performed poorly, especially in patients with an elevated $C_t$. The $C_t$ is strongly correlated with quantitative nasal cultures. Clinical and infection control applications of the $C_t$ have yet to be defined and warrant further evaluation.
Screening for methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization is done in an attempt to control the spread of MRSA within healthcare settings and prevent subsequent MRSA infection. The Veterans Health Administration (VHA) was an early adopter of active universal nasal MRSA colonization surveillance for all patients admitted to acute care facilities, with many hospitals and states subsequently following course (8, 13).

Most active surveillance programs rely on rapid detection methods using direct chromogenic agar cultures and/or real-time PCR to minimize delays in the identification and isolation of carriers in an attempt to prevent nosocomial transmission of MRSA. Although less costly, direct chromogenic agar cultures are insensitive compared to PCR (15, 22). Broth enrichment in typtic soy broth (TSB) prior to plating has been shown to increase the sensitivity of chromogenic techniques at the expense of increased workload and time to result for routine use in the clinical microbiology (11). Therefore, PCR has become the gold standard in determining colonization status.

Few studies have evaluated the impact of nasal MRSA colonization burden and results of agar-based screening tests. In 2009, Wolk et al. demonstrated a lower MRSA bacterial colonization burden is associated with discordant screening tests (positive PCR test and negative agar test) and the MRSA target cycle threshold \( (C_t) \) of the Xpert MRSA assay (Cepheid, Sunnyvale, CA) from the concordant samples was statistically lower than those from discordant samples (21). In our study, we examined the variation between PCR and direct culture screening methods for nasal MRSA colonization among hospitalized veterans, compared the \( C_t \) in patients with discordant admission and discharge screening results and evaluated the quantitative abilities of the Xpert MRSA PCR assay.
In October of 2007, the VHA and the Centers for Disease Control and Prevention developed a MRSA bundle to be instituted in all United States VHA medical centers. The MRSA bundle utilized, in addition to other measures, active surveillance of nasal MRSA colonization for all patients admitted to the hospital, transferred between units, and upon discharge from the hospital. The Atlanta VA Medical center (AVAMC) is a large, tertiary care medical center serving over 80,000 veterans. The patients admitted to the AVAMC are primarily male (≈95%) and either Caucasian (≈54%) or African American (≈44%) with significant medical and surgical co-morbidities. Like many VHA medical centers, admission and transfer screening is done with a PCR-based test, while discharge screening is done by directly plating nasal swabs onto chromogenic agar. ExTRANsual sites are not routinely screened for MRSA and MRSA decolonization is rarely implemented.

MRSA screening at the AVAMC is performed with one double swab (liquid Stuart Copan swabs; Cepheid) inserted (together) 1 cm into each nasal vestibule and rotated 4 revolutions while maintaining even contact with the nasal mucosa. Swabs are sent directly to the microbiology laboratory for immediate testing using one swab for PCR or culture with the partner swab saved as a backup. Admission and transfer screening is performed by the Xpert MRSA PCR assay according to the manufacturer’s instructions (which includes initial vortexing of the swab in the PCR cassette) and a $C_t$ from 15 – 36 considered positive. Discharge screening cultures are performed by direct inoculation (without initial vortexing) onto Spectra™ MRSA chromogenic agar (Remel, Lenexa, KS) (16) as rapid detection is unnecessary upon discharge and culture is significantly less costly.

From October 2007 through January 2008, 2,237 admission or transfer MRSA nasal screens were performed at the AVAMC and 369 (16.5%) were positive; corresponding to 272
admissions with either a positive admission and/or transfer screen (many admissions had multiple positive nasal screens). The $C_t$ value for all positive MRSA admission or transfer screens was recorded. A corresponding discharge nasal surveillance culture was performed in 181 of the 272 patients (the remaining 91 patients did not have a discharge nasal swab performed due to poor compliance with obtaining discharge swabs early in the VHA MRSA Directive). Of the 181 admissions with a positive admission or transfer screen, 62 (34.3%) had a negative discharge culture for MRSA. Of these 62 MRSA discordant patients (positive PCR test and negative agar test), 39 partner nasal discharge swabs were available for additional PCR testing by the Xpert MRSA assay. Of the 39 partner nasal discharge swabs, 16 (41.0%) swabs were PCR-positive and 23 (59.0%) swabs were PCR-negative. The remaining 23 unavailable swabs had either been used by the clinical lab for retesting or had been inadvertently discarded prior to retesting. These results demonstrate both the poor testing characteristics of direct plating without prior broth enrichment or vortexing (41.0% false negative rate among discordant samples) and the intermittent nature of MRSA nasal colonization (59% negative on repeat PCR testing) in a subgroup of patients.

The mean admission $C_t$ of the 62 patients with positive PCR admission swabs and negative discharge cultures was compared to the mean admission $C_t$ of the 119 patients with positive PCR admission and discharge culture with a two-sample T test (SAS version 9.2, Cary, North Carolina). The mean $C_t$ was significantly lower in those that remained positive at discharge compared to those that were negative at discharge (26.1 vs. 30.1, $p < 0.0001$). This is consistent with previous studies demonstrating higher nasal bacterial burden in patients persistently colonized with S. aureus (14).
Five MRSA hospital acquired infections (HAI) occurred during the above 272 hospital admissions (1.84% of admissions). The admission Ct of those patients that developed a HAI was not different than those that did not develop a HAI (25.5 vs. 27.2, p = 0.4674). The very few HAI in this cohort limits our power to detect a significant difference between the two groups and warrants further evaluation with a dedicated case-control study.

To ensure clinical factors (i.e. antibiotics) were not contributing to the discrepancies seen between admission/transfer PCR and discharge culture results, we analyzed partner nasal swabs by PCR and culture simultaneously. Partner swabs for 204 consecutive positive admission or transfer PCR screens were collected from January – May 2011 and directly inoculated onto Spectra MRSA agar by cross-streaking in four quadrants to yield 1+ to 4+ semi-quantitative culture results, identical to the method used in discharge nasal screens (23). All swabs were inoculated onto Spectra agar within 48 hours of a positive Xpert assay. The mean Ct was 27.3 for all positive swabs with a standard deviation of 5.0. Only 146/204 (71.6%) were positive by direct inoculation to Spectra™ MRSA agar. The mean Ct of those positive by both PCR and direct agar culture was 25.6 compared to a Ct of 31.4 in those positive by PCR and negative on direct inoculation (figure 1, p < 0.005).

In an attempt to assess the quantitative capabilities of the Xpert MRSA assay, 76 PCR-positive admission partner swabs were placed into 500 μl of tryptic soy broth (Becton Dickinson and Co., Sparks, MD) and vortexed for 10 seconds to simulate the initial step in the Xpert assay. Serial dilutions of the broth were spiral plated onto Spectra™ MRSA agar; colonies were counted at 24 hours and converted to log10 CFU/mL (12). Using this method, 73/76 PCR-positive partner swabs grew MRSA. A strong correlation between Ct and log10 CFU/mL was observed with a
Pearson’s correlation coefficient of -0.89 (p value < 0.0001, Figure 2). The following linear regression line was fit to the data: \( \log_{10} (\text{CFU/ml}) = 10.54 - 0.26 \, C_t \).

Using a preexisting MRSA surveillance system, we have demonstrated that the MRSA Ct on the Xpert MRSA assay is a reliable marker of nasal MRSA colonization burden, that direct plating of nasal swabs is a less reliable test in patients with low MRSA nasal burden, and a higher MRSA nasal burden is associated with subsequent positive nasal screens.

Nasal \( S. \, aureus \) colonization clearly increases the risk of developing subsequent \( S. \, aureus \) infections (3, 4, 6, 7, 18). Nasal colonization is now commonly being determined by PCR-based testing (1, 8, 9) due to ease of implementation, reliability, and rapid results. An additional advantage of PCR-based tests is an easily obtained measure of quantification in the \( C_t \).

Quantification of nasal \( S. \, aureus \) colonization was described over 50 years ago and has been linked to the persistently colonized state (14, 20), an increased risk of contamination of the environment (5, 17), an increased risk of infection (10, 19), and a higher likelihood that other body sites (besides the nares) are colonized (12). In these studies, quantification of \( S. \, aureus \) carriage was measured by labor and time-intensive techniques not easily implemented in a clinical practice. Now, with an easily obtained and reliable measure of nasal quantification, further evaluation of the clinical and infection control implications of nasal \( S. \, aureus \) burden can be explored.

The Xpert MRSA assay targets the staphylococcal cassette chromosome \( mec \) (SCCmec)-orf\( X \) junction and does not specifically target the \( mecA \) gene. Because of this, methicillin-sensitive \( S. \, aureus \) (MSSA) isolates with empty cassettes may test positive with the Xpert MRSA assay. A recent report evaluated this phenomenon and reported a false positive rate of...
7.7% of positive nasal MRSA screens (2). We did not attempt to isolate MSSA from swabs that were PCR positive and culture negative and this is a limitation of our study. However, the large discrepancy seen between PCR testing modalities and direct plating is not likely all due to this phenomenon. As our data demonstrates, negative direct cultures are most likely due to the inability of MRSA to transfer from swab to agar and not a limitation of the agar itself. This failure of transmission likely causes the majority of discordant results. Vortexing nasal swabs prior to plating greatly enhanced culture results.

Since the AVAMC uses direct plating for all discharge cultures in an attempt to measure MRSA acquisition in the hospital, this technique will likely miss a significant proportion of patients with low-level MRSA nasal colonization and calls into question the reliability of this protocol and cost savings. The potential role of nasal MRSA quantification in infection control and clinical care is unknown and deserves further study.
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Potential Conflicts of Interest: None to declare.
Figure 1: Box plot of cycle thresholds (Xpert MRSA assay) among patients with a positive Xpert nasal screen and simultaneous positive direct agar screen (concordant) and those with a positive Xpert nasal screen and a simultaneous negative direct agar screen (discordant). T test for comparing two means, \( P = <0.005 \).
Figure 2: Relationship of cycle threshold (Xpert MRSA assay) and quantitative cultures with corresponding fitted line (Pearson’s correlation coefficient, $r = -0.89$, $p < 0.0001$). Regression equation: $\log_{10} (\text{CFU/ml}) = 10.54 - 0.26 C_t$. 

![Graph showing the relationship between cycle threshold and log (CFU/ml)]


