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Major Article

Methicillin-resistant *Staphylococcus aureus* has greater risk of transmission in the operating room than methicillin-sensitive *S aureus*

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogenic *S aureus* strain characteristic associated with increased patient morbidity and mortality. The health care system needs to understand MRSA transmissibility in all settings to improve basic preventive measures to generate sustained reductions in invasive MRSA infections. Our primary aim was to compare intraoperative transmissibility of MRSA versus methicillin-sensitive *S aureus* (MSSA) isolates.

Methods: *S aureus* isolates (N = 173) collected from 274 randomly selected operating room environments (first and second case of the day in each operating room, a case pair) at 3 hospitals underwent systematic-phenotypic and genomic processing to identify clonally related transmission events. Confirmed transmission events were defined as at least 2 *S aureus* isolates obtained from ≥2 distinct intraoperative reservoirs sampled within or between cases in a study unit that were epidemiologically and clonally related. We explored the relationship between clonal transmission and methicillin resistance with Poisson regression analysis.

Results: We identified 58 clonal transmission events. MRSA isolates were associated with increased risk of clonal transmission compared with MSSA isolates (adjusted incidence risk ratio [IRR], 1.68; 95% confidence interval [CI], 1.13-2.49; $P = .010$; unadjusted IRR, 1.85; 95% CI, 1.23-2.77; $P = .003$, respectively).

Conclusions: MRSA isolates are associated with increased risk of intraoperative transmission. Future work should examine the impact of the attenuation of intraoperative MRSA transmission on the incidence of invasive MRSA infections.

Staphylococcus aureus has caused human disease since Hans Christian Gram developed the stain that bears his name. The pathogenicity of *S aureus* is caused in part by ongoing acquisition of genetic traits that enhance antimicrobial resistance, virulence, and survival.[1-4] Because *S aureus* is the most likely disease-causing pathogen for surgical site infections (SSIs) and is a leading cause of bloodstream and respiratory infections,[5-10] health care systems must gain an improved understanding of the epidemiology of *S aureus* transmission to facilitate infection prevention.[9]

S aureus pathogens dispersed from provider hands and patient skin surfaces can contaminate aerosolized particles, equipment, and tools such as laryngoscope blades, laryngoscope handles, anesthesia machines, and ventilators.[11] Because *S aureus* has been shown to survive on environmental surfaces for up to 360 days,[11] factors that lead to increased survival of transmitted pathogens could facilitate the establishment of institutional reservoirs. In turn, institutional reservoirs could lead to perpetual environmental transmission to patient and provider hosts in the patient care arena and, ultimately, to infectious disease outbreaks.[12]

Methicillin-resistant *S aureus* (MRSA) is associated with more severe and prolonged infections. Invasive MRSA infections are

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associated with an 18% mortality rate even among healthy patients in the community,[1-4] and recent evidence concluded that the incidence of invasive MRSA infections in 2015 was no different from an established 2010-2011 baseline.[13] We must gain a better understanding of MRSA transmission dynamics in all health care settings to generate sustained reductions in MRSA transmission and subsequent infection development.

The overall goal of this study was to provider further insight into intraoperative MRSA transmission dynamics. Our primary aim was to examine the association of MRSA with clonal transmission. We hypothesized that the MRSA strain characteristic would be associated with increased risk of intraoperative clonal transmission events. Our hope was that this work would lead to the establishment of infection control measures targeting intraoperative MRSA spread.

MATERIALS AND METHODS

Background and general description

Because study activity was limited to analysis of deidentified data from a previous institutional review board–approved project (no. 201507774, Assessment of Routine Intraoperative Horizontal Transmission of Potentially Pathogenic Bacterial Organisms II), the University of Iowa waived the need for institutional review board–approved review and declared that the additional analysis did not meet the definition of human subjects research. We observed 274 randomly selected case pairs (first and second case of the day in each operating room environment) across 3 hospitals over a 1-year period (March 2009-February 2010). The randomized study design resulted in the inclusion of a wide variety of surgical procedures, patient comorbidities, infection control measures, and health care providers. The 1-year study period addressed seasonal variation.[10,14] Systematic intraoperative bacterial reservoir collection from 274 study units yielded 173 *S aureus* isolates implicated in ≥1 possible transmission events (defined as at least 2 isolates from 2 distinct reservoirs within or between cases in a case pair) which were included in this study.

We monitored institutional infection control policies during the study period at each of the 3 hospital sites. Hospital site 1 used surface disinfection wipes and quaternary ammonium disinfectants for routine cleaning of between-case environmental surfaces. Hospital sites 0 and 2 used only quaternary ammonium disinfectants. All hospitals provided wall-mounted, 62% alcohol dispensers or 70% alcohol dispensers located on anesthesia carts for intraoperative hand decontamination. A machine-mounted, foam-based alcohol dispenser was also available at hospital site 1. Gloves were immediately available for use throughout patient care episodes at all hospitals, whereas use of preoperative chlorhexidine baths and nasal mupirocin by patients was infrequent across all sites. There were no changes in these standardized procedures during the study period.[10,14]

S aureus *collection*

We systematically sampled intraoperative bacterial reservoirs via use of a proven model for study of intraoperative bacterial cross contamination[14] that has previously led to reduction in intraoperative bacterial transmission and subsequent infection development.[15-17] Reservoir sites included anesthesia provider hands (attending and resident physicians and certified registered nurse anesthetists) throughout patient care, the adjustable pressure-limiting valve and agent dial of the anesthesia machine, the internal lumen of the intravenous stopcock set, and the patient nasopharynx and axilla. Air was a continuous medium potentially affecting all measured reservoirs through settling of aerosolized particles. The reservoir sampling sequence for case 1 included baseline environmental samples, provider hands prior to patient care, the patient nasopharynx and axilla after induction of anesthesia and patient stabilization, provider hands throughout care (hands were sampled from any provider who entered the anesthesia work area during the case including departures and returns), the same environmental sites at case end, and the internal lumen of the patient intravenous stopcock set at case end (proven invariably negative on removal of the packaging material).[14] This process was repeated for the second case in an observational unit, except that environmental sites were not decontaminated prior to culture so residual contamination after routine cleaning procedures between cases could be assessed. The specific methods of culture acquisition and handling for this process are previously described.[10,14]

S aureus *isolate processing*

Class of pathogen

Bacterial isolates were initially identified by colony morphology, gram stain, and simple rapid tests.[10,14,18]

Analytical profile indexing

Bacterial organisms were then identified and isotypes specified using the commercially available bioMérieux API identification system (Marcy l'Etoile, France), resulting in an identification number that was cross referenced using the Analytical Profile Index database to obtain the final organism biotype.[10,14,17]

Antibiotic susceptibility

We used disk diffusion antibiotic susceptibility testing (methicillin, ampicillin, cefazolin, cefepime, ceftazidime, cefuroxime, ciprofloxacin, clindamycin, gentamicin, meropenem, penicillin, piperacillin-tazobactam, sulfamethoxazole trimethoprim, linezolid, tetracycline, and vancomycin) analysis as previously described.[10,14] Bacterial sensitivity was recorded and subsequently analyzed as sensitive or resistant (including intermediate resistance).[18] MRSA and vancomycin-resistant *Enterococcus* were also confirmed by agar dilution minimal inhibitory concentration.[10,14,18]

Next-generation sequencing

Extracted *S aureus* isolate DNA underwent next-generation sequencing via the Illumina platform at the Iowa Institute of Human Genetics. DNA samples (1.2 μg/60 μL) were sheared to approximately 400-bp fragments on the Covaris E220 (Covaris, Woburn, MA). Sequencing libraries were prepared from the sheared DNA (1 μg/50 μL) using the KAPA Hyper Library Prep on the PE Caliper Sciclone (Rosche Diagnostics, Indianapolis, IN). Each library was prepared using an adapter that carries a unique barcode (Integrated DNA Technologies, Coralville, IA). Libraries were analyzed on a fragment analyzer, and equimolar amounts of the libraries were pooled based on fragment analyzer results for a smear analysis of 450-670 bp. A size range of 450-670 bp was recovered from the pool on the Blue Pippin (Sage Science, Beverly, MA). The KAPA library quantification kit for Illumina platforms was used to determine the molar concentration of the size-selected pool. The pool was loaded on the cBot (Illumina) for cluster generation, and the flow cell was loaded on the HiSeq4000 (Illumina) for sequence analysis.

Multilocus sequence typing and single nucleotide variant analysis

S aureus sequence reads were generated and downloaded into the CLC Genomics Workbench Module (Version 1.1; Qiagen Aarhus, Germantown, MD). CLC Genomics Workbench Plugin was used to trim and to remove adapters and broken pairs from *S aureus* sequence reads, and K-mer spectra analysis was used to identify a best match to *S aureus* isolates. *S aureus* 252 (MRSA252, NC_002952) was