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Molecular epidemiology of *Staphylococcus aureus* in post-earthquake northern Haiti

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SUMMARY

Background: Knowledge of nasal carriage is important in predicting staphylococcal infection, and no information exists regarding the endemicity of *Staphylococcus aureus* in Haiti.
Methods: We performed a cross-sectional analysis of *S. aureus* nasal screening in an acute care, a subacute rehabilitation, and a community setting, with a brief medical and epidemiological history. PCR-positive *S. aureus* screening nasal cultures underwent molecular analysis for *spa* type, SCC*mec* type, and virulence genes (Panton–Valentine leukocidin (PVL), toxic shock syndrome toxin (TSST), and arginine catabolic mobile element (ACME)), and were evaluated for antibiotic susceptibility using commercial tests.
Results: Overall carriage rates of 8.4% methicillin-susceptible *S. aureus* (MSSA) and 2.8% methicillin-resistant *S. aureus* (MRSA) were identified, with a high rate of tetracycline resistance. TSST and PVL genes were identified in MSSA. MRSA isolates contained no virulence markers. Unique MSSA phenotypes (i.e., linezolid-resistant, vancomycin-sensitive/daptomycin non-susceptible) were identified, as were two PVL-positive ST152 MSSA colonization isolates, previously geographically limited to Africa.
Conclusions: We found a low *S. aureus* carriage rate with complete vancomycin susceptibility and high tetracycline resistance, which has important public health implications with regard to treatment. Additionally, the finding of PVL-positive MSSA isolates, including the expansion of a previously described limited 'divergent' clone, ST152, warrants further evaluation.

1. Introduction

On January 12, 2010 a devastating magnitude 7.2 earthquake destroyed Port-au-Prince, Haiti, leaving over 222 000 dead and 300 000 injured.[1] Two million residents were displaced from their homes; many found temporary shelter in unsanitary and potentially unsafe conditions, such as tent cities. Injuries were treated at Hôpital Adventiste in Port-au-Prince, in addition to many make-shift field hospitals.[2–4] Victims were transported from the epicenter to Hôpital Sacré Coeur in Milot, some 138 km, or 7 h ground travel over rough terrain. Hôpital Sacré Coeur quickly became inundated with patients, and its maximum capacity was surpassed, necessitating the use of temporary tents which functioned as patient wards and pre- and postoperative patient care units. It was hypothesized that, similar to the situation following the devastating earthquake in 2008 in Wenchuan

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County, Sichuan, China, the most common pathogen in injuries and wound cultures would be *Staphylococcus aureus*.[5] It is accepted that nasal carriage is an important risk factor for staphylococcal infection, whether through endogenous acquisition (i.e., self-inoculation) or nosocomial transmission via the hands or instruments of the health-care delivery system.[6,7] Knowledge of *S. aureus* epidemiology is important for public health treatment and prevention strategies when facing natural disasters, such as earthquakes.

Minimal information is known regarding the endemicity of both methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA, respectively) in Haiti, especially in the post-earthquake period. A review of the literature revealed only one prior report regarding the clinical significance of *S. aureus* in Haiti, which concerned a neonatal HIV population with *S. aureus* bacteremia.[8]

The purpose of this study was to determine the distribution of *S. aureus* nasal carriage and self-reported or documented infections in patients, visitors, and health-care workers at three unique locations in post-earthquake northern Haiti: (1) currently hospitalized patients, caregivers/visitors, and health-care workers at an acute care hospital, (2) patients, visitors, and health-care workers at a subacute rehabilitation facility, and (3) patients, visitors, and staff seeking care from a community dispensary (clinic). We sought to describe the molecular epidemiology of nasal carriage isolates based on genotyping of staphylococcal protein A (*spa*), staphylococcal cassette chromosome *mec* (SCC*mec*), and putative virulence factors, as well as to evaluate automated methicillin susceptibility profiles to determine intrinsic carriage rates of MRSA.

2. Methods

This internally funded, institutional review board approved, cross-sectional study utilizing microbiological and molecular epidemiological methods took place in June 2012 in northern Haiti, 75 nautical miles north of Port-au-Prince. Three locations (hereafter identified as locations A, B, and C) were chosen. Location A, an acute care 88-bed hospital at the time of the earthquake, increased capacity to nearly 400 patients at the peak post-earthquake. This hospital has operating rooms, an intensive care unit, and a maternity ward, and was the main site for study participation. Adult patients housed in 8–10-bed separate male and female medical and surgical wards and a maternity ward were approached. Overflow patients were housed along the hallway corridors. Family members collected medication prescriptions at the centralized hospital pharmacy (including intravenous sets and syringes). Location B, a 30-bed long-term rehabilitation facility and national referral center located 24 km from location A, includes a multi-bed single room unit with a separate physical therapy and rehabilitation area. Location C, a community dispensary/clinic, serves the local community on a walk-in basis.

Using a Creole-speaking interpreter, patients, visitors, and employees at the three locations were invited to participate in an anonymous survey and submit to nasal screening for the assessment of *S. aureus* risk and carriage. Informed consent was implied by voluntary participation in this anonymous survey and screening, after an introduction to the purpose, method, and design of the study was provided verbally to each patient, visitor, and staff member, at each location, and all questions had been answered. Voluntary, non-remunerated participants were assigned anonymized serial study numbers. A brief medical history was taken and a questionnaire administered verbally to participants to collect data on medical and surgical conditions, antibiotic usage history, and place of residence during the 2010 earthquake, as well as contact or care of earthquake victims. Translated responses were recorded on-site by participating authors (MR, JS, LP, and AS) prior to obtaining nasal cultures.

Cultures were obtained by inserting a commercial dual-tipped culturette swab into bilateral anterior nares and rotating five times in a clockwise direction prior to returning the swab to the transport tube, which contained Stuart's medium (BBL Culture Swabs; Becton Dickinson). Numbered swabs were collected and placed in a refrigerated unit in the hospital laboratory of location A within 4 h of collection and were stored for up to 1 week. Swabs were transported on ice to the Public Health Research Institute at Rutgers University in Newark, New Jersey, USA by study personnel. All swabs were then plated on non-selective Luria Broth (LB) medium. Total bacterial DNA was isolated from overnight cultures using a boiling lysis method.

Real-time PCR with molecular beacons specific for the staphylococcal protein A (*spa*) gene was used to identify nasal screens containing *S. aureus*.[9,10] Bacterial isolates with positive *spa* PCR for *S. aureus* were then plated on selective CHROMagar (BBL media). Three mauve *S. aureus* colonies were picked from CHROMagar and re-streaked on LB plates. DNA isolation was accomplished using previously published methods.[11] Genetic characterization by sequencing the variable number tandem repeats in the protein A gene (*spa* typing) with the use of eGenomics software, as described previously, allowed for assignment to clonal complexes (CCs).[11,12] Both the US eGenomics-based *spa* typing assignment and the European Ridom StaphType, denoted by 't', are reported.[13,14] Any unassigned eGenomics or Ridom sequences were submitted for *spa* type assignment (http://tools.egenomics.com and http://spa.ridom.de/submission.shtml, respectively). The detection of genes for the arginine catabolic mobile element (ACME), Panton–Valentine leukocidin (PVL), and staphylococcal toxic shock syndrome toxin (TSST) was done using PCR-based techniques completed in triplicate.

Automated susceptibility determination for standard antibiotics (VITEK2; bioMérieux, Durham, NC, USA), or manual testing using Etest strips for non-automated antibiotics (such as daptomycin), was performed according to standard laboratory procedures. Clindamycin susceptibility was confirmed for inducible resistance using the D test.[15] The following antibiotics were tested: penicillin, oxacillin, gentamicin, ciprofloxacin, erythromycin, clindamycin, quinupristin–dalfopristin, linezolid, vancomycin, tetracycline, tigecycline, rifampin, trimethoprim–sulfamethoxazole, and daptomycin. Standard published Clinical and Laboratory Standards Institute (CLSI) breakpoints were used for interpretation. As daptomycin breakpoints are not established, non-susceptibility was defined as a minimum inhibitory concentration (MIC) $\geq$1.5.

2.1. Statistical methods

Continuous variables were summarized using the mean and range, and categorical variables were summarized using the frequency and percentage. Fisher's exact test was used to compare the proportions between groups. The two-sided *p*-value was reported for each test, with $p < 0.05$ considered an indication of statistical significance.

3. Results

Of 150 persons approached at the three distinct locations, 143 agreed to participate; more than half of these persons were female (62%, 89/143). One hundred six patients, visitors, and staff from location A, 24 from location B, and 13 from location C, completed the questionnaire and agreed to nasal swabs. Baseline demographics are shown in Table 1. Birthdates were approximated