

year. *S. pyogenes* infections disproportionately affect low-income countries where routine surveillance is not available. The objective of this study was to investigate the molecular epidemiology and antibiotic resistance of clinically relevant *S. pyogenes* isolates in Ulaanbaatar, Mongolia, to better understand the burden in this under-served population.

**Methods.** Clinical *S. pyogenes* isolates ( $n = 41$ ) collected at the Bacteriological Reference Laboratory, National Center for Communicable Diseases, Ulaanbaatar, Mongolia, were cultured and characterized using PCR techniques. The *emm* gene was sequenced and *emm* type was assigned as per Centers for Disease Control and Prevention (CDC) methods and guideline. Multi-locus sequence typing (MLST) was carried out on selected isolates ( $n = 15$ ). Antibiotic susceptibility testing (AST) was done via the Vitek-2 system as per manufacturer's instructions.

**Results.** We observed 18 distinct *emm* types among the 41 *S. pyogenes* isolates. stG6792.0 was the most common *emm* type, accounting for more than one-third of the isolates (15/41) followed by *emm*2.0 (ST55) (5/41) and *emm* 82.0 (ST314) (2/41). A total of seven sequence types (STs) were detected among 15 tested isolates. The most common ST type was ST55 accounting for one-third of the isolates (5/15). Most of the isolates were susceptible to all tested drugs.

**Conclusion.** The findings of this study provided some insights regarding the molecular characteristics of *S. pyogenes* in Mongolia that will be crucial for future surveillance studies. Five isolates of this study had similar *emm* types (*emm*74.0, *emm*66.0, stG480.0, *emm*83.1, *emm*89.0) compared with a previous surveillance study. *emm*89.0 (ST101) was a major epidemiological isolate in the United States between 2000 and 2004. *emm*89.0 was also implicated with a recent single-clone outbreak in China. This information suggests the possibility of a shifting epidemiological trend of *S. pyogenes* on the global stage. The information about antibiotic susceptibility patterns and molecular types can help to devise better treatment strategies for *S. pyogenes* infections, and potentially inform vaccine development.

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### 231. Microbial cell-free DNA Sequencing to Detect *Borrelia burgdorferi* DNA in the Plasma of Pediatric Patients with Lyme Disease

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**Background.** Diagnosing Lyme disease often involves laboratory evaluation, yet available tests have limitations. Serology remains negative for weeks after infection occurs, and may then remain positive for years. *Borrelia burgdorferi* blood PCR testing has low sensitivity, rendering it unhelpful. We sought to determine whether an emerging technology, next-generation sequencing (NGS) of microbial cell-free DNA (mcfDNA), can detect *B. burgdorferi* DNA in the plasma of pediatric patients with erythema migrans (EM).

**Methods.** Patients aged 1–17 years with a clinically-identified single or multiple EM were enrolled. Two clinical investigators were required to agree on the EM finding, with no evidence of an alternative diagnosis. Subjects were excluded if they previously had Lyme disease, had received antibiotics within 30 days prior to enrollment, or if the rash had resolved before the first blood draw. Three blood samples were taken during the study period: one before antibiotics were administered, then 1–3 weeks and 2–3 months later. At enrollment, plasma was tested for Lyme disease using C6 antibody with reflex to Western Blot and mcfDNA sequencing (Karius, Inc., Redwood City, CA). Briefly, mcfDNA was extracted from plasma and NGS performed. Human reads were removed and remaining sequences were aligned to a curated microbial database. Only mcfDNA testing was performed at follow-up visits.

**Results.** We enrolled 5 subjects (ages 4–15 years old, median age 4). Four subjects had a single EM and negative Lyme serology. One subject had approximately 20 EMs and positive serology (C6-antibody=7.52 (Positive >1.09), 3/3 IgM, 2/10 IgG). All 14 plasma samples, including five pre- and nine post-antibiotic samples, were negative for *B. burgdorferi* DNA by mcfDNA sequencing. No other infections, including other tick-borne infections, were detected.

**Conclusion.** NGS of mcfDNA did not identify *B. burgdorferi* DNA in the plasma of pediatric patients with active EM rashes. This approach is unlikely to be helpful in diagnosing early localized Lyme disease. This may be because spirochetes are localized to the periphery of the rash in EM and spirochetemia likely occurs at later stages of infection. Follow-up studies are planned to investigate how NGS of mcfDNA performs during early and late disseminated Lyme disease.

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### 232. Genomic Evidence for Dissemination of *Mycobacterium marinum* in an HIV Patient with Multifocal Cutaneous Disease

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**Background.** Hematogenous dissemination has been proposed to explain multifocal cutaneous disease caused by *Mycobacterium marinum* in certain patients. Treatment duration for disseminated disease is often months longer than for skin infection alone. However, distinguishing multiple independent inoculation events from dissemination has relied primarily on clinical judgement. Additionally, whether temperature-sensitive non-tuberculous mycobacteria such as *M. marinum* are indeed capable of invading the vascular space at core body temperature is debated. Here we used whole-genome sequencing (WGS) of serial isolates from a single patient with multifocal cutaneous *M. marinum* infection to distinguish dissemination of a clonal strain from multiple inoculation events.

**Methods.** A 35-year-old male with HIV (CD4 of 66 cells/ $\mu$ L) presented with a two-month history of a non-healing *M. marinum* wound on his left elbow (isolate MM0). This was followed a month later after initiation of antiretroviral therapy by a second *M. marinum* lesion on the right heel (MM1) without history of repeat inoculation, and increased swelling and erythema of the wound on the left arm (MM2) consistent with paradoxical immune reconstitution inflammatory syndrome. A PacBio genome was generated for MM0 and short read Illumina genomes were generated for MM1 and MM2.

**Results.** All isolates were found to be closely related, with MM1 and MM2 distinguished from MM0 by one and five single-nucleotide variants (SNVs), respectively. Given the substantial genetic heterogeneity among environmental *M. marinum* strains, such close relatedness of these isolates suggests common origin, and provides strong evidence for dissemination of a clonal strain in this patient. The SNVs included a frameshift mutation in the *purT* gene, which encodes a formate-dependent phosphoribosylglycinamide formyltransferase involved in de novo purine synthesis, and missense mutations in *atsA* and the DNA methylase *hsdM*. All isolates grew at 35°C, compared with the optimal growth temperature of 30°C typically observed for *M. marinum*, suggesting thermotolerance permissive for dissemination.

**Conclusion.** These results demonstrate the potential role of WGS for providing supportive evidence of disseminated infection with *M. marinum*.

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### 233. The Epidemiology, Genomics, and Evolution of *Staphylococcus aureus* in Northeast Ohio

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**Background.** Infections due to *S. aureus* result in significant morbidity, mortality, and healthcare expense. We sought to identify the strains of *S. aureus* causing infections in hospitalized patients in Northeast Ohio and determine whether they are reflective of the *S. aureus* strains present in the surrounding environment.

**Methods.** The study was approved by the Institutional Review Board at Cleveland Clinic Akron General. Clinical *S. aureus* isolates ( $n = 300$ ) were cultured and PCR was used to amplify the staphylococcus protein A (*spa*), Pantone-Valentine Leukocidin (PVL), and *mecA* genes. The clinical *spa* types were compared with ones from our data base of *S. aureus* strains previously collected and sequenced from the community and environment in Northeast Ohio.

**Results.** A total of 51 *spa* types were detected from 129 *S. aureus* clinical isolates (discriminatory index, 0.876; 95% confidence interval [CI], 0.827–0.925; Table 1). The most common *spa* types were t008 (42/129, 32.6%), t002 (16/129, 12.4%), and t334 (6/129, 4.7%). In comparison, the most frequently detected *spa* types from the environmental samples were t189 (40/257, 15.6%), t002 (16/257, 6.2%), and t008 (11/257, 4.3%). Among the *S. aureus* isolates ( $n = 146$ ), 45 were PVL-positive (30.8%) and 94 (66.7%) carried *mecA*. Of the 42 t008 (ST8/USA300; a common community-associated strain) isolates, 35 (83.3%) were methicillin-resistant *S. aureus* (MRSA) (based on the presence of the *mecA* gene) and 25 (59.5%) were PVL-positive. Thirteen of the sixteen (81.2%) t002 (ST5/USA100; a common hospital-associated strain) were MRSA and only one (6.2%) was PVL-positive.

**Conclusion.** There is considerable overlap of *S. aureus* strains present in clinical samples with those found in the environment. This finding should draw attention to the need for more effective prevention strategies to reduce the risk of transmission of *S. aureus*, including MRSA, in the environment to humans.