Characterization of *Ureaplasma* Isolates from Neonates in the Global Multi-Center Child Health and Mortality Prevention Surveillance Network

JL Waller,1, E Yang,2,3, JAP Hamlin,3,4, MH Díaz,2,5, JM Winchell,2, Child Health and Mortality Prevention Surveillance Consortium6

1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2WIRC, Inc., Atlanta, GA, USA, 3Emory Global Health Institute, Atlanta, GA, USA

**BACKGROUND**

*Ureaplasma* species, including *U. urealyticum* and *U. parvum*, commonly colonize the female urogenital tract and are transmissible to neonates during birth or to the fetus during pregnancy. Recent studies in South Asia and South Africa revealed *Ureaplasma* species as a leading cause of severe neonatal infections. However, diagnostic testing of clinical specimens and comprehensive genomic studies of *Ureaplasma* are limited.

**METHODS**

Whole blood, cerebrospinal fluid (CSF), and tissue specimens were collected from neonates post-mortem via a minimally invasive tissue sampling (MITS) procedure at seven Child Health and Mortality Prevention Surveillance (CHAMPS) sites in sub-Saharan Africa and South Asia from May 2017 to October 2021. *Ureaplasma* species were detected by real-time reverse transcription polymerase chain reaction (RT-qPCR) using custom TaqMan Array Cards for multipathogen detection. Specimens from CHAMPS cases in which *Ureaplasma* was detected were transferred to CDC for culture, real-time PCR (qPCR) identification of *Ureaplasma* species from primary specimens and isolates when obtained, and isolate whole genome sequencing using Illumina MiSeq. Genome assemblies were analyzed to evaluate strain diversity and the potential for antimicrobial resistance attributed to known resistance mediating mutations.

**RESULTS**

RT-PCR performed on 22 primary specimens from CHAMPS cases enrolled in South Africa revealed *U. urealyticum* in 9 (41%), *U. parvum* in 6 (27%), and both species in 2 (9%); neither species was determined in 5 (23%) specimens. Ten isolates (5 *U. urealyticum*, *U. parvum*) were recovered from 22 primary specimens, including lung tissue (n=5), blood (n=2), and CSF (n=3), from 6 CHAMPS cases; *Ureaplasma* was detected but not attributed to the cause of death in these cases. Isolate species matched the primary specimen species when the specimen was not a mixed species. In one case *U. parvum* was recovered from both blood and CSF while *U. urealyticum* was recovered from tissue; both species were identified in primary blood and CSF specimens from this case. Genome assemblies were generated for 8 isolates and species identified by WGS matched species identification for all isolates. Expanded multi-locus sequence typing revealed diversity of isolates. Four (50%) isolates matched known sequence types clustering in clonal complexes corresponding to species. Novel allelic profiles were identified in 4 (50%) isolates. Sequence evaluation for potential AMR revealed 1 isolate harboring tet (M), an indicator of tetracycline resistance. Additionally, all isolates had mutations in at least one gene or protein investigated but these were different from the known resistance-mediating mutations.

**Ureaplasma species are an underrecognized cause of neonatal infections.** Genome assemblies revealed allelic profile diversity and potential indication of tetracycline resistance. Additional sequencing could uncover unique features impacting illness severity.