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Evaluation of Azithromycin Resistance in *Treponema pallidum* Specimens from Madagascar

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Syphilis is an important public health concern in Madagascar. We have observed high syphilis prevalence levels among sex workers (16 to 31%), women who reported occasional sex trading (13%), women seeking care for genito-urinary infections (6 to 29%), and pregnant women (12%).[1–4]

According to the Center for Disease Control and Prevention Sexually Transmitted Disease Guidelines, the standard of care for treatment of early syphilis infection is benzathine penicillin administered as a single intramuscular injection.[5] Though the cost is low and treatment failure uncommon, there are also disadvantages. First, patients may have allergy to penicillin.[6–10] Second, intramuscular injection is painful and requires injection equipment and medically trained personnel, imposing logistical barriers to rapid and efficient administration of therapy in resource-poor areas, especially in field settings. Third, there are risks associated with transmission of blood-borne infections when injection equipment is reused.

The macrolide antibiotic azithromycin has been used successfully to treat primary and secondary syphilis and has the advantage of a single oral dose regimen.[11–14] Because azithromycin is well tolerated[3] and easier to administer than benzathine penicillin, use of azithromycin for syphilis treatment may improve syphilis treatment coverage in Madagascar and other settings.

Of concern, however, are therapeutic failures that have been reported with azithromycin. Resistance to azithromycin can be conferred by a A2058G mutation in the 23S ribosomal RNA (rRNA) gene of *Treponema pallidum*.[15] This mutation has been detected in specimens from a high proportion of syphilis-infected patients on the west coast of the United States (US), in Canada, and in Dublin.[15–19] Additional evaluation of samples from the western US and Canada suggests that the prevalence of the mutation has been increasing over time.[16–19] Azithromycin usage for syphilis treatment in regions with known high prevalence of

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resistance could compromise treatment. However, in countries like Madagascar that have a high syphilis burden and insufficient public health resources, azithromycin might be an important tool for infection control and prevention. Because monitoring of azithromycin resistance will be needed if use of the antibiotic becomes common, we performed a baseline assessment among a sample of persons with early syphilis in Madagascar.

The research we report here was conducted as part of a large ongoing, multi-center, Phase III randomized controlled trial conducted to evaluate the effectiveness of azithromycin delivered as a single oral dose for treatment of early syphilis in the US and Madagascar.[20] The efficacy of azithromycin (one 2g oral dose) *versus* benzathine penicillin (one 2.4 million unit dose, administered intramuscularly) for treatment of primary, secondary, or early latent syphilis among HIV-negative volunteers aged 18 to 55 years is determined at six months clinically and serologically (rapid plasma reagin test and *T. pallidum* particle agglutination assay). The study was described in detail, and those who chose to enroll were asked to provide written informed consent. The ethical review boards of the Ministry of Public Health, Antananarivo, Madagascar; the University of North Carolina at Chapel Hill; the University of Washington; and the University of Alabama at Birmingham approved the research. The trial is registered at ClinicalTrials.gov (Identifier #: NCT00031499).

We evaluated the presence of *T. pallidum* containing the macrolide resistance mutation in a sub-sample of patients from the 3 study sites in Madagascar (Antananarivo, Toamasina, and Mahajanga) where 82% of trial participants have been enrolled. Swab samples were obtained from ulcer lesions or condylomata lata (N=186). The presence or absence of the A2058G mutation was determined by nested polymerase-chain-reaction (PCR) amplification of one 23S ribosomal DNA region, followed by restriction digestion of the amplicon, as has been described previously.[15]

A total of 464 Malagasy participants were enrolled in the syphilis randomized controlled trial. Study participants were 61% male, had a median age of 24 years at recruitment (range: 18–53 years), nearly universally reported heterosexual sex only (98%), and reported a median of two sexual partners per year. Thirty-one percent of participants were diagnosed with primary syphilis, 42% with secondary syphilis, and 27% with early latent syphilis.

T. pallidum DNA could be detected in 146 (78%) of 186 specimens received, as measured by amplification of the TpN47 gene. Of the 146 samples containing detectable *T. pallidum* DNA, the 23S rRNA gene could be amplified by PCR in 141 specimens, and the A2058G mutation was not observed in any of these. In five specimens, the 23S rRNA gene could not be amplified. The 141 specimens with amplifiable *T. pallidum* DNA which were included in the resistance study were obtained from a sub-group of participants who differed somewhat from the main trial participants; they were more likely to be male (79%) and 78% were diagnosed with primary syphilis and 28% with secondary syphilis.

These results, based on evaluation of the first large case series of specimens from Africa, demonstrate the absence of the macrolide resistance mutation common in several North American and European populations. There are several important considerations. First, the absence of the mutation in our study may be limited to Madagascar, which is an island country. No data have been reported from the African continent, although a randomized controlled trial in Tanzania showed equivalence of azythromycin compared with benzathine penicillin for early syphilis treatment.[14] Second, our study population, nearly universally heterosexual and HIV-negative, may represent only a subset of individuals at risk for syphilis. Previous studies that indicated a high prevalence of azithromycin resistance in *T. pallidum* were conducted in the west among small samples, primarily composed of men who

have sex with men including men with HIV infection.[15,17,18] *T. pallidum* strains that are resistant to azithromycin may be circulating among other populations in Madagascar.

Because of the risk of increasing *T. pallidum* resistance to macrolides, emerging resistance must be prospectively evaluated in the community. In addition, follow-up of patients with syphilis for treatment failure needs to be assiduous, whether benzathine penicillin or azithromycin is administered. The potential for treatment failure and/or reinfection in patients with syphilis has long been recognized.[15–18] Because macrolide resistance can be conferred by additional 23S rRNA gene mutations, novel mutations in patients who fail therapy need to be detected and correlated with treatment outcome(s). This point presents the greatest challenge, because molecular sequencing techniques needed to identify novel mutations are not currently available in Madagascar. However, monitoring such as this is feasible through global partnerships. Finally, it is important to emphasize that azithromycin should not be used in pregnant women to prevent congenital syphilis.[21]

What is the implication of these findings for use of azithromycin to treat early syphilis in Madagascar? Because syphilis is common in Madagascar, there is an urgent need for an effective, safe, well-tolerated syphilis treatment alternative. Combined with evidence from the parent randomized clinical trial that azythromycin was equivalent to benzathine penicillin[20] for treatment of early syphilis, we argue that azithromycin can be used as an alternative treatment to penicillin in Madagascar and during the initial phase of a public health campaign to reduce early syphilis in Madagascar.

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