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The worldwide spread of ciprofloxacin-resistant *Shigella sonnei* among HIV-infected men who have sex with men, Taiwan

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Abstract

Ciprofloxacin-resistant shigellosis outbreaks among men who have sex with men (MSM) have not been reported in Asia. During 3 March to 6 May 2015, the Notifiable Disease Surveillance System detected nine non-imported *Shigella sonnei* infections among human immunodeficiency virus (HIV) -infected Taiwanese MSM. We conducted a molecular epidemiological investigation using a 1 : 5 matched case–control study and laboratory characterizations for the isolates. Of the nine patients, four reported engagement in oral–anal sex before illness onset. Shigellosis was associated with a syphilis report within 12 months (adjusted odds ratio (aOR) 8.6; 95% CI 1.05–70.3) and no HIV outpatient follow-up within 12 months (aOR 22.3; 95% CI 2.5–201). *Shigella sonnei* isolates from the nine patients were all ciprofloxacin-resistant and the resistance was associated with S83L and D87G mutations in *gyrA* and S80I mutation in *parC*. The nine outbreak isolates were discriminated into two closely related pulsed-field gel electrophoresis (PFGE) genotypes and seven 8-locus multilocus variable-number tandem repeat analysis (MLVA8) types that suggest multiple sources of infections for the outbreak and possible under-recognition of infection among Taiwanese MSM. The outbreak isolates were characterized to be variants of the intercontinentally transmitted SS18.1 clone, which falls into the globally prevalent phylogenetic sub-lineage IIIb. Inter-database pattern similarity searching indicated that the two PFGE genotypes had emerged in the USA and Japan. The epidemiological characteristics of this outbreak suggest roles of risky sexual behaviours or networks in *S. sonnei* transmission. We urge enhanced surveillance and risk-reduction interventions regionally against the interplay of HIV and shigellosis among MSM.

Keywords: Bacillary, ciprofloxacin resistance, dysentery, HIV infections, homosexuality, male, molecular epidemiology, *Shigella sonnei*
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Introduction

Shigellosis, an acute gastroenteritis caused by *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*, can be transmitted through direct or indirect oral–anal exposures and outbreaks among men who have sex with men (MSM), human immunodeficiency virus (HIV) -infected or not, have been reported worldwide [1–6]. Resistance to fluoroquinolones, the recommended drugs for shigellosis treatment, is more commonly identified among MSM [1,4]. However, ciprofloxacin-resistant shigellosis outbreaks among MSM have only been reported in North America, Australia and Europe, not in Asia [6].

Shigellosis is nationally notifiable through the web-based Taiwan Centres for Disease Control (TCDC) -operated Notifiable Disease Surveillance System (NDSS). From 2005 to 2014, 134–342 cases were reported annually in Taiwan, a country with a population of 23 million. More than 33% of the cases occurred in persons who had resided in or visited areas

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were shigellosis was endemic within ≤7 days of illness onset and were determined as imported cases (i.e. acquired abroad) based on public health investigations. *Shigella sonnei* has become the most prevalent cause for the disease since 2000. Before 2015, only four cases were reported among HIV-infected MSM, one acquired in the Philippines (infected by *S. flexneri* 4a) in 2011 and one in Thailand (*S. flexneri* 3a) in 2014, and the other two domestically acquired in 2008 (*S. flexneri* 2a) and 2014 (*S. flexneri* 3b), respectively. Between 3 March and 6 May 2015, the NDSS detected nine *S. sonnei*-infected cases among HIV-infected MSM.

In this study, we investigated the molecular epidemiology of this outbreak. We conducted a 1 : 5 matched case–control study using the NDSS database. The bacterial isolates were characterized using antimicrobial susceptibility testing to obtain the resistance profiles, pulsed-field gel electrophoresis (PFGE) and multilocus variable-number tandem repeat (VNTR) analysis (MLVA) on eight highly variable VNTR loci (MLVA8) to investigate the relationships of infections among the cases [7], and MLVA on 18 less variable loci (MLVA18) and single nucleotide polymorphism (SNP) typing to identify the clonality and phylogeny of the isolates [8,9]. The resistance mechanisms to ciprofloxacin in the *S. sonnei* isolates were determined and the emergence of the *S. sonnei* strains involved in the outbreak in other countries was investigated through PFGE pattern searching in the national databases of the USA and Japan.

Materials and Methods

Surveillance of shigellosis, HIV, syphilis and gonorrhoea

Shigellosis has been listed in the Communicable Diseases Control Act as a nationally notifiable condition since 1999. Healthcare providers are required to report culture-positive shigellosis cases ≤24 h of diagnosis and submit *Shigella* isolates to TCDC for confirmation and molecular subtyping. For all reported shigellosis cases, local health department staff administered telephone or face-to-face interviews within ≤48 h of reporting and used a standardized questionnaire to collect information on foodborne and waterborne exposures, recent travel to endemic areas, and engagement in oral–anal sex ≤7 days before illness onset.

Surveillance of HIV infection, syphilis and gonorrhoea through the NDSS has been described [10,11]. For all reported HIV-positive men, information on sexual behaviours was collected through face-to-face interviews. The NDSS also collected the patients' CD4 count, plasma HIV RNA load and antiretroviral therapy (ART) use every 3–6 months through hospitals designated for HIV care.

Matched case–control study

Each *S. sonnei* case was matched to five HIV-infected MSM without shigellosis reported in the NDSS on age (± 3 years), HIV diagnosis date (± 30 days) and county/city of residence at HIV diagnosis. The matching ratio was determined as 1 : 5 to increase the statistical power (estimated as 0.70 at the design stage) while following the general rule that little statistical power is gained by increasing the ratio to more than 1 : 5. In each pair, the end of observation was defined as the case-patient's shigellosis diagnosis date. If more than five men could be identified as controls for a case-patient, we selected those men whose age at HIV diagnosis best matched that of the case-patient. To ensure a similar observation period in each pair, people were not eligible to be controls if they died before the date of the corresponding case-patients' shigellosis diagnosis. The demographics, CD4 count, plasma viral load, antiretroviral therapy use, date of outpatient visit for HIV care, and history of syphilis and gonorrhoea reporting were collected from the NDSS.

Genotyping

Nine *S. sonnei* isolates from the nine cases were subjected to genotyping using a PulseNet standardized PFGE protocol with restriction enzymes *Xba*I (PFGE-*Xba*I) and *Not*I (PFGE-*Not*I), an MLVA protocol based on 26 VNTR loci, and an SNP typing scheme [12–14]. PFGE patterns were saved in .tif image format and analysed using BioNumerics software version 6.6 (Applied Maths, Kortrjk, Belgium). A dendrogram for *S. sonnei* isolates was constructed with the composite of PFGE-*Xba*I and PFGE-*Not*I patterns using an Unweighted Pair Group Method with Arithmetic Mean. MLVA8 is based on the eight highly variable VNTR loci and has a higher discriminatory power than PFGE in discerning very closely related *S. sonnei* strains [13]. MLVA18 is based on analysing the 18 less variable loci and is suitable when investigating clonal relationships among strains that have evolved over a longer timescale [7].

Antimicrobial susceptibility testing

The MICs for *S. sonnei* isolates were measured using the broth microdilution method with a custom-made 96-well Sensititre MIC panel (Trek Diagnostic Systems Ltd., West Grinstead, UK). The panel comprised 15 antimicrobials included ampicillin, cefotaxime, cefoxitin, ceftazidime, chloramphenicol, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline and sulfamethoxazole/trimethoprim. Testing results were interpreted using the 2013 CLSI criteria [15], except in the case of streptomycin, for which MICs of ≥64, 32 and ≤16 mg/L were defined as thresholds for resistant, intermediate, and susceptible results, respectively.

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