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Clinical Science and Epidemiology

Genomic Drivers of Multidrug-Resistant *Shigella* Affecting Vulnerable Patient Populations in the United States and Abroad

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ABSTRACT Multidrug-resistant (MDR) *Shigella* infections have been identified globally among men who have sex with men (MSM). The highly drug-resistant phenotype often confounds initial antimicrobial therapy, placing patients at risk for adverse outcomes, the development of more drug-resistant strains, and additional treatment failures. New macrolide-resistant *Shigella* strains complicate treatment further as azithromycin is a next-in-line antibiotic for MDR strains, and an antibiotic-strain combination confounded by gaps in validated clinical breakpoints for clinical laboratories to interpret macrolide resistance in *Shigella*. We present the first high-resolution genomic analyses of 2,097 U.S. *Shigella* isolates, including those from MDR outbreaks. A sentinel shigellosis case in an MSM patient revealed a strain carrying 12 plasmids, of which two carried known resistance genes, the pKSR100-related plasmid pMHMC-004 and spA-related plasmid pMHMC-012. Genomic-epidemiologic analyses of isolates revealed high carriage rates of pMHMC-004 predominantly in U.S. isolates from men and not in other demographic groups. Isolates genetically related to the sentinel case further harbored elevated numbers of unique replicons, showing the receptivity of this *Shigella* lineage to plasmid acquisition. Findings from integrated genomic-epidemiologic analyses were leveraged to direct targeted clinical actions to improve rapid diagnosis and patient care and for public health efforts to further reduce spread.

IMPORTANCE Multidrug-resistant *Shigella* isolates with resistance to macrolides are an emerging public health threat. We define a plasmid/pathogen complex behind infections seen in the United States and globally in vulnerable patient populations and identify multiple outbreaks in the United States and evidence of intercontinental transmission. Using new tools and sequence information, we experimentally identify the drivers of antibiotic resistance that complicate patient treatment to facilitate improvements to clinical microbiologic testing for their detection. We illustrate the use of these methods to support multiagency efforts to combat multidrug-resistant *Shigella* using publicly available tools, existing genomic data, and resources in clinical microbiology and public health laboratories to inform credible actions to reduce spread.

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Sexually transmitted *Shigella sonnei* and *S. flexneri* have been reported among men who have sex with men (MSM) in the United States since 1974 (1–3). HIV-positive MSM are at increased risk for sporadic shigellosis, and infection with HIV is among a range of factors that can contribute to transmission of shigellosis among MSM (4). Multiple U.S. outbreaks of shigellosis among MSM have occurred over the past decade, resulting in calls for active additional testing, surveillance, and health education efforts focused on MSM, patients, and health care professionals to address this public health threat (5–8).

Antimicrobial resistance (AMR) in *Shigella* isolates is frequently mediated by plasmid-borne genes in addition to chromosomal determinants (9). Isolates of multidrug-resistant (MDR; defined as resistant to at least three classes of antibiotics [10]) *Shigella* from MSM in New York City have increasingly shown decreased susceptibility to the macrolide azithromycin (11), and a large proportion of *Shigella* isolates with decreased susceptibility to azithromycin have been shown among HIV-positive men (12). These findings are concerning given azithromycin's role in empirical treatment of infectious diarrhea and use for *Shigella* resistant to fluoroquinolones, penicillins, and sulfamethoxazole/trimethoprim (13). However, in contrast to these other antibiotic classes, macrolides are not included in most automated clinical microbiology tests for drug resistance in *Enterobacteriaceae*. The lack of Clinical and Laboratory Standards Institute (CLSI) validated clinical breakpoints for interpretation of macrolide results in *Shigella* further confounds timely interpretation and reporting of resistance (14).

Azithromycin-resistant *S. sonnei* isolates carrying the *mph*(A) gene have been found in the United States since 1987 (15). Plasmid pKSR100, recently associated with *S. sonnei* and *S. flexneri* infections among MSM, carries *mph*(A) and has been identified as a primary driver of macrolide resistance in *Shigella* spp. and strongly associated with infections in MSM generally (16–19). Recent research on pKSR100 plasmids in Great Britain shows fluctuations in case numbers among different clades of *Shigella*, indicating that this plasmid/pathogen complex continues to evolve (20). Molecularly, insertion sequence 26 (IS*26*) has been identified as an important factor in the evolution of *mph*(A) within this plasmid family, with significant plasmid sequence diversity seen in Canada (19).

We identified an MSM patient with MDR *S. sonnei* that demonstrated resistance to multiple antibiotic classes, including macrolides (10). Integrated genomic and epidemiologic analyses of 2,097 U.S.-based *Shigella* cases, with functional *in vitro* studies, identified strain- and plasmid-specific drivers of drug resistance, risks for spread, and potential for further acquisition of resistance. The clear identification of strain- and plasmid-level drivers of resistance and spread directed targeted clinical actions to improve rapid diagnosis and to support public health efforts to further reduce spread.

RESULTS

Clinical case. A 50- to 60-year-old HIV-positive self-reported MSM reported 2 weeks of watery, blood-streaked diarrhea, left-lower quadrant abdominal pain, and 25 pounds of weight loss over a month, without fever or chills. He had been off antiretroviral therapy for 4 years, had traveled to Central America 1 year prior, and had recent same-sex sexual activity prior to the onset of symptoms. The patient's white blood cell count was 6,000/μl with 19% eosinophils and a CD4 cell count of $<$50/μl. His HIV viral load was $>$150,00 copies/ml, and he tested positive for cytomegalovirus viremia and *Strongyloides* IgG. An abdominal computerized tomography scan showed proctocolitis.

S. sonnei SBJ-9962 was isolated from stool culture. The patient was initially treated with ciprofloxacin but switched to intravenous (i.v.) ceftriaxone after susceptibility testing showed resistance to ciprofloxacin ($>$32 μg/ml), ampicillin ($>$32 μg/ml), and

sulfamethoxazole-trimethoprim (>320 μg/ml), with susceptibility to ceftriaxone (<1 μg/ml). Upon request, an azithromycin Etest showed a MIC of >256 μg/ml. Symptoms resolved after 4 days, and he was transitioned to oral cefpodoxime. Antiretroviral therapy was resumed.

The patient was discharged to home on cefpodoxime, but several days later he developed a recurrence of diarrhea and abdominal pain. Repeat stool cultures again isolated *S. sonnei* with the same susceptibilities, producing the isolate SBJ-9961. He was continued on oral cefpodoxime, but symptoms persisted. At that time, he was restarted on 2 g of ceftriaxone i.v. daily. Symptoms improved after 1 week of therapy, and repeat stool cultures were negative. After two additional weeks of intravenous ceftriaxone, stool cultures remained negative.

National infrastructure for pathogen genomic surveillance identifies diverse plasmid replicons in MSM *Shigella*. The isolates SBJ-9962 and SBJ-9961 were submitted to the FDA's GenomeTrakr Network (21) and to CDC's PulseNet network (22), distributed networks of laboratories in the United States and abroad that utilize whole-genome sequencing for pathogen detection and analysis. Deidentified genomic data from isolates were deposited into NCBI from both sources for access by local hospital, public health, and governmental personnel to support surveillance and active outbreak investigations from local through international levels.

A high-resolution, closed genome of strain SBJ-9962 identified 12 distinct plasmids, including pMHMC-004, which showed >99% sequence similarity to the original pKSR100 draft sequence (GenBank accession no. LN624486.1) but with three gaps: one in the replication machinery, one at IS*1R*, and one between IS*6100* and IS*15DI*. pMHMC-004 was nearly identical to pSF2, a pKSR100 family member seen first in Canada, where a gap in alignment exists next to IS15*DI* (Fig. 1; see also Fig. S1 in the supplemental material) (19). The second MDR plasmid, pMHMC-012, a plasmid with widespread distribution among *S. sonnei* isolates, showed sequence similarity to plasmid spA from *S. sonnei* strain Ss046 (23, 24). Six plasmids, including the MDR plasmids pMHMC-004 and pMHMC-012, contained known origins of transfer (Fig. S1). Both pMHMC-003 and pMHMC-004 encoded putatively intact type IV secretion systems. Genomic single-nucleotide polymorphism (SNP) comparison and clinical phenotypes showed that the patient's two isolates, SBJ-9962 and SBJ-9961, are clonally related, with no clinical antibiotic resistance differences found and only 3 SNPs found in chromosomal coding regions.

Functional studies of the host range for the two MDR plasmids showed that pMHMC-012 could be transferred by conjugation to *S. sonnei* and *Escherichia coli*, while pMHMC-004 could only be transformed into several *S. sonnei* strains, including recently isolated clinical strains, by electroporation, but not *E. coli* DH5α or *E. coli* J53 (Data Set S2).

Antibiotic resistance profile. *S. sonnei* SBJ-9961 demonstrated resistance to seven antibiotic classes: aminoglycosides, fluoroquinolones, macrolides, penicillins, sulfonamides, and tetracyclines (Table S1). Based on plasmid electroporation into the putatively pan-susceptible strain *S. sonnei* ATCC 25931, pMHMC-004 conferred resistance to macrolides, penicillins, sulfonamides, and sulfamethoxazole-trimethoprim, while pMHMC-012 conferred resistance to tetracycline, streptomycin, and sulfonamides. Both plasmids alone confer an MDR phenotype, defined as resistant to antimicrobials in at least three antibiotic classes. All strains remained susceptible to cephalosporins, colistin, beta-lactam combination agents, and several other classes of antimicrobials (Table S1) (10).

Antimicrobial resistance determinants. SBJ-9962 carried chromosomal *dfrA1* and *sat2* AMR genes in a class 2 integron in Tn*7* downstream of *glmS*, which promote resistance to trimethoprim and streptothricin, respectively, but without *aadA1*, which is usually present in this integron (25). The chromosome also carries the fluoroquinolone resistance mutations *gyrA* S83L and D87G and *parC* S80I, mutations also identified in California outbreak strains in 2014 (15). Two of the 12 plasmids carried known resistance genes: pMHMC-004, which encodes *erm*(B), *mph*(A), *sul1*, *qacE*Δ*1*, *aadA5*, *dfrA17*, and bla_{TEM-1}; and pMHMC-012, which encodes *aph*(6)-Id, *tet*(A), *sul2*, and *aph*(3″)-Ib (Fig. 1). pMHMC-004 harbored four intact ISs, three of which belong to the IS*6* family and one to the IS*1* family (Fig. 1C). IS*26* and IS*6100*, both IS*6* family members, flank *mph*(A) (Fig. 1C).

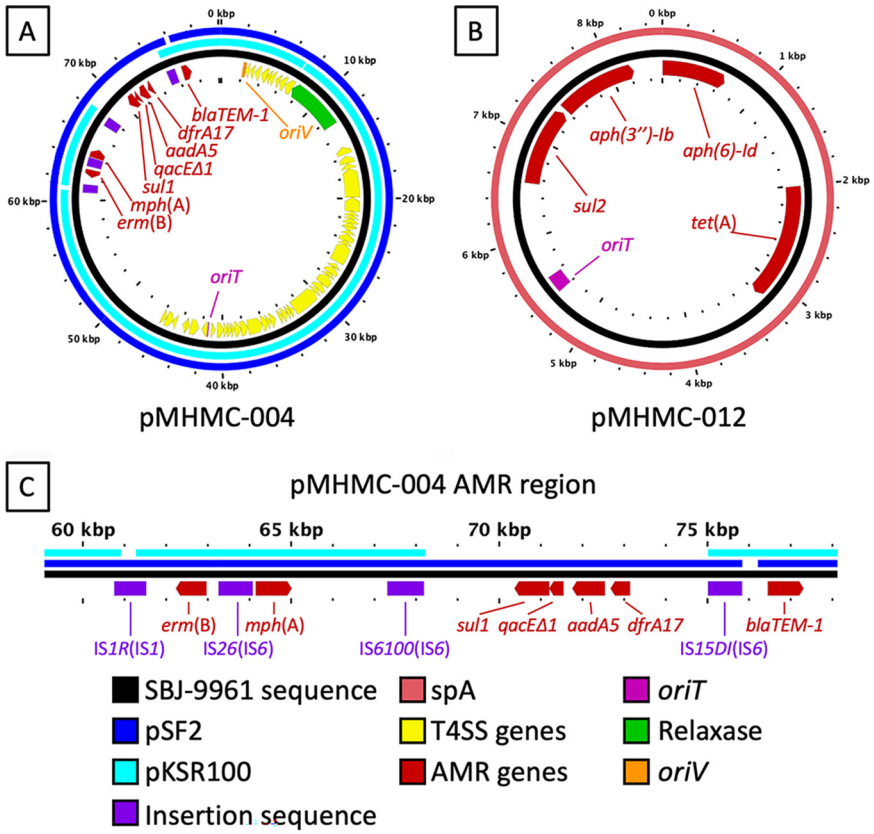


FIG 1 pMHMC-004 and pMHMC-012 are related to known multi-AMR gene plasmids. Plasmid BLAST identity over 80% with a minimum length of 100 bp is shown by colored bars exterior to the plasmid backbone in black. Genes and genetic features are labeled as directional arrows and boxes, respectively, in the inner circle. (A) pMHMC-004. (B) pMHMC-012. (C) Detail of the AMR gene region of pMHMC-004 with IS elements labeled; the IS element family is in parentheses. There were no putatively intact IS sequences found outside this region, and none were found in pMHMC-012.

SBJ-9962 belongs to an *S. sonnei* clade that harbors high plasmid replicon counts. The NCBI Pathogen Detection program (https://www.ncbi.nlm.nih.gov/pathogens/) calculates groups of genetically related isolates by genomic SNP content. The genome sequences enabled improved analyses of *S. sonnei* outbreak strains in the United States and abroad, particularly of SNP cluster PDS000033428. Members within this cluster averaged 5.6 unique replicons among their plasmids (Fig. 2). However, the subclade within PDS000033428 that includes SBJ-9962, referred to here as clade A, showed 6.9 different replicons on average ($n = 718$), including the 8 unique replicons found in SBJ-9962, versus 4.1 different replicons on average for those not in clade A ($n = 598$, $P < 0.001$). The distribution of unique replicons within the overall cluster is bimodal with respect to this phylogenetic distinction. The patient isolates contain an IS*Sfl4* insertion in *cas6e*, an insertion seen in other *Shigella* isolates (26).

Distribution of pMHMC-004 among *S. sonnei*, *S. flexneri*, and *E. coli*. Approximately 20% of all *S. flexneri* and *S. sonnei* isolate genomes listed in the Pathogen Detection Isolates Browser showed carriage of pMHMC-004 or pKSR100 homologs (Table S2). In contrast, only 2 of 42,465 *E. coli* isolates harbored any of these plasmids, one of which was adherent-invasive *E. coli* (AIEC) LF82 (NC_011993.1), and the other was cultured from an asymptomatic case of bacteriuria (ABU), *E. coli* 83972 (NC_017631.1) (27, 28).

Among *Shigella*, pMHMC-004 occurred in all *Shigella* clades (Fig. S2), with multiple instances of plasmid acquisition and loss. Additional strains across clades carried gene regions with homology to portions of pMHMC-004 but at thresholds below those for calling pMHMC-004 or pKSR100 presence. Given the known sequence diversity for the

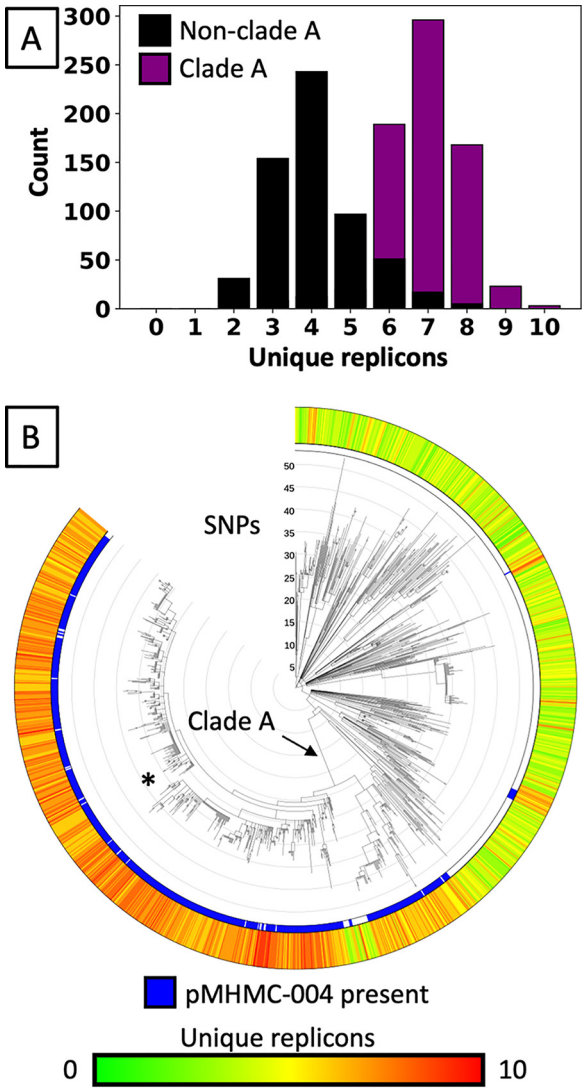
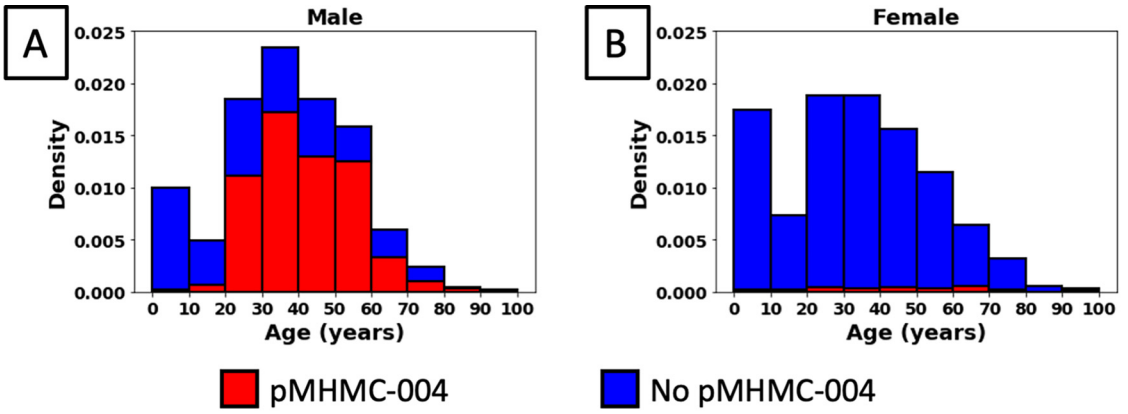


FIG 2 Unique replicon counts within PDS000033428. (A) Histogram of the number of different replicons detected in each isolate in PDS000033428.133 using the PlasmidFinder database. Isolates from a phylogenetically defined subgroup, clade A, average more distinct replicons. (B) The SNP cluster PDS000033428.133, which includes SBJ-9962 (*) with 8 different replicons, contains clade A with an elevated number of plasmid replicons. Clade A has 718 isolates.

pKSR100-related plasmids, heterogenous plasmid populations exist with broadly shared genetic cassettes (18, 19).

Intercontinental strain and plasmid transmission. *Shigella* isolates among all SNP clusters defined by NCBI at the time of access originated primarily from the United Kingdom (2,130 isolates), the United States (2,097 isolates), and Australia (321 isolates), with 132 isolates from other countries or that were of unspecified origin (Fig. S2). Isolates within each SNP cluster occurred across the three predominant countries, and subclades within these clusters showed strong geographical biases, suggesting localized spread with genetic divergence from a common ancestor. Cluster PDS000033428, which includes SBJ-9962, is particularly diverse in origin, with the repeated occurrence of the strain between the United States and the United Kingdom. These findings illustrate the occurrence of plasmid/strain complexes across continents and demonstrate putative instances of intercontinental transmission.

pMHMC-004 and related plasmids have association with adult males in the United States. Epidemiologic analyses of 1,883 U.S.-based *Shigella* cases with known demographic data identified strong association of the MDR *S. sonnei* strain and



C

	With pMHMC-004	No pMHMC-004	p-value
Age	41.3 ± 13.8	33.0 ± 19.9	$9.94e^{-22, t}$
Demographic			
Adult male (N, %)	604 (32.1%)	653 (34.7%)	
Adult female (N, %)	31 (1.7%)	287 (15.2%)	$4.07e^{-70, \chi}$
Children (N, %)	11 (0.6%)	297 (15.8%)	

$\chi = \chi^2$ test, t = t-test

FIG 3 Demographic distribution of pMHMC-004-containing isolates. (A) Normalized histogram showing the proportion of isolates with or without pMHMC-004 by age for male patients. (B) Normalized histogram showing the proportion of isolates with or without pMHMC-004 by age for female patients. (C) Univariate analysis of plasmid carriage likelihood by age and demographic groups.

plasmids with adult males (Fig. 3). The average age of patients linked to isolates with pMHMC-004 was 41.3 ± 13.8, while that of patients linked to isolates without the plasmid averaged 33.0 ± 19.9 ($P = 9.9 \times 10^{-22}$). The discrepancy in age was largely due to higher numbers of children (ages 0 to 18) in the latter category, where only 11 of 308 (3.6%) isolates carried the plasmid. Similarly, of cases involving adult females, only 31 of 318 (9.7%) isolates carried pMHMC-004. However, among isolates from adult males, 604 of 1,257 (52%) cases carried the plasmid. In total, of all cases involving the plasmid, 604 out of 646 cases (93%) with both age and sex recorded involved adult males. The observed difference in this distribution was highly significant ($\chi^2 = 320$, df = 2, $P = 4.1e^{-70}$).

The largest SNP cluster, PDS000033428, which includes SBJ-9962, has five large clades that are primarily associated with adult males (>80%). Adult male-associated clades 1, 2, 3, and 4 demonstrated high prevalence of pMHMC-004-related sequence, a trait that adult male-associated clade 5 does not demonstrate (Fig. 4, Fig. S3, Data Set S3). The clade of non-adult male-associated strains does not have strong pKSR100 family sequence representation.

pMHMC-004 and pMHMC-012 show variable carriage of AMR genes over time. Significant variation occurred in the AMR genes seen in clades associated with cluster PDS000033428 (Fig. 4, Fig. S3, Data Set S4). After points of acquisition of macrolide resistance genes, sporadic isolates lacking *mph*(A) or *erm*(B) occurred, suggesting genetic excision events involving the three IS*6* family ISs. Several strains lacked some or all genes bounded by these elements, indicative of multiple possible rearrangements involving these IS*6* elements. The variability in MDR genes illustrates the degree of heterogeneity in the pKSR100 family of plasmids, attributes that are not unique to this or other *Shigella* plasmids, including spA/pMHMC-012.

DISCUSSION

We present the first functional and genomic epidemiologic analyses of *Shigella* strains seen predominantly among men in the United States, suggesting transmission

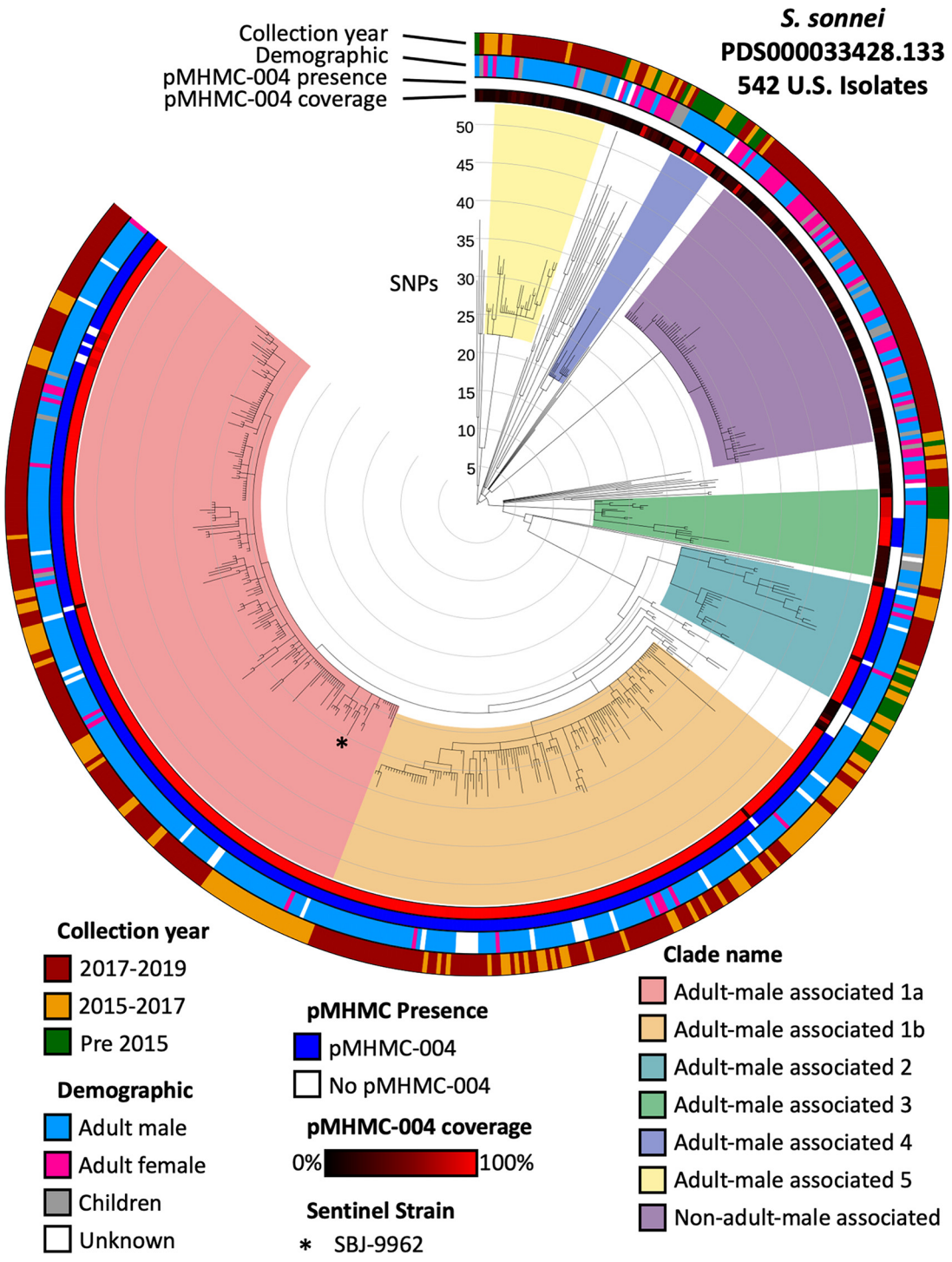


FIG 4 SNP cluster PDS000033428.133 includes isolates from a recent and large clade of MDR *Shigella sonnei* in the United States. The rings, from interior to exterior, represent pMHMC-004 coverage, pMHMC-004 presence, demographic group, and year of isolation. Clades of interest within this SNP cluster are color coded directly on the tree. Adult male-associated clades have >80% of isolates from adult males.

among MSM, and we further define how these components fit more broadly within *Shigella* cases globally (29, 30). *Shigella* spp. can quickly acquire and develop antibiotic resistance (31). Notably, strains seen among men can carry many different plasmids, as highlighted by the 12 unique plasmids identified in SBJ-9961. The patient isolates contain an insertion in *cas6e*, a component of the CRISPR-Cas system that may play a role

in its capacity to maintain a high plasmid count (26). pMHMC-004 belongs to an emerging family of MDR plasmids that includes pKSR100 and has shown frequent acquisition and loss of antimicrobial resistance genes commonly mediated by recombination events among homologous insertion sequences and other acquired repetitive elements (17, 19).

pMHMC-004 showed significant association in *Shigella* cases of adult males, including the sentinel MSM case that started our investigation, and not in other patient populations. This combined plasmid/pathogen complex significantly complicated our patient's treatment. Chromosomal resistance to fluoroquinolones combined with plasmid-mediated resistance to ampicillin, sulfamethoxazole-trimethoprim, and azithromycin rendered the usual oral therapies ineffective and required intravenous treatment. The receptivity of *Shigella* to plasmid acquisition raises further concerns for the acquisition of additional resistance genes, including those for extended-spectrum and other beta-lactamase enzymes, that could further limit treatment options (32–35).

While pMHMC-004 encodes a putative type IV secretion system, we were unable to conjugatively transfer the plasmid to susceptible recipients under laboratory conditions. However, pMHMC-012 was transferrable by conjugation, demonstrating the existence of functioning conjugative machinery in this isolate. Electroporation experiments demonstrated a primary host range limited to other *Shigella* species and not to other *E. coli* strains, a finding further supported by genomic epidemiologic analyses that identified only two instances of pMHMC-004 homologs in >40,000 *E. coli* genomes, strengthening the idea that this plasmid has a limited host range (Table S2).

Relatives of the pMHMC-004 plasmid (18, 19) demonstrate variation in their antibiotic resistance gene profiles, suggesting that this family of plasmids provides an adaptable backbone for the transmission of antibiotic resistance across *Shigella*. Clinical microbiologic phenotypic testing for antibiotic resistance in strains infecting vulnerable patient populations is paramount to ensure proper treatment and, when appropriate during outbreaks, the implementation of measures for prevention. Furthermore, ongoing national and international efforts for genomic surveillance provide means to actively monitor AMR gene acquisition to inform clinical, public health, and food safety agencies for appropriate monitoring, diagnostic, and therapeutic options to ensure optimal and timely treatment. As part of surveillance activities, the collection of demographic and behavioral data is essential to further characterize populations disproportionately impacted by MDR *Shigella*.

Our sentinel strain and others in the United States among adult males had putative macrolide resistance mediated via *mph*(A) (17). Given the current lack of CLSI clinical breakpoints to call macrolide resistance in *Shigella* species, laboratories may opt to proactively test *Shigella* with MIC-based methods to provide a minimum antibiotic concentration to direct clinical therapy. Longer term, understanding the current drivers of antimicrobial resistance is best incorporated in ongoing national and international efforts to provide timely frameworks for diagnostic laboratories in support of patient care. These issues also highlight the need for reflex cultures if culture-independent diagnostic testing (CIDT) is used as a primary modality to diagnose *Shigella* infections, particularly for patients who do not clear infection or who may be at higher risk for infection with MDR strains.

We note that drug-resistant cases of *Shigellosis* are not unique to MSM patient populations (5, 7, 17, 18). Healthcare providers should consider the potential for drug-resistant *Shigella* in gastroenteritis patients who are immunocompromised and have not recovered or in any case are failing to respond to antimicrobial therapy. Education for patients with MDR shigellosis to prevent transmission through a range of modes, including food, water, and both sexual and nonsexual person-to-person contact, are important to further limit pathogen spread. Among MSM, where the disease appears to be more prevalent, physicians should take sexual histories into account in providing prevention information. While multiple pathogens and clinical diseases can

present with such symptoms, standard clinical microbiologic methods for identifying enteric pathogens and for susceptibility testing of identified *Shigella* are needed to provide appropriate diagnostic information to guide patient care and management.

MATERIALS AND METHODS

Clinical case. An MDR *S. sonnei* infection was identified in the Brigham and Women's Hospital Clinical Microbiology Laboratory and flagged by Infection Control for genomic analyses through the Partner's Pathogen Genomic Surveillance program (IRB protocol 2011-P002883; L. Bry) to identify drivers of antimicrobial resistance and relatedness with outbreak *Shigella* seen in the Northeast (36, 37). Details about this case and the two isolates from it are presented in Results.

Bacterial isolation and maintenance. *Shigella* isolates were isolated by stool culture on Hektoen enteric agar (Remel, Lenexa, KS) and species determined by Vitek 2 (bioMérieux, Durham, NC). Rifampin-resistant mutants of clinical strains, used for functional studies, were created by plating a 10-μl loopful of cells from LB agar (Becton, Dickinson and Company, Sparks, MD) onto LB agar with rifampin overnight at 37°C. The following antibiotics were used for selections: ampicillin (MilliporeSigma, Saint Louis, MO), 200 μg/ml; rifampin (G-Biosciences, Saint Louis, MO), 100 μg/ml; and tetracycline (MilliporeSigma, Saint Louis, MO), 15 μg/ml. Bacterial strains used in this study are listed in Table S3 in the supplemental material.

Plasmid transfer analyses. Bacterial transformations were done as previously described (38), with additional screening done on CHROMagar MH orientation agar (CHROMagar, Paris, France) and MacConkey enteric agar (Remel, Lenexa, KS) to confirm species and strain phenotypes.

For conjugation and electroporation studies of plasmid transfer, donor and recipient strains were grown overnight on LB agar at 37°C with selective antibiotics whenever appropriate (Table S3). Cells were suspended in sterile LB to an optical density at 600 nm (OD_{600}) of 0.1, and then donor and recipient were mixed 1:1 and 50 μl was plated onto LB agar. The reaction mixtures were incubated 16 to 20 h at 37°C, 30°C, or room temperature to evaluate the temperature dependence of conjugation. Reaction mixtures were resuspended into 5 ml sterile LB using a sterile loop and diluted before plating. Thirty microliters of dilution was plated onto half a selective or nonselective agar plate for determining cell concentrations.

Electroporation was performed as described previously, with the following modifications (39). Cells were grown on solid media and resuspended in 300 mM sucrose (MilliporeSigma, Saint Louis, MO), and 2-mm cuvettes (Thermo Fisher Scientific, Waltham, MA) were used with a 2.5-kV, 25-mF, 200-Ω program and 100 μl of prepped cells. DNA for electroporation was purified using a QIAfilter plasmid midi kit (Qiagen, Germantown, MD) with 100 ml of late-log-phase-growth culture grown in dual selection with ampicillin and tetracycline at 37°C. One microliter of plasmid DNA solution (400 ng) was used in electroporation reactions.

Antibiotic susceptibility testing. Kirby-Bauer, Etest, and broth microdilution studies were performed within Clinical and Laboratory Standards Institute (CLSI) guidelines (14, 40, 41). Azithromycin resistance levels were determined by Etest (bioMérieux, Durham, NC).

Genomic analyses. The Partners Pathogen Genomic Surveillance program is a node on the FDA's GenomeTrakr Network and submitted the strain for genomic analysis. DNA isolation and Illumina MiSeq sequencing were performed as described previously (36). PacBio sequencing was performed as previously described (42). Sequence accession numbers used in genomic analyses are listed in Data Set S1.

Sequences were analyzed for resistance genes using the Bacterial Antimicrobial Resistance Reference Gene Database (PRJNA313047) and BLAST (43). AMR genes were screened for using the thresholds of 80% protein sequence identity and 60% gene length, keeping the best hit per genome locus. All hits had >95% protein sequence identity and length. Replicons were identified using the PlasmidFinder reference sequences and with homologous plasmids called at 80% nucleotide identity and 60% coverage (44). An independent *t* test was used to determine if differences in averages of the number of different replicons in isolates between two groups were significant. In the case of pMHMC-011, which had 92% sequence identity and 53% replicon coverage for Col(MG828), the replicon is interrupted by the beginning of the sequence. Plasmid-encoded type IV secretion system elements were identified using oriTfinder (45). GenBank files with all features annotated were created using Biopython (46).

Sequencing reads from the *E. coli* and *Shigella* isolate genomes in the NCBI Pathogen Detection Isolates Browser (https://www.ncbi.nlm.nih.gov/pathogens) were downloaded from the Sequence Reads Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) in May 2019. There are tools available for comparing these to known clusters, including those previously seen in the United Kingdom (K. Holt, J. Hawkey, and K. Paranagama, Sonneityping, https://github.com/katholt/sonneityping) (20, 23). The reads were *de novo* assembled using SPAdes with the –careful –cov-cutoff auto options to generate draft-level genome assemblies (47). Putative plasmids were evaluated using BLASTn comparisons between the complete plasmid sequences and the assembled genomes. Eighty percent sequence-level identity was used as a cutoff for mapping reads to plasmids in calculating the coverage of reference plasmid sequence in draft genomes. Due to the nature of similarity between the plasmids pMHMC-004 and pKSR100, a minimum plasmid coverage of 95% was used. The *dfrA17-sul1* resistance region was also used to differentiate pMHMC-004 and pKSR100 type plasmids (Fig. 1C) (17). Kraken2 was used to confirm strain species assignments (48).

For comparisons of pMHMC-004 to related plasmids pKSR100 and pSF2, GView was used on default settings of greater than 80% nucleotide sequence identity and an E value of less than $1e^{-10}$ to

determine overall sequence alignment and coverage of pMHMC-004 to the two reference plasmids (49). Plasmid sequence composition comparisons were done using BLASTn (43). Intact ISs were detected using ISfinder's BLAST function using 80% reference sequence coverage and 80% nucleotide identity as cutoffs for pMHMC-004 and pMHMC-012 (50).

Genomic SNP comparison of the patient isolates was performed using BLASTn with nucleotide features of SBJ-9962 (CP053751.1) used to search the NCBI SBJ-9961 assembly AAVBEV000000000.1 (43).

Phylogenetic analyses. Phylogenetic relationships were obtained from the NCBI Pathogen Detection Isolates Browser (https://www.ncbi.nlm.nih.gov/pathogens/isolates/). Visualizations with plasmid sequence alignment were made with the Interactive Tree of Life (51).

Epidemiological data management and statistics. U.S. isolates had year of isolation, patient age, and patient sex information collected for analysis when available. Sexual orientation or sexual behavior data were not available for U.S. isolates. Patients were classified into one of three demographic groups: adult males are patients who were of male sex and at least 18 years of age, adult females are patients who were of female sex and at least 18 years of age, and children are all patients under 18 years of age. The proportion of adult males was used as an indicator to suggest MSM transmission. Previous work used gender distributions to identify excess cases of shigellosis among men, which can suggest MSM transmission (29, 30). For determination of the significance of patient age by isolate pMHMC-004 carriage, a *t* test was used. A χ^2 test was used to evaluate the significance of demographic group between patient isolate pMHMC-004 carriage levels. *t* test and χ^2 tests were performed using SciPy (52).

Data accessibility. The sequences determined in the course of this work have been deposited in NCBI under BioSample accession numbers SAMN11948688 (*S. sonnei* 726), SAMN07450846 (*S. sonnei* SBJ-12001), SAMN05510450 (*S. sonnei* SBJ-13001), SAMN07662568 (*S. sonnei* SBJ-9961), SAMN07663113 (*S. sonnei* SBJ-9962).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

DATA SET S1, XLSX file, 0.2 MB.

DATA SET S2, XLSX file, 0.03 MB.

DATA SET S3, PDF file, 2.3 MB.

DATA SET S4, PDF file, 3.3 MB.

FIG S1, PDF file, 0.5 MB.

FIG S2, PDF file, 1 MB.

FIG S3, PDF file, 0.7 MB.

TABLE S1, DOCX file, 0.02 MB.

TABLE S2, DOCX file, 0.01 MB.

TABLE S3, DOCX file, 0.02 MB.

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Jay Worley performed biological experiments, performed bioinformatic and statistical analyses, crafted figures, and wrote the manuscript. Kiran Javkar, Errol Strain, and Mihai Pop performed bioinformatic analyses and provided computational support. Maria Hoffmann and Marc Allard sequenced the bacteria and the plasmids. Kristen Hysell provided the case report. Amanda Garcia-Williams, Kaitlin Tagg, and Louise Francois Watkins provided epidemiological data and analysis. Louis Francois Watkins and Amanda Garcia-Williams provided public health information and messaging. Sanjat Kanjilal provided case data and research guidance. Lynn Bry wrote and edited the manuscript and provided direction.

We have no competing interests to declare.

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