

ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015

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In England in 2015, *Shigella sonnei* isolates from men who have sex with men produced extended-spectrum β -lactamases and exhibited macrolide resistance. Whole-genome sequencing showed a close relationship among the isolates, which harbored a plasmid that was previously identified in a shigellosis outbreak among this population but has acquired a mobile element.

Historically, shigellosis is an infection associated with travel to countries in which this infection is endemic. In England in 2009, *Shigella flexneri* 3a infections dramatically increased among men who have sex with men (MSM) and were thought to be associated with sexual transmission (1). Since 2011, an emerging epidemic of *S. sonnei* infections in England among men has occurred (2). We describe an investigation of a cluster of multidrug-resistant *S. sonnei* infections among MSM in England during 2015.

The Study

In November 2015, Public Health England (PHE) identified a cluster of *S. sonnei* isolates by analyzing whole-genome sequencing data from specimens collected during September and October from 4 adult men residing in London and having no known travel history. Clinical reporting data indicated that 2 of the men were MSM. The isolates were later found to exhibit high levels of antimicrobial drug resistance to amoxicillin, ceftriaxone, tetracycline, sulfonamides, trimethoprim, and azithromycin and to produce extended-spectrum β -lactamase (ESBL) (Table 1). The isolates were sensitive to quinolones. PHE convened an out-

break control team to identify additional cases, characterize cases, capture exposure information, rule out a point source for the outbreak, and provide evidence for additional targeted public health efforts.

Since August 2015, PHE has conducted whole-genome sequencing of $\approx 70\%$ of *S. sonnei* isolates (those voluntarily referred from hospital laboratories) (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/22/11/16-0653-Techapp1.pdf>). Single nucleotide polymorphism (SNP) clustering is performed, as previously described (3). Readings (in FASTQ format) from all sequences in this study can be found at the PHE Pathogens BioProject (National Center for Biotechnology Information project no. PRJNA315192).

DNA from isolate 183660 was used to elucidate plasmid sequencing (online Technical Appendix). The multidrug-resistance phenotype was explained by the presence of an 89,000-bp IncFII plasmid designated p183660 (GenBank accession no. KX008967). This plasmid had $>95\%$ nt identity to pKSR100, a multidrug-resistance plasmid from an MSM case of *S. flexneri* 3a infection (GenBank accession no. LN624486) that had acquired the previously described pKSR100 integron (*bla*_{TEM-12}, *df*rA17, *df*rA1, *su*l1, *aadA5*) (4) and a novel mobile element harboring *bla*_{CTX-M-27} (Figure 1). Macrolide resistance was conferred by *ermB* and *mphA*.

For this investigation, we defined a case-patient as a resident of England with laboratory-confirmed *S. sonnei* infection belonging to a 5 SNP cluster with the SNP address 1.3.3.9.207.212.%, a resistance profile characterized by the presence of the plasmid p183660, and a specimen collected during September 1, 2015–February 29, 2016. We identified 9 case-patients (Figure 2) from fecal specimens; all had no known travel history in the 2 weeks before illness. Median age was 33 (range 28–83) years. Gastrointestinal symptom onset ranged from early September 2015 to late December 2015. No patients were bacteremic. Treatment failure was reported for 2 patients; both were hospitalized and 1 died, although *Shigella* was not recorded as a cause of death. Duration of illness was not systematically collected. Diagnoses occurred in general practice (n = 5), sexual health clinics (n = 2), and hospitals (n = 2). Seven patients

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Table 1. Results of the phenotypic antimicrobial drug resistance profile of 4 isolates of *Shigella sonnei*, England, 2015*

Isolate ID	MIC for antimicrobial drugs tested, µg/mL†																	
	AMP	AMC	CTX	CRO	CAZ	CFP	GEN	TOB	TMP	SEP	ATM	CIP	COL	ERT	MER	FOS	TEM	AZT
182834	>1,052	1	>32	>32	2	0.38	0.5	0.38	>32	64	0.38	<0.032	0.19	0.008	0.06	1	<1	>256
184494	>1,052	1	>32	>32	2	0.5	0.38	0.38	>32	128	0.5	<0.032	0.125	0.008	0.06	1.5	<1	>256
183660	>1,052	1	>32	>32	1	0.38	0.38	0.38	>32	128	0.38	<0.032	0.125	0.008	0.06	1	<1	>256
164679	>1,052	1	>32	>32	1	0.38	0.38	0.38	>32	64	0.38	<0.032	0.19	0.008	0.06	1	<1	>256

*TEM was included in the panel to aid detection of OXA-48-like carbapenemases. AMC, amoxicillin/clavulanate; AMP, ampicillin; ATM, aztreonam; AZT, azithromycin; CAZ, ceftazidime; CFP, cefepime; CIP, ciprofloxacin; COL, colistin; CRO, ceftriaxone; CTX, cefotaxime; ERT, ertapenem; FOS, fosfomycin; GEN, gentamicin; ID, identification; MER, meropenem; SEP, trimethoprim/sulfamethoxazole; TEM, temocillin; TMP, trimethoprim; TOB, tobramycin.
 †Phenotypic antimicrobial drug susceptibility testing was performed by using Etest (bioMérieux, Marcy l’Etoile, France) and interpreted by using breakpoints for *Enterobacteriaceae* established by the European Committee on Antimicrobial Susceptibility Testing (<http://www.eucast.org/>). Production of extended-spectrum β-lactamase was confirmed by using CTX + clavulanic acid and CAZ + clavulanic acid in combination (bioMérieux).

resided in London; 2 resided elsewhere in England, 1 of whom reported recent travel to London. Of 8 patients with reported ethnicity, 6 self-identified as white.

In-depth questionnaires administered by telephone interviews collected information on sexual history, food exposures, and travel history; 8 patients completed the interviews. Seven answered detailed sexual history questions (including the 2 patients identified as MSM from clinical reporting data); all 7 reported having male partners. Six reported exposures considered to be high risk for *Shigella* transmission during the incubation period (e.g., fisting, sex under the influence of recreational drugs [6], oral-anal contact, attendance at sex parties, or use of sex toys with a partner) (Table 2). Reported number of sexual partners during the 2 weeks before illness was 0–5. The 7 patients

who completed the sexual history questions reported previous high-risk sexual exposures; 6 of these patients and 1 patient who partially completed the questionnaire reported a history of other sexually transmitted infections, including gonorrhea, chlamydia, syphilis, nonspecific urethritis, hepatitis C, and pubic lice. Five reported using mobile apps to meet partners at some time in the past; none reported a prior shigellosis diagnosis. All had visited sexual health services (Table 2). Some patients were HIV positive (frequency is omitted to prevent deductive disclosure).

No epidemiologic links were identified among patients, and no point sources were identified, although food exposure data was collected for 5 patients only. Two reported attending the same sex-on-premises venue in London 3 months apart (1 reported no sexual contact at the venue). None

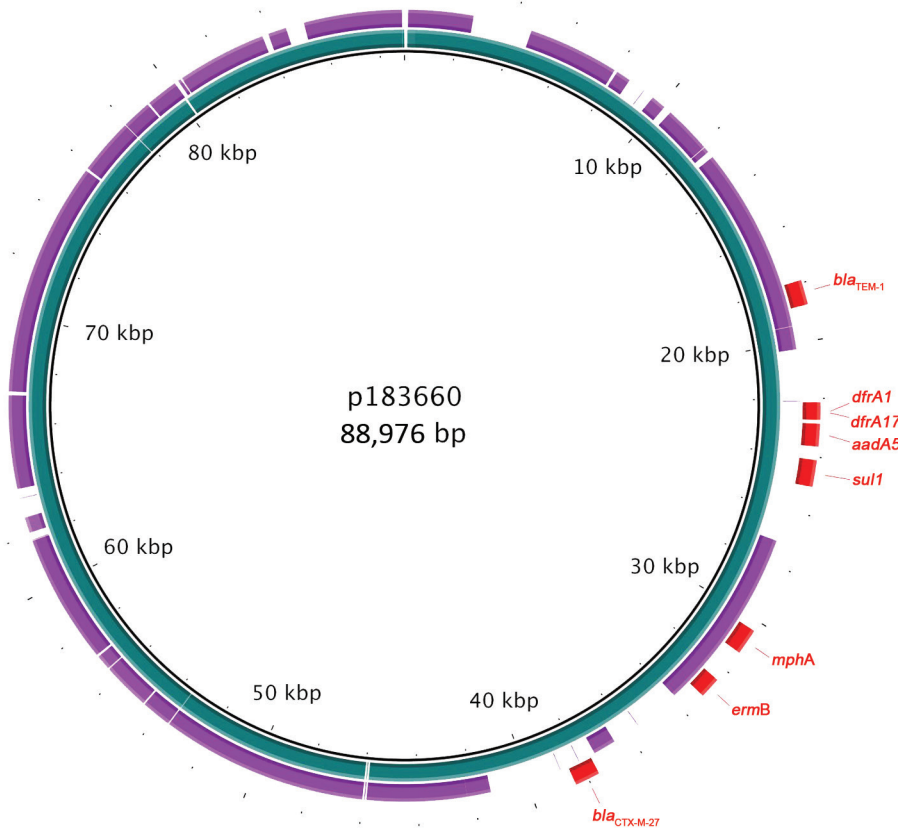


Figure 1. Genomic plot of multidrug-resistance plasmid p183660 (inner ring, blue) from a man in England infected with *Shigella sonnei* compared with pKSR100 (outer ring, purple), a multidrug-resistance plasmid from a case of *S. flexneri* 3a infection occurring among men who have sex in men (4). Drug-resistant elements from p183660 are shown in red. Plot produced by using BLAST Ring Image Generator (5).

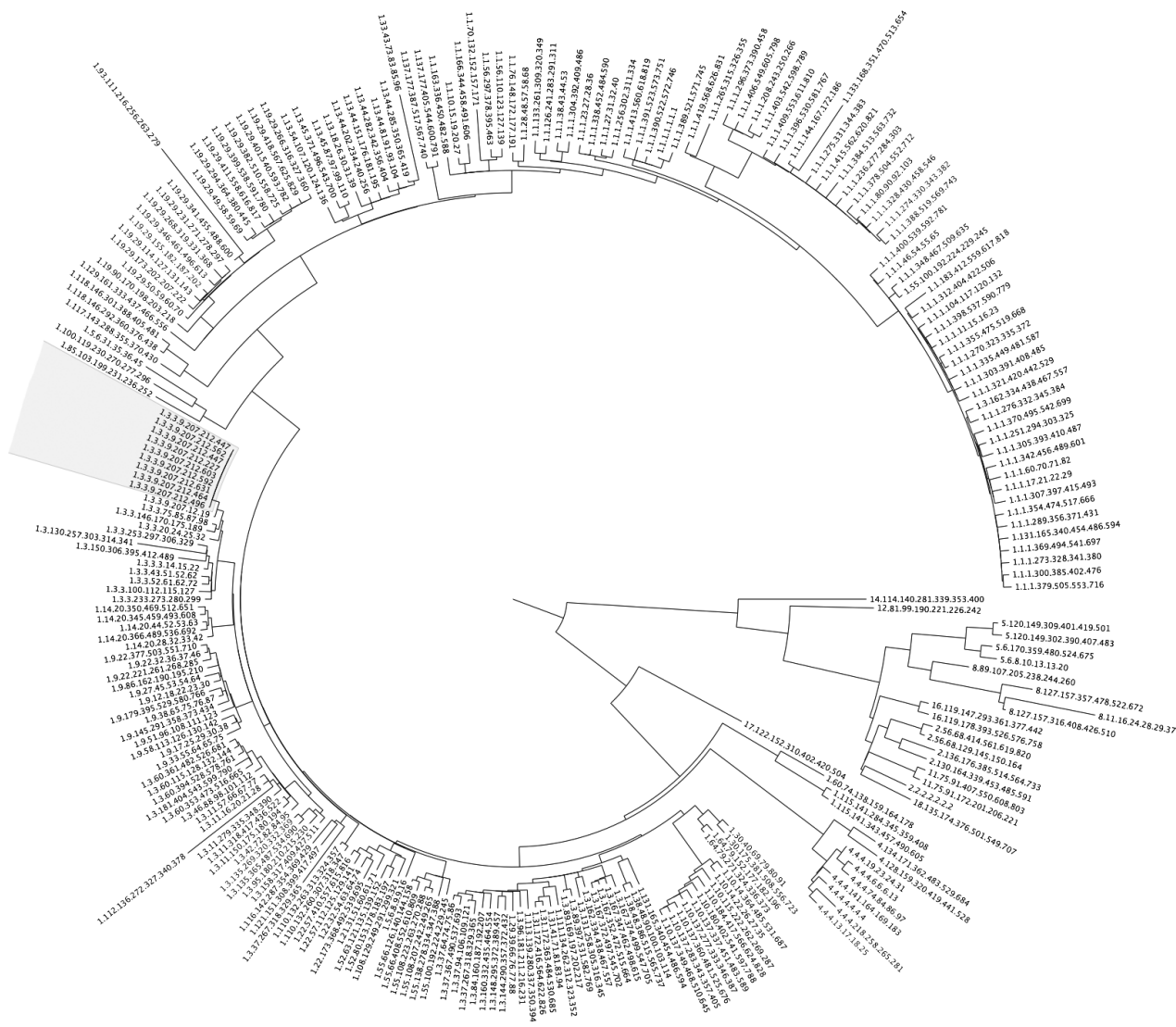


Figure 2. Maximum-likelihood phylogeny showing isolates from a cluster of 9 cases (gray shading) of *Shigella sonnei* infection among men who have sex with men in England, 2015. For context, 246 *S. sonnei* isolates that are representatives from each t25 cluster were included in the comparison. Isolates are labeled by single nucleotide polymorphism address.

reported awareness of possible infection with *Shigella* spp. or of contact with others with gastrointestinal symptoms. Three thought they had acquired their infections from food. Patients reported that they accessed health promotion messages at sexual health clinics and on the internet.

Conclusions

We describe a phylogenetic cluster of ESBL-producing *S. sonnei* infections in MSM in England that raises concerns about the ability to manage the spread of resistant *Shigella* infection in this population. Whole-genome sequencing revealed that the multidrug-resistance phenotype is conferred by acquisition of an IncFII plasmid (p183660) known to be

circulating in *S. flexneri*-affected MSM (4); this plasmid has acquired *bla*_{CTX-M-27}.

Patient-reported behaviors were similar to those reported in an earlier *S. flexneri* epidemic among MSM: high numbers of sexual partners, high levels of condomless sex, attendance at sex parties, sex under the influence of recreational drugs, and prior HIV infection (6). We identified no point source, further indicating that sexual contact was the dominant mode of transmission.

Although *S. sonnei* causes self-limiting diarrhea in most patients, life-threatening invasive infections in patients co-infected with HIV have occurred (7). Quinolone and azithromycin resistance have been observed in recent

Table 2. Reported exposure history for 9 case-patients with *Shigella sonnei* infection in the cluster with ESBL production and macrolide resistance, England, 2015*

Risk factor	No. (%) exposed	No. (%) unexposed	No. unknown†
Exposure ever before onset			
Self-identified as a man who has sex with men	7 (100)	0	2
Fisting	2 (29)	5 (71)	2
Sex under the influence of recreational drugs	4 (57)	3 (43)	2
Scat play	0	7 (100)	2
History of other sexually transmitted infections	7 (88)	1 (13)	1
Use of apps to meet partners	5 (71)	2 (29)	2
Awareness of <i>Shigella</i> infections	0	7 (100)	2
Previous <i>Shigella</i> diagnosis	0	8 (100)	1
Known to sexual health services	7 (100)	0	2
Exposure during the 2 weeks before onset			
Fisting	1 (14)	6 (86)	2
Sex under the influence of recreational drugs	3 (43)	4 (57)	2
Oral–anal contact	3 (43)	4 (57)	2
Condomless sex‡	5 (71)	2 (29)	2
Attended sex parties or live sex premises	3 (43)	4 (57)	2
Use of sex toys with partner	1 (14)	6 (86)	2
Scat play	0	7 (100)	2

*ESBL, extended-spectrum β -lactamase; scat play, sexual arousal or activity linked to feces.

†Unknowns were not included in percentage calculations; 7 of 9 patients completed and 1 partially completed interviews about sexual behavior.

‡Any condomless sexual contact.

outbreaks of *S. sonnei* infections in MSM; cephalosporins are recognized as a suitable therapeutic choice for invasive or prolonged infections (8,9).

Primary public health concerns include possible treatment failure for severe shigellosis in immunocompromised patients; further spread of the multidrug-resistant IncFII plasmid to other enteric pathogens in MSM, with possible implications for treatment among the immunocompromised; rapid spread of drug-resistant *S. sonnei* infections among MSM, including outside the United Kingdom, as happened with *S. flexneri* 3a (4), especially among the HIV infected; and transmission through food handling and childcare centers, with potential to cause outbreaks of drug-resistant *S. sonnei* infections in other populations. Low awareness of *Shigella* infections among patients in our study suggests that prior awareness campaigns targeting high-risk MSM in England (10) have not been fully successful. Future campaigns being planned to improve awareness of *Shigella* infection and transmission routes will target social media, sexual health clinics, and primary care workers to increase awareness among MSM and healthcare staff. Frontline sexual health clinicians and microbiologists in England have been made aware of emerging drug resistance in *Shigella* spp. among this vulnerable group and of the need to perform antimicrobial drug susceptibility testing if treatment is considered necessary.

Acknowledgments

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Dr. Mook is an epidemiologist with the Field Epidemiology Service, Public Health England. His research interests include gastrointestinal disease surveillance and outbreak response.

References

- Borg ML, Modi A, Tostmann A, Gobin M, Cartwright J, Quigley C, et al. Ongoing outbreak of *Shigella flexneri* serotype 3a in men who have sex with men in England and Wales, data from 2009–2011. *Euro Surveill.* 2012;17:pii:20137.
- Simms I, Field N, Jenkins C, Childs T, Gilbert VL, Dallman TJ, et al. Intensified shigellosis epidemic associated with sexual transmission in men who have sex with men—*Shigella flexneri* and *S. sonnei* in England, 2004 to end of February 2015. *Euro Surveill.* 2015;20:pii:21097. <http://dx.doi.org/10.2807/1560-7917.ES2015.20.15.21097>
- Ashton P, Nair S, Peters T, Tewolde R, Day M, Doumith M, et al. Revolutionising public health reference microbiology using whole genome sequencing: salmonella as an exemplar. *bioRxiv.* 2015;033225.
- Baker KS, Dallman TJ, Ashton PM, Day M, Hughes G, Crook PD, et al. Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study. *Lancet Infect Dis.* 2015;15:913–21. [http://dx.doi.org/10.1016/S1473-3099\(15\)00002-X](http://dx.doi.org/10.1016/S1473-3099(15)00002-X)
- Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics.* 2011;12:402. <http://dx.doi.org/10.1186/1471-2164-12-402>
- Gilbart VL, Simms I, Jenkins C, Furegato M, Gobin M, Oliver I, et al. Sex, drugs and smart phone applications: findings from semistructured interviews with men who have sex with men diagnosed with *Shigella flexneri* 3a in England and Wales. *Sex Transm Infect.* 2015;91:598–602. <http://dx.doi.org/10.1136/sextrans-2015-052014>
- Baer JT, Vugia DJ, Reingold AL, Aragon T, Angulo FJ, Bradford WZ. HIV infection as a risk factor for shigellosis. *Emerg Infect Dis.* 1999;5:820–3. <http://dx.doi.org/10.3201/eid0506.990614>
- Hoffmann C, Sahly H, Jessen A, Ingiliz P, Stellbrink H-J, Neifer S, et al. High rates of quinolone-resistant strains of *Shigella*

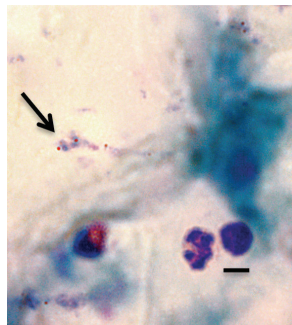
- sonnei* in HIV-infected MSM. *Infection*. 2013;41:999–1003. <http://dx.doi.org/10.1007/s15010-013-0501-4>
9. Bowen A, Eikmeier D, Talley P, Siston A, Smith S, Hurd J, et al.; Centers for Disease Control and Prevention (CDC). Notes from the field: outbreaks of *Shigella sonnei* infection with decreased susceptibility to azithromycin among men who have Sex with men—Chicago and Metropolitan Minneapolis–St. Paul, 2014. *MMWR Morb Mortal Wkly Rep*. 2015;64:597–8.

10. Public Health England. *Shigella*: leaflet and poster for men who have sex with men. 2013 Dec 1 [cited 2016 Feb 10]. <https://www.gov.uk/government/publications/shigella-leaflet-and-poster>

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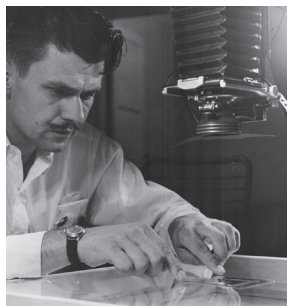
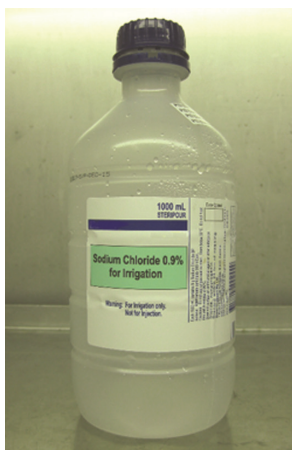


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ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015

Technical Appendix

Detailed Methods

Public Health England conducted whole-genome sequencing of $\approx 70\%$ of *S. sonnei* isolates voluntarily referred from hospital laboratories. In brief, DNA is extracted from cultures of *Shigella sonnei* isolates for sequencing on the HiSeq 2500 (Illumina, San Diego, USA). High-quality reads are mapped to the *S. sonnei* reference genome Ss046 (GenBank accession no. NC_007384.1) by using the Burrows-Wheeler Aligner's Smith-Waterman Alignment algorithm (1). Single nucleotide polymorphisms (SNPs) are identified by using the Genome Analysis Toolkit (2) in unified genotyper mode. Core genome positions with a high-quality SNP ($>90\%$ similarity, minimum depth [minimum number of reads needed to call a genotype] $\times 10$, genotype quality >30) in at least 1 isolate are extracted, and maximum likelihood phylogeny is derived by using RaxML (3).

When necessary to elucidate plasmid sequence, DNA is extracted using the molecular Invisorb Spin Mini Kit (Stratec Biomedical) and is diluted to a concentration of 1 μg of genomic DNA in 50 μL of water. A MinION library (Oxford Nanopore Technologies, Oxford, UK) is prepared by using the SQK-MAP006 genomic sequencing kit (Nanopore), according to the manufacturer's instructions, and sequencing is performed on an Mk1 MinION device (Nanopore) with an Mk1 flow cell. A FASTA file of the readings is extracted with poretools (4) and SPAdes version 3.6.2 (5), which is used to produce a hybrid.

References

1. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2010;26:589–95 <http://dx.doi.org/10.1093/bioinformatics/btp698>

2. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20:1297–303 <http://dx.doi.org/10.1101/gr.107524.110>.
3. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 2014;30:1312–3 <http://dx.doi.org/10.1093/bioinformatics/btu033>.
4. Loman NJ, Quinlan AR. Poretools: a toolkit for analyzing nanopore sequence data. *Bioinformatics.* 2014;30:3399–401 <http://dx.doi.org/10.1093/bioinformatics/btu555>.
5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* 2012;19:455–77 <http://dx.doi.org/10.1089/cmb.2012.0021>.