Detection of OXA-181 Carbapenemase in *Shigella flexneri*

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We report the detection of OXA-181 carbapenemase in an azithromycin-resistant *Shigella* spp. bacteria in an immunocompromised patient. The emergence of OXA-181 in *Shigella* spp. bacteria raises concerns about the global dissemination of carbapenem resistance in Enterobacterales and its implications for the treatment of infections caused by *Shigella* bacteria.

Shigella flexnerii infection leads to shigellosis, an acute gastrointestinal disease. Shigellosis affects socioeconomically disadvantaged and densely populated communities that have unsafe water, poor sanitation, and poor hygiene (*1*). *Shigella* spp. bacteria are major contributors to acute bloody diarrhea worldwide, adding to disease numbers and death in children under 5 years of age (*2*). The emergence of multidrug-resistant *Shigella* strains is a concerning trend. Multidrug-resistant strains resist multiple first-line oral antimicrobials (i.e., ampicillin, trimethoprim/sulfamethoxazole, and ciprofloxacin). The situation is further complicated by enzyme-mediated β-lactam resistance in *Shigella* bacteria, further impacting empiric therapy and making the isolates extensively drug-resistant (*2*). Although extensively drug-resistant isolates have remained susceptible to carbapenem therapy, carbapenem resistance in *Shigella* spp. through imipenemase-type metallo-β-lactamase, New Delhi metallo-β-Lactamase, and Verona integron-encoded metallo-β-lactamase has been reported (*3*,*4*).

We report a case of OXA-181–producing *S. flexneri* bacteria recovered from the stool of an immunocompromised patient with B-cell acute lymphoblastic leukemia (B-ALL). OXA-181 is a subtype of the OXA-48–like carbapenemase enzymes, classified as an Ambler class D β-lactamase, that primarily hydrolyzes penicillins and carbapenems. Those enzymes are usually transmitted on plasmids and are typically associated with Enterobacterales such as *Klebsiella pneumoniae* and *Escherichia coli* bacteria (*5*).

A 2-year-old girl, born in a rural area near Hyderabad, India, was diagnosed with standard-risk B-ALL. Her chemotherapy treatment was complicated by 2 episodes of culture-negative febrile neutropenia and acute gastroenteritis. Her diarrhea was presumed to be allopurinol-induced and was managed with supportive care. Her care team discovered evidence of a B-ALL relapse. The patient recovered from the fever and diarrhea, and her family immigrated to Canada, where the patient was admitted to a hospital to establish care for her relapsed B-ALL.

The patient was afebrile and did not have diarrhea until day 3 in the hospital, when she had onset of febrile neutropenia, nonbloody diarrhea, and abdominal pain. In accordance with the hospital's infection prevention protocol, we collected a stool sample for carbapenemase-producing Enterobacterales (CPE) screening. It exhibited growth of non–lactose fermenting colonies on the OXA side of a Chromid Carba Smart plate (bioMérieux, http://www.biomerieux.com), which we confirmed to be *S. flexneri* bacteria type 2a by using a biochemical panel and serotyping. We performed a stool PCR by using Seegene Allplex GI-EB gastrointestinal multiplex assay (SeeGene Inc., https://www.seegene.com) that showed the presence of *Shigella* spp. bacteria and astrovirus. We also isolated *S. flexneri* bacteria from a stool culture by using molecular detection (Appendix).

We began treatment for febrile neutropenia with piperacillin/tazobactam and vancomycin, in addition to azithromycin because of the detection of *S. flexneri* bacteria from the patient stool samples. Both isolates from the CPE screen and stool culture demonstrated a similar susceptibility profile (Table 1). Although the meropenem MIC was susceptible according to Clinical and Laboratory Standards Institute breakpoints, it was higher than 0.12 mg/L, the CPE screening cutoff in our laboratory protocol (*6*). We used the CARBA-5 assay (NG Biotech, https://www.ngbiotech.com) to further evaluate the antibiotic susceptibility, and the results indicated the presence of an OXA-48–like enzyme. The Public Health Ontario Laboratory verified the presence of OXA-48–like gene by using multiplex PCR (*7*). Because of the azithromycin resistance, we modified the treatment to trimethoprim/sulfamethoxazole.

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Table 1. Antibiotic-susceptibility results using 4 different methodologies for the *Shigella flexneri* bacteria cultured from an immunocompromised patient, Canada*

Antibiotic	BD Phoenix,† mg/L	Broth microdilution, mg/L	Agar dilution, mg/L	CLSI breakpoints for susceptibility, mg/L	Kirby–Bauer disk diffusion, mm
Azithromycin	NA	NA	≥32	≤8	NA
Ceftriaxone	0.5	0.5	NA	≤1	28
Ceftazidime	0.5	1	NA	≤4	30
Ertapenem	>1	1	≤0.5	≤0.5	24
Meropenem	0.5	0.5‡	≤0.12	≤1	24
Ciprofloxacin	2	>2	NA	≤0.25	NA
TMP/SMX	≤0.5	2/38	NA	≤2/38	NA
Colistin	NA	NA	≤0.25	≤2§	NA

*CLSI, Clinical and Laboratory Standards Institute; NA, not available; TMP/SMX, trimethoprim/sulfamethoxazole.
†Becton Dickinson, https://www.bd.com.
‡Lowest concentration for meropenem on methodology used (Gram negative sensititer panel).
§Intermediate susceptibility.

After treatment, the patient experienced rapid defervescence and resolution of the diarrhea. We repeated the stool culture after 2 weeks of treatment, and the culture resulted in no growth of *S. flexneri* bacteria.

We conducted whole-genome sequencing (Appendix). We extracted DNA from the bacterial isolate by using easyMag (bioMérieux) and sequenced on a GridION system with a R10.4.1 flow cell (Oxford Nanopore Technologies, https://nanoporetech.com). We analyzed data with MinKNOW 23.04.5 (Oxford Nanopore Technologies) to construct a consensus genome. We analyzed the isolate's genome and plasmid with the Comprehensive Antibiotic Resistance Database (CARD) (http://arpcard.mcmaster.ca), identifying 5 resistance genes on the plasmid (Table 2), including OXA-181 with ≥95% identity and length within the plasmid. The plasmid has a size of 91,956 bp and carries all the genes for the resistance profile (Appendix). We deposited the plasmid gene sequence into GenBank (accession no. PP417752).

Given the low-hydrolytic activity of OXA-48–like enzymes, microbiology laboratories face difficult challenges in accurately detecting these enzymes in Enterobacterales. The Clinical and Laboratory Standards Institute breakpoints for meropenem are not suited for CPE surveillance, potentially missing OXA-48–like producers (*8*). Our laboratory has adopted a meropenem MIC breakpoint of >0.12 mg/L for CPE screening, in line with European Committee on Antimicrobial Susceptibility Testing recommendations (*9*). This approach is crucial for identifying isolates that require further CPE investigation, especially considering the reduced activity of OXA-48–like enzymes against cephalosporins.

Identification of an OXA-181 carbapenemase in a plasmid carried by *S. flexneri* bacteria is an alarming finding and concerning for the spread of this resistance profile in densely populated low- and middle-income communities. The detection of OXA-181 in *Shigella* spp. bacteria increases concerns about the broad dissemination of carbapenem resistance among other Enterobacterales (*10*). This finding emphasizes the need for vigilant and targeted surveillance for CPE in at-risk patients.

Table 2. Antibiotic-resistance genes detected within the plasmid recovered from a *Shigella flexneri* bacteria cultured from an immunocompromised patient sample, based on the Comprehensive Antibiotic Resistance Database,* Canada

Gene	Phenotypic resistance
OXA-181	Carbapenems
qnrS1	Fluoroquinolones
mrx	Macrolides
mphA	Macrolides
ermB	Macrolides

*Available at http://arpcard.mcmaster.ca.

Dr. Dhabaan is finishing his clinical microbiology fellowship at the University of Toronto. His interests include leveraging artificial intelligence alongside genomics and clinical data to advance infectious disease management.

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SARS-CoV-2 IgG Levels as Predictors of XBB Variant Neutralization, Israel, 2022 and 2023

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Although a vaccine against SARS-CoV-2 Omicron-XBB.1.5 variant is available worldwide and recent infection is protective, the lack of recorded infection data highlights the need to assess variant-specific antibody neutralization levels. We analyzed IgG levels against receptor-binding domain–specific SARS-CoV-2 ancestral strain as a correlate for high neutralizing titers against XBB variants.

Since the beginning of 2023, SARS-CoV-2 Omicron XBB variants have led as the cause of global SARS-CoV-2 infections (*1*,*2*). SARS-CoV-2 mRNA vaccines based on the ancestral variant were shown to be less effective against Omicron variants, with reduced neutralization efficiency (*3*,*4*). Because of this reduced neutralization efficiency, updated mRNA vaccines, like the monovalent XBB1.15 vaccine, were developed and distributed (*5*). High levels of neutralizing and receptor-binding domain (RBD) binding IgG levels are known to be correlated with protection from infection or severe disease (*6*,*7*). The evasiveness of Omicron variants against neutralizing antibodies induced by vaccination or infection with previous variants demonstrated the importance of determining variant-specific neutralizing antibodies (*4*). In this study, we investigated the utility of measuring RBD IgG levels against the SARS-CoV-2 ancestral (wild-type [WT]) strain to predict titers of XBB-specific neutralizing antibodies.

During February 2022–August 2023, we obtained 1,070 samples from 373 study participants at Sheba Medical Center in Ramat Gan, Israel, and tested the samples for levels of IgG against WT-RBD and XBB-specific neutralizing antibody levels (Appendix, https://wwwnc.cdc.gov/EID/article/30/5/23-1739-

Table. Sex, age range, and COVID-19 history of patient participants who provided samples for testing IgG against SARS-CoV-2 ancestral strain and Omicron XBB-specific neutralizing antibody levels in 2022 and 2023, Israel*

Variable	Value
Sex	
F	251 (67)
M	122 (33)
No. COVID-19 vaccinations received	
0	1 (0.3)
1	13 (3.5)
2	5 (1.3)
3	102 (27)
4	215 (58)
5	36 (9.7)
6	1 (0.3)
No. COVID-19 infections	
0	227 (61)
1	120 (32)
2	22 (5.9)
3	3 (0.8)
4	1 (0.3)

*Values are no. (%) except as indicated.

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Appendix

Shigella flexneri isolation methodology

From the carbapenemase-producing Enterobacterales (CPE) surveillance specimen, the stool specimen was inoculated on CHROMID® CARBA SMART Agar. The next day, scant gray colonies grew on the OXA side of the plate. The organism was identified as *Escherichia coli* by matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics). However, because the colonies were gray, not pink on the agar, and due to a known limitation that MALDI-TOF cannot distinguish *E. coli* from *Shigella*, serotyping using *Shigella* antisera and biochemical reactions were set up. Kligler iron agar was alkaline over acid with no H2S and produced little gas. The organism was negative for lysine, ornithine, indole, and citrate. Motility was negative, and the isolate showed agglutination with *S. flexneri* group B antisera, types 1–6, and no agglutination with antisera for other *Shigella* groups. The identification was confirmed by the BD Phoenix system, which gave a 99% *S. flexneri* identification of the isolate. The isolate was forwarded to the Provincial laboratory and was serotyped as *S. flexneri* type 2a. Stool culture ordered as a reflex test after detection of *Shigella* species by multiplex PCR identified the organism when it grew as non-lactose fermenting colonies on MacConkey agar. All biochemical and serotyping results were identical to the isolate from the CPE surveillance specimen described previously.

Isolate whole genome sequencing methodology

DNA was extracted from an overnight culture of the bacterial isolate using the easyMag system (bioMérieux). Purified DNA was prepared for sequencing using the Rapid Barcoding

Sequencing Kit SQK-LSK114 (Oxford Nanopore Technologies, UK). Sequencing occurred on a GridION system using a R10.4.1 flow cell (FLO-MIN114, Oxford Nanopore Technologies) with High-accuracy model basecalling with data captured over 26 hours. Data was evaluated with the MinKNOW software 23.04.5. A consensus genome was constructed using Flye 2.9.

Identification of antibiotic resistance genes

The consensus genome for both the bacterial isolate and plasmid were analyzed through the Resistance Gene Identification tool within the Comprehensive Antibiotic Resistance Database (*1*). Hits demonstrating a ≥95% identity and ≥95% length of reference sequence were included in analysis. This database identified 5 resistance genes present within the plasmid (Appendix Table). The plasmid consensus sequence was visualized and annotated using Geneious Prime v2023.0.1 (Biomaters, U.S.).

Appendix Table. Resistance Gene Identification obtained through the CARD database for the isolated plasmid.

RGI Criteria	ARO Term	% Identify of Matching Region	% Length of Reference Sequence
Perfect	OXA-181	100	100
Perfect	QnrS1	100	100
Perfect	Mrx	100	100
Perfect	MphA	100	100
Strict	ErmB	97.96	98.79

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