Detection of OXA-181 Carbapenemase in Shigella flexneri

Ghulam Dhabaan, Hassan Jamal, Danielle Ouellette, Sarah Alexander, Karen Arane, Aaron Campigotto, Manal Tadros, Pierre-Philippe Piché-Renaud


DOI: https://doi.org/10.3201/eid3005.231558

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 flexneri 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 CÄRBÄ-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.

These authors contributed equally to this article.
After treatment, the patient experienced rapid defer-
vence and resolution of the diarrhea. We repeated
the stool culture after 2 weeks of treatment, and the
culture resulted in no growth of *Shigella flexneri* bacteria.

We conducted whole-genome sequencing (Ap-
pendix). 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
We analyzed data with MinKNOW 23.04.5 (Oxford
Nanopore Technologies, a GridION system with a R10.4.1 flow cell (Oxford
Nanopore Technologies,)). We analyzed the isolate’s genome and plas-
mid 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
Enterobacteriaceae. The Clinical and Laboratory Stan-
dards 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 An-
timicrobial 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 en-
zymes against cephalosporins.

Identification of an OXA-181 carbapenemase in
a plasmid carried by *Shigella 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 dissemina-
tion of carbapenem resistance among other Enterobacte-
rales (10). This finding emphasizes the need for vigilant
and targeted surveillance for CPE in at-risk patients.

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.

### References:

1. World Health Organization. Water, Sanitation and Hygiene (WASH) [cited 2024 Feb 26]. https://www.who.int/
health-topics/water-sanitation-and-hygiene-wash.

2. Centers for Disease Control and Prevention Health Alert
Network. Increase in extensively drug-resistant shigellosis
in the United States [cited 2024 Feb 26]. https://emergency.
cdc.gov/han/2023/pdf/CDCHAN_486.pdf

3. Abdelaziz NA. Phenotype-genotype correlations among
carbapenem-resistant Enterobacteriaceae recovered from
eight Egyptian hospitals with the report of SPM
https://doi.org/10.1186/s13756-022-01061-7

4. Thamizhmani R, Rhagavan R, Sugunan AP, Vijayachari P.
VIM- and IMP-type metallo-β-lactamase-producing *Shigella*
spp. in childhood diarrhea from Andaman Islands. Infect
23744235.2015.1022874

5. Pitout JDD, Peirano G, Kock MM, Strydom KA, Matsumura Y.
The global ascendency of OXA-48-type carbapenemases. Clin
CMR.00102-19

6. Clinical & Laboratory Standards Institute. Performance
standards for antimicrobial susceptibility testing: thirty-third

---

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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>BD Phoenix, †</th>
<th>Broth microdilution, mg/L</th>
<th>Agar dilution, mg/L</th>
<th>CLSI breakpoints for susceptibility, mg/L</th>
<th>Kirby–Bauer disk diffusion, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>NA</td>
<td>NA</td>
<td>≥32</td>
<td>≤8</td>
<td>NA</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.5</td>
<td>0.5</td>
<td>NA</td>
<td>≥1</td>
<td>28</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>0.5</td>
<td>1</td>
<td>NA</td>
<td>≤4</td>
<td>30</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;1</td>
<td>1</td>
<td>≤0.5</td>
<td>≥0.5</td>
<td>24</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.5</td>
<td>0.5‡</td>
<td>≤0.12</td>
<td>≤1</td>
<td>24</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>≥2</td>
<td>NA</td>
<td>≤0.25</td>
<td>NA</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>&lt;0.5</td>
<td>2/38</td>
<td>NA</td>
<td>≤2/38</td>
<td>NA</td>
</tr>
<tr>
<td>Colistin</td>
<td>NA</td>
<td>NA</td>
<td>≤0.25</td>
<td>&gt;2/38</td>
<td>NA</td>
</tr>
</tbody>
</table>

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

---

**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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotypic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA-181</td>
<td>Carbapenems</td>
</tr>
<tr>
<td>qnrS1</td>
<td>Fluoroquinolones</td>
</tr>
<tr>
<td>mrx</td>
<td>Macrolides</td>
</tr>
<tr>
<td>mphA</td>
<td>Macrolides</td>
</tr>
<tr>
<td>ermB</td>
<td>Macrolides</td>
</tr>
</tbody>
</table>

*Available at http://arpcard.mcmaster.ca.
SARS-CoV-2 IgG Levels as Predictors of XBB Variant Neutralization, Israel, 2022 and 2023

Yaniv Lustig, Michal Canetti, Victoria Indenbaum, Yovel Peretz, Yael Weiss-Ottolenghi, Ili Margalit, Keren Asraf, Tai Levin, Neta Zuckerman, Enosh Tomer, Michal Mandelboim, Ram Doolman, Noam Barda, Gili Regev-Yochay

Author affiliations: Sheba Pandemic Research Institute, Ramat-Gan, Israel (Y. Lustig, M. Canetti, Y. Peretz, Y. Weiss-Ottolenghi, I. Margalit, N. Zuckerman, G. Regev-Yochay); Tel-Aviv University Faculty of Medical and Health Sciences, Tel Aviv, Israel (Y. Lustig, M. Canetti, I. Margalit, M. Mandelboim, G. Regev-Yochay); Central Virology Laboratory, Public Health Services, Ministry of Health, Ramat-Gan (Y. Lustig, V. Indenbaum, T. Levin, N. Zuckerman, E. Tomer, M. Mandelboim); The Dworman Automated-Mega Laboratory, Ramat-Gan (K. Asraf, R. Doolman); ARC Innovation Center, Ramat-Gan (N. Barda); Ben-Gurion University of the Negev, Be’er Sheva, Israel (N. Barda)

DOI: https://doi.org/10.3201/eid3005.231739

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 higher 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 receptor-binding domain–specific SARS-CoV-2 ancestral strain as a correlate for high neutralizing titers against XBB variants.

### 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F 251 (67)</td>
</tr>
<tr>
<td>No. COVID-19 vaccinations received</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>1</td>
<td>13 (3.5)</td>
</tr>
<tr>
<td>2</td>
<td>5 (1.3)</td>
</tr>
<tr>
<td>3</td>
<td>102 (27)</td>
</tr>
<tr>
<td>4</td>
<td>215 (58)</td>
</tr>
<tr>
<td>5</td>
<td>36 (9.7)</td>
</tr>
<tr>
<td>6</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>No. COVID-19 infections</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>227 (61)</td>
</tr>
<tr>
<td>1</td>
<td>120 (32)</td>
</tr>
<tr>
<td>2</td>
<td>22 (5.9)</td>
</tr>
<tr>
<td>3</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>4</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

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