



# Antibiotic profiles and draft genome sequences of four *Shigella flexneri* recovered from untreated community wastewater samples in Ghana

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**ABSTRACT** Genomic data on clinically relevant bacteria of public health concern from sub-Saharan Africa are limited. In this study, we present the phenotypic profiles and genomic characteristics of four *Shigella flexneri* isolates recovered from untreated community wastewater samples in Ghana.

**KEYWORDS** *Shigella flexneri*, Ghana, wastewater, antimicrobial resistance, Africa, genome

Wastewater surveillance combined with whole-genome sequencing enables high-resolution monitoring of antimicrobial resistance and supports public health responses (1–7). In Africa, *Shigella flexneri* isolates show widespread extended-spectrum  $\beta$ -lactamase (ESBL) production and high levels of multidrug and carbapenem resistance (8). Here, we report on the draft genome sequences and antibiogram of four *S. flexneri* recovered from untreated community wastewater samples.

A total of 277 untreated community wastewater samples were archived from 13 sentinel sites across seven regions in Ghana. These samples were concentrated at the Virology Department of the NMIMR and stored in 2 mL cryovials for poliovirus detection. The 277 archived concentrates were then retrieved and cultured on MacConkey agar (Oxoid, UK) and incubated at 37°C for 18–24 hours. The isolates obtained were subcultured onto nutrient agar (Oxoid, UK) and identified to the species level using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Germany). Antimicrobial susceptibility testing was performed on isolates using the Kirby-Bauer disk diffusion method with 13 antimicrobial agents. Detection of extended-spectrum  $\beta$ -lactamase production was performed using the double-disk diffusion method. Measured zone sizes were interpreted using CLSI (2024) guidelines (9).

DNA extraction and purification were performed using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Quantification of DNA was done using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA). Libraries were prepared using the Illumina DNA Preparation (M) Tagmentation Kit (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. The process included the enzymatic fragmentation of DNA using bead-linked transposons, index addition, amplification, and bead-based size selection. Library fragment sizes and concentrations were assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and quantitative PCR (KAPA SYBR Fast qPCR kit), respectively. Libraries were normalized to 2 nM, pooled, and then sequenced using 2 × 150 bp paired-end chemistry on the Illumina NextSeq 2000 system (Illumina

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TABLE 1 Antimicrobial resistance profiles and genomic characteristics of isolates<sup>a</sup>

| Characteristics                          | Isolate information                                                                                                                                                                                                           |                                                                                                                                                                                                                                   |                                                                                                                                                                                        |                                                                                                                                                             |
|------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample ID                                | 470_24_S308                                                                                                                                                                                                                   | 543_24_S307                                                                                                                                                                                                                       | 511_23_S251                                                                                                                                                                            | 636_23_S239                                                                                                                                                 |
| Strain ID                                | SF001                                                                                                                                                                                                                         | SF002                                                                                                                                                                                                                             | SF003                                                                                                                                                                                  | SF004                                                                                                                                                       |
| Geographical location                    | Nima Freetown (5.58° North Latitude and 0.2° West Longitude)                                                                                                                                                                  | Ahinsan (6.66° North Latitude and –1.6° West Longitude)                                                                                                                                                                           | Sunyani Zongo (7.34° North Latitude and –2.32° West Longitude)                                                                                                                         | Shiabu (5.53° North Latitude and –0.25° West Longitude)                                                                                                     |
| Antimicrobial agents (Zone diameters/mm) |                                                                                                                                                                                                                               |                                                                                                                                                                                                                                   |                                                                                                                                                                                        |                                                                                                                                                             |
| Amikacin (30 µg)                         | 20 (S)                                                                                                                                                                                                                        | 20 (S)                                                                                                                                                                                                                            | 18 (I)                                                                                                                                                                                 | 18 (I)                                                                                                                                                      |
| Ampicillin (10 µg)                       | 6 (R)                                                                                                                                                                                                                         | 6 (R)                                                                                                                                                                                                                             | 6 (R)                                                                                                                                                                                  | 6 (R)                                                                                                                                                       |
| Cefotaxime (30 µg)                       | 10 (R)                                                                                                                                                                                                                        | 10 (R)                                                                                                                                                                                                                            | 10 (R)                                                                                                                                                                                 | 10 (R)                                                                                                                                                      |
| Cefoxitin (30 µg)                        | 6 (R)                                                                                                                                                                                                                         | 26 (S)                                                                                                                                                                                                                            | 25 (S)                                                                                                                                                                                 | 8 (R)                                                                                                                                                       |
| Ceftazidime (30 µg)                      | 18 (I)                                                                                                                                                                                                                        | 17 (R)                                                                                                                                                                                                                            | 18 (I)                                                                                                                                                                                 | 6 (R)                                                                                                                                                       |
| Cefuroxime (30 µg)                       | 6 (R)                                                                                                                                                                                                                         | 6 (R)                                                                                                                                                                                                                             | 6 (R)                                                                                                                                                                                  | 6 (R)                                                                                                                                                       |
| Chloramphenicol (30 µg)                  | 18 (S)                                                                                                                                                                                                                        | 24 (S)                                                                                                                                                                                                                            | 26 (S)                                                                                                                                                                                 | 25 (S)                                                                                                                                                      |
| Ciprofloxacin (5 µg)                     | 19 (R)                                                                                                                                                                                                                        | 20 (R)                                                                                                                                                                                                                            | 14 (R)                                                                                                                                                                                 | 21 (R)                                                                                                                                                      |
| Gentamicin (10 µg)                       | 19 (S)                                                                                                                                                                                                                        | 17 (I)                                                                                                                                                                                                                            | 18 (S)                                                                                                                                                                                 | 15 (I)                                                                                                                                                      |
| Meropenem (10 µg)                        | 22 (I)                                                                                                                                                                                                                        | 30 (S)                                                                                                                                                                                                                            | 34 (S)                                                                                                                                                                                 | 11 (R)                                                                                                                                                      |
| Norfloxacin (10 µg)                      | 23 (S)                                                                                                                                                                                                                        | 15 (I)                                                                                                                                                                                                                            | 20 (S)                                                                                                                                                                                 | 20 (S)                                                                                                                                                      |
| Tetracycline (30 µg)                     | 6 (S)                                                                                                                                                                                                                         | 7 (S)                                                                                                                                                                                                                             | 6 (R)                                                                                                                                                                                  | 7 (R)                                                                                                                                                       |
|                                          | 6 (R)                                                                                                                                                                                                                         | 6 (R)                                                                                                                                                                                                                             | 6 (R)                                                                                                                                                                                  | 28 (S)                                                                                                                                                      |
| Trimethoprim-Sulfamethoxazole (25 µg)    |                                                                                                                                                                                                                               |                                                                                                                                                                                                                                   |                                                                                                                                                                                        |                                                                                                                                                             |
| ESBL                                     | Positive                                                                                                                                                                                                                      | Positive                                                                                                                                                                                                                          | Positive                                                                                                                                                                               | Negative                                                                                                                                                    |
| Resistance genes                         |                                                                                                                                                                                                                               |                                                                                                                                                                                                                                   |                                                                                                                                                                                        |                                                                                                                                                             |
| Aminoglycosides                          | <i>aph (6)-I<sub>d</sub></i> , <i>aph (3'')-I<sub>b</sub></i> , and <i>aadA5</i>                                                                                                                                              | ND                                                                                                                                                                                                                                | <i>aph (6)-I<sub>d</sub></i> and <i>aph (3'')-I<sub>b</sub></i>                                                                                                                        | ND                                                                                                                                                          |
| β-Lactams                                | <i>bla<sub>CTX-M-15</sub></i>                                                                                                                                                                                                 | <i>bla<sub>CTX-M-15</sub></i>                                                                                                                                                                                                     | <i>bla<sub>CTX-M-15</sub></i> and <i>bla<sub>TEM-1B</sub></i>                                                                                                                          | <i>bla<sub>TEM-1B</sub></i>                                                                                                                                 |
| Folate pathway antagonists               | <i>sul2</i> , <i>sul1</i> , and <i>dfrA17</i>                                                                                                                                                                                 | ND                                                                                                                                                                                                                                | <i>sul2</i> and <i>dfrA14</i>                                                                                                                                                          | ND                                                                                                                                                          |
| Macrolides                               | <i>erm(B)</i> and <i>mph(A)</i>                                                                                                                                                                                               | ND                                                                                                                                                                                                                                | ND                                                                                                                                                                                     | ND                                                                                                                                                          |
| Quinolones                               | <i>qnrD1</i> , <i>qnrS1</i> , <i>emrR</i> , and <i>emrB</i>                                                                                                                                                                   | <i>qnrS1</i> , <i>emrR</i> , <i>emrA</i> , <i>emrB</i> , and <i>mdtB</i>                                                                                                                                                          | <i>qnrS1</i> , <i>emrB</i> , <i>emrR</i> , and <i>mdtH</i>                                                                                                                             | <i>qnrS1</i> , <i>emrB</i> , and <i>emrR</i>                                                                                                                |
| Tetracyclines                            | <i>tet(A)</i> and <i>emrY</i>                                                                                                                                                                                                 | <i>tet(A)</i>                                                                                                                                                                                                                     | <i>tet(A)</i> and <i>emrY</i>                                                                                                                                                          | <i>tet(A)</i>                                                                                                                                               |
| Nitroimidazole                           | <i>msbA</i>                                                                                                                                                                                                                   | <i>msbA</i>                                                                                                                                                                                                                       | <i>msbA</i>                                                                                                                                                                            | <i>msbA</i>                                                                                                                                                 |
| Peptide                                  |                                                                                                                                                                                                                               | <i>bacA</i> and <i>pmrF</i>                                                                                                                                                                                                       | <i>pmrF</i>                                                                                                                                                                            | <i>yojI</i> , <i>pmrF</i> , and <i>bacA</i>                                                                                                                 |
| Disinfecting agents and antiseptics      | <i>qacEdelta1</i>                                                                                                                                                                                                             | ND                                                                                                                                                                                                                                | ND                                                                                                                                                                                     | ND                                                                                                                                                          |
| Phosphonic acid                          | <i>mdtG</i>                                                                                                                                                                                                                   | ND                                                                                                                                                                                                                                | <i>mdtG</i>                                                                                                                                                                            | <i>mdtG</i>                                                                                                                                                 |
| Multiple drugs <sup>b</sup>              | <i>acrS</i> , <i>gadX</i> , <i>mdtE</i> , <i>evgA</i> , <i>acrA</i> , <i>acrB</i> , <i>cpxA</i> , and <i>marA</i>                                                                                                             | <i>acrS</i> , <i>acrE</i> , <i>acrA</i> , <i>acrB</i> , <i>mdtN</i> , <i>mdtP</i> , <i>evgA</i> , <i>marA</i> , <i>acrE</i> , <i>acrS</i> , <i>mdtE</i> , <i>cpxA</i> , <i>gadX</i> , <i>mdtF</i> , <i>mdtE</i> , and <i>marA</i> | <i>acrB</i> , <i>acrA</i> , and <i>cpxA</i>                                                                                                                                            | <i>mdtP</i> , <i>mdtN</i> , <i>acrE</i> , <i>acrA</i> , <i>acrB</i> , <i>cpxA</i> , <i>gadX</i> , <i>mdtF</i> , <i>mdtE</i> , <i>marA</i> , and <i>evgA</i> |
| Plasmids                                 | Col3M, IncFIA, IncFIB, IncFII, and IncR                                                                                                                                                                                       | ND                                                                                                                                                                                                                                | IncY                                                                                                                                                                                   | IncY                                                                                                                                                        |
| Virulence genes                          | <i>anr</i> , <i>capU</i> , <i>csgA</i> , <i>fdeC</i> , <i>gad</i> , <i>hha</i> , <i>hlyE</i> , <i>iss</i> , <i>nlpl</i> , <i>sitA</i> , <i>terC</i> , <i>traT</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , and <i>yehD</i> | <i>capU</i> , <i>csgA</i> , <i>fdeC</i> , <i>gad</i> , <i>hha</i> , <i>hlyE</i> , <i>iss</i> , <i>nlpl</i> , <i>sitA</i> , <i>terC</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , <i>yehD</i> , and <i>yghJ</i>                  | <i>capU</i> , <i>csgA</i> , <i>fdeC</i> , <i>fimH</i> , <i>gad</i> , <i>hlyE</i> , <i>nlpl</i> , <i>terC</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , <i>yehD</i> , and <i>yghJ</i> | <i>clpK1</i> , <i>csgA</i> , <i>fdeC</i> , <i>fimH</i> , <i>gad</i> , <i>hha</i> , <i>hlyE</i> , <i>nlpl</i> , <i>sitA</i> , <i>terC</i> , and <i>yghJ</i>  |
| Genome size (bp)                         | 4,743,972                                                                                                                                                                                                                     | 4,586,942                                                                                                                                                                                                                         | 4,817,493                                                                                                                                                                              | 4,510,260                                                                                                                                                   |
| Sequence type                            | ST1598                                                                                                                                                                                                                        | ST1421                                                                                                                                                                                                                            | ST224                                                                                                                                                                                  | ST46                                                                                                                                                        |
| N <sub>50</sub> (bp)                     | 82,775                                                                                                                                                                                                                        | 82,809                                                                                                                                                                                                                            | 86,580                                                                                                                                                                                 | 84,358                                                                                                                                                      |
| GC content (%)                           | 50.73                                                                                                                                                                                                                         | 50.82                                                                                                                                                                                                                             | 50.83                                                                                                                                                                                  | 50.78                                                                                                                                                       |
| No. of reads                             | 7,576,788                                                                                                                                                                                                                     | 7,329,186                                                                                                                                                                                                                         | 6,642,772                                                                                                                                                                              | 8,680,358                                                                                                                                                   |

(Continued on next page)

TABLE 1 Antimicrobial resistance profiles and genomic characteristics of isolates<sup>a</sup> (Continued)

| Characteristics             |                 | Isolate information |                 |                 |
|-----------------------------|-----------------|---------------------|-----------------|-----------------|
| No. of contigs              | 279             | 198                 | 77              | 145             |
| Size of largest contig (bp) | 278,327         | 229,652             | 977,979         | 235,020         |
| No. of coding sequences     | 4,473           | 4,263               | 4,430           | 4,186           |
| No. of rRNAs                | 9               | 3                   | 9               | 2               |
| No. of tRNAs                | 80              | 80                  | 80              | 77              |
| Coverage (×)                | 241             | 241                 | 208             | 291             |
| BioProject accession no.    | PRJNA1338482    | PRJNA1338482        | PRJNA1338482    | PRJNA1338482    |
| Genome accession no.        | JBRNXC000000000 | JBRNXB000000000     | JBSCCP000000000 | JBSCCM000000000 |
| BioSample accession no.     | SAMN52447488    | SAMN52447489        | SAMN53088219    | SAMN53088222    |
| SRA accession no.           | SRR35793167     | SRR35793166         | SRR35980169     | SRR35980168     |

<sup>a</sup>R, resistant; I, intermediate; S, susceptible; ND, not detected.<sup>b</sup>Genes conferring resistance to multiple drug classes. The drug classes can be found at <https://doi.org/10.6084/m9.figshare.31078558>.

Inc., San Diego, CA, USA). Indexes and reads with quality scores <20 were trimmed using Trimmomatic v0.39 (10), quality-checked with FastQC v1.0 (11), and assembled using Unicycler v0.5.0 (12). The assemblies were evaluated using Quast v5.2.0 (13). All genomes met established quality thresholds ( $Q \geq 20$ ,  $\geq 20\times$  coverage, and contig length  $\geq 200$  bp). Species confirmation, virulence profiling, plasmid identification, and multilocus sequence typing were performed using KmerFinder v3.0.2 (14), VirulenceFinder v2.0.5 (15), PlasmidFinder v2.0.1 (16), and MLST v2.0.9 (17), respectively. Antibiotic resistance was determined using ResFinder v4.7.2 (18) and CARD v4.0.1 (19). Genome annotation was performed using NCBI PGAP v6.10 (20). Default settings were utilized except where mentioned. Table 1 presents a summary of the results.

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Validation, Visualization, Writing – original draft, Writing – review and editing | Alex Awentirim Akayiri, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Emmanuel Ohene Kyei McKeown, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Daniel Kwaku Baka, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | William Boateng, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Christian Owusu-Nyantakyi, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Grebstad Rabbi Amuasi, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Quaneeta Mohktar, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Gabriella Acquah, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Maa Lankai Quaye, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Alfred Bortey, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Emmanuel Gberbi, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Angella Yayra Kampo, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Agnes Oclu, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Justice Danso, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Blessing Kofi Adu Tabi, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Rhodalyn Tagoe, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Evangeline Obodai, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Christa Twyford Gibson, Funding acquisition, Project administration, Writing – review and editing | Pernille Nilsson, Funding acquisition, Project administration, Writing – review and editing | Rene S. Hendriksen, Funding acquisition, Project administration, Writing – review and editing | Bright Adu, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | John Kofi Odoom, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – review and editing

## DATA AVAILABILITY

The genomes were uploaded to the National Center for Biotechnology Information (NCBI) database under BioProject accession number [PRJNA1338482](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1338482) and assigned unique accession numbers as provided in Table 1.

## ETHICS APPROVAL

The study was approved by the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (FW00001824).

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