

Antibiotic resistance and draft genome profiles of 10 *Streptococcus pneumoniae* and 3 *Streptococcus pseudopneumoniae* strains isolated from the nasopharynx of people living with human immunodeficiency virus in Ghana

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ABSTRACT Genomic data on clinically important bacteria species such as *Streptococcus pneumoniae* and *Streptococcus pseudopneumoniae* from low- and middle-income countries, including Ghana, are scarce. In this study, we provide data on antimicrobial resistance (AMR) and whole-genome profiles of a collection of streptococci species to support AMR surveillance efforts in the country.

KEYWORDS Africa, *Streptococcus pneumoniae*, *S. pseudopneumoniae*

Streptococci are a major cause of a wide range of diseases including pneumoniae, bacteremia, and meningitis (1). The similarities between *Streptococcus pseudopneumoniae* and *Streptococcus pneumoniae* make distinguishing them using traditional typing methods challenging (2, 3). Information on the epidemiology and genomic characteristics of *S. pseudopneumoniae* is scarce in sub-Saharan countries, including Ghana. Like *S. pneumoniae*, *S. pseudopneumoniae* is frequently associated with high rates of antimicrobial resistance, particularly to penicillin, macrolides, co-trimoxazole, and tetracycline (3). This article details the antimicrobial susceptibility and genomic profiles of 10 *S. pneumoniae* and 3 *S. pseudopneumoniae* isolates recovered from nasopharyngeal swabs collected from HIV-positive individuals during a cross-sectional study conducted at three hospitals in the Greater Accra Region of Ghana: University of Ghana Hospital, LEKMA Hospital, and Korle Bu Teaching Hospital. The study received approval from the University of Ghana, College of Health Sciences Ethical and Protocol Review Committee (Protocol number: CHS-Et/M.7-P 4.3/2022–2023). The nasopharyngeal swab samples were plated on 5% sheep blood agar (Oxoid) and incubated anaerobically for 24 hours at 37°C. Bacteria identification was done using the matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer. Antimicrobial susceptibility testing was performed on MH agar with 5% sheep blood using seven antibiotics (chloramphenicol, clindamycin, erythromycin, linezolid, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin) in a disk diffusion assay and interpreted according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2023). *S. pneumoniae* ATCC 49619 strain was used as control.

Following the manufacturer's instructions, DNA was extracted from pure overnight cultures using the QIAamp DNA Mini Kit (Qiagen, Germany) after suspending the colonies in lysozyme. The extracted DNA was then quantified using the Qubit 4.0 fluorometer and the High Sensitivity dsDNA Assay Kit. Libraries were generated using the Illumina DNA library preparation kit (Illumina Inc., San Diego, CA, USA). The quality and

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TABLE 1 Antimicrobial resistance profiles and genomic characteristics of isolates^a (Continued)

Isolate ID	Strain	Hospital	Genome accession no.	SRA accession no.	Biosample accession no.	Antibiotic resistance profile	Resistance genes	Virulence genes	Sequence type	Coverage (%)	Genome size (bp)	No. of contigs	NS0 (bp)	GC content (%)	No. of reads	CDS	No. of genes	rRNA	tRNA	tmRNA	No. of
HRV-23-443	SA9	LEKMA	JBBKAB0000000000	SRR28419016	SAMN40566645	TE and SXT	<i>ataA</i> , <i>patB</i> , <i>pmrA</i> , and <i>tet(M)</i>	<i>cbpD</i> , <i>cbpG</i> , <i>hysA</i> , <i>lytA</i> , <i>lytB</i> , <i>lytC</i> , <i>nanB</i> , <i>pavA</i> , <i>pce</i> , <i>ptfA</i> , <i>ply</i> , and <i>psaA</i>	ST700	101	2,057,773	69	82,415	39.5	970,696	2,016	2,055	3	35	1	1
HRV-23-66	SA10	LEKMA	JBBKAA0000000000	SRR28419015	SAMN40566646	C, TE, and SXT	<i>ataA</i> , <i>patB</i> , <i>pmrA</i> , <i>catpC194</i> , <i>pspC</i> , <i>cbpA</i> and <i>tet(M)</i>	<i>cbpD</i> , <i>cbpG</i> , <i>hysA</i> , <i>lytA</i> , <i>lytC</i> , <i>nanB</i> , <i>pce</i> , <i>ptfA</i> , <i>ply</i> , <i>psaA</i> , and <i>pspC</i>	ST13456	47	2,052,751	84	39,511	39.6	464,844	2,001	2,035	3	30	1	1
HRV-23-71	SA11	LEKMA	JBBJZ0000000000	SRR28419014	SAMN40566647	TE and SXT	<i>ataA</i> , <i>patB</i> , <i>pmrA</i> , and <i>tet(M)</i>	<i>cbpD</i> , <i>cbpG</i> , <i>cps4A</i> , <i>cps4B</i> , <i>cps4C</i> , <i>cps4D</i> , <i>hysA</i> , <i>lytA</i> , <i>lytB</i> , <i>lytC</i> , <i>nanB</i> , <i>pavA</i> , <i>pce</i> , <i>ptfA</i> , <i>ply</i> , and <i>psaA</i>	ST18376	84	2,115,087	90	64,833	39.5	847,416	2,083	2,120	3	33	1	1
<i>S. pseudopneumoniae</i>																					
HRV-23-029	SA12	LEKMA	JBBJZY0000000000	SRR28419013	SAMN40566648	ERY, CLL, and SXT	<i>patB</i>	<i>cbpD</i> , <i>lytA</i> , <i>lytC</i> , <i>pavA</i> , <i>pce</i> , <i>ply</i> , and <i>psaA</i>	UNK	46	1,941,672	47	75,591	40.3	387,928	1,769	1,795	2	25	1	1
HRV-23-34	SA13	LEKMA	JBBJZX0000000000	SRR28419012	SAMN40566649	TE and SXT	<i>ataA</i> , <i>patB</i> , <i>pmrA</i> , and <i>tet(M)</i>	<i>cbpD</i> , <i>hysA</i> , <i>lytC</i> , <i>pavA</i> , <i>pce</i> , and <i>psaA</i>	UNK	91	2,155,673	292	20,469	39.9	918,962	1,983	1,999	2	13	1	1
HRV-23-36	SA14	LEKMA	JBBJZW0000000000	SRR28419020	SAMN40566650	TE and SXT	<i>hysA</i> , <i>lytA</i> , <i>lytC</i> , <i>pavA</i> , <i>pce</i> , <i>ply</i> , and <i>psaA</i>	<i>patA</i> , <i>patB</i> , <i>pmrA</i> , and <i>tet(M)</i>	UNK	135	2,057,522	45	131,504	40.0	1,208,424	1,876	1,917	2	38	1	1

^aERY, erythromycin; CLL, clindamycin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole; C, chloramphenicol; UNK, unknown; LEKMA, LEKMA Hospital; KBTH, Korle Bu Teaching Hospital.

concentration of the libraries were subsequently assessed using the 2100 bioanalyzer system (Agilent) and Qubit 4.0 fluorometer, respectively. The libraries underwent dilution to reach a concentration of 2 nM, subsequently pooled, and subjected to sequencing on the Illumina Miseq platform (Illumina Inc., San Diego, CA) using a 2 × 300 bp chemistry. After sequencing, Trimmomatic v.0.39 (4) was employed in the trimming of adaptors and reads with a quality score below 20. FastQC v.1.0 (<https://www.bioinformatics.babraham.ac.uk>) was used in the quality control checks of the reads. Unicycler v.0.5.0 (5) was used in the assembly of the trimmed reads and subsequently assessed using Quast v.5.2.0 (6). All genomes had a Q-score greater than 30, a minimum coverage of 40×, less than 300 contigs, and a minimum contig size exceeding 200 bp. Post assembly, Kmer-Finder v.4.1 (<https://cge.food.dtu.dk/services/KmerFinder/>) (7) was employed in bacterial species identification. ResFinder v.4.1 (<https://cge.food.dtu.dk/services/ResFinder/>) (8), CARD v.3.2.9 (<https://card.mcmaster.ca/>) (9), VirulenceFinder v.2.0 (<https://cge.food.dtu.dk/services/VirulenceFinder/>) (10), and MLST v.2.0 (<https://cge.food.dtu.dk/services/MLST/>) (11) were used in the determination of sequence types, resistance, and virulence genes using default settings. The assembled genomes were annotated using Prokka v.1.14.6 (12).

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Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Beverly Egyir, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

The genomes were deposited in the National Center for Biotechnology Information database with BioProject number [PRJNA952500](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA952500). Table 1 summarizes the antibiotic profile and genomic characteristics of the isolates.

REFERENCES

1. Sadowy E, Hryniewicz W. 2020. Identification of *Streptococcus pneumoniae* and other Mitis streptococci: importance of molecular methods. *Eur J Clin Microbiol Infect Dis* 39:2247–2256. <https://doi.org/10.1007/s10096-020-03991-9>
2. Bogaerts B, Winand R, Van Braekel J, Hoffman S, Roosens NHC, De Keersmaecker SCJ, Marchal K, Vanneste K. 2021. Evaluation of WGS performance for bacterial pathogen characterization with the Illumina technology optimized for time-critical situations. *Microb Genom* 7:000699. <https://doi.org/10.1099/mgen.0.000699>
3. Garriss G, Nannapaneni P, Simões AS, Browall S, Subramanian K, Sá-Leão R, Goossens H, de Lencastre H, Henriques-Normark B. 2019. Genomic characterization of the emerging pathogen *Streptococcus pseudopneumoniae*. *MBio* 10:e01286-19. <https://doi.org/10.1128/mBio.01286-19>
4. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
5. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>
6. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>
7. Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Møller N, Aarestrup FM. 2014. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *J Clin Microbiol* 52:139–146. <https://doi.org/10.1128/JCM.02452-13>
8. Florensa AF, Kaas RS, Clausen P, Aytan-Aktug D, Aarestrup FM. 2022. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microb Genom* 8:1–10. <https://doi.org/10.1099/mgen.0.000748>
9. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK, et al. 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the comprehensive antibiotic resistance database. *Nucleic Acids Res* 51:D690–D699. <https://doi.org/10.1093/nar/gkac920>
10. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52:1501–1510. <https://doi.org/10.1128/JCM.03617-13>
11. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 50:1355–1361. <https://doi.org/10.1128/JCM.06094-11>
12. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>