Georgia Public Health Laboratory (GPHL)

Carbapenemase-Producing Organisms (CPO) Sequencing Report – MOCK version

Tatyana Kiryutina and Dr. Arunachalam Ramaiah

07/17/2024

Figure 1: Distribution of 9 CPO isolates collected during 4/1/2024 – 6/20/2024.

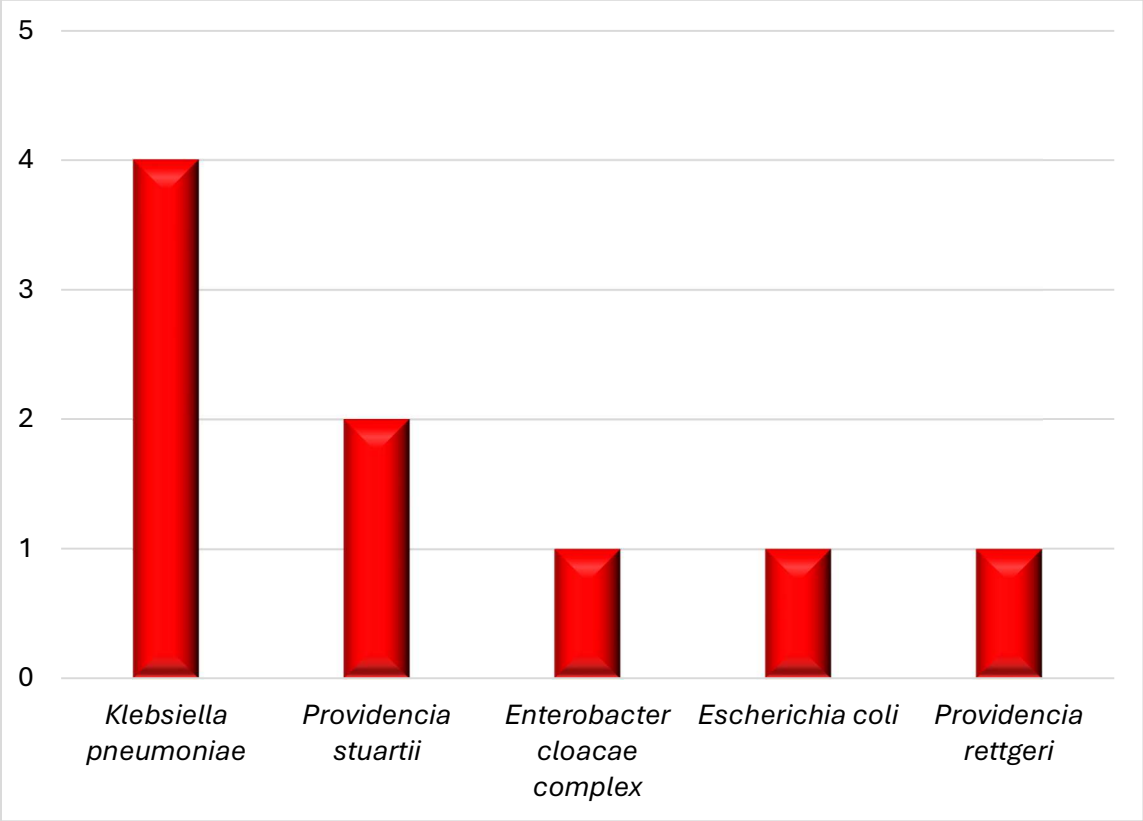

Table 1: Bioinformatic data analysis results on 9 CPO specimens studied.

WGS_ID	Accession_ID	Collection_Date	Source	Organism	MLST_ST
2024LY####1	24C00###10	4/1/2024	bone	*Enterobacter cloacae*	78
2024LY####2	24C00###11	5/2/2024	blood	*Klebsiella pneumoniae*	1691
2024LY####3	24C00###12	5/3/2024	sacrum drainage	*Escherichia coli*	410
2024LY####4	24C00###13	5/4/2024	blood	*Providencia stuartii*	Not identified
2024LY####5	24C00###14	5/5/2024	urine	*Klebsiella pneumoniae*	147
2024LY####6	24C00###15	5/6/2024	bone	*Klebsiella pneumoniae*	16
2024LY####7	24C00###16	6/1/2024	urine	*Providencia stuartii*	Not identified
2024LY####8	24C00###17	6/2/2024	urine	*Klebsiella pneumoniae*	234
2024LY####9	25C00###18	6/20/2024	bone	*Providencia rettgeri*	Not identified

Table 2: PHoeNIx pipeline was used to identify GAMMA Beta Lactam Resistance Genes (column 1) in the specimens studied. The red color cells highlight the presence of AMR genes in the given specimens.

	2024LY####1	2024LY####2	2024LY####3	2024LY####4	2024LY####5	2024LY####6	2024LY####7	2024LY####8	2024LY####9
blaACT-24	0	1	0	0	0	0	0	0	0
blaCTX-M-15	0	0	1	1	0	0	0	0	0
blaEC-15	0	0	1	0	0	0	0	0	0
blaIMP-27	0	0	0	0	0	0	0	0	1
blaKPC-4	0	1	0	0	0	0	0	0	0
blaNDM-1	1	1	0	0	1	1	0	1	0
blaNDM-5	0	0	1	1	0	0	0	0	0
blaOXA-181_blaOXA-48-like	0	0	0	0	0	0	1	0	0
blaOXA-1_blaOXA-1-like	0	1	1	0	0	0	0	0	0
blaSHV-122-partial	0	0	0	0	0	0	0	1	0
blaSHV-1	0	0	0	0	0	0	1	0	0
blaSHV-26	1	0	0	0	0	0	0	0	0
blaSHV-30	0	0	0	0	1	0	0	0	0
blaSHV-39-partial	0	0	0	1	0	0	0	0	0
blaSHV-7	0	1	0	0	0	0	0	0	0
blaTEM-176	0	0	0	0	0	0	1	0	0
blaTEM-1	0	1	0	1	0	0	0	1	0

Results:
From mid-June, GPHL has sequenced and submitted raw sequencing data for 9 quality CPO pathogens to NCBI with specimen collection date range of 4/1/2024 – 6/20/2024 (Figure 1). NCBI submissions of the data were conducted using de-identified whole-genome sequencing (WGS) IDs (Table 1).

Multi-locus sequence typing (MLST) was performed both by the FLAQ-AMR and PHoeNIx pipelines against traditional PubMLST typing schemes. The final decision for MLST was based on matching genus and results from both tools for the sake of consistency. Overall, results from multiple schemes were generated by PHoeNIx for *Escherichia coli* and *Acinetobacter* spp. For these organisms, the Achtman scheme is selected for *E. coli* and the Pasteur scheme for *Acinetobacter* spp.

Antimicrobial resistance (AMR) gene detection: The PHoeNIx pipeline summarized the beta-lactam resistance genes present in each isolate from hits against three databases: AMRFinderPlus, ARG-ANNOT, and ResFinder. The gene presence-absence matrix is included in Table 2.

Analysis pipelines and bioinformatics tools:

- FLAQ-AMR (Florida Assembly Quality - AMR Detection): https://github.com/BPHL-Molecular/flaq_amr. Within this analysis pipeline, the following tools generate key results:
    - MLST: https://github.com/tseemann/mlst
- CDC's 🔥🐦‍🔥PHoeNIx: A short-read pipeline for healthcare-associated and antimicrobial resistant pathogens (https://github.com/CDCgov/phoenix/wiki).
    - This pipeline also characterizes isolates and in addition, conducts automatic quality control checks (QC) based on the Antimicrobial Resistance Laboratory Network (ARLN) guidance for Whole-Genome Sequencing (WGS) of HAI AR pathogens v2.
    - GAMMA: https://github.com/rastanton/GAMMA (Gene Allele Mutation Microbial Assessment) for AMR gene detection.
- Ribosomal MLST: https://pubmlst.org/species-id for any additional species confirmation.
- Raw sequencing data can be found in the CDC HAI-Seq Gram-negative bacteria BioProject on NCBI: https://www.ncbi.nlm.nih.gov/bioproject/288601 (Accession PRJNA288601).

DISCLAIMER: The identification methods used and the data summarized are for public health surveillance or outbreak investigational purposes only and must NOT be communicated to the patient, their care provider, or placed in the patient's medical record. These results should NOT be used for diagnosis, treatment, or assessment of individual patient health or management. This report is for internal DPH use ONLY.