

# WGS as an Analytical Tool in the Investigation of a *Pseudomonas aeruginosa* Outbreak in Artificial Tears

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## Abstract

In December 2022, the FDA began collaborating with the CDC to investigate a multistate outbreak of antibiotic-resistant *Pseudomonas aeruginosa* infections associated with artificial tears. As part of this investigation, the FDA collected samples from lots of opened and unopened product. The samples were sent to FDA medical products laboratories for testing where *Pseudomonas aeruginosa* was recovered from both open and unopened products. The genomic DNA of fifteen isolates was extracted and sequenced using Illumina MiSeq systems. Bioinformatic analyses were performed using tools available in GalaxyTrakr. The sequence data was subsequently submitted to the NCBI database. The CFSAN SNP Pipeline was used to compare the FDA and outbreak isolates. Thirteen isolates clustered with the outbreak isolates with a mean of 5.27 SNPs. The isolates had a sequence type (ST) 1203 and exhibited one of two distinctive antimicrobial resistance (AMR) profiles. Seven isolates encoded the *bla*<sub>VIM-80</sub> and *bla*<sub>GES-9</sub> genes which was the profile associated with the outbreak and six isolates encoded the *bla*<sub>VIM-2</sub> and *bla*<sub>GES-9</sub> genes. Additionally, two isolates matched each other but did not cluster with the outbreak isolates. These isolates had an ST 357 and did not encode any of the *bla*<sub>VIM</sub> and *bla*<sub>GES</sub> genes. Antibiotic-resistant *Pseudomonas aeruginosa* is a major healthcare-associated pathogen worldwide. The variants associated with this outbreak had not been previously detected in the US. This investigation led to a nationwide recall of all lots of this product and underscored the value of WGS as an analytical tool for the FDA medical products labs.

## Introduction

Ophthalmic drug products, such as eye drops, pose a heightened risk of harm to users because drugs applied to the eyes bypass some of the body's natural defenses. FDA regulations for over-the-counter (OTC) ophthalmic drugs require that multi-dose products contain preservatives if the product does not contain inherent antimicrobial activity. The product implicated in this outbreak was a preservative-free solution produced by a foreign manufacturer, packaged in multi-dose bottles, and sold to US consumers over the internet and to US healthcare facilities through medical product distributors.

On January 2023, CDC recommended that clinicians and patients stop using and discard Brand A artificial tears due to potential bacterial contamination. On January 2023, FDA began collecting product samples and sending them to the medical products labs for analysis. The manufacturer issued a voluntary nationwide recall for all unexpired lots of Brands A artificial tears on 2 February 2023. The FDA began an onsite inspection of the manufacturer on 20 February 2023 and found significant violations of Current Good Manufacturing Practice (CGMP) regulations for finished pharmaceuticals. A warning letter was issued to the manufacturer in October 2023.

FDA response by the numbers:

- ✓ 43 samples collected from medical facilities, distributors & consumers
- ✓ 18 unopened and 6 open lots of product
- ✓ 14 unopened lots were found non-sterile
- ✓ 6 lots were contaminated with *Pseudomonas aeruginosa*
- ✓ 700+ isolates from multiple species were recovered
- ✓ 150+ isolates were analyzed by WGS

**Table 1.** Bacterial species most recovered from Brand A artificial tears.

Species	Number of lots
<i>Pseudomonas aeruginosa</i>	6
<i>Pseudomonas putida</i>	11
<i>Pseudomonas plecoglossicida</i>	2
<i>Bacillus licheniformis</i>	2
<i>Bacillus paralicheniformis</i>	2
<i>Bacillus altitudinis</i>	1
<i>Bacillus infantis</i>	1

## Materials and Methods

**Sterility testing:** Opened and unopened containers were tested as per United States Pharmacopeial (USP) Chapter <71> Sterility Tests. All unique bacterial morphologies were identified by Sanger sequencing.

**Sanger sequencing:** All recovered bacterial isolates were identified using the MicroSEQ® Microbial Identification System targeting the 16S rDNA gene.

**WGS:** DNA was extracted from bacterial isolates using the QIAcube system with DNeasy Blood and Tissue Kit. Libraries were prepared using Illumina DNA Prep Kits. WGS was performed on MiSeq systems using V2 and V3 chemistries.

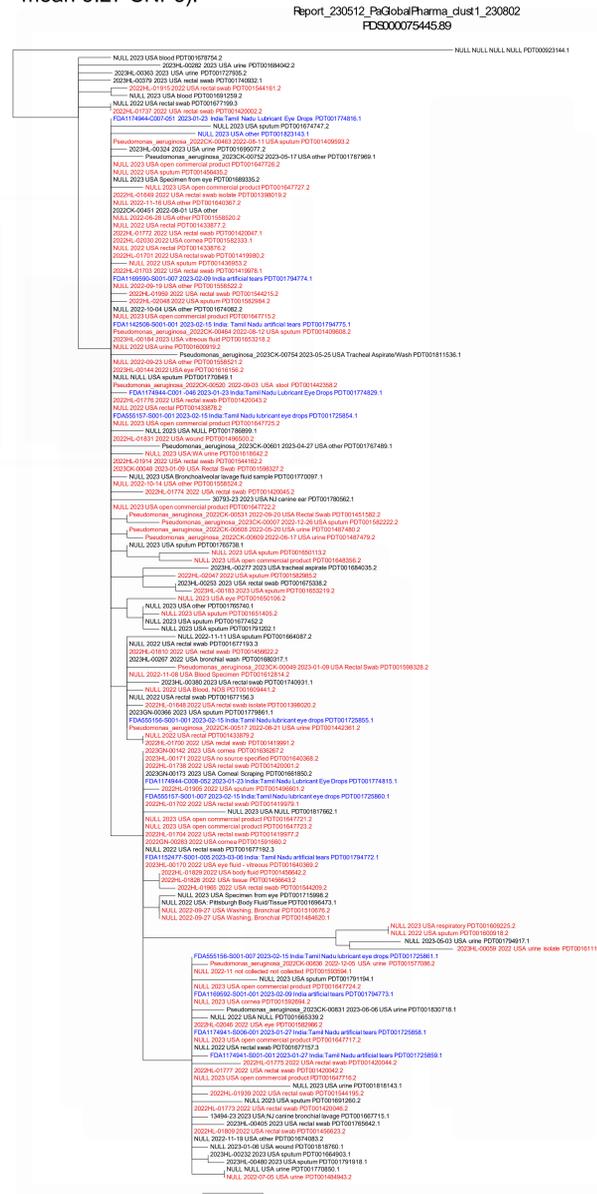
**Data analysis:** Trimming, assembly, quality assessments and MLST were performed using MicroRunQC v1.1. Taxonomic assignments were performed using Kraken2 (Galaxy Version 2.1.1+galaxy1) and Sendsketch (Galaxy Version 38.73). Antimicrobial resistance genes were identified using AMRFinderPlus (Galaxy Version 3.11.11+galaxy1). Genetic relatedness was assessed using the CFSAN SNP Pipeline Version: v2.2.1.

**Data Sharing:** Sequencing data was uploaded to the Sequence Read Archive (SRA) at the NCBI under BioProject PRJNA874709.

**Disclaimer:** Mention of commercial products does not constitute an FDA endorsement.

## Results

**Figure 1.** CFSAN SNP Pipeline analysis of *Pseudomonas aeruginosa* ST1203 with *bla*<sub>VIM-80</sub> and *bla*<sub>GES-9</sub> and *P. aeruginosa* with ST1203 with *bla*<sub>VIM-2</sub> and *bla*<sub>GES-9</sub>. Clust1 (PDS000075445.89; *Pseudomonas aeruginosa*) includes 13 of the isolates analyzed (blue labels). They are part of a large group of matching isolates that also includes other product and clinical isolates (isolates associated with this outbreak are labelled red; mean 5.27 SNPs).



**Table 2.** Antimicrobial resistance genes identified by AMRFinder Plus in the *Pseudomonas aeruginosa* isolates.

Antibiotic Class	Antibiotic Subclass	Gene Symbol
Beta-lactam	Carbapenem	<i>bla</i> <sub>VIM-80</sub>
	Carbapenem	<i>bla</i> <sub>VIM-2</sub>
	Beta-Lactam	<i>bla</i> <sub>OXA-395</sub>
	Cephalosporin	<i>bla</i> <sub>OXA-10</sub>
	Cephalosporin	<i>bla</i> <sub>GES-9</sub>
Aminoglycoside	Streptomycin	<i>aadA1</i>
	Aminoglycoside	<i>rmtF2</i>
	Streptomycin	<i>aph(6)-Ic</i>
	Streptomycin	<i>aph(3'')-Ib</i>
Phenicol	Kanamycin	<i>aph(3')-Ib</i>
	Gentamicin	<i>aac(6')-Ib</i>
Fluoroquinolone	Fluoroquinolone	<i>crpP</i>
	Sulfonamide	Chloramphenicol/Florfenicol
Chloramphenicol/Florfenicol		<i>floR</i>
Chloramphenicol		<i>catB</i>
Chloramphenicol		<i>catB7</i>
Tetracycline	Tetracycline	<i>tet(G)</i>
	Fosfomycin	Fosfomycin
Macrolide		Macrolide
	Trimethoprim	Trimethoprim

## Conclusion

According to the CDC, more than 2.8 million antimicrobial-resistant infections occur in the U.S. each year and more than 35,000 people die as a result. The strains recovered in this outbreak harbored genes conferring resistance to carbapenems. This beta (β)-lactam class of antibiotics are a last-line of defense used to treat serious multidrug-resistant infections. These genes are encoded on mobile genetic elements that can be transferred between strains and across bacterial species, making them a serious public health threat due to the potential for rapid, widespread dissemination. This multistate outbreak of antibiotic-resistant *Pseudomonas aeruginosa* ultimately affected more than 80 patients and led to 4 patient deaths and at least 14 cases of vision loss.

Analysis of unopened units found that 14 lots were non-sterile revealing that the product was intrinsically contaminated with microorganisms. The isolates recovered were further characterized using whole genome sequencing and compared to isolates in the NCBI national database. *P. aeruginosa* isolates from 6 different lots of product were found to be close genetic matches to clinical isolates associated with this outbreak. Furthermore, the isolates shared the same rare antibiotic resistance genes.