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Short Communication

FIRST COMPLETE GENOME SEQUENCE OF OCULAR ISOLATE CARRYING BLA$_{VIM-2}$ MEDIATED MULTI-DRUG-RESISTANT(MDR) *PSEUDOMONAS AERUGINOSA* VRFPA04 FROM INDIA AND TWO DRAFT GENOME SEQUENCE OF OCULAR ISOLATES *PSEUDOMONAS AERUGINOSA* VRFPA03 AND VRFPA05 HARBORING NOVEL BLA$_{DIM-1}$ AND BLA$_{GES-9}$ RESPECTIVELY

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ABSTRACT

Objective: Ocular infections caused by Multi-Drug resistant (MDR) *Pseudomonas aeruginosa* are rare but increasingly identified recently. In this context, we have applied Next Generation Sequencing (NGS) based whole genome analysis on three ocular isolates of MDR *P. aeruginosa* to explore the drug resistance determinants and genomic level variations.

Methods: Three ocular isolates namely *P. aeruginosa* VRFP03, VRFPA04 from two different Keratitis patients (corneal button) and VRFPA05 isolated from the intraocular specimen (Vitreous humor) collected from an endophthalmitis patient was included in this study. Phenotypically VRFPA03, VRFPA04, VRFPA05 showed resistant to a wide group of antibiotics and hence they were taken up for Ion Torrent-PGM based whole genome study.

Results: Here, we report the first complete genome sequence of MDR *P. aeruginosa* VRFPA04 isolated from the Indian keratitis patients clinical specimen (corneal button) submitted to L & T Microbiology Research Centre, Vision Research Foundation, Sankara Nethralaya, Chennai, Tamil Nadu, India. The circular chromosome of *P aeruginosa* VRFPA04 was published under the NCBI accession number CP008739.2. Two Draft genome sequences of MDR *P. aeruginosa* VRFPA03 and VRFPA05 were published under the NCBI accession number ATNK01000000.1 and AXZJ01000000.1 respectively.

Conclusion: Preliminary genomic analysis on *P. aeruginosa* VRFPA03, VRFPA04 and VRFPA05 revealed the presence of Metallo Beta-lactamase (MBL) genes bla$_{Dim-1,}$ blaV$_{im-2}$ and bla$_{Ges-9}$ genes respectively. These MBL genes were concordance with a phenotypic pattern of carbapenem resistance among VRFPA03, VRFPA04 and VRFPA05 strains, respectively. We are proud to share that this is the first of its kind to report bla$_{DIM-1}$ from an Indian isolate especially from an Ocular origin.

Keywords: Complete Genome, Pseudomonas aeruginosa, Multi-Drug Resistant, Genomics.

Pseudomonas aeruginosa (P. aeruginosa) is an important nosocomial, gram-negative, opportunistic pathogen infecting immuno-compromised humans. *P. aeruginosa* contributes to a significant proportion of bacterial keratitis, accounting for 6–39% of the cases in the United States and 8–21% in South India [1, 2]. Contact lens wearers were more prone to get *P. aeruginosa* infection due to the ubiquitous nature of the bacterium, and it is a common contaminant of contact lens cases leading to ocular infections [3]. Occasionally, severe *Pseudomonas* keratitis can invade intraocular tissues either by infection or by trauma, resulting into endophthalmitis, a sight-threatening condition [4]. The presence of the multidrug-resistant (MDR) *P. aeruginosa* in patients with ocular infections seems to be increasing in consonance with the increase of drug resistance among systemic bacterial infections [5].

In this context, we report the first complete genome sequence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* VRFPA04 from India, and two draft genome sequence of *P. aeruginosa* VRFPA03 and VRFPA05. *P. aeruginosa* VRFP03 and VRFPA04 were isolated from two different keratitis patients (corneal button) and VRFPA05 was isolated from vitreous humor sample of a patient clinically suspected with postoperative endophthalmitis. Phenotypically, *P aeruginosa* VRFPA03, VRFPA04 and VRFPA05 exhibited higher resistant to almost all commonly used drugs, including carbapenems, except aztreonam and colistin. Whereas, VRFPA03 and VRFPA05 were additionally susceptible to imipenem drug confirmed by Minimum inhibitory Concentration (MIC) and standard Kirby-Bauer methods [6-8]. Hence, they were taken up for whole genome sequencing using in-house Ion Torrent (PGM) sequencer with 400-bp read chemistry (Life Technologies). The Sequencing protocol was followed as per previous report [9-11] and it produced 2.3 million reads (2,369,461) [VRFPA03], 2.6 million reads (2,631,848) [VRFPA04] and 1.8 million reads (1,895,953) [VRFPA05] respectively under optimal parameters. The best-assembled result using optimal parameter produced 150, 80 and 170 contigs with 46x, 81X and 65x genome coverage, for VRFPA03, VRFPA04 and VRFPA05 genome respectively. Whole genome sequence length of 7,037,729 bp, 6,998,695 bp and 7,050,363 bp for VRFPA03, VRFPA04 and VRFPA05 respectively was obtained and they were submitted and published in NCBI under the draft genome accession no ATNK01000000.1, AWWZ01000000.1. and AXZJ01000000.1 respectively.

Since, VRFPA04 genome resulted in lowest contig number and its highly drug-resistant comparable to VRFPA03 and VRFPA05, it was chosen for genome finishing. Enrichment of VRFPA04 draft genome sequences by Mauve analysis yielded 78 contigs and the gaps between 78 contigs were closed by designing 77 set of primers using CLC Genomics Workbench-Genome Finishing module software version 6.5.1 (CLC bio, Germantown, MD) and the gaps were filled in as per earlier report [7]. The assembled data was subjected to RAST annotation [12] and Prokaryotic Genome Automatic Annotation Pipeline (PGAAP). Closing the gaps of VRFPA04 draft genome with additional parameters yielded a circular complete chromosome of 6,818,030 bp (6.8 Mb) genome size were the first of its kind to report from India on complete circular chromosome of MDR *P. aeruginosa* and published under the NCBI accession no CP008739.2, which happen to be the third largest complete genome available in the Genbank to date. Complete genome sequence of *P. aeruginosa* VRFPA04 published in NCBI on 21st August 2014 and it was

considered as the largest genome available in the NCBI as on august 2014. *P. aeruginosa* strain Carb01-63 (CP011317.1) and *P. aeruginosa* NCGM1984 (AP014646.1) (unpublished data) was now considered as the two largest genome published in NCBI after VRFPA04. Despite, draft genomes with more than 6.8Mb are available, they were incomplete and the higher genome size may be due to duplication of same regions covered multiple times during the process. In our study, duplicate sequences were trimmed during the analysis of complete genome process of VRFPA04. PGAAP annotation deciphered, *P. aeruginosa* VRFPA04 chromosome consisted of 5,939 protein-encoding genes (excluding 64 pseudogenes), 5,778 coding sequences (CDS), 69 tRNA genes, 27 rRNA genes, and 1 noncoding RNA. Whereas *P. aeruginosa* VRFPA03 and VRFPA05 comprised of 5,973 and 6,155 protein-coding genes, with 57 and 66 tRNA genes followed by 5 and 18 ribosomal encoding rRNA genes, respectively. Three non-coding RNAs (ncRNAs) were found in the VRFPA03 genome and no ncRNAs were found in the VRFPA05 genome. Identity of the strains were confirmed by *in silico* multilocus sequence typing (MLST) (http://cge. cbs. dtu. dk/services/) using the *Pseudomonas aeruginosa* MLST database targeting seven potential loci (*acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE*) and classified the *P. aeruginosa* VRFPA03 as novel unknown sequence type and VRFPA04 under (ST)-823 and VRFPA05 under ST-1203. The preliminary genome-wide analysis of drug resistance mechanism using Resfinder tool [13] predicted the presence of novel bla_{Dim-1} in VRFPA03 genome, which is the first of its kind to report of this ($bla_{Dim-1)}$ Dutch imipenamase gene from India, especially from an ocular isolate. Whereas bla_{Vim-2} gene identified from VRFPA04 and bla_{Ges-9} from VRFPA05 defend the MDR mechanism of resistance against cephalosporins and carbapenem drugs. Further, in-depth analysis of these strains will be carried out and published in near future.

Nucleotide sequence accession numbers. The complete Genome Sequence of *P. aeruginosa* strain VRFPA04 has been deposited at DDBJ/EMBL/GenBank under accession CP008739.2 and the draft genome sequence of VRFPA03, VRFPA04, and VRFPA05 has been deposited at DDBJ/EMBL/GenBank under accession no. ATNK01000000.1, AWWZ01000000.1. and AXZJ01000000.1 respectively.

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CONFLICT OF INTERESTS

Declared None

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