Virulence genome analysis of *Pseudomonas aeruginosa* VRFPA10 recovered from patient with scleritis

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**A B S T R A C T**

Infectious keratitis is a major cause of blindness, next to cataract and majority of cases are mainly caused by gram negative bacterium *Pseudomonas aeruginosa* (*P. aeruginosa*). In this study, we investigated a *P. aeruginosa* VRFPA10 genome which exhibited susceptibility to commonly used drugs in vitro but the patient had poor prognosis due to its hyper virulent nature. Genomic analysis of VRFPA10 deciphered multiple virulence factors and *P. aeruginosa* Genomic Islands (PAGIs) VRFPA10 genome which correlated with hyper virulence nature of the organism. The genome sequence has been deposited in DDBJ/EMBL/GenBank under the accession numbers LFMZ01000001-LFMZ01000044.

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*Pseudomonas aeruginosa*
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Ocular infection

2. Background

*Paeruginosa* is the predominant gram negative bacterium often associated with ocular infections such as keratitis and scleritis. Scleritis is a severe painful condition caused by inflammatory process in the sclera, which may involve cornea, adjacent episclera and underlying uvea may turn into blindness condition [1]. In the current study, we investigated a 38 years old male patient with history of injury caused by foreign body and he was initially diagnosed and treated for perforated corneal ulcer. But, the patient subsequently developed into scleritis condition, despite appropriate medical management.

3. Materials and methods

The specimen was collected by an ophthalmologist as per standard method [2]. In brief, edge of the ulcer was firmly scraped using Bard Parker blade No. 15 after removal of debris or discharge in the vicinity. Several scrapings were collected and used in a sequence to inoculate culture media. Wherein, “C” curve on blood agar, MacConkey agar and inoculated on Brain Heart Infusion Broth (BHB) initially. Upon confirmation of *Paeruginosa* by biochemical methods, it was subcultured on Mueller Hinton agar plates to assess pigment production and was incubated at 37°C for 24 h.

After 24 h colonies greenish pigmented colonies morphologically resembling *P. aeruginosa* had grown in all the culture plates and identification was using a combination of colonial morphology with bluish
green pigmentation on MHA plate, non lactose fermenting colonies in MacConkey agar, presence of motility, positive reaction for oxidase, catalase, simmon’s citrate medium, nitrate reduction and mannitol sugar test and negative reaction for urease, indole, Methyl Red, Vogues Prosker test, sucrose, lactose and maltose sugar tests were observed [3, 4].

Further the organism was genotypically confirmed up to species level using 16s ribosomal RNA gene based sequencing result against blast tool available at NCBI database revealed 99% homology to all the existing P. aeruginosa strains in the database inclusive of our previously reported strains VRFPAP01-VRFPAP09 [5–10], hence the strain isolated from scleritis was designated as P. aeruginosa VRFPAP10. Irrespective of the fact that P. aeruginosa VRFPAP10 was phenotypically susceptible but the patient finally underwent Therapeutic Penetrating Keratoplasty (TPK). Whole genome study was undertaken by utilizing Ion Torrent (PGM) sequencer with 400-bp read chemistry (Life Technologies) accordace with manufacturer’s instructions. In brief, genomic DNA from VRFPAP10 was isolated from the overnight cultures with DNeasy miniprep kit (Qiagen, Hilden, Germany) and the sequencing protocol was followed as per previous study [5–10].

4. Genomic analysis

It is to be noted that there are no or scanty study available on Virulence factor and molecular mechanism of pathogenesis in P. aeruginosa mediated scleritis [11–14]. Henceforth, we undertook this study to analyze VRFPAP10 whole genome to unveil the genomic nature of virulence mechanism and drug resistance genes which may be involved in drug resistance in in vitro condition but may show susceptibility in in vi tro tests.

Data of 56× coverage was produced after initial quality analysis and reference based assembly with P. aeruginosa VRFPAP04 (CP0008739.2) yielded 44 contigs with 7,728,786 bp (7.7 Mb genome size). The VRFPAP10 genome was published in NCBI under the accession number LFMZ00000000.1. The genes were annotated by NCBI Prokaryotic Genome Reference guide assembly with manufacturer’s instructions. In brief, genomic DNA from VRFPAP10 was isolated from the overnight cultures with DNeasy miniprep kit (Qiagen, Hilden, Germany) and the sequencing protocol was followed as per previous study [5–10].

| Table 1
| Genomic Features of P. aeruginosa VRFPAP10 |
| Features | VRFPAP10 |
| Specimen | Scleral scraping |
| NCBI accession no | LFMZ00000000.1 |
| Genome size | 7,728,786 bp |
| No of contigs | 44 |
| No of proteins | 5252 |
| No of genes | 6431 |
| CRISPR arrays | 0 |
| Ribosomal RNA | 73 |
| rRNA | 80 |
| Noncoding RNA | 51 |
| Pseudo genes | 501 |
| Frameshifted genes | 555 |
| Genome coverage | 56× |
| Reference guided assembly | CP0008739.2 |
| NCBI Accession WGS ID | LFMZ00000000.1 |
| MLST Type | ST-313 |
| Beta lactamas | blaTem |
| Gene | Ctx-M-15 |
| Total no of phages | ND |
| Aminoglycoside genes | ahp(3)fol |
| Fusfomycin | fosA |
| Phenicol | CatB7 |
| Tetracycline | ND |
| Trimethoprine | ND |
| Genomic Island | PAGI-1-2,9 (partial) |
| Integron | ND |
| Pathogenic island | ND |

ND - Not detected.

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