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Short Communication

Draft genome sequence of bla_{Veb-1}, bla_{oxa-10} producing multi-drug resistant (MDR) *Pseudomonas aeruginosa* strain VRFPA09 recovered from bloodstream infection.

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) bacteremia causes significant mortality rate due to emergence of multidrug resistant (MDR) nosocomial infections. We report the draft genome sequence of *P. aeruginosa* strain VRFPA09, a human bloodstream isolate, phenotypically proven as MDR strain. Whole genome sequencing on VRFPA09, deciphered betalactamase encoding bla_{veb-1} and bla_{OXA-10} genes and multiple drug resistance, virulence factor encoding genes.

Key words: *Pseudomonas aeruginosa*, extended spectrum betalactamases, next generation sequencing, blood.

Introduction

Bloodstream infections caused by *Pseudomonas aeruginosa* are serious infections with significant patient mortality and health-care costs (Micek *et al.*, 2005). The mortality rates for patients with Multi-drug-resistant (MDR) is 34% and drug susceptible *P. aeruginosa* is 22%. The high mortality and morbidity due to infections associated with *P.aeruginosa* drug resistance urges increased resource utilization leading to increased cost and time (Nathwani *et al.*, 2014). Emergence of acquired resistance during anti-pseudomonal therapy among initially susceptible isolates accomplished to life threatening infectious disease with limited or no further treatment option (Micek *et al.*, 2005).

Herein, we announce the draft genome sequence of MDR *P. aeruginosa* VRFPA09 strain isolated from human blood specimen at L & T Microbiology Research Centre, Vision Research Foundation, Sankara Nethralaya, Chennai, India. Phenotypically, VRFPA09 showed resistance to more than one agent in three or more antibiotic groups such as penicillins, cephalosporins, aminoglycosides and fluoroquinolones. Admitting, VRFPA09 susceptible to imipenem drug but it showed resistance to almost all the commonly used drugs including meropenem and tested positive for Extended spectrum Beta lactamases (ESBLs) production by CLSI method (Jiang *et al.*, 2006).

In this context, we have selected VRFPA09 isolate for whole genome sequencing based genomic analysis owing to multi drug resistance, virulence factors, intrinsic and extrinsic genomic factors involved in VRFPA09 genome. Ion Torrent (PGM) sequencer with 400-bp read chemistry (Life Technologies) was used to sequence the isolate, according to manufacturer's instructions. Genomic DNA from VRFPA09 was isolated from overnight cultures with DNeasy miniprep kit (Qiagen, Hilden, Germany). Initial identification and confirmation of the monoclonality of the strain VRFPA09 was verified through 16s ribosomal RNA gene based Sanger sequencing. The NGS sequencing protocol has been followed as mentioned in our previous study (Malathi *et al.*, 2013; Murugan *et al.*, 2014). The generated data with phred score ≤ 30 was filtered and the raw data was assembled by both *de novo* and reference based method using both Mira v. 3.4.1.1 embedded in Torrent suite server version 4.0.12 and CLC Genomics Workbench software version 6.5 (CLC bio, Germantown, MD) against reference strain *P. aeruginosa* VRFPA04 (NCBI Accession no: CP008739.1). Upon assembling the raw reads, 80 contigs

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with 75x coverage was obtained with N50 value as 6,165,520. The assembled contigs were published in the NCBI under the accession no JAAO00000000.1. The genes were annotated by NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP, http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). The genomic features of VRFPA09 included genome size of 6,711,239 bp (6.7MB) containing 6,568 coding sequences with 6,014 proteins and a total of 65 RNA genes including 56 tRNA, 6 rRNA and 1 non-coding RNA.

Genomic analysis carried out using Web server ResFinder (Zankari *et al.*, 2012) and manual examination detected the following resistance genes aadA1, aph(3')Iib (aminoglycoside), Sul1 (sulfonamide), CatB7 (chloramphenicol), TetG (tetracycline), *dfrB5 (* Trimethoprim) and *fosA* (fosfomycin) in VRFPA09 genome. In addition, a novel integron designated as In1147 comprised of $bla_{Veb\text{-}1}$,$bla_{OXA\text{-}10,}$ dfrB2, aacA7, aadA1genes in array of gene cassettes which confers broad spectrum resistance to third generation cephalosporins, aminoglycosides and meropenem drug was detected (Yin *et al.*, 2008). Analysis using Blast revealed the presence of Type 3 Secretion system (T3SS) cytotoxin encoding exoenzyme U gene (Gawish *et al.*, 2013). However, further study on VRFPA09 genome is required for detecting multifactorial resistance genes and its molecular mechanism of resistance.

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