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Mini Review

Profilling of Virulence Determinants of Methicillin Resistant *Staphylococcus aureus* Recovered from Dog in India

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen causing a number of diseases in animals as well as humans. Therefore, whole-genome sequencing of an MRSA strain isolated from dog suffering from pyoderma, was performed to better characterise the strain and to characterize the mechanism of antimicrobial resistance and virulence determinants. MRSA isolate AMD was sequenced on an Illumina MiSeq platform. The genome was assembled with CLC genomic workbench 23.0.5 and was annotated using PGAP v.4.3. The strain was characterised using spaTyper 1.0, SCCmecFinder v.1.2 and MLST 2.0 server. Antimicrobial resistance genes and virulence determinants were identified using AMRFinderPlus and VFDB, respectively. MRSA strain AMD has an estimated genome size of 2694681 bp with a GC content of 32.77% and harbours 4 antimicrobial resistance genes, 2 Point mutations and 60 virulence determinants. This strain having more virulence determinants so could be used as a reference strain for comparative genomic analysis of other MRSA strains isolated from dogs in India.

Keywords: Antimicrobial Resistance; MRSA; Pyoderma; Virulence

Staphylococcus aureus is an opportunistic pathogen causing numerous skin and soft tissue infections in companion animals [1]. *S. aureus* from animals are also known to carry several antimicrobial resistance determinants that can be transferred to humans and other animals [2]. MRSA is a group of *S. aureus* strains that have developed resistance to methicillin and to the majority of the beta-lactam antibiotics following the acquisition of a *mecA* gene [3]. MRSA is recognized as one of the leading pathogens responsible for nosocomial and community-associated infections worldwide [4].

Here we report a draft genome of MRSA strain AMD, isolated from dog suffering from pyoderma presented at Veterinary Clinical Complex, Navsari, Gujarat, India. The isolate was cultured on mannitol salt agar (Himedia, Ltd.) at 37_C. DNA was extracted from the isolated pure cultured colonies using the QIAamp DNA mini kit (Qiagen). The quality of the extracted DNA was checked using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The library preparation was performed using a Nextera XT DNA Library Preparation kit. After library preparation, quantity and quality were checked using a Qubit 3.0 Fluorometer and Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) using high sensitivity DNA 1000 chip, respectively. Sequencing was performed using Illumina's reagent kit v2 on an Illumina MiSeq benchtop sequencer (Illumina, San Diego, CA, USA). The quality of raw data was checked using FastQC (version 0.11.9), while the low quality sequence data were removed using PRINSEQ-lite (version 0.20.4). Cutadapt (version 3.3) was used for the adaptor trimming. De novo assembly was performed on the CLC genomic workbench 23.0.5.

De novo assembly generated 131 contigs, with the largest contig of 84188 bp and an N50 value of 36097 bp. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.4.3 was used for annotation, which determined a total genome length of 2694681 bp with 2633 coding sequences (CDSs), 2694 genes and 2569 coding genes. Strain AMD has a GC content of 32.77%, 52 tRNAs, 5 rRNAs (two 5S, two16S and one 23S rRNAs), 4 ncRNAs and 64 pseudogenes.

Molecular typing using spaTyper 1.0 (https://cge.cbs.dtu.dk/services/spatyper/) and SCCmecFinder v.1.2 (https://cge.cbs.dtu.dk/ services/SCCmecFinder/) showed that strain AMD belongs to spa type t304 and having SCC*mec*_type_Iva (2B). The MLST 2.0 server (https://cge.cbs.dtu.dk/services/MLST/) identified sequence type ST-6 for strain AMD.

The NCBI's AMRFinderPlus [5] detected the methicillin resistance gene *mecA* and *mecR1*, the fosfomycin resistance gene *fosB*, the tetracycline resistance gene *tet (38)*, as well as point mutations in the genes *gyrA* and *parC* conferring resistance to fluoroquinolones. The Virulence Factor Database (VFDB) [6] identified 60 virulence determinants, including hemolysins (*hld, hly/hla, hlgB, hlgC, hlb*), leukocidin (*lukF-PV*), ica operon (*icaA, icaB, icaC, icaD, icaR*), capsular polysaccharide synthesis enzymes (*cap8A, cap8B, cap8C, cap8D, cap8E, cap8F, cap8G, cap8H, cap8I, cap8J, cap8K, cap8L, cap8M, cap8N, cap8O, cap8P*), adherence factors (*map, fnbA, fnbB, sdrD, sdrE, ebp, cna*), various enzymes (*aur, hysA, geh, lip, sspC, sspB, sspA, vWbp*), immune evasion factors (*adsA, sbi, esxA, esaA, essA, esaB, essB, essC, esaC, esxB*) and iron regulation proteins (*isdA, , isdB, isdC, isdD, isdE, isdF, isdG, srtB*).

Till date very less data available for whole genome sequencing of *Staphylococcus aureus* recovered from dog in India, so this report provides a base for further detailed study of MRSA.

Accession numbers

The genome sequence of strain AMD has been deposited in NCBI GenBank with the accession no. JAWDCT000000 under BioProject accession no. PRJNA1022430._

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Conflict of Interests

The authors declare no conflict of interest.

Ethical Approval

Not required.

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