

DATA NOTE

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Whole genome sequence of *Staphylococcus aureus* strain 37 M isolated from cows with subclinical mastitis in Uganda

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Abstract

Objective This study presents the whole genome sequence of *Staphylococcus aureus* strain 37M isolated from cows with subclinical mastitis in Kiruhura district, Uganda. The objective was to characterize the genome to understand the isolate's resistance and virulence potential. The genome sequence accompanying metadata are available under the accession GCA_052439145.1.

Data description The final assembled genome has a genome size of 2,795,296 bp, Contig N50 (53.2 kb), L50 16, Genome coverage 2.79534e + 06x, Assembly level contigs and a GC content of 32.5%. It contains 2,748 CDSs, 2,819 genes, 2,584 predicted protein-coding genes, 57 tRNA genes, 3 complete rRNAs (23 S), 5 non-coding RNAs and 159 Pseudo Genes. Quality analysis of the genome using CheckM analysis (v1.2.4) revealed 96.72% completeness, and 0.7% contamination. Average Nucleotide Identity (ANI) analysis revealed that the closest type strain to our isolate was *Staphylococcus aureus* (GCA_000330825.2) with an ANI of 99.04%, 92% assembly coverage, 93.3% type assembly coverage, confirming species-level identity and close relatedness. Screening with the Comprehensive Antibiotic Resistance Database (CARD) and the Virulence Factor Database (VFDB) revealed several genes conferring resistance to different classes of antibiotics: antibiotic (tet(K) and tet(38)- tetracyclines, mepA, aminoglycosides, fluoroquinolones, norA- Fluoroquinolones, LmrS- Macrolides, aminoglycosides, lincosamide, oxazolidinones and blaZ- Beta-lactams, regulatory gene (mepR, arlR and arlS). Virulence factors included: Adherence (icaA, icaB, icaC, icaD, icaR, clfA, ebp, fnbA, fnbB, map, sdrC, sbi, spa,,), Secretion system (esaA, esaB, esaC, esaD, esaE, esaF, esaG, esaH, esaI, esaJ, esaK, esaL, esaM, esaN, esaO, esaP, esaQ, esaR, esaS, esaT, esaU, esaV, esaW, esaX, esaY, esaZ, esaAA, esaAB, esaAC, esaAD, esaAE, esaAF, esaAG, esaAH, esaAI, esaAJ, esaAK, esaAL, esaAM, esaAN, esaAO, esaAP, esaAQ, esaAR, esaAS, esaAT, esaAU, esaAV, esaAW, esaAX, esaAY, esaAZ, esaBA, esaBB, esaBC, esaBD, esaBE, esaBF, esaBG, esaBH, esaBI, esaBJ, esaBK, esaBL, esaBM, esaBN, esaBO, esaBP, esaBQ, esaBR, esaBS, esaBT, esaBU, esaBV, esaBW, esaBX, esaBY, esaBZ, esaCA, esaCB, esaCC, esaCD, esaCE, esaCF, esaCG, esaCH, esaCI, esaCJ, esaCK, esaCL, esaCM, esaCN, esaCO, esaCP, esaCQ, esaCR, esaCS, esaCT, esaCU, esaCV, esaCW, esaCX, esaCY, esaCZ, esaDA, esaDB, esaDC, esaDD, esaDE, esaDF, esaDG, esaDH, esaDI, esaDJ, esaDK, esaDL, esaDM, esaDN, esaDO, esaDP, esaDQ, esaDR, esaDS, esaDT, esaDU, esaDV, esaDW, esaDX, esaDY, esaDZ, esaEA, esaEB, esaEC, esaED, esaEE, esaEF, esaEG, esaEH, esaEI, esaEJ, esaEK, esaEL, esaEM, esaEN, esaEO, esaEP, esaEQ, esaER, esaES, esaET, esaEU, esaEV, esaEW, esaEX, esaEY, esaEZ, esaFA, esaFB, esaFC, esaFD, esaFE, esaFF, esaFG, esaFH, esaFI, esaFJ, esaFK, esaFL, esaFM, esaFN, esaFO, esaFP, esaFQ, esaFR, esaFS, esaFT, esaFU, esaFV, esaFW, esaFX, esaFY, esaFZ, esaGA, esaGB, esaGC, esaGD, esaGE, esaGF, esaGG, esaGH, esaGI, esaGJ, esaGK, esaGL, esaGM, esaGN, esaGO, esaGP, esaGQ, esaGR, esaGS, esaGT, esaGU, esaGV, esaGW, esaGX, esaGY, esaGZ, esaHA, esaHB, esaHC, esaHD, esaHE, esaHF, esaHG, esaHH, esaHI, esaHJ, esaHK, esaHL, esaHM, esaHN, esaHO, esaHP, esaHQ, esaHR, esaHS, esaHT, esaHU, esaHV, esaHW, esaHX, esaHY, esaHZ, esaIA, esaIB, esaIC, esaID, esaIE, esaIF, esaIG, esaIH, esaII, esaIJ, esaIK, esaIL, esaIM, esaIN, esaIO, esaIP, esaIQ, esaIR, esaIS, esaIT, esaIU, esaIV, esaIW, esaIX, esaIY, esaIZ, esaJA, esaJB, esaJC, esaJD, esaJE, esaJF, esaJG, esaJH, esaJI, esaJJ, 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esaPA, esaPB, esaPC, esaPD, esaPE, esaPF, esaPG, esaPH, esaPI, esaPJ, esaPK, esaPL, esaPM, esaPN, esaPO, esaPP, esaPQ, esaPR, esaPS, esaPT, esaPU, esaPV, esaPW, esaPX, esaPY, esaPZ, esaQA, esaQB, esaQC, esaQD, esaQE, esaQF, esaQG, esaQH, esaQI, esaQJ, esaQK, esaQL, esaQM, esaQN, esaQO, esaQP, esaQQ, esaQR, esaQS, esaQT, esaQU, esaQV, esaQW, esaQX, esaQY, esaQZ, esaRA, esaRB, esaRC, esaRD, esaRE, esaRF, esaRG, esaRH, esaRI, esaRJ, esaRK, esaRL, esaRM, esaRN, esaRO, esaRP, esaRQ, esaRR, esaRS, esaRT, esaRU, esaRV, esaRW, esaRX, esaRY, esaRZ, esaSA, esaSB, esaSC, esaSD, esaSE, esaSF, esaSG, esaSH, esaSI, esaSJ, esaSK, esaSL, esaSM, esaSN, esaSO, esaSP, esaSQ, esaSR, esaSS, esaST, esaSU, esaSV, esaSW, esaSX, esaSY, esaSZ, esaTA, esaTB, esaTC, esaTD, esaTE, esaTF, esaTG, esaTH, esaTI, esaTJ, esaTK, esaTL, esaTM, esaTN, esaTO, esaTP, esaTQ, esaTR, esaTS, esaTT, esaTU, esaTV, esaTW, esaTX, esaTY, esaTZ, esaUA, esaUB, esaUC, esaUD, esaUE, esaUF, esaUG, esaUH, esaUI, esaUJ, esaUK, esaUL, esaUM, esaUN, esaUO, esaUP, esaUQ, esaUR, esaUS, esaUT, esaUU, esaUV, esaUW, esaUX, esaUY, esaUZ, esaVA, esaVB, esaVC, esaVD, esaVE, esaVF, esaVG, esaVH, esaVI, esaVJ, esaVK, esaVL, esaVM, esaVN, esaVO, esaVP, esaVQ, esaVR, esaVS, esaVT, esaVU, esaVV, esaVW, esaVX, esaVY, esaVZ, esaWA, esaWB, esaWC, esaWD, esaWE, esaWF, esaWG, esaWH, esaWI, esaWJ, esaWK, esaWL, esaWM, esaWN, esaWO, esaWP, esaWQ, esaWR, esaWS, esaWT, esaWU, esaWV, esaWW, esaWX, esaWY, esaWZ, esaXA, esaXB, esaXC, esaXD, esaXE, esaXF, esaXG, esaXH, esaXI, esaXJ, esaXK, esaXL, esaXM, esaXN, esaXO, esaXP, esaXQ, esaXR, esaXS, esaXT, esaXU, esaXV, esaXW, esaXX, esaXY, esaXZ, esaYA, esaYB, esaYC, esaYD, esaYE, esaYF, esaYG, esaYH, esaYI, esaYJ, esaYK, esaYL, esaYM, esaYN, esaYO, esaYP, esaYQ, esaYR, esaYS, esaYT, esaYU, esaYV, esaYW, esaYX, esaYY, esaYZ, esaZA, esaZB, esaZC, esaZD, esaZE, esaZF, esaZG, esaZH, esaZI, esaZJ, esaZK, esaZL, esaZM, esaZN, esaZO, esaZP, esaZQ, esaZR, esaZS, esaZT, esaZU, esaZV, esaZW, esaZX, esaZY, esaZZ).

Keywords Bacterial genomics, Antimicrobial-resistant genes, Subclinical mastitis, Genomic annotation, Minlon MK1B sequencing

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Objective

Subclinical mastitis (SCM) is a burden for the dairy sector due to its asymptomatic nature [1]. *Staphylococcus aureus* is the most contagious bacteria that causes subclinical mastitis (SCM) in dairy cattle [2]. *S. aureus* intramammary infections (IMI) is believed to occur during the milking process [3]. Hand milking is one of the most obvious possibilities for transmission [4]. Antibiotics for human and veterinary use are readily available over the counter in developing countries [5]. This high prevalence has been linked to the irrational use of antibiotics without laboratory confirmation as well as an increase in farmers handling SCM cases with little professional assistance [6]. These factors have led to an emergency of multidrug resistant (MDR) strains of *S. aureus* that are linked to financial losses and food insecurity [7].

This study aimed to describe the genetic characteristics of *S. aureus* strain 37M isolated from subclinical mastitis affected cows in Uganda's Kiruhura area. The purpose of whole genome sequencing (WGS) was to learn more about the organism's overall genomic architecture as well as its genes for virulence and antibiotic resistance. Subclinical mastitis prevention and treatment approaches will be guided by these data, which will also enhance continuing work in genomic surveillance of MDR pathogens.

No scientific paper had previously published this genomic sequence. The information displayed here was produced as a result of scholarly studies looking into the molecular epidemiology and microbiology of subclinical in Uganda's Kiruhura district. Reference to this data note will be made in future papers from the original study that examine comparative genomes and resistance evolution across various isolates. The assembled genome and raw data have both been uploaded to NCBI public resources to guarantee transparency and reusability.

Data description

The data set presented includes the whole-genome sequence data of *Staphylococcus aureus* strain 37M, isolated from cows with subclinical mastitis in Kiruhura district, Uganda. Data include raw sequence reads (dataset 1), draft genome assemblies (dataset 2), and annotated genome data (dataset 3), all deposited in NCBI.

Strain 37M was isolated from a milk sample collected from cows with Sub-clinical mastitis in Kiruhura district, Uganda. The milk sample was cultured on blood agar, observing the beta hemolysis on blood agar, suspected isolates were sub-cultured on Mannitol salt agar (Himedia, India), (MSA) plates incubated aerobically at 37 °C for 24 h. Yellow colonies indicative of *Staphylococcus aureus*, were streaked onto a fresh differential media MSA plates for further purification. Pure *S. aureus* isolates were further confirmed by following a positive catalase, coagulase, and Staphaurex latex agglutination test (Thermo Fisher Scientific) as per the manufacturer's instructions. Antibiotic sensitivity testing was conducted on obtained pure isolates using Kirby-Bauer, standard disk diffusion method on Mueller-Hinton agar (MHA) with a dilution adjusted in compliance with the 0.5 McFarland standard (1.5×10^8 cfu/ml) in line with CLSI guidelines [8, 9]. The strain was resistant to multiple antibiotics, including penicillin (10 units; 6 mm), erythromycin (15 µg; 10 mm), tetracycline (30 µg; 12 mm), and cefoxitin (30 µg, 18 mm), indicating methicillin resistance.

Pure *Staphylococcus aureus* isolates were cultured on Luria-Bertani (LB) broth incubated in shaker at 200 rpm for 18–24 h at 37 °C and DNA was extracted using a column-based extraction using the NEB Monarch® Spin gDNA Extraction Kit, including the extra lysozyme pre treatment step for efficient lysis of Gram-positive cells [10]. The integrity of the extracted DNA was assessed by 1% (w/v) agarose gel electrophoresis (Fig. 1), DNA

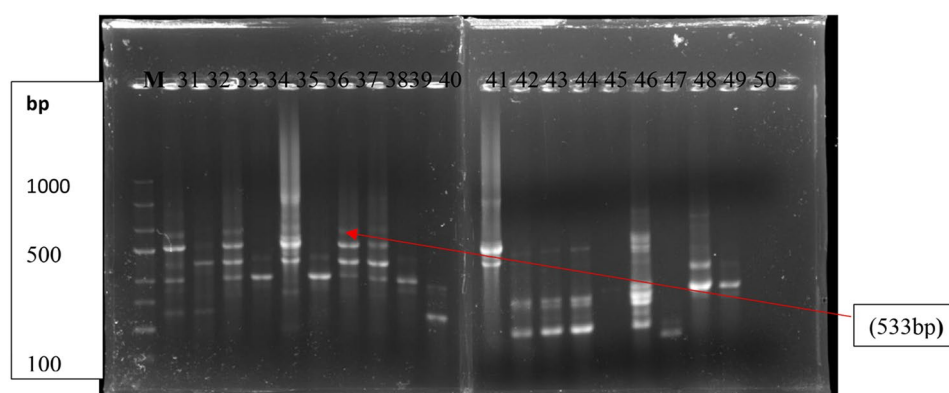


Fig. 1 An agarose gel image showing *mecA* gene from selected multi-drug-resistant *Staphylococcus aureus* isolated from cows with subclinical mastitis, determined using colony PCR, well 1 is the DNA ladder (100 to 1000bp), well 2 (Positive control, *S. aureus* ATCC 33591), wells 3–6 (32M–36M), well 8 (37M), wells 9–20 (38M–49M) samples, well 21 (Non template control)

Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1	Raw sequence reads	FASTQ (.fastq)	NCBI SRA http://identifiers.org/insdc.sra:SRR35137992 [24]
Data set 2	Draft genome sequence	FASTQ (.fastq)	NCBI GenBank http://identifiers.org/nucleotide:JBQVMI000000000.1 [25]
Data set 3	Annotated genome (NCBI PGAP results)	GenBank/Annotation Files	NCBI GenBank http://identifiers.org/insdc.ca:GCA_052439145.1 [26]

concentration and purity were also measured using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific) with Quant-iT dsDNA Assay Kit, High Sensitivity (Invitrogen) and Nano Drop ND 1000 spectrophotometer (thermos Fisher Scientific). The MinION MK1B (MIN-101B) (Oxford NANOPORE technology) platform was used for whole-genome sequencing producing 1D reads [11, 12]. Library preparation was conducted using the Rapid barcoding kit 96V14(SQK-RBK114.96) (Oxford NANOPORE technologies). FLO-MIN114, MinIONor GridION Flow cell R10 version-lot 11,004,111 by Oxford NANOPORE technologies was loaded with prepared library [13].

The length of the assembled genome was 2,795,296 bp, 89 contigs, 53,181 N50, 14,429 N90, 16 L50, 54 L90, 32.73% GC, 97.3% depth coverage and 0.7% minimum coverage. Raw reads were quality checked with FastQC (v0.12.1) before and after trimming [14]. Adapter sequences and low-quality bases were trimmed using Trimmomatic (v0.39) [15]. All high-quality trimmed reads were used for de novo assembly with Unicycler (v0.5.1), base calling with (*SUP*, v4.3.0) and polished with Medaka [16–18]. All assembled reads passed quality assessment using QUAST (v5.3.0) and assembly completeness was determined using BUSCO (v5.8.3) [19, 20]. Resulting in 188390 total sequences, 131.3Mbp total bases, 60–71,894 sequence length, GC 34% before trimming, after trimming with 141908 total sequences, 104.7Mbp total bases, 20-71703sequence length, GC 32%. The assembled genome was submitted and annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP (v6.10) [21]. And yielded a genome length of 2,795,296 bp, 89 contigs with an N50 53,181, 16 L50 and GC 32.5% after submission as shared in Table 1.

AMR genes were found using the Comprehensive Antibiotic Resistance Database (CARD) [22] and ResFinder v4.6.0. Likewise, BLAST was used with default parameters to query the Virulence Factor Database (VFDB) [23] in order to find possible virulence genes. Significant hits were defined as those with ≥ 90% identification and ≥ 60% coverage.

Limitations

Not Applicable.

Abbreviations

SCM	Subclinical Mastitis
DNA	Deoxyribonucleic acid
NCBI	National Center for Biotechnology Information
MDR	Multi Drug Resistant

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12863-025-01402-6>.

Supplementary Material 1

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Author contributions

Conceptualization, RM, VS, GR; investigation, RM VS EE, GR, SA, DR; resources, MA, RM, DM, PPD; writing, RM, NAT TP, DM, VS; supervision, VS, GR, EE and RM. The authors read and approved the final manuscript.

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Data availability

The information in this data note is publicly available on NCBI under JBQVMI000000000. For more information and data links, please refer to Table 1 and references 24–26.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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