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Whole genome sequencing reveals identification of new *Acinetobacter* spp. (maqsudiensis) from local meat samples collected from Dhaka, Bangladesh

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ABSTRACT

The advent of next-generation technology has paved way for the discovery of new species which could otherwise be misidentified using conventional cultural and serotyping methods. *Acinetobacter* is an important nosocomial pathogen frequently isolated from raw and undercooked meat. In this study we announce the draft genome sequence of a newly identified *Acinetobacter* species cross-reacting with *E. coli* serotype O157:H7. Initially, isolates collected from local meat samples in Dhaka, Bangladesh were cultured in EMB and SMAC for the presumptive identification of O157:H7. However, whole genome and Average Nucleotide Identity (ANI) analyses identified the isolate as a new species within the *Acinetobacter* genus. The whole genome sequence of this isolate will help to identify potential marker/s of intervention. Further genomic analyses might also shed light onto the virulence properties of this newly identified *Acinetobacter* species which has been provided the new name of *Acinetobacter maqsudiensis*.

KEYWORDS:

Acinetobacter; Bangladesh; next-generation sequencing; average nucleotide identity

GENOME REPORT

Different pathogenic bacterial strains isolated from raw and undercooked meat have been associated with many outbreaks of foodborne diseases worldwide (1–5). Major bacterial strains isolated from meat sources include *Staphylococcus aureus*, *Streptococcus* spp., *Listeria monocytogenes*, *Bacillus* spp., *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Acinetobacter* spp., *Aeromonas* spp. and *Pseudomonas* spp. (2,3,6–9). *Acinetobacter* species are important nosocomial pathogens (10,11) and infections caused by these organisms include pneumonia, endocarditis, meningitis, skin and wound infections, peritonitis in patients receiving peritoneal dialysis, UTI and bacteremia (10,11). In this investigation we carried out whole genome sequencing of *E. coli* O157:H7 isolated from meat samples collected from local vendors. However, the characterization of whole genome sequence of one of the suspected *E. coli* isolates resulted in the identification of a new *Acinetobacter* spp. We are currently carrying out in depth genomic characterization and other required investigations to confirm this isolate as a new *Acinetobacter* species.

Initially samples were investigated for the presence of *E. coli* O157:H7 isolated from 50 samples of raw chicken, and meat samples. Meat samples were randomly collected (simple random sampling procedure) from the butchers and shopping centers of different parts of Dhaka City, Bangladesh. All isolates were transferred to NSU Genome Research Institute (NGRI) at North South University, Bangladesh. The specimens were inoculated initially on differential media EMB (HiMedia) and selective media SMAC (Oxoid) for presumptive identification of *E. coli* O157:H7. Green metallic sheen lines on EMB which had corresponding white colonies on SMAC were selected for latex agglutination test. The isolates were stored in BHI medium containing 15% glycerol at -20°C. Six of these suspected *E. coli* 0157:H7 isolates were selected for whole genome sequencing. The genomic library was constructed and 300-bp paired-end data was generated using whole-genome sequencing using an Illumina MiSeq platform (Illumina, San Diego, CA, USA) at NGRI. The raw reads were generated (~15×coverage) and assembled using SPAdes version 3.11.

Serendipitously one of the six isolates i.e., B51 was confirmed as an *Acinetobacter* spp. based on its whole genome sequence analysis. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession PJRJ00000000. Based on the 16s rRNA phylogeny we initially identified this isolate as *Acinetobacter johnsonii*, however, analysis of Average Nucleotide Identity (ANI) by GenBank groups provided the information that this isolate could potentially be a new species (Table 1). The ANI calculation suggested that the genome was misidentified and therefore could possibly be a new *Acinetobacter* species. As this similarity value was lower than the 98.65% threshold recommended to define a new species (12), we suggest the creation of a new species within the genus *Acinetobacter* named *Acinetobacter maqsudiensis*, after Muhammad Maqsud Hossain (Director, NGRI) who led the discovery of this new strain.

The findings yet need to be confirmed by further comprehensive analyses using other biochemical parameters and MALDI-TOF-MS spectrum.

TABLE 1 ANI calculation results of *Acinetobacter* species whole genome

82.324 (20.8 20.3) 3330008 assembly *Acinetobacter gandensis* (GCA 001678755.1, ASM167875v1)
81.866 (20.4 21.8) 596228 assembly *Acinetobacter _ towneri* (GCA 000368785.1, Acin town CIP 107472 V1)
81.755 (20.2 22.0) 1066968 assembly *Acinetobacter _ towneri* (GCA 000688495.1, ASM68849v1)
81.553 (20.2 19.5) 841088 assembly *Acinetobacter_ indicus* (GCA 000488255.1, Acin indi CIP110367 V2)
82.008 (20.1 17.4) 595858 assembly *Acinetobacter johnsonii* (GCA 000368045.1, Acin john CIP 64 6 V1)
81.760 (19.9 17.7) 596678 assembly *Acinetobacter variabilis* (GCA 000369625.1, Acin sp NIPH 2171 V1)
81.333 (19.9 20.1) 1506848 assembly *Acinetobacter _ indicus* (GCA 000830155.1, ASM83015v1)
81.856 (19.7 17.6) 1677758 assembly *Acinetobacter johnsonii* (GCA 000949655.1, ASM94965v1)
82.128 (19.5 18.0) 432348 assembly *Acinetobacter_ lwoffii* (GCA 000248355.2, ASM24835v2)
81.525 (19.4 16.5) 595798 assembly *Acinetobacter bohemicus* (GCA 000367925.1, Acin sp ANC 3994 V1)
82.217 (19.2 17.7) 596148 assembly *Acinetobacter schindleri* (GCA 000368625.1, Acin schi CIP107287 V1)
81.125 (19.3 14.7) 774958 assembly *Acinetobacter_ tandoii* (GCA 000400735.1, Acin tand CIP 107469 V1)
81.089 (19.2 14.7) 993398 assembly *Acinetobacter_ tandoii* (GCA 000621065.1, ASM62106v1)
82.276 (18.7 18.1) 596398 assembly *Acinetobacter lwoffii* (GCA 000369105.1, Acin lwof CIP 64 10 V1)
81.263 (18.5 18.6) 3397888 assembly *Acinetobacter _ celticus* (GCA 001707755.1, ASM170775v1)
81.034 (16.2 15.0) 603628 assembly *Acinetobacter bouvetii* (GCA 000373725.1, ASM37372v1)
82.479 (15.9 16.1) 2383068 assembly *Acinetobacter equi* (GCA 001307195.1, ASM130719v1)
80.900 (16.0 16.2) 3378228 assembly *Acinetobacter _albensis* (GCA 900095025.1, IMG-taxon 2671180230 annotated assembly)
82.347 (15.1 12.5) 3383468 assembly *Acinetobacter defluvii* (GCA 001704615.1, ASM170461v1)
80.692 (15.3 16.6) 1465898 assembly *Acinetobacter harbinensis* (GCA 000816495.1, ASM81649v1)

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