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Whole genome sequencing reveals identification of new *Acinetobacter* spp. (maqsudiensis) from cow fecal sample collected from Dhaka, Bangladesh

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ABSTRACT In this study we announce the draft genome sequence of a newly identified *Acinetobacter* species cross-reacting with *E. coli* serotype 0157:H7. The advent of Next-Generation technology has paved to way to discover new species which could otherwise be misidentified using conventional cultural and serotyping methods. The whole genome sequence of this isolate will help to identify potential marker/s of intervention and further genomic analysis might also shed light onto the virulence properties of this newly identified Acinetobacter species which has been provided the new name of *Acinetobacter maqsudiensis*.

GENOME REPORT

Different pathogenic bacterial strains have been isolated from raw and undercooked meat and they have been associated with many outbreaks of foodborne diseases worldwide (1–5). Major bacterial strains isolated from meat sources include *Staphylococcus aureus*, *Streptococcus* species, *Listeria monocytogenes*, *Bacillus* spp., *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Acinetobacter* spp., *Aeromonas* spp., *Pseudomonas* spp. and etc. (2, 3, 6–9)*Acinetobacter* species are are important nosocomial pathogen (10, 11) and infections caused by this organism 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 cow fecal collected from local vendors, however, the characterization of whole genome sequence of one of the suspected *E. coli* 0187:H7 isolates resulted in the identification of new *Acinetobacter* spp. We are currently carrying out in depth genomic characterization of this new *Acinetobacter* spp. as well as required investigation to confirm this as a new *Acinetobacter* species.

Initially samples were investigated for the presence of *E. coli* 0157:H7 isolated from 50 samples of raw chicken, meat samples, and cow fecal samples. Samples were randomly collected (simple random sampling procedure) from the butchers, shopping, and dairy farms from different parts of the Dhaka City, Bangladesh. All isolates were transferred to the NSU Genome Research Institute (NGRI) at North South University, Bangladesh.

For identification of *E. coli* 0157:H7 the specimens were inoculated initially on differential media EMB (HiMedia) and selective media SMAC (Oxoid) for presumptive identification of 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 the Genome Research Institute of North South University, Bangladesh. The raw reads were generated (~15× coverage) and assembled using SPAdes version 3.11.

Serendipitously one of these isolates i.e. B51 collected from cow fecal sample was confirmed as *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. The analysis of Average nucleotide identification (ANI) by GenBank provided the information that the isolate potentially could be a new species and details of which provided in Table 1.

Based on the 16s rRNA phylogeny we initially identified this as *Acinetobacter johnsonii* , however, the average nucleotide identity (ANI) calculation suggested that the genome was misidentified and the could possibly be a new *Acinetobacter* species.

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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 (maqsud is the group leader Muhammad Maqsud Hossain, Director, NSU Genome Research Institute, who led the discovery of this new strain).

The finding yet need to be confirmed by further comprehensive analysis using other biochemical parameters and MALDI-TOF-MS spectrum prior to the submission of the new isolate to recognized culture collection center such as American Type Culture Collection (ATCC).

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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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