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Use of whole-genome sequencing to characterize a cluster of Gram-negative bacilli of the family Xanthomonadaceae infecting humans by zoonotic transmission

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Background: Nine isolates collected over a 41 year period (1971- 2012) recovered from human infections associated with animal bites were studied. These were closest to each other (>99% identity) by 16S rRNA gene sequencing but distinct from other genera in the family Xanthomonadaceae by that approach. Strains were extensively characterized using conventional methods as well as by whole genome sequencing (WGS). They appeared to have caused at least localized infections in humans after being bitten by an animal and so may represent novel zoonotic agents.

Material/methods: Bacteria studied had been tested biochemically using standard methods, by API panels, for cellular fatty acids (CFAs) after growth on blood agar and by 16S rRNA gene sequencing (16S). Several strains were also assessed for ubiquinone type by HPLC. MICs were assessed by broth microdilution (Sensititre). MALDI-TOF analysis and Mass Spectral Profiles (MSPs) were done on a Bruker system. Draft whole genome sequences of the nine stains were compared to each other using Average Nucleotide Identities by Blastn, *in silico* DNA-DNA hybridization and single nucleotide variation (SNV) analysis. A comparison of genes within the strains was done using GView Blast Atlas and unique signatures were identified using Neptune.

Results: All isolates were Gram-negative bacilli or coccobacilli which grew well aerobically, in 5% CO_2 but not anaerobically. Optimal growth was obtained at 35°C but they also grew well at 25°C and 42°C. Colonies were pinpoint sized at 24h at 35°C but were 1-3mm with yellowish pigment and often slightly mucoid, at 72h. All were non-reactive with respect to TSI, OF sugars and API panel sugars and towards most substrates tested. All were catalase and oxidase positive and hydrolyzed gelatin.

Motility was observed for some strains. Ubiquinone 8 was detected. MICs were low, suggesting susceptibility to 15 antibiotics (one exception). MALDI-TOF spectra were unique and could be used to identify these bacteria. By WGS, these bacteria fell into 5 taxon groups, one with 4 strains and the rest represented by single isolates. Genome sizes and GC content were similar for eight of the nine strains (2.16 to 2.39 Mb; G+C of 60.06 to 60.46%) by WGS, which was similar to that described for other genera in the Xanthomonadaceae. One strain had a larger genome at 3.24 Mb and a higher G+C % of 69.25. This strain was unique by WGS and MALDI-TOF analyses.

Conclusions: After genomic level comparisons, this cluster appeared to be heterogenic but strains were otherwise very difficult to discern biochemically, by CFAs and by 16S. Ultimately, this work will serve as a basis for the description of a novel genus with several new species of bacteria transmitted by zoonotic means which can cause human infections.