Acinetobacter baumannii Sampled from Cattle and Pigs Represent Novel Clones

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ABSTRACT  Acinetobacter baumannii is a very important human pathogen. Nonetheless, we know very little about nonhuman isolates of A. baumannii. Here, we determine the genomic identity of 15 Scottish cattle and pig isolates, as well as their antibiotic and virulence genetic determinants, and compare them with 148 genomes from the main human clinical international clones. Our results demonstrate that cattle and pig isolates represent novel clones well separated from the major international clones. Furthermore, these new clones showed fewer antibiotic resistance genes and may have fewer virulence genes than human clinical isolates.

IMPORTANCE  Over the last decades, huge amounts of information have been obtained for clinical isolates of A. baumannii and the clones they belong to. In contrast, very little is known about the genomic identity and the genomic basis for virulence and resistance of animal isolates. To fulfill this gap, we conducted a genomic epidemiology study of 15 Scottish cattle and pig isolates in the context of almost 150 genomes belonging to the main international clones of A. baumannii. Our findings show that these animal isolates represent novel clones clearly different from the major international clones. Furthermore, these new clones are distinct in nature considering both antibiotic resistance and virulence when compared with their human clinical counterparts.

KEYWORDS  genome epidemiology, Acinetobacter baumannii, antibiotic resistance, animal isolates, bacterial clones, One Health

Acinetobacter baumannii is a Gram-negative opportunistic bacterial pathogen, notorious for being associated with high morbidity and mortality due to its highly drug-resistant nature. While A. baumannii can be isolated from clinical samples, its natural environment is less clear. Animals have been suggested as a potential host or reservoir for A. baumannii. Birds, in particular White Storks, have been proposed as a reservoir (1), though this does not seem to apply to other bird species (2), and as A. baumannii can be released into the environment from hospital effluent (3) it is not clear the degree to which wild animals are acquiring the bacteria from contaminated soil and water. However, it is clear that A. baumannii should be considered a One Health issue, as some nonhuman isolates have important antibiotic resistance genes (4). A. baumannii seems to be fairly common in domestic livestock, particularly cattle (5, 6), where isolates tend to have a generally susceptible antibiotic resistance profile and appear to be genetically distinct from clinical strains by molecular typing methods. In a previous study, 16 A. baumannii isolates were collected from cattle and pigs that had been recently slaughtered, and were shown by pulsed-field gel electrophoresis (PFGE) to cluster separately from the three major clones of A. baumannii prevalent at the time; furthermore, they carried different oxaAb (blaOXA-51-like) variants (7). Here, we sequenced the genomes of these 16 isolates to determine how genetically similar they are to human clinical isolates.
Total DNA was extracted from overnight broth cultures with a Promega Wizard Genomic DNA Purification kit (Promega, UK), quality checked by nanodrop and quantity assessed by Qubit. Purified DNA was paired-end sequenced on an Illumina platform. The sequences were trimmed with Trim Galore and assembled via SPAdes, as described previously (10). The genomes were annotated employing Prokka and genotyped by the Pasteur Multilocus Sequencing Typing (MLST) scheme using the PubMLST online database (13). The genome quality was assessed with CheckM and only the genomes with more than 95% completeness and less than 5% contamination were considered for downstream analyses. One isolate from a pig fecal sample (PF33) was discarded as it showed a high percentage of contamination (>60%). For the phylogenetic analysis, we also included 148 human-related A. baumannii genomes previously genotyped in Hernández-González et al. (15). These genomes were chosen as they are part of the eight main international clones (ICs). Table S1 provides the BioSample ID for all the isolates and also some other information such as host, isolation source, geographic location, ST assignation, etc. A maximum likelihood (ML) core phylogeny was built using the strategy described in Graña-Miraglia (16). Briefly, the genes present in a single copy in all the genomes (single-gene families) were identified with Roary and tested for recombination using PhiPack. We found 759 single-gene families without recombination, which represent 47.8% of the core genome, and these were concatenated to build a phylogeny with RAxML, the tree was annotated using iTOL. The antibiotic resistance genes prediction on the genome assemblies was carried out with the Comprehensive Antibiotic Resistance Database (CARD) (21), and ampC alleles were identified using the PubMLST database.

The ML core genome phylogeny of the 15 animal isolates alongside a collection of 148 clinical isolates (22) representing the major international clones showed that the animal isolates formed three well-separated clades, each of which was distinct and very distant from any of the clinical isolates (Fig. 1). The pig fecal isolates formed a single clade, two of the cattle fecal isolates (CF233 and CF234) formed a second clade, and the remaining four cattle fecal isolates formed a clade with the two cattle nostril isolates. Considering the Pasteur MLST genotyping, these three clades corresponded to sequence type (STPAS) 162, STPAS1014, and STPAS492, respectively. As described previously, the isolates belonging to STPAS1014 carried the oxaAb variant oxaAb(150), and the STPAS492 isolates carried oxaAb(148) (7). However, the STPAS162 strains carried oxaAb(51), which is considered diagnostic of international clone (IC) 4 isolates (23). IC4 isolates typically belong to STPAS15, which only shares a single allele in common with STPAS162, and the STPAS162 and IC4 isolates are very clearly separated in the core genome phylogeny (Fig. 1). It is interesting to note that incongruence between MLST ST and oxaAb allele has been observed previously for STPAS162, and warrants further investigation (24). Of note is that all published STPAS162 isolates, and all of those in the PubMed database, are from South American countries (Brazil and Chile), geographically very distant from the Scottish isolates reported here. Only two STPAS492 isolates are listed in the PubMed database, from Lebanon and Russia, and one of these is an animal isolate. There are no other STPAS1014 isolates in the PubMed database, but there are 11 isolates that match six loci, and of these, two are listed as coming from animals, two from food, and one from the environment, suggesting that these STs are commonly isolated from nonclinical sources. Collectively, these results show that the pig and cattle isolates form well-differentiated groups and they are not closely related to the major international clones.

As expected from the previously reported generally antibiotic sensitive nature of the isolates, only chromosomally encoded resistance genes such as oxaAb, ampC, and efflux systems were identified, with no acquired antibiotic resistance genes present (Fig. 2). Of note, we identified novel ampC alleles in the isolates: ampC-84 was present in CF233 and CF234; ampC-85 was found in CN26, CN35, CF251, CF254, CF258, and CF260; and ampC-86 was present in the rest of the isolates. It had previously been described for these animal isolates that they did not carry ISAbat upstream of the oxaAb genes or the ampC genes, where it can provide a promoter for their expression.
Of the 15 isolates, seven had no substantial match to any insertion sequences in the ISFinder database (accessed 09/03/2022). Of the remaining eight, one isolate (CN26) had a short contig with a partial match to IS1411, while all seven pig fecal isolates contained a 184-bp fragment with 97% identity to the 5′ end of ISAjo2, and a 279 bp fragment with 84% similarity to the 3′ end of ISAcsp2. We, therefore, did not detect any complete IS elements in these strains. In A. baumannii, IS elements are thought to be a major mechanism through which the bacteria regulate gene expression and mobilize genes, and are a common feature of clinical isolates. Their almost complete absence from these animal isolates highlights how different in nature they are from clinical isolates, and that IS-mediated adaptation may be a feature of successful clinical strains rather than a general characteristic of the species.

In order to assess whether the animal isolates differed in their complement of virulence factors, the genome assemblies were analyzed using VFanalyzer alongside all 15 available genomes included in the VFanalyzer database (14 clinical and one human louse isolate; Table S2) (25). These genomes represent six different MLSTPAS STs, including eight STPAS2 and three STPAS1 genomes. Animal isolates had a significantly smaller complement of capsule-related genes, averaging 17, whereas the clinical isolates averaged 22 (t test, \( P = 0.000016 \)). The six STPAS492 isolates differed from the other animal
isolates in that they lacked 8 genes involved in heme utilization, including hemO (Table S2). Variation in the carriage of these genes is common, with seven out of the 14 clinical and one louse strains also lacking these genes. Thus, these data suggest that the animal isolates may have fewer virulence factors than human clinical isolates.

In conclusion, our study shows that these cattle and pig isolates represent three novel clones well-separated from the major international clones. Furthermore, these new clones are distinct in nature considering both antibiotic resistance and virulence when compared with their human clinical counterparts. In a broader context, our findings highlight the need for further studies on the genomic epidemiology, and also surveillance of animals isolates of this bacterial species.

Data availability. The animal isolates were submitted to the NCBI under the BioProject number PRJNA819013. In addition, the BioSample number for each isolate is listed in Table S1.

SUPPLEMENTAL MATERIAL
Supplemental material is available online only.
SUPPLEMENTAL FILE 1, XLSX file, 0.02 MB.
SUPPLEMENTAL FILE 2, XLS file, 0.1 MB.

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