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MLST typing of *Enterococcus faecium* and *Enterococcus faecalis* recovered from healthy pigs and chickens across Europe and sequence type comparisons with the international MLST database

Simjee S[*1,6], Blackman Northwood J[2], Knight D R[2], Shryock T R[3,6], de Jong A[4,6], Willems R J L[5] and EASSA Study Group[6]
[1]Elanco Animal Health, UK, [2]QB BioResearch, UK, [3]Elanco Animal Health, USA, [4]Bayer Animal Health, Germany, [5]University Medical Center Utrecht, NL and [6]EASSA Study Group, CEESA, Brussels, Belgium

CEESA
Centre Européen d'Etudes pour la Santé Animale
European Animal Health Study center
Association Internationale • International Association

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Background

Molecular epidemiological studies of *E. faecium* using multilocus sequence typing (MLST) revealed the existence of host-specific lineages and a distinct genetic subpopulation named clonal complex 17 (CC17) that is responsible for the majority of hospital-related infections and outbreaks and that has spread globally. Until now, less has been known about the population structure of *E. faecalis*. Ruiz-Garbajosa *et al.* identified 55 sequence types (ST) and four major CCs, two of which, CC2 and CC9, were significantly enriched among nosocomial isolates and were considered to represent hospital-adapted complexes, equivalent to *E. faecium* CC17.

The aim of this study was to determine the genetic relatedness of randomly selected *E. faecium* (*Em*) and *E. faecalis* (*Es*) isolates, from chickens and pigs, by multi-locus sequence typing (MLST) and comparisons against an international MLST database including STs of human isolates.

Methods

From the EASSA collection epidemiologically-unrelated 32 *Em* and 29 *Es* isolates from chickens and pigs were randomly selected. DNA was extracted from pure culture and purified using ethanol based reagent kits and silica filter columns. PCR primers, parameter and expected fragment sizes are as listed on the enterococci MLST website (http://efaecium.mlst.net/misc/info.asp and http://efaecalis.mlst.net/misc/info.asp).

Sequencing primers were the same as those used for the PCR. Sequencing was performed using both the forward and reverse primers, the consensus of these used to determine the DNA sequence of the loci. *Em* control strain ATCC 19434 (MLST ST 160) and *Es* control strain JH2-2 (MLST ST 8) were included in each run of PCR and DNA sequencing.

MLST-based allelic profiles were clustered with international databases (http://efaecium.mlst.net/) using the eBURST algorithm and sequence types (STs) of the individual isolates were compared with STs in the database. Isolates in this database originated from hospitalized patients (surveillance, clinical and outbreak-associated isolates) and from the community (humans, pigs, chickens, calves, other animals, food, and the environment)

Results and Discussion

Assignment of alleles and STs

The following new alleles were assigned: *Em adk*-24; *gdh*-40; *atpA*-56; *gdh*-41 and *Es gdh*-54, *pstS*-55, *aroE*-58, *yqiL*-57; *pstS*-56; *aroE*-59, *yqiL*-58; *yqiL*-59. Four alleles could not be assigned due to poor sequence trace files.

The following STs were assigned: *Em* ST-486; ST-487; ST-488; ST-489; ST-487; ST-490; ST-491; ST-492; ST-490; ST-489 and *Es* ST-289; ST-290; ST-291; ST-292. For four *Em* strains, no ST could be assigned because of incomplete allelic profiles.

Clustering and comparison of allelic profiles of *E. faecium*

Allelic profiles of 32 *Em* strains from chickens and pigs and one control isolate (ATCC 19434) were clustered with profiles of isolates contained in the international database using the eBURST algorithm. The *Em* population snapshot generated by eBURST is characterized by the presence of a specific Clonal Complex (CC)17 in which the majority of invasive (clinical) and hospital outbreak isolates group together (Fig. 1). Of the 32 animal-derived *Em* isolates only one chicken isolate grouped in CC17. All other chicken and pig isolates clustered outside CC17.

When STs of the pig isolates were compared in detail with STs from the database, 4 STs, representing 5/13 pig isolates with a complete allelic profile (38%), were identical to STs found among isolates from hospitalized patients, mainly to hospital surveillance isolates. In contrast, 8 STs, representing 11/13 isolates (84%) were identical to STs in the community, mainly to pig isolates. Two pig isolates had a unique profile.

Of the three pig isolates with an incomplete profile, one isolate had a unique profile, and the profile of the two others closely resembles profiles of both hospital and non-hospital isolates.

Eight (47%) of 15 chicken isolates had a unique ST, two isolates (20%) had a ST also found among clinical isolates of hospitalized patients, and seven isolates (47%) had a ST found among community isolates, mainly poultry isolates. The incomplete allelic profile of the one chicken isolate was not present in the database.

Clustering and comparison of allelic profiles of *E. faecalis*

Allelic profiles of 29 *Es* strains from chickens and pigs and one control isolate (JH2-2) were clustered with profiles of isolates contained in the international database using the eBURST algorithm. The *Es* population snapshot generated by eBURST is characterized by the presence of a number of Clonal Complexes in which hospital-derived isolates are enriched and which may be considered as equivalent to the *Em* CC17. Two of these so-called *Es* High Risk Enterococcal Clonal Complexes (HiRECC), CC2 and CC9, have been described previously.

Analysis of isolates currently contained in the *Es* MLST database indicate that in addition to CC2 and CC9, CC11 and CC88 could also be classified as HiRECC (Fig. 2). Of the 29 animal-derived *Es* isolates only one pig isolate, grouped in one of the *Es* HiRECC, CC11. All other chicken and pig isolates clustered outside these *Es* HiRECC.

When STs of the *Es* pig isolates were compared in detail with STs from the database, 5 STs, representing 8 of 17 pig isolates (47%), were identical to STs found among isolates from hospitalized patients, mainly to clinical isolates. In contrast, 5 STs, representing 12/17 isolates (71%) were identical to STs in the community, mainly to pig isolates. Two pig isolates had a unique profile.

Two (17%) out of 12 chicken isolates had a unique ST, 9 isolates (75%) comprising 4 STs had a ST also found among hospitalized patients, mainly clinical isolates, and six isolates (50%) had three different STs found among community isolates, mainly human isolates. One isolate with ST95 had an ST identical to an isolate in the database from unknown source.

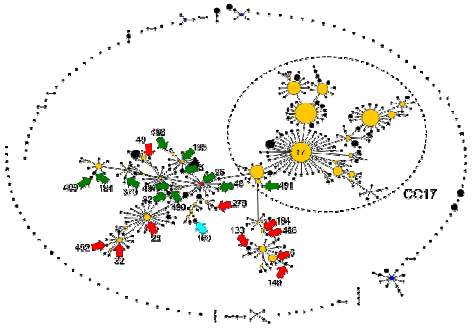

Fig. 1. *Em* eBURST-based population snapshot based on 1670 isolates from various human and non-human sources from multiple countries and continents. ST26 is set as primary founder. Circles represent STs and the area of each circle in the eBURST diagram corresponds to the abundance of the isolates of the ST in the input data.

STs that differ only in one of the seven loci, the so-called single locus variants (SLVs) are linked. Clusters of linked isolates correspond to clonal complexes. Primary founders (blue) and user-defined founder (red) are positioned centrally in the cluster, and subgroup founders are shown in yellow. CC17 (dotted circle) and ST17 (17) are indicated.

Arrows points towards STs of the *Em* isolates analyzed in this study: Red arrows = pig isolates, green arrows = chicken isolates and light-blue arrow = ATCC 19434. Numbers near the arrows indicate ST numbers.

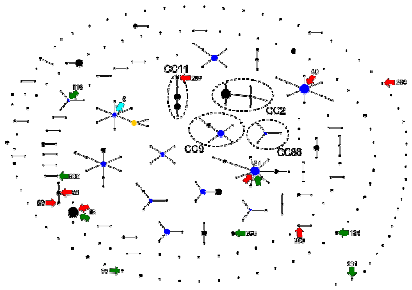

Fig. 2. *Es* eBURST-based population snapshot based on 776 isolates from various human and non-human sources from multiple countries and continents. Circles represent STs and the area of each circle in the eBURST diagram corresponds to the abundance of the isolates of the ST in the input data.

STs that differ only in one of the seven loci, the so-called single locus variants (SLVs) are linked. Clusters of linked isolates correspond to clonal complexes. Primary founders (blue) are positioned centrally in the cluster, and subgroup founders are shown in yellow. CC2, CC9, CC11, and CC88 (dotted circles) are indicated.

Arrows points towards STs of the *Es* isolates analyzed in this study: Red arrows = pig isolates, green arrows = chicken isolates and light-blue arrow = JH2-2. Numbers near the arrows indicate ST numbers.

Comparison of MLST data of the chicken and pig *Em* and *Es* isolates from the EASSA project with isolates contained in the international databases indicated that with two exceptions, one *Em* and one *Es* isolate, the animal-derived isolates did not group with previously and newly defined HiRECC represented by *Em* CC17 and *Es* CC2, CC9, CC11 and CC88.

For the *Em* isolates this means that with one exception the animal-derived isolates were genotypically distinct from clones causing the vast majority of invasive infections and hospital-outbreaks. It is highly probably that this one isolate that clustered with CC17 was the result of eroneous linkage due to high recombination vs. mutation rate (Willems *et al* 2011).

The population structure of *Es* is more complicated. A single dominant lineage of invasive and outbreak isolates, like *Em* CC17, does not seem to exist. On the other hand there are some smaller CCs, like CC2, CC9, CC11 and CC88 that are significantly enriched in invasive and outbreak isolates and it is clear from the data that with one exception the animal-derived *Es* isolates do not group in these CCs. However, since invasive *Es* isolates are much more genetically diverse thus more dispersed in the MLST-based population snapshot, it is not obvious from the clustering that the animal-derived *Es* isolates characterized in this study are genotypically distinct from the majority of invasive isolates.

The fact that only two of the animal-derived isolates from this study co-cluster in *Em* or *Es* HiRECC does not mean that the animal-derived isolates studied here are completely genotypically distinct from hospital isolates from the database.

For the *Em* isolates analyzed here 10 (36%) had unique STs, no isolates had STs that were exclusively found among hospital isolates, 10 (36%) had STs that were exclusively found among non-hospital isolates and 8 (29%) had STs that were found both in hospital and non-hospital isolates in the database.

For the *Es* isolates analyzed here 4 (14%) had unique STs, 6 (21%) isolates had STs that were exclusively found among hospital isolates, 6 (21%) had STs that were exclusively found among non-hospital isolates and 12 (41%) had STs that were found both in hospital and non-hospital isolates in the database.

The observation that STs of the *Em* animal-derived isolates were less frequently encountered in both hospital and non-hospital isolates than it was the case for the *Es* isolates reflects partly the difference in the population structure between *Em* and *Es*, with more extensive host-specific grouping in *Em* than in *Es*.

References

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