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Antimicrobial susceptibility of enterococci recovered from healthy cattle, pigs and chickens in nine EU countries (EASSA Study) to critically important antibiotics

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ABSTRACT

The European Antimicrobial Susceptibility Surveillance in Animals (EASSA) program collects zoonotic and commensal bacteria from food-producing animals at slaughter and tracks their susceptibility to medically important antibiotics. Results of commensal enterococci species (2013–2014) are presented here. Intestinal content from cattle, pigs and chickens were randomly sampled (5–6 countries/host; ≥4 abattoirs/country; 1 sample/animal/farm) for isolation of enterococci, MICs of 9 antibiotics were assessed by CLSI agar dilution in a central laboratory. Clinical breakpoints (CLSI) and epidemiological cut-off values (EUCAST) were applied for data interpretation.

In total 960 *Enterococcus faecium* and 779 *Enterococcus faecalis* strains were recovered. Seven porcine *E. faecium/faecalis* strains of Spanish origin were resistant to linezolid. One avian *E. faecalis* and one porcine *E. faecium* strain were non-wild type (MICs 8 mg/L) to daptomycin. Clinical vancomycin resistance was absent; 2 poultry *E. faecium* and 1 bovine *E. faecalis* strains were non-wild type, all with MICs of 8 mg/L. None of the strains tested were clinically resistant to tigecycline. Little clinical resistance to ampicillin or gentamicin was observed. Clinical resistance of *E. faecium* to quinupristin/dalfopristin was slightly higher (2.2–12.0%) but 61.9–83.2% of the strains were classified as non-wild type. Very high percentages resistance to tetracycline (67.4–78.3%) and to erythromycin (27.1–57.0%) were noted for both *E. faecium* and *E. faecalis* in pigs and chickens compared to cattle (5.2–30.4 and 9.0–10.4%, respectively). Similar non-wild type results were observed for *E. hirae* (n = 557), *E. durans* (n = 218) and *E. casseliflavus* (n = 55) including percentage non-wild type for daptomycin, linezolid, tigecycline being absent and for vancomycin low. For these species percentage non-wild type to erythromycin was lower as compared to *E. faecalis/faecium*. This pan-EU survey shows high variability in antibiotic susceptibility of commensal enterococci from healthy food animals. Clinical resistance to critically important antibiotics for human medicine was absent or low, except for erythromycin.

1. Introduction

The potential for transfer of antimicrobial resistance from enteric bacteria in animals to humans is a global public health concern. It is therefore important to monitor antimicrobial resistance of zoonotic and commensal bacteria in animals. There are many national surveillance programs to monitor antimicrobial susceptibility of zoonotic and commensal bacteria in food-producing animals e.g., CIPARS in Canada, JVARM in Japan, MARAN in The Netherlands, NORM-VET in Norway, NARMS in USA. The European Food Safety Authority (EFSA) annually analyzes and reports information on antimicrobial resistance in bacteria from animals submitted by various European Union (EU) Member

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States (EFSA/ECDC, 2015). However, international surveys are scarce (Schrijver et al., 2018). Regarding the commensal bacteria, *Escherichia coli* and enterococci species are included as indicator organisms for the Gram-negative and Gram-positive flora, respectively. The level of antimicrobial resistance in bacteria inhabiting the intestinal tract can directly reflect the selection pressure as a result of the use of antibiotics, especially over time. This paper focuses on enterococci.

The European Antimicrobial Susceptibility Surveillance in Animals (EASSA) project is an ongoing pan-EU program to monitor the *in vitro* antimicrobial susceptibility of zoonotic (*Salmonella, Campylobacter*) and commensal (*E. coli, Enterococcus*) bacteria isolated from healthy food-producing animals (broiler chickens, slaughter pigs, beef cattle) at slaughter. The survey is based on uniform sampling and bacterial isolation procedures performed in local laboratories, and MIC determination in a central laboratory to panels of antimicrobials commonly used in human medicine (de Jong et al., 2013). The findings of previous EASSA studies (1998–2001, EASSA I; 2002–2006, EASSA II; 2008–2009, EASSA III) have been reported elsewhere (Bywater et al., 2004, 2005; de Jong et al., 2009, 2012; Simjee et al., 2013). Here, the susceptibility results of enterococci including primarily *E. faecium, E. faecalis* and *E. hirae* recovered during 2013–2014 (EASSA IV) are reported.

2. Materials and methods

The design of the EASSA program including collection criteria such as animal population and sampling procedures were described previously (e.g., Bywater et al., 2004; de Jong et al., 2013; El Garch et al., 2018). In brief, samples of colon (caecum for chicken) contents were randomly collected from between 4 and 20 abattoirs per country in 5 or 6 EU countries per host species (see Table 1 for the countries per host). Standardized sampling (*ca.* 200 samples per host with few exceptions) was conducted throughout the two year collection period. The total number of samples taken amounted to 862, 1146 and 1024 for cattle, pigs and chickens, respectively. Each herd or flock was sampled only once. Isolation and phenotypic identification of enterococci was performed using standardized procedures in national microbiology laboratories (de Jong et al., 2009). MALDI-ToF was used for 6.4% of the isolates to confirm strain identity at the central laboratory (LGC, Fordham, UK) if isolates were only identified to genus level by the collecting country or if growth characteristics raised doubts on the original identification. Only one isolate of each *Enterococcus* species was retained from each sample. Overall for a minority of the enterococci-positive samples, isolates of two enterococci species were recovered; in rare cases the samples yielded three species. Agar dilution MIC testing was performed at the central laboratory according to CLSI recommendations (CLSI, 2013). Daptomycin testing was performed using the broth microdilution method with Mueller Hinton Broth fortified with 50 mg/L calcium. The following antibiotics (test ranges expressed as mg/L), representing nine antimicrobial classes (9 human-use antimicrobials/antimicrobial combinations either classified (WHO, 2017) as Critically Important Antibiotics (7) or Highly Important Antibiotics (2)), were tested: ampicillin (0.06–64), daptomycin (0.12–16), erythromycin (0.06–256), gentamicin (1–1024), linezolid (0.25–8), quinupristin/dalfopristin (Q/D; 0.25–32), tetracycline (0.03–256), tigecycline (0.03–8) and vancomycin (0.25–256). *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 served as quality control strains.

Results were interpreted based on two criteria, i.e., clinical breakpoints and epidemiological cut-off values (ECOFFs) (www.eucast.org), similar to previous EASSA studies (Moyaert et al., 2014; de Jong et al., 2016; 2017). Clinical resistance was determined in case of *E. faecium* and *E. faecalis* for each antimicrobial, host species and country according to CLSI breakpoints (CLSI, 2017), the only exceptions being Q/D for *E. faecium* and tigecycline (European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints; www.eucast.org). For daptomycin, and Q/D in *E. faecalis,* clinical breakpoints have not been set. The population non-wild type was based on ECOFFs, as defined by EFSA (2012) and confirmed by the European Commission (EC, 2013). For two antibiotics (erythromycin and linezolid) clinical breakpoints and ECOFFs are identical resulting in identical percentages resistance and non-wild type; for the other seven antibiotics breakpoints and ECOFFs differ at least one dilution step. For non-*E. faecium* and non-*E. faecalis* enterococci species only percentage non-wild type has been calculated. Decreased susceptibility is calculated as the percentage non wild-type subtracted by the percentage clinical resistance. Resistance breakpoints and ECOFFs are indicated in Tables 1 and 2. Multi-drug resistance (MDR) is defined as occurrence of clinical resistance to molecules of at least three different antimicrobial classes (Magiorakos et al., 2012).

3. Results

The total number of *Enterococcus* spp. strains recovered was 2669 including 960 *E. faecium* (36.0%; cattle 134, pigs 328, chickens 498), 779 *E. faecalis* (29.2%; cattle 115, pigs 176, chickens 488) and 557 *E. hirae* (20.9%; cattle 234, pigs 286, chickens 37). The remainder of the enterococci species (14.0%; 373) comprised *E. durans* (218; 8.2%), *E. casseliflavus* (88; 3.3%), *E. gallinarum* (24; 0.9%), *E. mundtii* (15; 0.6%), *E. thailandicus* (15; 0.6%), *E. avium* (4; 0.1%), *E. villorum* (4; 0.1%), *E. devriesei* (2; 0.1%), *E. saccharolyticus* (2; 0.1%) and one *Enterococcus* isolate unidentified by MALDI-ToF. The results comprising $MIC_{50/90}$, percentage resistance and percentage non-wild type are summarized for *E. faecium* and *E. faecalis* in Tables 1 and 2; for non-*E. faecium*/*E. faecalis* species $MIC_{50/90}$ and percentage non-wild type are indicated in Tables 3 (*E. hirae*) and Table 4 (*E. durans, E. casseliflavus*). Sample size for the other enterococci species precluded any detailed analysis such as the analysis per country (Table 4). The overall isolation rates of *E. faecium* and *E. faecalis* were 30.6 and 25.7%, and predominantly recovered from pigs and chickens. The MICs of the quality control strains were within the acceptable CLSI ranges.

3.1. *E. faecium;* Table 1

Overall, little clinical resistance to ampicillin and gentamicin was observed for *E. faecium* strains (on average 0.0–7.6% and 0.0–1.6% across host species, respectively). Nine isolates displayed high-level gentamicin resistance. All isolates but one (German isolate with MIC 8 mg/L) were wild type for daptomycin. All except 3 porcine isolates from Spain were susceptible to linezolid. Clinical resistance to neither tigecycline nor vancomycin was observed. In contrast, the resistance prevalence to erythromycin and tetracycline was much higher, on average 5.2–77.9% across host species. MIC frequency distributions for these two compounds exhibit a bimodal pattern (Figs. 1A and Figure 2A). Overall resistance to Q/D varied from 2.2 to 12.0% per host species, but percentage non-wild type – based on provisional ECOFFs (www.eucast.org) – was very high (61.9–83.2%). Only in this case decreased susceptibility (number of isolates with MICs > ECOFF but < clinical breakpoint) was very high (59.7–80.8%); for all critically important antibiotics decreased susceptibility was negligible. Marked country differences were observed for several antibiotics, however isolate numbers per country were too low in some cases to allow reliable comparisons to be made. MDR in *E. faecium* amounted to 1.5, 2.1 and 16.1% for cattle, pigs and chickens, respectively. The most frequent MDR phenotypes observed representing resistance to three compounds were erythromycin,Q/D,tetracycline (n = 48) and ampicillin,erythromycin, tetracycline (n = 25). Other combinations with three compounds were erythromycin,gentamicin,tetracycline (n = 4) and ampicillin,gentamicin,tetracycline (n = 1). Eleven isolates were resistant to four compounds with phenotype ampicillin, erythromycin,Q/D,tetracycline (n = 5), ampicillin,erythromycin,gentamicin,tetracycline (n = 4), and ampicillin/erythromycin/linezolid/tetracycline (n = 2).