

Acknowledgments

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Porcine and Human Community Reservoirs of *Enterococcus faecalis*, Denmark

To the Editor: *Enterococcus faecalis*, which exists commensally in the gut in warm-blooded animals and humans, is an opportunistic pathogen that causes a variety of community-acquired and health care-associated infections, such as urinary tract and intraabdominal infections, bacteremia, and endocarditis (1). Only a few studies have assessed the relationships between clinical *E. faecalis* strains; strains endemic to the health care setting; and community strains residing in humans, animals, or animal-origin food (2).

Recently we showed that the emergence of high-level gentamicin-resistant (HLGR) *E. faecalis* among patients with infective endocarditis (IE) coincided with an increase in HLGR *E. faecalis* in the pig population in Denmark (3). The majority of isolates belonged to the same clonal group (sequence type [ST] 16), suggesting that pigs constitute a community reservoir of HLGR *E. faecalis*.

We investigated human and porcine community reservoirs of other *E. faecalis* clonal types associated with IE in humans in Denmark.

A total of 20 consecutive gentamicin-susceptible *E. faecalis* isolates were obtained from IE patients in North Denmark Region during 1996–2002 (online Appendix Table, wwwnc.cdc.gov/EID/article/17/12/10-1584-TA1.htm). Cases of IE were classified as definite ($n = 12$) or possible ($n = 8$) according to the modified Duke criteria (4). A case of community-acquired *E. faecalis* infection ($n = 6$) was defined in accordance with strict criteria applied for methicillin-resistant *Staphylococcus aureus* (5); otherwise, cases were deemed to be health care associated ($n = 14$) (online Appendix Table). HLGR ST16 isolates recovered from 2 IE patients during the study period have been characterized (3) and were excluded from the present study.

Using multilocus sequence typing (6), we identified 14 STs among the 20 IE isolates (online Appendix Table), then compared them with STs from 2 collections of *E. faecalis* isolates collected as part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (www.danmap.org): 1) all 14 isolates recovered from community-dwelling humans in North Denmark Region during 2002–2006 with approval from the local ethics committee ([KF] 01-006/02), which were classified into 10 STs in this study (online Appendix Table); and 2) 19 pig isolates from 2001 that were shown in a previous study to belong to 12 STs (7).

Among the 14 STs identified in IE isolates, 4 (ST19, ST21, ST72, and ST306) and 2 (ST40 and ST97) were also found among isolates from community-dwelling humans and pigs, respectively (online Appendix Table). Isolates belonging to these 6 STs were further characterized by pulsed-field gel electrophoresis (PFGE) by using *Sma*I and grouped

into PFGE pulsotypes as described (3). STs and PFGE pulsotypes (A–F) were largely concordant (ST97:A, ST72:B, ST19:C, ST40:D, ST21:E, and ST306:F), except for 2 isolates belonging to ST72 and ST40, for which PFGE banding patterns (U1 and U2, respectively) were unrelated to the major PFGE pulsotypes (A–F), and 1 ST306 isolate exhibiting the ST21-like PFGE banding pattern E (online Appendix Table).

These findings confirm the genetic relatedness of IE isolates with those from community-dwelling humans (ST72:B, ST19:C, ST21:E, and ST306:F) and pigs (ST97:A and ST40:D). Seven (64%) of 11 IE isolates belonging to these 6 clonal types originated from IE patients with health care-associated risk factors (online Appendix Table), which suggests that health care users are predisposed to colonization and infection with *E. faecalis* strains residing in human and porcine community reservoirs.

Previous reports have shown that epidemiologically distinct *E. faecalis* populations differ in terms of biofilm formation, virulence gene content, and antimicrobial drug susceptibility profiles (2,8). Therefore, we characterized all isolates with respect to these traits. Isolates were categorized into strong, medium, weak, and non-biofilm formers by using the method of Mohamed et al. (8). The presence of 12 virulence-associated and pathogenicity island genes (*ebpA*, *gelE*, *ef1824*, *hylA*, *ef1896*, *ef2347*, *ef2505*, *hylB*, *ace*, *cbh*, *esp*, and *ef0571*) was investigated by using colony lysates and probes that have been described elsewhere (9). The antimicrobial drug susceptibility profiles (ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, linezolid, penicillin, streptomycin, teicoplanin, tetracycline, and vancomycin) were determined by the Sensititre system (Trek Diagnostic Systems, East Grinstead, UK) in ac-

cordance with Clinical and Laboratory Standards Institute guidelines (10). The isolates were generally homogenous within each clonal type in terms of biofilm formation, presence of virulence-associated and pathogenicity island genes, and resistance profiles (online Appendix Table), further supporting that IE isolates are genetically related to those from community-dwelling humans and pigs, respectively. Notably, most IE isolates were susceptible to ampicillin (100%), penicillin (100%), vancomycin (100%), high-level gentamicin (100%), and high-level streptomycin (80%), which are the drugs of choice in therapeutic regimens for *E. faecalis* endocarditis.

In conclusion, our results suggest that the normal intestinal microflora of humans and pigs are community reservoirs of clinical *E. faecalis* and link 2 porcine-origin clonal types of gentamicin-susceptible *E. faecalis*, ST97:A, and ST40:D to IE in humans in Denmark. This finding strengthens existing evidence that pigs can be a source of serious infections in humans.

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West Nile Fever Outbreak in Horses and Humans, Spain, 2010

To the Editor: *West Nile virus* (WNV) is a member of the genus *Flavivirus* within the Japanese encephalitis antigenic complex. The enzootic virus cycle involves transmission between avian hosts and ornithophilic mosquitoes, whereas humans and horses are considered

dead-end hosts. Given the recent increase of WNV infection in humans and horses in Europe, concern has been raised regarding public and animal health.

In Spain, WNV seropositivity has been reported for humans (2001), horses (2005–2008), and wild birds (2007–2008) (1–3). Clinical disease has been described for humans (2004) and raptors (2001–2005) (4,5) but not for horses. We report the main epidemiologic and clinical findings of a WNV outbreak in horses and humans in Spain in 2010.

After the first clinical case of West Nile fever was detected in a horse in September 2010 in Andalusia (southern Spain), a control program for WNV was initiated that included symptomatic treatment of animals, protection of horses in shelters during hours of higher vector activity, vaccination (not mandatory), vector control using pyrethroid-based insecticides, and elimination of mosquito breeding habitats. Horses with neurologic signs were confirmed as WNV positive by detection of serum IgM against WNV by using a competitive ELISA (IDEXX IgM WNV Ab; IDEXX Laboratories, Westbrook, ME, USA). To assess level of WNV infection within affected herds, samples from sick and clinically healthy unvaccinated horses were collected 2 months after the last case. Serum was tested for IgG against WNV by using a blocking ELISA (Ingezim West Nile compac R.10. WNV.K3; Ingenasa, Madrid, Spain). Positivity was confirmed by a serum microneutralization test (SNT) against WNV (strain Eg101) according to World Organisation for Animal Health guidelines. Blood and cerebrospinal fluid samples from clinically affected horses were analyzed by real-time reverse transcription PCR (6).

IgM against WNV was detected in 51 (50%) of 102 clinically ill horses; 15 died and 3 were euthanized. The most common clinical signs were