Short Communication

Survey of *Enterococcus faecalis* in Diseased Swine in Hunan Province, China

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Abstract

The prevalence of *Enterococcus faecalis* was investigated in diseased swine in Hunan Province, China between February 2007 and February 2011. A total of 385 diseased swine dissected in prosectorium from 10 representative administrative regions in Hunan Province were examined for the presence of *E. faecalis*. Forty-two out of 385 (10.91%) diseased swine were found to be infected with *E. faecalis* by bacteria isolations. *E. faecalis* was mainly recovered from the lung and joint cavity. Identification of the organisms was based on biochemical assays and sequencing of the partial 16S rRNA. The results of the present investigation have implications for the ongoing control of *E. faecalis* infections in swine and humans in Hunan Province, China.

Keywords: China, diseased swine, *Enterococcus faecalis*, survey

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บทคัดย่อ

การสำรวจเชื้อ *Enterococcus faecalis* ในสุกรป่วยของมณฑลหูหนาน ประเทศจีน

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ทำการสำรวจอุบัติการณ์ของการติดเชื้อ *Enterococcus faecalis* ในสุกรป่วยของมณฑลหูหนาน ประเทศจีน ระหว่างเดือนกุมภาพันธ์ ปี ค.ศ. 2007-2011. ทำการเก็บตัวอย่างจำนวน 385 ตัวอย่าง จากอวัยวะภายในของสุกรป่วยโดยการสุ่มคัดเลือกพื้นที่ จำนวน 10 แห่ง ผลการศึกษา สามารถเพาะแยกเชื้อ *E. faecalis* ได้จำนวน 42 ตัวอย่าง (10.91 %) จากปอด และข้อต่อของสุกรป่วย การจำแนกชนิดของเชื้อดังกล่าวกระทำโดย การตรวจทางชีวเคมี และการตรวจรหัสพันธุกรรมของ 16S rRNA ซึ่งผลการศึกษานี้สามารถนำไปใช้ในการควบคุมการติดเชื้อ *E. faecalis* ในสุกรและมนุษย์ได้ในเขตมณฑลหูหนาน ประเทศจีน

คำสำคัญ: ประเทศจีน สุกรป่วย *Enterococcus faecalis* การสำรวจ

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Introduction

Enterococcus faecalis is a common component of the microflora community in many warm-blooded animal species, including humans (Lebreton et al., 2009). The organism can withstand in harsh environment. Thus, it is not surprising that they can be found in soil, water, sewage and food (Cohen, 2000; Cohen, 2002; Paulsen et al., 2003). *E. faecalis* is an opportunistic pathogen that often causes bacteremia, endocarditis, meningitis, prostatitis, pneumonia, surgical wound infection, orthopedic prosthesis infection, root canal infection and eye infection in humans and animals (Butaye et al., 2001; Creti et al., 2004). A recent report revealed that prevalence of *E. faecalis* infection in swine was higher than previous report (Zou et al., 2011).

With significant socio-economic development, China is now the largest pork producer in the world (all data supplied by FAO). Limited surveys have shown that *E. faecalis* infection in swine can cause health problems in humans such as endocarditis, meningitis, peritonitis through food chain (Ruiz-Garbajosa et al., 2006). Moreover, genetic analysis of *E. faecalis* demonstrated that the clinic isolates are closely to isolates from other sources (Petersson-Wolfe et al., 2008). This suggests that in some cases, the bacteria in humans come from animals such as bovine, poultry etc. through food chain. Only a few case reports revealed that *E. faecalis* was isolated from animals in Henan, Xinjiang, Guangxi province but in Hunan province, there has not been any reports. The objective of the present study was to determine the prevalence of *E. faecalis* infection in diseased swine in China's central Hunan Province.

Materials and Methods

The survey took place between February 2007 and February 2011. All diseased swine which showed symptoms in 10 regions of Hunan province (Table 1) were randomly sampled. All diseased swine were submitted to the College of Veterinary Medicine, Hunan Agricultural University. Some of the diseased swine showed emaciation, severe fever (temperature range: 38-39.5°C), depressed and diarrhea. At necropsies, lung emphysema, and hemorrhage of the kidneys were found. All tests were performed in triplicate. Bacteria isolation was carried out with freshly prepared medium (Tryptic soy broth, TSB, Fluka, USA) by streaking inoculation on separate occasions. In addition, the blood agar plate, and the broth cultures were used for the identification of the bacteria. After 18-24 hours of incubation at 37°C in an aerobic environment on the agar plate, it grew gray, non-transparent, smooth colonies of 0.5-1 mm in diameter. Then, picking colony from the plate to Gram stain, Gram-positive, spherical-shaped bacteria were observed (data not shown). All 42 strains grew in the presence of 6.5% NaCl, at temperature ranging from 10°C to 45°C, at pH 9.6, as well as on bile-esculin agar.

Genomic DNA was extracted from 42 individual *E. faecalis* representing 10 geographical regions. The 16S rRNA gene sequences of the isolated bacteria were amplified by PCR with a thermal cycler (Biometra). The PCR reactions contain 20 µl solution consisting of 10 mM Taq reaction buffer (pH 8.4), 200 mM each of dNTP, 50 pmol of each primer and 2 U Taq polymerase (TIANGEN). The oligonucleotide primers used for the 16S rRNA gene were 16SFP (5'-

AGAGTTTGATCCTGGCTCAG-3′) and 16SRP (5′-CGGTTACCTTGTTACGACTT-3′) which were designed based on conserved regions of 16S rRNA gene (Weisburg et al., 1991). The thermocycle program was as follows: an initial denaturation at 94°C for 5 min, then 94°C for 30 sec (denaturation), 59°C for 30 sec (annealing), 72°C for 60 sec (extension) for 30 cycles, followed by 72°C for 5 min (final extension). Each amplicon (5 µl) was examined by agarose gel electrophoresis to validate amplification efficiency. Then, the partial 16S rRNA gene amplicons were submitted to Sangon Biotech Co., Ltd (Shanghai, China) for sequencing from both directions by using primers used in the PCR amplifications. The sequences of the PCR products were compared with those of closely related species in GenBank by multiple sequence alignment using ClustalX 1.83 (Thompson et al., 1997).

Results and Discussion

E. faecalis was found in 42 out of 385 (10.91%) diseased swine. A large proportion of *E. faecalis* was found in the lung and joint cavity (69% and 14% respectively). The infection rate ranged from 5.77% to 17.5% in diseased swine in different geographical locations (Table 1). Diseased swine in Yongzhou country had the highest infection rate (17.5%) (Kaneene et al., 2002), this is more likely due to difference in swine infection disease and swine husbandry practices.

Table 1 Infection of diseased swine with E. faecalis in Hunan Province, China.

Geographical locations	Infected No.	Specimen Number	Prevalence (%)
Lake area			
Yueyang	3	48	6.25
Yiyang	3	42	7.14
Mountain area			
Chenzhou	4	43	9.30
Huaihua	3	22	13.64
Hengyang	3	28	10.71
Yongzhou	7	40	17.5
Loudi	5	35	14.29
Shaoyang	3	19	15.79
Hill area			
Changsha	8	56	14.29
Zhuzhou	3	52	5.77
Total	42	385	10.91

The partial 16S rRNA sequences were between 1270-1400 bp in length. The A+T contents of the sequences were 45.75-46.18%, consistent with previous reports of the AT bias of *E. faecalis* in humans (Phyllis et al., 2008; Monstein et al., 1998). Sequence variations in the 16S rRNA gene among all *E. faecalis* isolates were 0.00-1.10%, which revealed that they were divided into the same species.

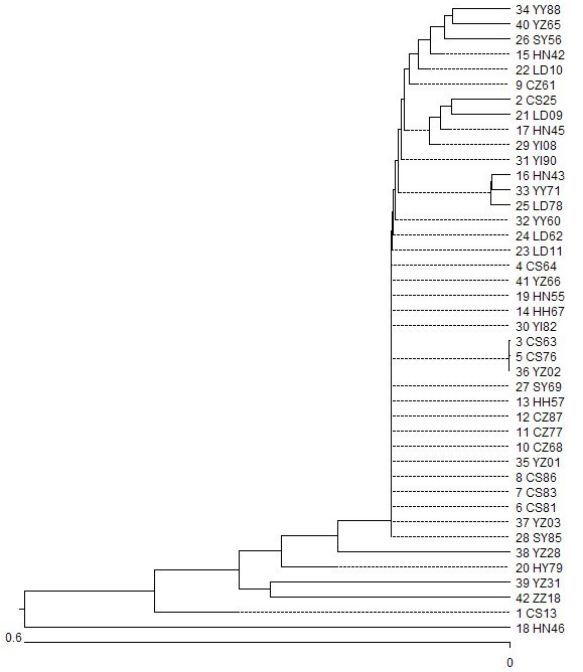

Figure 1 Phylogenetic tree of 16S rRNA of E.faecalis

The sequences of 16S rRNA of bacterial isolates in this study were identified to the *E. faecalis* reported (GenBank accession number EU168401.1) with similarity >98%. All sequencing results were restored in GenBank and their phylogenetic tree (Fig 1) did not show any distinct regularity between different place (Fig 1). Most of those strains were isolated from lung (69%), which may be involved in pneumonia (Creti et al., 2004). The other strains were isolated from joint cavity (14%) followed by lymph nodes and brain (7%). *Strepcoccus spp.*, *E. coli*, *E. faecium* and other bacteria were found in most of the specimens, which indicated that most of the cases were mixed infection (Singh et al., 2007).

This is the first report of *E. faecalis* infection of swine in Hunan Province. Further study observation revealed that diseased swine are susceptible to *E. faecalis*, with high infection rate (5.77-17.5%). The results of the present survey indicated that *E. faecalis* infections are highly prevalent in Hunan Province, China. Swine are the host of a number of bacteria such as Enterococci and Salmonella which can infect a number of domestic animals and humans, resulting in significant economic losses and public health threats. In addition, *E. faecalis* is also associated with human infection including nosocomial infections and their increasing resistance to common used antibiotics, especially vancomycin which has not been used in clinic (van den Bogaard and Stobberingh, 1999; Arciola et al., 2008;). Additionally, *E. faecalis* can harbor antibiotics resistance genes, virulence factors and other mobile elements and transfer to other bacteria as a gene reservoir, through the food chain (Roberts et al., 2006; Newell et al., 2010). Therefore, survey and monitor *E. faecalis* in foods and animals is very necessary for public health.

In conclusion, the present study demonstrated the occurrence of *E. faecalis* in Hunan province, which has implications for the ongoing control of *E. faecalis* in humans and animals.

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References

Arciola, C.R., Baldassarri, L., Campoccia, D., Creti, R., Pirini, V., Huebner, J. and Montanaro, L. 2008. Strong biofilm production, antibiotic multi-resistance and high gelE expression in epidemic clones of *Enterococcus faecalis* from orthopaedic implant infections. Biomaterials. 29(5): 580-586.

Butaye, P., Devriese, L.A. and Haesebrouck, F. 2001. Differences in antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals. Antimicrob Agents Chemother. 45(5): 1374-1378.

Cohen, M.L. 2000. Changing patterns of infectious disease. Nature. 406(6797): 762-767.

Creti, R., Imperi. M., Bertuccini. L., Fabretti, F., Orefici, G., Di Rosa, R. and Baldassarri, L. 2004. Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. J Med Microbiol. 53(Pt 1): 13-20.

Kaneene, J.B., Bruning-Fann, C.S., Granger, L.M., Miller, R. and Porter-Spalding, B.A. 2002. Environmental and farm management factors associated with tuberculosis on cattle farms in northeastern Michigan. J Am Vet Med Assoc. 221(6): 837-42.

Lebreton,F., Eliette, R.B., Serror,P., Sanguinetti, M., Posteraro,B., Torelli, R., Hartke, A., Auffray, Y. and Giard, J.C. 2009. Ace, which encodes an adhesin in *Enterococcus faecalis*, is regulated by Ers and Is involved in virulence. Infect Immun. 77(7): 2832–2839.

Monstein, H.J., Quednau, M., Samuelsson, A., Ahrné, S., Isaksson, B. and Jonasson, J. 1998. Division of the genus Enterococcus into species groups using PCR-based molecular typing methods. Microbiology. 144 (Pt 5): 1171-1179.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., van der Giessen, J. and Kruse, H. 2010. Food-borne diseases- The challenges of 20 years ago still persist while new ones continue to emerge. Int J Food Microbiol. 139(Suppl 1): S3-S15.

Paulsen, I.T., Banerjei, L., Myers, G.S., Nelson, K.E., Seshadri, R., Read, T.D., Fouts, D.E., Eisen, J.A., Gill, S.R., Heidelberg, J.F., Tettelin, H., Dodson, R.J., Umayam, L., Brinkac, L., Beanan, M., Daugherty, S., DeBoy, R.T., Durkin, S., Kolonay, J., Madupu, R., Nelson, W., Vamathevan, J., Tran, B., Upton, J., Hansen, T., Shetty, J., Khouri, H., Utterback, T., Radune, D., Ketchum, K.A., Dougherty, B.A. and Fraser, C.M. 2003. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. Science. 299(5615): 2071-2074.

Petersson-Wolfe, C.S., Adams, S., Wolf, S.L. and Hogan, J.S. 2008. Genomic typing of Enterococci isolated from bovine mammary glands and environmental sources. J Dairy Sci. 19(2): 615-619

Phyllis, A.W.M., Elizabeth, A.M. and Dawn, E.G.R. 2008. Microbial combinatorics: A simplified approach for isolating insecticidal bacteria. Biocontrol Sci Technol. 18(3): 291-305

Roberts, A.P., Davis, I.J., Seville, L., Villedieu, A. and Mullany, P. 2006. Mullany characterization of the ends and target site of a novel tetracycline resistance-encoding conjugative transposon from *Enterococcus faecium* 664.1H1. J Bacteriol. 188(12): 4356-4361.

Ruiz-Garbajosa, P., Bonten, M.J., Robinson, D.A., Top, J., Nallapareddy, S.R., Torres, C., Coque, T.M., Cantón, R., Baquero, F., Murray, B.E., del Campo, R. and Willems, R.J. 2006. Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic complexes in a background of high rates of recombination. J Clin Microbiol. 44(6): 2220-2228.

Singh, K.V., Nallapareddy, S.R. and Murray, B.E. 2007. Importance of the ebp (endocarditis- and biofilm-associated pilus) locus in the pathogenesis of *Enterococcus faecalis* ascending urinary tract infection. J Infect Dis. 195(11): 1671-7.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25(24): 4876-4882.

van den Bogaard, A.E. and Stobberingh, E.E. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs. 58(4): 589-607.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 173(2): 697-703.

Wheeler, A.L., Hartel, P.G., Godfrey, D.G., Hill, J.L. and Segars, W.I. 2002. Potential of *Enterococcus faecalis* as a human fecal indicator for microbial source tracking. J Environ Qual. 31(4): 1286-1293

Zou, L.K., Wang, H.N., Zeng, B., Li, J.N., Li, X.T., Zhang, A.Y., Zhou, Y.S., Yang, X., Xu, C.W. and Xia, Q.Q. 2011. Erythromycin resistance and virulence genes in *Enterococcus faecalis* from swine in China. New Microbiol. 34(1): 73-80.