

Prevalence of *esp*, Encoding the Enterococcal Surface Protein, in *Enterococcus faecalis* and *Enterococcus faecium* Isolates from Hospital Patients, Poultry, and Pigs in Denmark

The enterococcal surface protein, Esp, originally found in *Enterococcus faecalis*, has been associated with increased virulence, colonization, and biofilm formation (2, 3, 5, 6). Recently, a variant *esp* gene was found in *Enterococcus faecium* isolates from humans (L. Baldassarri, L. Burtuccini, M. G. Ammendolia, G. Gherardi, and R. Creti, Letter, Lancet 357:1802, 2001; R. J. Willems, W. Homan, J. Top, M. van Santen-Verheuevel, D. Tribe, X. Manziros, C. Gaillard, C. M. Vandembroucke-Grauls, E. M. Mascini, E. van Kregten, J. D. van Embden, and M. J. Bonten, Letter, Lancet 357:853-855, 2001; N. Woodford, M. Soltani, and K. J. Hardy, Letter, Lancet 358:584, 2001). The *esp* gene encodes a protein with inferred global structural similarity to Rib and C alpha proteins from group B streptococci and R28 from group A streptococci (3, 4).

In the present study, the prevalence of *esp* was investigated by PCR with primers published by Woodford et al.: Esp-p1, 5'-GGT CAC AAA GCC CAA CTT GT-3', and Esp-p2, 5'-ACG TCG AAA GTT CGA TTT CC-3' (Woodford et al., letter). Twenty-four *E. faecalis* and 29 *E. faecium* isolates from blood culture from patients in 13 different Danish hospitals were tested together with 75 *E. faecium* and 75 *E. faecalis* isolates from Danish pigs and 66 *E. faecium* and 60 *E. faecalis* isolates from Danish poultry (Table 1).

Among isolates from hospital patients, 10 of 24 (41.7%) *E. faecalis* isolates and 10 of 29 (34.5%) *E. faecium* isolates were *esp* positive (Table 1). The prevalence of *esp*-positive *E. faecalis* blood culture isolates was higher than that found by Shankar et al. (29%) (2) but lower than that reported by Waar et al. (45%) (6). Shankar et al. (2) did not find any *esp*-positive *E. faecium* isolates, whereas 56.5% of blood culture isolates were positive in the study by Woodford et al. (Woodford et al., letter). Among animal isolates, none of the *E. faecium* isolates were *esp* positive. This is consistent with previous studies (Woodford et al., letter; Willems et al., letter).

Six of the 75 (8.0%) *E. faecalis* isolates from pigs were *esp* positive, whereas none of the *E. faecalis* isolates from poultry were positive (Table 1). The 407-bp *esp* amplicons from two pig isolates of *E. faecalis* were sequenced. No variations were

found in comparison with GenBank AF034779. To our knowledge, *esp* has not previously been reported in *E. faecalis* isolates of animal origin. Expression of the Esp protein was not investigated.

Willems et al. found that *esp* was associated with epidemic vancomycin-resistant *E. faecium* clones (Willems et al., letter). In our study none of the *esp*-positive isolates from humans and animals were resistant to vancomycin or to quinupristin-dalfopristin. However, 9 of 10 *esp*-positive *E. faecium* isolates from humans, 3 of 10 *esp*-positive *E. faecalis* isolates from humans, and 1 of 6 *esp*-positive *E. faecalis* isolates from pigs were resistant to gentamicin, encoded by *aac(6')*-Ie-*aph(2'')*-Ia. All gentamicin-resistant, *esp*-positive isolates were also resistant to erythromycin, streptomycin, and kanamycin, encoded by *erm(B)*, *aadE*, and *aphA*, respectively. This suggests that a DNA element like the *erm(B)*-Tn5405-like element, recently found in *Staphylococcus intermedius* (1), could be present in some gentamicin-resistant, *esp*-positive enterococci.

The *esp* gene has been detected in 40% of *E. faecalis* isolates from the feces of healthy humans (6). This observation, taken together with the finding of the gene in pig isolates in this study, increases the need for more investigation of the function of this protein.

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TABLE 1. Prevalence of the *esp* gene in *E. faecalis* and *E. faecium* isolates from human, pigs, and poultry

Origin and sp.	No. of isolates tested	No. of isolates PCR positive for <i>esp</i> (%)
Humans		
<i>E. faecium</i>	29	10 (34.5)
<i>E. faecalis</i>	24	10 (41.7)
Pigs		
<i>E. faecium</i>	75	0 (0)
<i>E. faecalis</i>	75	6 (8)
Poultry		
<i>E. faecium</i>	66	0 (0)
<i>E. faecalis</i>	60	0 (0)