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Risk factors in enterococci isolated from foods in Morocco: Determination of antimicrobial resistance and incidence of virulence traits

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ABSTRACT

A collection of enterococci isolated from meat, dairy and vegetable foods from Morocco including 23 *Enterococus faecalis* and 15 *Enterococcus faecium* isolates was studied. All isolates were sensitive to ampicillin, penicillin, and gentamicin. Many *E. faecalis* isolates were resistant to tetracycline (86.95%), followed by rifampicin (78.26% ciprofloxacin (60.87%), quinupristin/dalfopristin (56.52%), nitrofurantoin (43.47%), levofloxacin (39.13%), erythromycin (21.73%), streptomycin (17.39%), chloramphenicol (8.69%), vancomycin (8.69%), and teicoplanin (4.34%). *E. faecium* isolates showed a different antibiotic resistance profile: a high percentage were resistant to nitrofurantoin (73.33%), followed by erythromycin (66.60%), ciprofloxacin (66.66%), levofloxacin (60.00%), and rifampicin (26.66%), and only a very low percentage were resistant to tetracycline (6.66%). One isolate was resistant to vancomycin and teicoplanin. The incidence of virulence factors was much higher among *E. faecalis* isolates, especially for genes encoding for sex pheromones, collagen adhesin, enterococcal endocarditis antigen, and enterococcal surface protein. Isolates with multiple factors (both antibiotic resistance and virulence traits) were also more frequent among *E. faecalis* isolates, in which one isolate cumulated up to 15 traits. By contrast, several isolates of *E. faecium* had only very few unwanted traits as compared to only two isolates in *E. faecalis*. The high abundance of isolates carrying virulence factors and antibiotic resistance traits suggests that the sanitary quality of foods should be improved in order to decrease the incidence of enterococci.

1. Introduction

Enterococci live as commensals of the gastrointestinal tract of warm-blooded animals, being the most abundant Gram-positive cocci in humans (Tannock and Cook, 2002). They can also be found in foods of animal origin (milk, cheese, fermented sausages), vegetables and plant materials because of their ability to survive heat treatments and adverse environmental conditions (Giard et al., 2001). They also play an important technological role in manufacture of meat and dairy products (Giraffa, 2003; Hugas et al., 2003; Foulquié Moreno et al., 2006) such as development of aroma and ripening of different cheeses. However, they have been involved in food spoilage (Franz et al., 1999), in food intoxication (Giraffa et al., 1997; Gardini et al., 2001), in nosocomial infections (Kayser, 2003) and in the spreading of antibiotic resistance through the food chain (Murray, 1990; Giraffa, 2002), rising concerns about the safety of strains found in foods. *Enterococcus faecalis* is the species most frequently involved in hospital infections (80–90%) followed by *Enterococcus faecium* (less than 10%). Enterococci may carry different genes directly or indirectly contributing to virulence (Eaton and Gasson, 2001; Franz et al., 1999, 2001, 2003; Kayser, 2003; Ben Omar et al., 2004). Enterococci can also acquire multiple antibiotic resistance, thanks to the different DNA exchange mechanisms they have (such as conjugative plasmid transfer, transposons and insertion sequences). The dual role played by these bacteria has raised concerns about the risks for spread of potentially virulent strains and their antibiotic resistance traits outside hospital environments, especially in foods (Franz et al., 2003).

In order to provide complementary data on enterococci from different geographic regions, this study was focused on enterococci isolated from foods in Morocco, for which no previous reports have been published on this topic. The purpose of the study was to determine the incidence of virulence factors and antibiotic resistance traits among a collection of enterococci isolated from different food sources.

2. Materials and methods

2.1. Strain isolation and identification

Food samples were obtained from local stores in Tanger, Marrakech, and Essawira (Morocco) and stored in their original envelopes under refrigeration until arrival to the laboratory. For selective isolation of enterococci, food samples (5 g each) were mixed with 45 ml sterile saline solution and homogenised for 30 s with a

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Stomacher 80 (Biomaster, Seward, UK) at low speed. Samples were spread on Slanetz agar (Scharlab, Barcelona, Spain) plates and incubated at 37 °C for 48 h. From each sample, 3 to 5 typical colonies were isolated and repurified on Slanetz agar. Isolates were kept in 20% glycerol at −80 °C. The following tests were carried out for presumptive identification of the isolates: observation of colony characteristics and cell morphology, Gram staining, catalase and oxidase production, growth at 10 °C and 45 °C, growth in the presence of 6.5% NaCl, at pH 9.6, as well as growth and esculin hydrolysis on bile-esculin agar (Scharlab). Genetic identification at species level was done by species-specific PCR and 16S rDNA sequencing as described elsewhere (Abriouel et al., 2005).

2.2. Antibiotic resistance

The antibiotic susceptibility of isolates was determined by using ATB ENTEROC 5 strips (BioMérieux, Marcy-l'Etoile, France). The test was performed following the manufacturer's instructions. Results were recorded after 24 h of incubation at 37 °C, and were evaluated according to the manufacturer's instructions.

2.3. Haemolysin production

Haemolysin activity was determined on Columbia Blood Agar (Oxoid, Basingstoke, England) containing 5% defibrinised horse blood after 48 h of incubation at 37 °C. Zones of clearing around colonies indicated β-haemolysin production.

2.4. PCR detection of virulence determinants

Total DNA of strains was used in PCR reactions to detect the presence of genes involved in the expression of cytolysin (*cylA*, *cylB* and *cylM*), the aggregation substance (*agg*), gelatinase (*gelE*), enterococcal surface protein (*esp*), cell wall adhesins ($efaA_{fs}$ and $efaA_{fm}$), and sex pheromones (*cpd*, *cob* and *ccf*) according to Eaton and Gasson (2001), and the collagen adhesin (*ace*) as described by Duprè et al. (2003). DNA from strain *E. faecalis* FI9190 (kindly provided by Drs. Eaton and Gasson, IFR, Norwich) was used as positive control in the corresponding PCR reactions.

3. Results and discussion

A total of 58 isolates from Slanetz agar plates corresponding to 18 food samples of different types, including vegetable, meat and dairy foods were selected for study (Fig. 1). After phenotypic characterization, 38 isolates with typical features of enterococci (sodium azide-resistant, catalase-negative Gram-positive cocci able to grow at 10 °C and 45 °C, at pH 9.0, in the presence of 6.5% NaCl, and to hydrolise esculin) were selected for identification at species level by species-specific PCR and 16S rRNA gene sequencing. A total of 23 isolates were identified as *E. faecalis* and 15 as *E. faecium* (Fig. 1).

The incidence of antimicrobial resistance and virulence factors for each isolate is shown in Fig. 1, and the corresponding percentage values are shown in Fig. 2. Among *E. faecalis,* a very high percentage of isolates (86.95%) were resistant to tetracycline (MIC > 8 μg/ml), followed by rifampicin (78.26%; MIC > 2 μg/ml), ciprofloxacin (60.87%; MIC > 2 μg/ml), quinupristin/dalfopristin (56.52%), nitrofurantoin (43.47%; MIC > 64 μg/ml), levofloxacin (39.13%; MIC > 4 μg/ml), erythromycin (21.73%; MIC > 4 μg/ml),

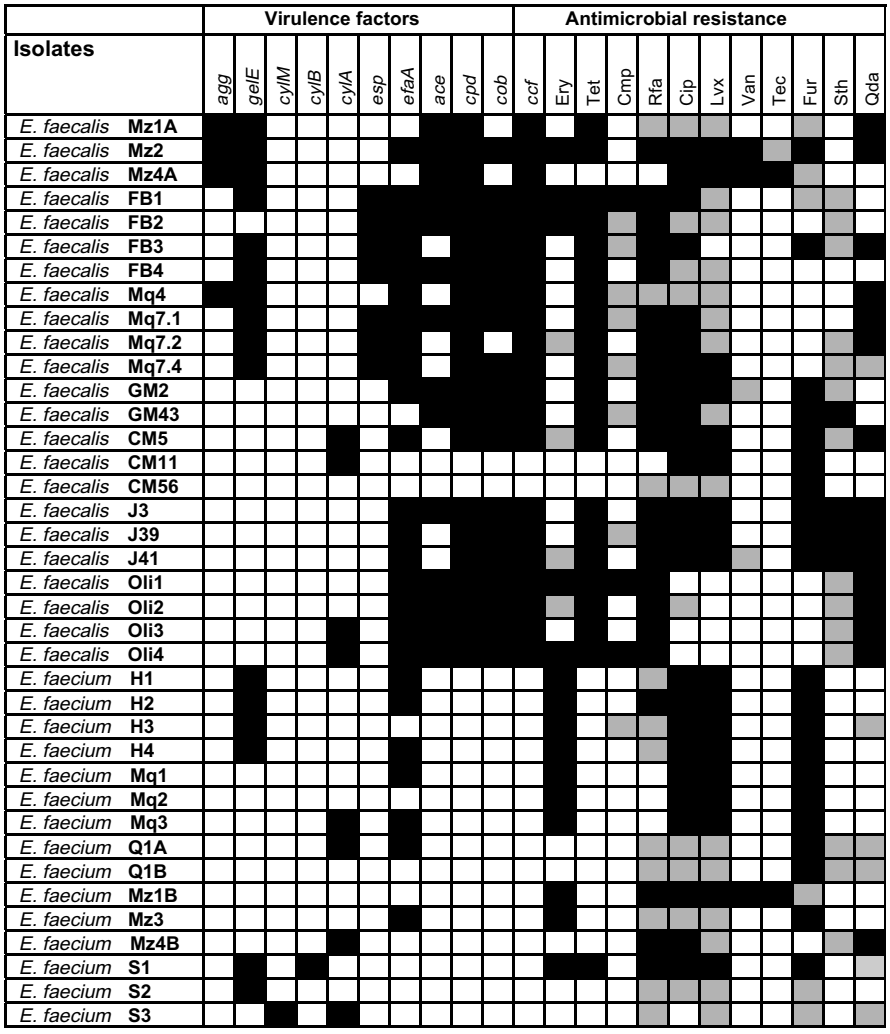

Fig. 1. Incidence of virulence factors and resistance to different antimicrobials for *E. faecalis* and *E. faecium* isolates of different types of foods from Morocco: ground pepper (harissa, H), table olives (Oli), salami from cow meat (Mz), turkey salami (S), goat milk (GM), cow milk (CM), commercial butter (Mq), jben traditional cheese (J), commercial goat cheese (FB), and commercial cow milk's cheese (Q). Positive and negative results are marked by black or white rectangles, respectively. Rectangles in gray indicate intermediate antibiotic resistance. Resistance to ampicillin, penicillin and gentamicin were not detected in any isolate.