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Report on the 2nd International ASM/FEMS Conference on Enterococci

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Conference Aims

The aim of the conference was to facilitate the exchange of current enterococci research amongst the leading enterococci researchers and those more recently involved in the area. The joint organisation of the conference between ASM and FEMS also aimed to promote international cooperation and exchange between researchers. Student and postdoctoral participation was also encouraged.

My personal aims were to interact with the world's leading enterococci researchers, learn about current enterococci research and how it can be applied to my work and to present my work amongst the international enterococci research community.

Program

Oral presentations were given mainly by the more prominent enterococci researchers and the people associated with them. Much of the conference content involved basic research, with a strong medical focus and had less applied research. As such, most of the work also focused on *Enterococcus faecalis* and *E. faecium*, which are the main species involved in clinical infection. Each oral session had four speakers over the space of 1.5 h.

Posters were exhibited for one full day and dedicated sessions were from 5-6:30 pm on Monday night and 7:30-9:30 pm on Tuesday night. My poster session was Tuesday night.

Session Details

Sunday, 28 August

Opening Session – FEMS Introduction (Eliora Ron), Historical perspective on enterococci research (Don Clewell), *Enterococcus*: A hub for antibiotic resistance genes (Patrice Courvalin)

Monday, 29 August

Session 1: Taxonomy, Identification and Typing
Session 2: Genome Information and Analysis
Session 3: Occurrence and Survival in the Environment
Session 4: Use of Enterococci in Food Production

Tuesday, 30 August

Session 5: Biology of Mobile Genetic Elements
Session 6: Horizontal Gene Transfer

Wednesday, 31 August

Session 7: Pathogenesis and Virulence
Session 8: Challenges in Clinical Settings
Session 9: Surveillance of Antimicrobial Resistance in Hospitals, the Community and Animals
Session 10: Evolution of Antimicrobial Resistance

Final Round Table Discussion
The chairs of each session (Figure 1) were asked for their comments on the conference.

Axel Hartke, Patrice Courvalin, Frank Aarestrup, Georgio Giraffa, Garry Dunny, Keith Weaver, Richard Facklam, Mike Gilmore, Barbara Murray, Neil Woodford, Lars Jensen

Figure 1. Round Table Discussion

Opening Session

The conference ran for four days, beginning with registration, three opening session presentations and the opening reception dinner. Lars Jensen, the Denmark Conference Organiser, opened the evening. He made reference to the setting of being in Helsingor (Elsinore), adjacent to Kronborg Castle, that instead of the opening to Shakespeare's Hamlet, "Something is rotten in the state of Denmark", that perhaps after the conference we could have "Something is fermenting in the state of Denmark", indicating the potential for exchange of ideas. Don Clewell, who is one of the longest standing researchers of streptococci and enterococci, presented an historical perspective of enterococci research, including early plasmid work and conjugation experiments. After this, Patrice Courvalin was also introduced as the "Granddaddy of enterococcal genetics", a title which he was not entirely thrilled about, as he did not consider himself to be finished yet!

Key Findings

Taxonomy

In the taxonomy presentations, DNA/DNA Reassociation was referred to as the Gold Standard for identifying new enterococcal species. The 16S rDNA sequencing technique was listed as the next most important, as it places the isolate in the genus. For phenotypic identification, reasonable results can usually be obtained by using a combination of the traditional tests. Laboratories should use the tests that work for them.

Genome Information, Mobile Genetic Elements and Horizontal Gene Transfer

One factor that has enabled enterococci to become an important nosocomial pathogen is the range of antibiotic resistances that they may carry, possibly due to their ability to acquire antibiotic resistance genes from elsewhere. This is demonstrated by the fact that V583, the first vancomycin resistant enterococci (VRE) isolated in the USA, contains 25% mobile or exogenously acquired DNA in its genome, as determined by analysis using micro arrays. Furthermore, the transfer of antibiotic resistance genes can occur even in the absence of antibiotic pressure. Several researchers are examining pheromone regulation and conjugative transfer in the tetracycline-resistance plasmid pCF10.

The transfer of *van*A vancomycin resistance in the human gut was investigated in a Danish study (Lester *et al*.) as this resistance is present in food animals in Europe. Six volunteers ingested vancomycin sensitive and vancomycin resistant strains of *E. faecium*. The vancomycin sensitive strain (10^9 cfu/mL) was ingested three hours prior to the ingestion of the resistant strain (10^7 cfu/mL). Vancomycin transconjugants were detected in the stools of three of the six people for the first seven days, illustrating that vancomycin transfer can occur in appropriate conditions in the human gut. The vancomycin resistant tranconjugants were not detected in the stool samples after 14 and 35 days, however there is the possibility that the vancomycin resistant isolates remained in the digestive tract of the volunteers but were below the testing detection limit. Also in that experiment, quinupristin/dalfopristin transferred in one person, although erythromycin failed to transfer. Some researchers at the conference, including Barbara Murray who is an MD, were sceptical of the ethics and safety of this experiment. In Australia, the predominant VRE are *van*B *E. faecium* VRE (Bell *et al.*, 1998), however, neither *van*A nor *van*B enterococci were detected in raw milk enterococci isolates previously tested at Food Science Australia.

Survival in the Environment

A Swedish study (Iversen *et al*.) looked at the presence of enterococci in sewage from hospitals and urban towns. Although enterococci with multiple antibiotic resistances (ampicillin, ciprofloxacin, gentamicin, tetracycline, erythromycin) were more commonly isolated from hospital sewage, VRE were more common in the urban sewage. An interesting case was found where sewage from an antroposophic village, in which the residents do not use antibiotics, had a higher level of tetracycline resistant enterococci than that found in the hospital sewage. In such a case, the enterococci would not have developed resistance due to selective antibiotic pressure within the individual residents and the residents probably acquired resistant enterococci from some other source such as food.

The presence of enterococci was examined in agricultural farmland soil in Denmark (Agersø *et al*.). Pig manure was added to the soil and *E. faecium* could be isolated from the soil two years after treatment. Tetracycline resistant *E. faecalis* was also added to the soil, but numbers declined rapidly until they were no longer detected after 45 days. However, horizontal gene transfer of *tet*M to *E. faecalis* and *E. faecium* was observed. Furthermore, *tet*M could be detected in the soil after the introduced tetracycline resistant enterococci could no longer be detected. It was mentioned that tetracycline degrades very slowly in soil and may accumulate over time. This could potentially explain the previous situation where tetracycline resistant enterococci were found in an antroposophic village. If the tetracycline remained in the soil due to former agricultural or medical practices, then the residing microorganisms, including enterococci, could develop resistance to tetracycline or maintain a previously obtained resistance. On a dairy farm, such microorganisms could enter the milk supply through lapses in hygiene.

Pathogenesis and Virulence

While antibiotic resistances make the treatment of enterococcal infections more difficult, virulence factors aid microorganisms in establishing and causing disease initially. Gelatinase, serine protease, cytolysin toxin and Ace and Acm adhesins were all mentioned, with most of the focus being on the gelatinase virulence factor. The production of gelatinase (GelE) has been recorded in *E. faecalis* isolates associated with infective endocarditis for over 100 years (Barbara Murray). It is predominantly produced by *E. faecalis* (Barbara Murray). While some work has found that gelatinase is critical for biofilm formation in some isolates of *E. faecalis* (Hancock *et al.*), other work has found that biofilm production was not different between gelatinase producers and non-producers (Barbara Murray). Still, there is the potential for the production of gelatinase to indicate possible biofilm capabilities of enterococci in other environments, such as those that occur in the dairy industry. Although studies have shown that both *E. hirae* and *E. faecium* can adhere to stainless steel (Wirtanen, 1995; Andrade *et al.*, 1998), the ability of enterococci to form biofilms in dairy environments has not been reported.

Enterococci in Food Production

Due to the association of enterococci with problematic nosocomial infections, the point was mentioned that the high incidence of enterococci in food could pose a risk, however disease usually only occurs in hospitals and non-healthy people (Holzapfel and Franz). Enterococci have been used in traditional foods such as Italian salami and German raw sausage, as well as non-starters in cheese in southern Europe (Giraffa). The approach in the food industry, then, is to take a strain-to-strain approach, avoiding the use of those strains with antibiotic resistance and suspected virulence factors (Giraffa). Microarray work has commenced comparing clinical, commensal and food enterococci isolates. Genes detected in the clinical and commensal isolates but not the food isolates could constitute new candidates for functional analysis related to host colonisation or virulence (Serror *et al*.). At this point, a debate arose between Barbara Murray and Mike Gilmore concerning what is a food isolate and whether or not they could have come from a human. Mike Gilmore presented the hypothesis that any *Enterococcus* could cause illness whereas Barbara Murray had the viewpoint that this might not be the case and that this is what research is trying to determine.

Poster Comments

Thierry Lambert, from the Laboratories Hospital St. Michel, in Paris, was interested in the fact that *E. hirae* were included in my poster. He commented that they had a patient with endocarditis caused by *E. hirae*. Although the isolate was both vancomycin and ampicillin sensitive, they were not able to eliminate it with antibiotics. This illustrates that species other than *E. faecalis* and *E. faecium* can cause serious illness in susceptible people.

Another delegate from eastern Europe was sceptical that *E. hirae* and *E. durans* were the main enterococci species that I detected in pasteurised milk. Her experience was that *E. faecalis* and *E. faecium* should be the main species detected. Upon further discussion, I discovered that the milk that she tested to be used in cheesemaking was only thermised and not pasteurised. The heat treatment in thermisation is 57-68°C for 15 s followed by refrigeration whereas pasteurisation involves a stronger heat treatment of 72°C for 15 s (Lewis, 2003). The milder heat treatment would allow the survival of more heat sensitive enterococci. In work conducted at Food Science Australia, *E. faecalis* and *E. faecium* were the most prevalent enterococcal species in raw milk, however isolates of *E. faecalis* and *E. faecium* were more heat sensitive than isolates of *E. durans* and one isolate of *E. hirae*, as determined by *z* values.

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Conference wrap-up

At the end of the conference, there was a round (elongated) table discussion where the session chairs made a few comments on their perspective. Richard Facklam queried whether or not haemolysis and gelatinase are actually virulence factors, although they contribute to pathogenicity. A comment was made by Keith Weaver that pathogenicity may be influenced by the phage content of enterococci and that the bacteriophage have been overlooked. Garry Dunny raised the idea that mobile genetic elements are possibly parasites of enterococci which have found a great place to live. Georgio Giraffa again mentioned that a strain by strain approach was needed for enterococci in food systems, with the ability of enterococci to express virulence genes, when present, and to transfer antibiotic resistance, being considerations. As different countries report data differently, the need for a way of comparing and improving the sharing of data was voiced by Frank Aarestrup. Finally, several of the presenters expressed the wish that there be some sort of central enterococci strain and plasmid collection, perhaps even free of charge. Patrice Courvalin then advised that there is a European proposal to generate a culture collection. A comment was made by Patrice Courvalin that many speakers did not keep to time, allowing very little time for questions, and that the purpose of such a conference should be to promote more discussion. At the final conference dinner, Lars Jensen made a toast to celebrate the finish of a successful conference (Figure 2)!

Figure 2. Lars Jensen, Denmark

Benefits of Attendance

I met and spoke with several of the leading enterococci researchers, including Barbara Murray, Richard Facklam, Patrice Courvalin, Lars Jensen and Georgio Giraffa. I also invited Georgio Giraffa to visit Food Science Australia if ever he was in Australia. Observing the mildly fiery debate between Barbara Murray and Milk Gilmore was a revelation as it demonstrated the contrasting views of leading enterococci researchers. The research on tetracycline indicating its survival in the environment gave weight to my findings of more prevalent tetracycline resistance compared to the resistances of other more frequently dispensed antibiotics. The association of the *gel*E gene with biofilm formation could be useful in further work on the transfer of antibiotic resistance in biofilm situations. Finally, the opportunity to present my work amongst the international enterococci research community is one of the highlights of my project on enterococci in the Australian dairy industry.

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