4th ASM Conference on Enterococci

In conjunction with the 8th International Symposium on Antimicrobial Resistance (ISAR)

March 5-7, 2014
Cartagena, Colombia
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Welcome to Cartagena! On behalf of the Program Committees for the 4th ASM Conference on Enterococci, and the 8th International Symposium on Antimicrobial Resistance, we want to thank you for your presence in this enchanting part of the world and invite you to take full advantage of the combined meetings discussing cutting edge clinical and basic science aspects of antimicrobial resistant pathogens with emphasis on enterococci. We have made an effort to bring the latest breaking science and presentations from top researchers around the world. The program has been designed to facilitate the exchange of science of the highest quality between researchers and clinicians dealing with challenging problems of antimicrobial resistance.

This joint meeting is special for two reasons:

First, this is the first time that the American Society for Microbiology is hosting a conference in Latin America, and we are delighted that this opportunity with the region starts in Colombia and in a magical place like Cartagena. We hope that this meeting would serve as a model to increase the involvement of ASM in Latin America, and stimulate the scientific development of the region to the highest level.

Second, we are honoring the memory of our beloved John P. Quinn, a friend, husband, researcher, clinician, and above all, a generous and committed physician-scientist who made a huge impact in our lives and in those around us and who, unfortunately, left us last year. Cartagena was always a special city for John and we feel that this is a perfect opportunity to honor his memory and immense legacy. Therefore, we are announcing that moving forward, ISAR will be named the “John P. Quinn International Symposium on Antimicrobial Resistance” and hope that you can join us in future versions.

We hope that you enjoy Cartagena, a jewel and world heritage place where the Caribbean winds mix with beautiful historic landmarks, tropical rhythms, exotic food and joyful people. Cartagena is one of the many fabulous faces of Colombia.

Thank you for joining us,

Cesar A. Arias, MD., MSc., PhD.
Chair, 4th ASM Conference on Enterococci

Maria Virginia Villegas, MD., MSc
Chair, 8th John P. Quinn ISAR
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*Indicates Committee Liaison for this Conference

ASM Conferences Mission

To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 500 people.

To encourage student and postdoctoral participation.

To recruit individuals in disciplines not already involved in ASM to ASM membership.

To foster interdisciplinary and international exchange and collaboration with other scientific organizations.
Program Committee

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Organizer, 8th Symposium on Antimicrobial Resistance
Executive Director, International Center for Medical Training and Research (CIDEIM)
Head, Bacterial Resistance Area, International Center for Medical Training and Research
Cali, Colombia
Acknowledgments

The 4th ASM Conference on Enterococci is made possible through the generous support of our collaborating partner, the 8th International Symposium on Antimicrobial Resistance, organized by the Centro Internacional de Entremiento e Investigaciones Medicas.

The Conference Organizers and the American Society for Microbiology acknowledge the following for their support of the 4th ASM Conference on Enterococci. On behalf of our leadership and members, we thank them for their financial support:

**Platinum Sponsor**

Cubist Pharmaceuticals

**Gold Sponsors**

Gunma University Graduate School of Medicine  Theravance, Inc.

**Silver Sponsor**

Universidad el Bosque

**Supporting Sponsors**

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General Information

REGISTRATION AND NAME BADGES
ASM Staff will be available at the registration desk in the Portal 4 in the Hotel Las Américas Convention Center during posted registration hours. Participants may collect name badges and program materials at the registration desk. A name badge is required for entry into all sessions and social events.

GENERAL SESSIONS
All Enterococci general sessions will be held in the Portal D and E in the Hotel Las Américas Convention Center. Joint sessions with the ISAR (as noted in the program schedule) are held in Portal A, B, C and F next door to the Enterococci conference session room.

POSTER SESSIONS
Poster boards are located in Portal 3 immediately adjacent to Portal D and E in the Hotel Las Américas Convention Center.

All Posters will be displayed for informal viewing throughout the conference. Odd-numbered posters (1,3,5…) will be officially presented during Poster Session A on Thursday afternoon. Even-numbered posters (2,4,6…) will be officially presented during Poster Session B on Friday afternoon.

Please check your assigned number in the abstract index. The same number is used for the presentation and board number.

EXHIBITS
All ASM Conference participants may visit the exhibits in the ISAR Exhibit Hall by showing the ASM conference name badge.

SOCIAL EVENTS
Registration includes attendance at the Opening Ceremony (joint with ISAR) on Wednesday, March 5. Ample time has been scheduled for participants to have lunch on their own.

CERTIFICATE OF ATTENDANCE
Certificates of Attendance can be found in the registration packet received at the registration desk.

Note: Certificates of Attendance do not list session information.

CAMERAS AND RECORDINGS POLICY
Audio/video recorders and cameras are not allowed in session rooms or in the exhibit and poster areas. Taking photographs with any device is prohibited.

CHILD POLICY
Children are not permitted in session rooms, poster sessions, conference meals or social events. Please contact the hotel concierge to arrange for babysitting services in your hotel room.
## Travel Grants

### STUDENT TRAVEL GRANTS

ASM encourages the participation of graduate students and new postdocs at ASM Conferences. To support the cost of attending the conference, ASM has awarded travel grants of $500 to each of the following individuals:

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<tr>
<th>Raghavendra Amachawadi</th>
<th>Diana Laverde</th>
<th>Fernanda Paganelli</th>
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<td>Laura Carrilero</td>
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<td>Chunhui Chen</td>
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<td>Anthony Gaca</td>
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<td>Ana Guzman Prieto</td>
<td>Maria Montealegre</td>
<td>Ana Tedim</td>
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<td>Margaret Lam</td>
<td>Veronika Oravcova</td>
<td>Xuewei Zhou</td>
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Scientific Program

Wednesday, March 5, 2014

8:00 am – 12:00 pm  Session 1: Genomics and Evolution
Portal D and E  Conveners: Michael Gilmore and Rob Willems

8:00 – 8:30 am  State-of-the-art Lecture:
The Evolving Biology of Enterococci as Revealed Through Genomics
Michael Gilmore; Departments of Ophthalmology, Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA

8:30 – 9:00 am  Comparative Genomics of the Broad Genus enterococcus
Francois LeBreton; Departments of Ophthalmology, Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA

9:00 – 9:30 am  Comparative E. faecium Genomics
Willem van Schaik; Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, NETHERLANDS

9:30 – 10:00 am  The E. faecium Resistome
George Weinstock; Jackson Laboratory for Genomic Medicine, Farmington, CT, USA

10:00 – 10:30 am  Break

10:30 – 11:00 am  Comparative Genomic Analysis of E. faecalis
Dag Anders Brede; Laboratory of Microbial Gene Technology and Food Microbiology, Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, NORWAY

11:00 – 11:30 am  Genetic Evolution of E. faecium and E. faecalis: Similarities and Differences
Rob Willems; Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, NETHERLANDS

11:30 – 11:45 am  The Enterococcus faecalis EbpA Start Codon AUU Plays a Role in the Translational Efficiency and Consequently in the Levels of EbpA Protein
Maria C. Montealegre; University of Texas Medical School at Houston, Houston, TX, USA
11:45 am – 12:00 pm Invading the Privacy of Enterococcal Invaders

Diana K. Morales; Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA

12:00 – 2:00 pm Lunch break (on your own)

2:00 – 5:30 pm Session 2: Enterococcal Virulence

Portal D and E

Conveners: Barbara E. Murray and Axel Hartke

2:00 – 2:30 pm Understanding Enterococcal Pathogenesis: Should We Stick to Adhesins?

Barbara E Murray; University of Texas Medical School at Houston, Houston, TX, USA

2:30 – 3:00 pm Understanding Enterococcal Pathogenesis: What are the Genes Actually in Use Inside the Host?

Axel Hartke; Université de Caen Basse-Normandie, Caen, FRANCE

3:00 – 3:30 pm siRNA: New Actors in the Stress Response and Virulence of Enterococcus faecalis

Jean-Christophe Giard; Equipe Antibio-résistance, Université de Caen, Caen, FRANCE

3:30 – 4:00 pm Break

4:00 – 4:30 pm Enterococcus faecalis Virulence: A Matter of Colonization and Adaptation

Pascale Serror; AgroParisTech, UMR Micalis, INRA, Micalis (Research Unit for Food and Gut Microbiology for Human Health), Jouy-en-Josas, FRANCE

4:30 – 5:00 pm Interactions between Enterococcus faecalis and Candida albicans Mutually Inhibit Virulence

Danielle Garsin; University of Texas, Houston, TX, USA

5:00 – 5:15 pm The Ebp Pilus Adhesin, EbpA, is Critical for E. faecalis’ Catheter-urinary Tract Infection

Ana L. Flores-Mireles; Washington University School of Medicine, St. Louis, MO, USA

5:15 – 5:30 pm Deletion of liaR Restores Daptomycin (DAP), Telavancin (TLV) and Tetracycline (TC) Susceptibility in Enterococcus faecalis

Jinnethe Reyes; Universidad El Bosque, Bogatá, COLOMBIA
6:00 – 8:00 pm  
Opening Ceremony  
Portal A, B, C, and F  
Joint Session with the 8th International Symposium on Antimicrobial Resistance  
Remarks from ISAR and ASM Representatives  
Keynote Address:  
Antibiotics, Microbiota and the Gut  
**Eric Pamer;** Memorial Sloan Kettering Cancer Center, New York, NY, USA  
Cultural Event  

Thursday, March 6, 2014  

8:00 am – 12:00 pm  
Session 3: Structure, Function and Evolution of Extrachromosomal Elements  
Portal D and E  
Conveners: Gary Dunny and Haruyoshi Tomita  

8:00 – 8:30 am  
State-of-the-art Lecture: Enterococcal Sex  
**Don Clewell;** Biologic and Materials Sciences, School of Dentistry and Microbiology and Immunology, The University of Michigan Medical School, Ann Arbor, MI, USA  

8:30 – 9:00 am  
Cell Surface Components of the pCF10 Conjugation Machine  
**Peter Christie;** Department of Microbiology and Molecular Genetics, University of Texas Medical School at Houston, Houston, TX, USA  

9:00 – 9:30 am  
Molecular Analysis of the pIP501 Type IV Conjugation System  
**Elisabeth Grohmann;** Division of Infectious Diseases, University Medical Center Freiburg, Freiburg, GERMANY  

9:30 – 10:00 am  
Post-transcriptional Mechanisms Amplify the Small Direct Effect of Sex Pheromones on Conjugation  
**Gary Dunny;** University of Minnesota, Minneapolis, MN, USA  

10:00 – 10:30 am  
Break  

10:30 – 11:00 am  
Composite Phages and Competitive Fitness  
**Breck A. Duerkop;** Department of Immunology, The Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA
11:00 – 11:30 am Analysis of Highly Conjugative pMG1-like Plasmids
Haruyoshi Tomita; Department of Bacteriology, Laboratory of Bacterial Drug Resistance, Gunma University Graduate School of Medicine, Maebashi, Gunma, JAPAN

11:30 – 11:45 am Evidence for Simultaneous Transfer of the esp-containing Pathogenicity Island and the vanB Resistance Locus in Enterococcus faecium
Jennifer Bender; Robert Koch-Institute, Wernigerode, GERMANY

11:45 am – 12:00 pm Characterization of YajC: A Transmembrane Protein Involved in Cell Wall Stability and Biofilm Formation in Enterococcus faecium
Fernanda L. Paganelli; UMC Utrecht, Utrecht, NETHERLANDS

12:00 – 2:00 pm Lunch Break (on your own)

2:00 – 4:00 pm Session 4: Vancomycin Resistance and Beyond
Portal D and E
Conveners: Patrice Courvalin and Louis Rice

2:00 – 2:30 pm State-of-the-art Lecture: Evolution of the vanA Operon and of Tn1546
Patrice Courvalin; Institut Pasteur, Unité des Agents Antibactériens, Paris, FRANCE

2:30 – 3:00 pm Cfr and Oxazolidinone Resistance
Alexander Mankin; Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, Chicago, IL, USA

3:00 – 3:30 pm From Single VanX, VanY to Bi-Functional VanXY Resistance Enzymes
Djalal Meziane-Cherif; Institut Pasteur, Unité des Agents Antibactériens, Paris, FRANCE

3:30 – 4:00 pm Strategies to Interrupt Gastrointestinal Colonization by Glycopeptide Resistant Enterococci
Louis Rice; Warren Alpert Medical School, Brown University/ Rhode Island and Miriam Hospitals, Providence, RI, USA

4:00 – 5:30 pm Poster Session A
Portal 3
(odd-numbered posters will be officially presented)
**Friday, March 7, 2014**

**8:00 am – 12:20 pm**

**Session 5: Mechanisms of Resistance and Therapy**

*Joint Session with the 8th International Symposium on Antimicrobial Resistance*

*Conveners: Cesar A. Arias and Michel Arthur*

8:00 – 8:30 am

State-of-the-art Lecture:

“Novel Peptidoglycan Assembly Pathways in the Enterococci: Contribution of Gene Acquisition and Modification of Preexisting Regulatory Circuits”

Michel Arthur; Centre de Recherche des Cordeliers, Université Pierre et Marie Curie—Paris 6, INSERM, Université Paris Descartes, Sorbonne Paris Cité, Paris, FRANCE

8:30 – 9:00 am

Novel Antimicrobial Combinations for the Treatment of Enterococcal Infections

George Sakoulas; Department of Pharmacology and Drug Discovery, University of California San Diego School of Medicine, La Jolla, CA, USA

9:00 – 9:30 am

Adaptive Evolution of Antibiotic Resistance in Enterococci

Yousif Shamoo; Department of Biochemistry and Cell Biology, Department of Ecology and Evolutionary Biology, Rice University, Houston, TX, USA

9:30 – 10:00 am

Enterococcal Vaccines

Johannes Huebner; Division of Pediatric Infectious Diseases, University of Munich, Munich, GERMANY

10:00 – 10:30 am

Break

10:30 – 11:00 am

Enterococcal Cell Membrane and Resistance to Antimicrobial Peptides

Cesar A. Arias; University of Texas, Houston, TX, USA / Molecular Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Bogotá, COLOMBIA

11:00 – 11:30 am

Targeting Virulence Factor Assembly Sites in *Enterococcus faecalis*

Kim Kline; Singapore Centre on Environmental Life Sciences, Engineering School of Biological Sciences, Nanyang Technological University, SINGAPORE
11:40 am – 12:20 pm  Keynote Address:
The Natural Selection of Antimicrobial Resistance
**Louis Rice;** Warren Alpert Medical School, Brown University/
Rhode Island and Miriam Hospitals, Providence, RI, USA

12:20 – 2:00 pm  Lunch break (on your own)

2:00 – 4:00 pm  **Session 6: Metabolism and Ecology**
Portal D and E

2:00 – 2:30 pm  State-of-the-art Lecture:
Ethanolamine Utilization in *E. faecalis*: Roles and Regulation
**Danielle Garsin;** University of Texas, Houston, TX, USA

2:30 – 3:00 pm  Peptide Transporters Contribute to Signaling in *Enterococcus faecalis* Biofilm Development
**Lynn Hancock;** Kansas State University, Manhattan, KS, USA

3:00 – 3:30 pm  (p)ppGpp Metabolism in *Enterococcus faecalis*: Beyond the Stringent Response
**Jose Lemos;** Center for Oral Biology, Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, USA

3:30 – 3:45 pm  Alleviation of CodY Regulation by (p)ppGpp is Central for the Activation of a Complex Stress Response Network in *Enterococcus faecalis*
**Anthony O. Gaca;** University of Rochester, Rochester, NY, USA

3:45 – 4:00 pm  Synthetic LTA-protein Conjugates Elicit Opsonic and Protective Antibodies
**Diane Laverde;** University Medical Center Freiburg / Dr. von Hauner Children’s Hospital, Ludwig-Maximilians-University, Munich, GERMANY

4:00 – 4:30 pm  **Funding Presentation:**
NIH Funding Opportunities for Antimicrobial Research
**Clayton Huntley;** NIH/NIAID, Rockville, MD, USA

4:30 – 6:00 pm  **Poster Session B**
Portal 3
(even-numbered posters will be officially presented)

**Conference Concludes**
Speaker Abstracts

**S1:1**

THE EVOLVING BIOLOGY OF ENTEROCOCCI AS REVEALED THROUGH GENOMICS

M. S. Gilmore;  

The enterococci are an ancient genus that evolved along with the tree of life. These intrinsically rugged bacteria are highly adapted members of the intestinal consortia of a range of hosts that spans the animal kingdom. Enterococci are also leading opportunistic hospital pathogens, causing infections that are often resistant to treatment with most antibiotics. Despite the importance of enterococci as hospital pathogens, the vast majority live outside of humans, and nearly all of their evolutionary history took place before the appearance of modern man. Because hospital infections represent evolutionary endpoints, traits that exacerbate human infection are unlikely to have evolved for that purpose. However, clusters of traits have converged in specific lineages that are well adapted to colonize the antibiotic-perturbed gastrointestinal tracts of patients, and thrive in the hospital environment. Comparative genomics is providing new insights into the genetic composition of hospital adapted enterococci, and how and when they arose.

**S1:2**

COMPARATIVE GENOMICS OF THE BROAD GENUS ENTEROCoccus

F. Lebreton;  
Harvard University, Boston, MA.

The Gram-positive bacterium Enterococcus faecium is usually a harmless commensal of the human intestinal tract. However, in the last twenty years it has emerged as a multidrug-resistant opportunistic pathogen in hospitalized patients. Genome sequencing of a large panel of E. faecium strains that were isolated from different niches revealed that the population structure of E. faecium can be resolved into different clades that are specifically associated with healthy individuals, animals and clinical infections. Each split in the E. faecium population coincided with the acquisition of metabolic capabilities and colonization factors that may have contributed to host specialization. The large number of E. faecium genome sequences allow genome-wide identification and functional characterization of genes and genetic elements that contribute to multi-drug resistance and the increased ability of clinical isolates to infect hospitalized patients. Whereas genetic studies in clinical E. faecium isolates were long thought to be nearly impossible, we have recently developed several genetic tools that allow for the efficient manipulation of E. faecium.

Using the available genome sequences and our genetic toolbox for E. faecium, we have identified a gene cluster encoding a phosphotransferase (PTS) system that is specifically present in clinical isolates. This PTS system contributes to gut colonization in a murine model in which the gut microbiota is disrupted by the administration of cephalosporins, suggesting that metabolic adaptations in clinical E.
faecium strains may contribute to the ability of clinical E. faecium strains to colonize the intestinal tract of patients at high levels. We also developed high-throughput transposon mapping approaches that have revealed genes that are involved in resistance to antibiotics, disinfectants, bile salts and serum in clinical E. faecium isolates. Finally, transcriptome profiling has revealed genes that are specifically expressed at 37°C. One of these genes, which encodes a LPxTG-type surface protein that is specific to E. faecium and closely related enterococci, is now the subject of further functional studies.

The combination of comparative and functional genomics has led to important novel insights into the biology of E. faecium, which may contribute to the development of novel therapeutics against this important nosocomial pathogen.

**S1:4**

**THE E. FAECIUM RESISTOME**

G. Weinstock;  
Jackson Laboratory for Genomic Medicine, Farmington, CT.

**S1:5**

**COMPARATIVE GENOMIC ANALYSIS OF ENTEROCOCCUS FAECALES**

D. A. Brede, S. Leanti La Rosa, L. G. Snipen, D. B. Diep, I. F. Nes;  
Norwegian University of Life Sciences, Ås, NORWAY.

Genome Sequencing has facilitated a quantum leap for research on E. faecalis. Large sequencing projects have generated enormous amounts of data, with more than 200 strains, clones and mutants being publicly available. These isolates represent a wide spectrum of biotypes and clonal lineages of environmental, human commensal, and clinical settings. Although the chromosomal backbone shows conserved characteristic features, E. faecalis genomic structure is highly variable and gene synteny is dramatically affected by mobile genetic elements. Particularly, presence of multiple plasmids and polylysogeny influence E. faecalis relationship with its host as these elements may confer advantageous properties to the bacterial strain. A number of genes have been identified to contribute to E. faecalis virulence; however, none of these seem indispensable for pathogenicity.

Here, we present a functional genomic analysis of microbe-host interaction of E. faecalis using a C. elegans model system. The virulence of 28 E. faecalis isolates representing 24 MLST types from human commensal and clinical, as well as animals and insect origin was assessed using C. elegans strain glp-4 (bn2ts); sek-1 (km4). This revealed that some E. faecalis isolates behaved commensalistic, while for nematocidal strains a wide dynamic range of virulence potential was observed. Using a combination of bioinformatics and statistical analysis, a genetic reference map of commensal or pathogenic interaction with C. elegans was constructed. Candidate virulence traits could subsequently be investigated by functional genomic analysis. This integrated approach facilitated a linear regression model which accounts for >85% of the observed difference in virulence potential between the strains.

**S1:6**

**GENETIC EVOLUTION OF ENTEROCOCCUS FAECIUM AND ENTEROCOCCUS FAECALIS: SIMILARITIES AND DIFFERENCES**

R. Willems;  
Medical Microbiology, University Medical Center Utrecht, Utrecht, NETHERLANDS.

Enterococci are ubiquitously found in nature as commensal of the gastro-intestinal tract of mammals, birds and insects. However, Enterococcus faecium and Enterococcus faecalis are also a leading cause of opportunistic infections in hospitalized patients. As a consequence of these different lifestyles enterococci need to adapt to different micro-environments all
exerting strong selective pressures. Despite the fact that *E. faecalis* and *E. faecium* are colonizing similar niches, it is remarkable that their sexual behavior, and therefore their population structure, is so different. A strategy for bacterial species to survive when confronted with a wide range of selective pressures is specialization in particular fitness peaks. That probably happened in *E. faecium* in which specific hospital-adapted lineages have evolved as a result of reduced recombination among hospital isolates due to either ecological or genetic isolation. Thus, *E. faecium* seems to inhabit a rugged fitness landscape in which characters of particular clones, like hospital clones, impose strong fitness differences. In contrast to *E. faecium*, no distinct clustering of *E. faecalis* hospital isolates was found. In *E. faecalis*, hospital isolates originate from multiple genetic backgrounds and their evolutionary background is not significantly different from that of *E. faecalis* isolates from other sources. The fact that there is limited specialization in *E. faecalis* correlates well with the observed higher rates of recombination in this species relative to *E. faecium* and points towards a more flat fitness landscape in which genes are continuously recycled through the entire population. As a consequence, adaptive elements that are acquired in one particular niche may readily be dispersed and provide selective advantage in other niches. An explanation for this difference in population structure between *E. faecalis* and *E. faecium* may reside in differences in population size of both species. *E. faecalis* seems to be more widespread and abundant in the intestines of animals and humans than *E. faecium*. The higher population density of *E. faecalis* relative to *E. faecium* in combination with its broader host-range may provide *E. faecalis* with more ample opportunities for genetic exchange and diversification, including the emergence of new alleles generated by recombination. We therefore hypothesize that *E. faecalis* species behaves as a collective organism, in which recombination-driven high-level genetic connectivity between strains is evolutionary associated with a more generalist lifestyle. This model can be considered a variant of the recently proposed Black Queen Hypothesis, in which collective genes shared in a highly recombinogenic structure acts as “common goods”, favoring that genes with leaky functions could become dispensable for individuals, provided they are not lost entirely from the community.

**S1:7**

**The Enterococcus faecalis EbpA Start Codon AUU Plays a Role in the Translational Efficiency and Consequently in the Levels of EbpA Protein**

*M. C. Montealegre*\(^1\), *S. Leanti La Rosa*\(^2\), *J. H. Roh*\(^1\), *B. R. Harvey*\(^1\), *B. E. Murray*\(^1\);

\(^1\)University of Texas Medical School, Houston, Texas, Houston, TX, \(^2\)The Norwegian University of Life Sciences, Ås, NORWAY.

**Background.** The endocarditis and biofilm-associated pili (Ebp) play an important role in the adherence of *E. faecalis* OG1RF to host extracellular matrix proteins, biofilm formation and in experimental models of infection. We recently demonstrated that EbpA localizes at the tip of the pilus fiber and that its deletion leads to extremely long pili compared to wild-type OG1RF, which suggests a role of EbpA in the termination of polymerization. We observed that the translational start codon of EbpA in OG1RF and other *E. faecalis* strains, is the rare triplet AUU, and it is estimated that less than 1% of *E. faecalis* proteins initiate translation from a codon other than AUG. This led us to investigate the contribution of the EbpA start codon AUU in the regulation of translation and surface display of the EbpA protein.

**Methods.** We performed site-directed mutagenesis to replace in situ the *ebpA* AUU initiation codon of *E. faecalis* OG1RF with AUG, and constructed translational fusions with each triplet to investigate the effect of the start codon on translational efficiency. In addition, quantitation of the surface-localized EbpA protein by ELISA and flow cytometry.
was performed. **Results.** Our results demonstrated that the translational reporter fusion carrying AUG (PebpA<sup>AUG</sup> :: lacZ) showed increased expression of the reporter protein compared to the corresponding construct carrying the triplet AUU as the initiator codon of translation (PebpA<sup>AUU</sup> :: lacZ) (P<0.0001). In addition, we found that the strain carrying AUG as the initiation codon of EbpA showed increased surface display of EbpA as measured by ELISA (P<0.001) and flow cytometry.

**Conclusion.** Our results using a translational fusion indicate that the rare initiation codon AUU is used to slow down the expression of the reporter protein, which correlates with the reduced level of EbpA protein on the surface of wild-type OG1RF, compared to the mutant strain carrying AUG as the initiation codon of EbpA.

**S1:8**

**INVADING THE PRIVACY OF ENTEROCOCCAL INVADERS**

**D. K. Morales**<sup>1</sup>, A. L. Hael<sup>1</sup>, A. Conery<sup>2</sup>, H. Vlamakis<sup>3</sup>, C. Cywes-Bentley<sup>3</sup>, T. Inoue<sup>4</sup>, G. B. Pier<sup>1</sup>, F. B. Ausubel<sup>2</sup>, M. S. Gilmore<sup>4</sup>, R. Kolter<sup>1</sup>;

<sup>1</sup>Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, <sup>2</sup>Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA, <sup>3</sup>Division of Infectious Diseases, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, <sup>4</sup>Department of Ophthalmology and Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA.

Enterococcus faecalis is a non-motile opportunistic pathogen that normally resides in the human gastrointestinal tract. Interest in enterococcal virulence has been prompted due to the fact that several strains are resistant to most of the currently available antimicrobial agents. Improvement in treating serious enterococcal infections will depend upon a clearer understanding of virulence traits and host-pathogen interactions. *E. faecalis* can adhere to and invade human tissues, a trait that is important for colonizing human sites different from the gastrointestinal tract. We have found that *E. faecalis* is also capable of invading semi-solid surfaces such as agar. This phenomenon is controlled by the composition of its growth environment as the presence of carbohydrates, such as glucose, prevents this invasive behavior. We screened an *E. faecalis* transposon mutant library for strains that were incapable of invading agar. By analyzing mutants defective in agar invasion, we found that invasiveness correlates with cell aggregation, and both phenomena were promoted by the production of the extracellular polysaccharide poly-N-acetylglucosamine (PNAG). Additionally, we found that invasion could be controlled by an “inversion-mediated” phase variation system, which has not been previously described in this microorganism. Corresponding with our hypothesis that invasiveness is required for virulence, invasion-defective mutants failed to kill the model host organism Caenorhabditis elegans. Our studies suggest that PNAG production; cell aggregation and invasion are required for enterococcal persistence within the host. Thus, this novel invasive behavior could be a useful tool for identifying new virulence traits in *E. faecalis*.

**S2:1**

**UNDERSTANDING ENTEROCOCCAL PATHOGENESIS: SHOULD WE STICK TO ADHESINS?**

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**S2:2**

**UNDERSTANDING ENTEROCOCCAL PATHOGENESIS: WHAT ARE THE GENES ACTUALLY IN USE INSIDE THE HOST?**

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*Enterococcus faecalis* is known to be part of the human intestinal microflora but also to cause serious diseases. The transformation of *E. faecalis* from a peaceful commensal to a pathogen is still poorly understood. A handful of major genetic factors influencing the capacity of this bacterium to cause severe infections have been identified and characterized in last decades. However, these virulence traits are not systematically detected in clinical isolates. This demonstrates that enterococcal virulence is still incompletely understood. Transcriptome analysis by RNA-sequencing (RNA-seq) is a recently introduced new approach which should be useful to study pathogens during the infection process. A major obstacle, however, is to isolate enough RNA from bacteria recovered from the infection sites. We will present in this communication the first in vivo RNA-seq transcriptome of *E. faecalis*. RNA was extracted from bacteria isolated after 24 hours of experimental peritonitis in mice. The in vivo expression data were compared to that of exponential growing and stationary phase cells. The number of filtered reads was between 3 and 5 x 10^6 and 50% to 65% of them mapped finally to the *E. faecalis* V583 genome sequence. All operons induced during infection were controlled by qPCR using new RNA extractions. Most of the genes with altered expression were observed for the categories “Hypothetical and Unknown Proteins” and “Metabolism”. The changes observed in this last category are of fundamental importance since invading pathogens have to proliferate in the host, which is particular puzzling for polyauxotrophic bacteria like *E. faecalis*. The results showed that the bacteria changed profoundly central metabolic pathways. For example, key enzymes of the upper part of the glycolysis were significantly down regulated. On the other hand, bacteria seem to favor reactions leading to the synthesis of reduced co-factors. In line with this observation is the concomitant induction of reactions leading to the regeneration of NADH. *E. faecalis* harbors a complex system for glycerol metabolism. Both catabolic pathways are strongly induced in the peritoneum. The study also demonstrates the in vivo induction of some prominent virulence factors mentioned above. Mutants affected in genes highly induced in vivo were constructed and will be tested in appropriate virulence models. The combined results demonstrate that RNA-seq is a powerful tool to define which sets of genes are actually in use during the infection process. Genes highly induced inside the host are expected to be interesting new targets for treatment or vaccine development.

**S2:3**

**SRNAS: NEW ACTORS IN THE STRESS RESPONSE AND VIRULENCE OF ENTEROCOCCUS FAECALIS**

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Small RNAs (sRNAs) occur in all kingdoms of life, and have become increasingly recognized as a novel class of gene expression regulators. The various biological roles of sRNAs elements encompass the regulation of metabolism, growth processes or adaptation to stress and some data show that regulatory RNAs play key roles in microbial pathogenesis. sRNAs have been recently identified in *Enterococcus faecalis* a major opportunistic pathogen responsible for nosocomial infections. Among those most expressed, we initiated a functional analysis of 6 sRNAs (5 members of the core genome and one located in the pathogenicity island (PAI)) by constructing mutants and complemented strains in order to determine whether they were implicated in the pathogenesis and the stress response in *E. faecalis*. It appeared that the one sRNA (ef0869-0870)
may be essential since construction of the corresponding mutant strain always failed. The five other sRNAs mutant strains (∆ef0408-0409, ∆ef0605-0606, ∆ef0820-0822, ∆ef1368-1369 and ∆ef3314-3315) were tested in the Galleria mellonella, urinary tract infection and intracellular macrophage survival models. For those that were involved in the virulence of *E. faecalis*, we looked at their phenotypes towards several stress conditions potentially encountered by the bacteria in its niches or during the infection process. Lastly, to identify the regulons, we performed 2-D PAGE technique in combination with mass spectrometry to compare the cytoplasmic proteome of sRNA mutants with the corresponding wild-type strain. This showed that the selected sRNAs controlled expression of proteins involved in diverse cellular processes and stress response. These results highlight for the first time the implication of certain sRNAs in the virulence and the stress response in the opportunistic pathogen *E. faecalis*. It has been shown that the post-transcriptional regulatory function of sRNA involves RNA-binding proteins such as Hfq which is present in some of Gram-positive and negative bacteria. However, despite the important role of Hfq, no homologous protein is present in low-GC Gram-positive bacteria genomes such as *S. pyogenes*, *L. lactis* or *E. faecalis*. In this context it was of interest to find RNA-binding proteins that may act as post-transcriptional regulators and compensate the lack of Hfq-like polypeptide. Thus, by co-precipitation experiments (RNA-protein), we identified CspR (for Cold Shock Protein RNA binding) and showed its involvement in the cold-shock response and bacterial virulence. In addition, this protein has a dual location (present in both intracellular and surface fraction) and antibodies raised against CspR protected the *G. mellonella* larvae to infections by *E. faecalis*. These results suggest that CspR may be an interesting candidate as a target for the development of an immune-strategy to prevent or fight *E. faecalis*.

### S2:4

**ENTEROCOCCUS FAECALIS VIRULENCE: A MATTER OF ADAPTATION AND COLONIZATION**

**P. Serror**; *Micalis* - INRA, Jouy en Josas, FRANCE.

**Title:** Enterococcus faecalis virulence: a matter of adaptation and colonization

Enterococci are generally harmless intestinal commensals of healthy humans; however some strains have emerged as multidrug-resistant pathogens responsible for opportunistic infections in immune-compromised and elderly patients. Enterococcal pathogenesis relies in part on the capacity of enterococci to colonize the gut and to disseminate to distant organs. Following disruption of the homeostasis of the gut microbiota, generally after antibiotic treatment, enterococci can overgrow, cross the intestinal barrier and invade the bloodstream; leading to consider them as human pathobionts of the intestinal tract.

Multidrug-resistant colonizing or invasive isolates of *Enterococcus faecalis* belonging to the clonal complex 2 (CC2) are widely disseminated in hospitals in the United States and in several European countries. Since little is known of *E. faecalis* CC2-enriched determinants important to pathogenesis, we used the vancomycin-resistant strain V583, a member of the CC2, to identify and study *E. faecalis* genes implicated in intestinal colonization and host invasion.

Selected mutants were screened for their capacity to colonize the gastrointestinal tract of mice after antibiotic treatment. Of the *E. faecalis* genes that promote intestinal colonization or persistence, *epaX* encodes a putative glycosyltransferase and is located in a highly variable region adjacent to the enterococcal polysaccharide antigen (*epa*) locus. We demonstrated that EpaX acts on Epa sugar composition promoting resistance to bile salts and cell wall integrity. Given that EpaX is likely to shape the decoration of the rhamnopolysaccharide, we propose that the genetic variability in the
The epa locus is a key determinant in the ability of *E. faecalis* strains to colonize the GIT. More importantly, EpaX may constitute an attractive bio-marker to monitor intestinal overgrowth of prominent hospital-adapted enterococci. V583 genome is enriched in prophage-related elements, suggesting that prophages may contribute to host or niche adaptation. Interestingly, some of these prophages encode platelet-binding-like proteins. Strains that harbor from one to all six non-ubiquitous prophages were created and used to analyze prophage activity and contribution to bacterial pathogenic traits. Besides unveiling intricate interactions between V583 prophages, we established a direct correlation between prophages and *E. faecalis* adhesion to human platelets, indicating that prophages might not only promote bacterial dissemination in the circulation but also platelet aggregation, which participates in the development of infective endocarditis. Altogether, this work brings new light on the molecular determinants involved in *E. faecalis* pathogenicity, opening novel perspectives for preventive strategies.

**S2:5**

INTERACTIONS BETWEEN *ENTEROCOCCUS FAECALIS* AND *CANDIDA ALBICANS* MUTUALLY INHIBIT VIRULENCE

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*Enterococcus faecalis*, and the fungus, *Candida albicans* are both found as commensals in many of the same niches of the human body, such as the oral cavity and gastrointestinal (GI) tract. However, both are opportunistic pathogens and have frequently been found as co-constituents of polymicrobial infections. Despite these features in common, there has been little investigation into whether these microbes affect one another in a biologically significant manner. Using a C. elegans model of polymicrobial infection, we discovered that *E. faecalis* and *C. albicans* negatively impact each other’s virulence. Much of the negative effect of *E. faecalis* on *C. albicans* was due to the inhibition of *C. albicans* hyphal morphogenesis, a developmental program crucial to *C. albicans* pathogenicity. The inhibition of *C. albicans* hyphal morphogenesis could be recapitulated when the microbes were grown together in an in vitro biofilm. We discovered that filament inhibition was partially dependent on the Fsr quorum-sensing system, a major regulator of virulence in *E. faecalis*. Specifically, two proteases regulated by Fsr, GelE and SerE, were partially required. Further characterization of the inhibitory signal revealed that it is secreted into the supernatant, is heat-resistant, is protease sensitive and is between 3 and 10 kDa. Based on these characteristics and a reverse genetics approach, we identified a candidate gene that may encode the activity. Overall, we demonstrate a biologically relevant interaction between two clinically important microbes that could affect treatment strategies as well as impact our understanding of interkingdom signaling and sensing in the human-associated microbiome.

**S2:6**

THE EBP PILUS ADHESIN, EBPA, IS CRITICAL FOR *E. FAECALIS*’ CATHETER-URINARY TRACT INFECTION

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Catheter-associated urinary tract infections (CAUTIs) account for approximately 40% of all hospital acquired infections (HAI) worldwide and are the most common cause of HAI. Associated with increased morbidity and mortality, CAUTIs are also the most common cause of secondary bloodstream infections. Enterococci are responsible for 30% of all CAUTIs and with the emergence of multi-antibiotic resistant *E. faecalis* strains, treatment of CAUTI is becoming more challenging.
As a critical component of CAUTI pathogenesis, *E. faecalis* forms a biofilm on the catheter that is essential for its persistence in the bladder and dissemination to the kidneys. In a murine CAUTI model, we found that the endocarditis and biofilm-associated (Ebp) pilus plays a critical role in CAUTI pathogenesis. Specifically, *E. faecalis* mutants lacking the EbpA pilus subunit or with a defect in EbpA’s MIDAS motif were attenuated for CAUTI. In this study we discovered the mechanism of EbpA mediated biofilm formation on silicon catheter implants in the murine CAUTI model. We found that EbpA is the adhesin of the Ebp pilus that recognizes Fg. Using whole cell ELISA and protein binding assays, we found that EbpA binds directly to Fg and that its MIDAS motif is essential for recognizing Fg, but not collagen. By immunostaining, we found that Fg is released upon catheter implantation and coats the implanted catheter in a time-dependent manner. Furthermore, *E. faecalis* binds to Fg deposited on the catheter and this interaction is essential for catheter biofilm formation. Paradoxically, *E. faecalis* OG1RF grows poorly in urine and does not form a biofilm on the same silicone catheters *in vitro* even when supplemented with glucose. We resolved this paradox by discovering that Fg promotes growth and biofilm formation in human urine *in vitro*, demonstrating that Fg plays a crucial role in multiple steps of CAUTI pathogenesis. Further analysis of the role of EbpA and Fg in CAUTI will provide information important for the development of effective drugs to prevent CAUTI and other Ebp pilus-related diseases.

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**S2:7**

**DELETION OF LIAR RESTORES DAPTOMYCIN (DAP), TELAVANCIN (TLV) AND TETRACYCLINE (TC) SUSCEPTIBILITY IN ENTEROCOCCUS FAECALIS**


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**Background:** The LiaFSR system is a three-component regulatory system that is highly conserved in Gram-positive pathogens, including enterococci. This system orchestrates the cell envelope response to different types of stress, including cell-wall acting antibiotics. We previously showed that changes in LiaFSR are implicated in DAP resistance in *E. faecalis*. Furthermore, homologs of this system have been shown to play a role in the development of oxacillin resistance and in the response to several antibiotics by different Gram-positive bacteria. In this work, we investigated the impact of the deletion of liaR, the response regulator of LiaFSR, on the susceptibility to different antibiotics in *E. faecalis*. **Methods:** We included i) a laboratory derived DAP-R mutant carrying a codon deletion in liaF, and changes in the phospholipid metabolism genes cls and gdpD (S613ΔliaF177gdpD170cls61), ii) a liaR deletion mutant constructed in the background of the above DAP-R mutant (S613ΔliaF177gdpD170cls61ΔliaR) and iii) the complemented strain of this liaR deletion mutant (S613ΔliaF177gdpD170cls61ΔliaR:liaR). MICs to DAP, TLV, TC, doxycycline (DC), and minocycline (MC) were performed by Etest. **Results:** As previously reported, S613ΔliaF177gdpD170cls61 was DAP-R (MIC 8 µg/ml). MICs of TLV (32 µg/ml), TC (128 µg/ml), MC (24 µg/ml), and DC (8 µg/ml) were all in the range of resistance. Deletion of liaR drastically reduced the MIC of DAP (0.094 µg/ml, 85 fold). Likewise, the MICs of TLV, DC, MC and TC were dra-
matically reduced (8, 42, >100 and >100 fold, respectively). Thus, the liaR deletion mutant became susceptible to all compounds tested. Reintroduction of liaR restored MICs similar to S613ΔliaF177gdpD170cls61. **Conclusion:** Our results corroborate that the LiaFSR system is critical in the development of DAP-R in *E. faecalis* and that deletion of liaR produces a DAP hyper-susceptible phenotype. Unexpectedly, turning off the LiaFSR system also had a major impact on the susceptibility profile to TLV and the TCs.

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**Opening Ceremony – Keynote Address**

**INTESTINAL MICROBIOTA AND IMMUNE DEFENSE AGAINST VRE INFECTION.**

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Infections caused by antibiotic-resistant bacteria generally begin with colonization of mucosal surfaces, in particular the intestinal epithelium. The intestinal microbiota provides resistance to infection with highly antibiotic-resistant bacteria by inducing the production of antimicrobial factors in intestinal epithelial cells. In mice, depletion of the microbiota with antibiotics diminishes the expression of Reg3, an antimicrobial C-type lectin that kills Gram-positive bacteria, thereby decreasing resistance to intestinal colonization by Vancomycin Resistant Enterococcus (VRE). Stimulation of TLRs can re-induce the expression of Reg3 and enhance resistance against VRE. Antibiotic administration dramatically alters the composition of the intestinal microbiota and can result in the long-term loss of many bacterial taxa. Loss of intestinal microbial diversity renders mice susceptible to infection with *Clostridium difficile*, the major cause of hospitalization-associated diarrhea. Parallel studies in patients undergoing allogeneic hematopoietic stem cell transplantation also suggest that loss of specific obligate anaerobic bacteria correlates with susceptibility to VRE and *C. difficile* infection. Metagenomic sequencing of the murine and human microbiota following treatment with different antibiotics is beginning to identify bacterial taxa that are associated with resistance to VRE taxa that are associated with resistance to VRE and *C. difficile* infection.

**S3:1 ENTEROCOCCAL SEX**

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Enterococci are well known for their abundance of plasmids, many of which are highly mobile and bear determinants for antibiotic resistance, bacteriocins, as well as virulence. This presentation provides, from an historical perspective, an overview of the transmissibility and maintenance of such elements with some emphasis on species such as *Enterococcus faecalis* and *Enterococcus faecium*. In addition to having various replicative behaviors, specific groups of conjugative enterococcal plasmids differ significantly with respect to how intercellular transfer occurs and is genetically regulated. Some plasmids move from donor to recipient at relatively high frequencies in liquid environments, whereas others exhibit significant transfer only if matings occur on solid surfaces. Transfer with reasonably good efficiency in broth (e.g. 10^-4 or better) may involve those responding to a recipient-produced peptide sex pheromone (e.g. pAD1 or pCF10); whereas others, such as the pMG1 group do not appear to involve a pheromone response. Those that tend to require solid surfaces for transfer, such as pAMβ1 or pIP501, exhibit a broad host range among Gram positives; whereas those that transfer well in broth appear thus-far to have a narrow host range (e.g. enterococci). Enterococci also commonly carry conjugative transposons, such as Tn916, which are usually found integrated in the bacterial chromosome, but which can excise, circularize, and transfer much like a plasmid. Tn916-like elements are able to insert at multiple sites in the recipient genome and exhibit a broad host-range. Certain conjugative
plasmids, as well as Tn916-like elements have been the subject of detailed analyses and much is known about their mechanisms of transfer and genetic regulation. With the rapidly increasing availability of complete genomic sequences, the detection of chromosome-borne segments of DNA bearing genes resembling those related to plasmids, transposons, integrases, etc. has facilitated the identification and demonstration of genomic islands appearing to have been acquired by horizontal transfer. Some of these exhibit conjugative potential and have been referred to as integrative conjugative elements (ICEs). Some of the latter encode virulence properties and are designated as pathogenicity islands (PAIs). These may also carry antibiotic resistance determinants and are particularly common among enterococci associated with nosocomial infections. The dynamic activity of the enterococcal mobilome is intimately connected to the emergence of enterococci as potential pathogens; and studies of relevant epidemiology reveal a growing evolutionary divergence resulting from numerous bacterial interactions and the influence of selective pressures associated with clinical treatment.

**S3:2**

**CELL SURFACE COMPONENTS OF THE PCF10 CONJUGATION MACHINE**

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*Enterococcus faecalis* plasmid pCF10 encodes a highly efficient conjugation system in response to pheromone-mediated transcriptional activation of the prg/prf transfer operon. The prg/prf operon codes for a set of three surface-anchored proteins, thought to be important for establishment of donor-recipient mating pairs, and a type IV secretion system (T4SS) that is responsible for plasmid delivery to recipient cells. In this study, we defined contributions of the pCF10-encoded surface proteins PrgA, PrgB (Aggregation Substance or AS), and PrgC to conjugation, biofilm formation, and virulence using the *C. elegans* model host. Through analyses of prgA, prgB, or prgC nonpolar mutants and complemented strains, we determined that even though prgB-encoded aggregation substance induces cellular clumping and contributes to biofilm formation, AS is completely dispensable for plasmid transfer and infection of *C. elegans*. By contrast, the two previously uncharacterized surface factors, PrgA and PrgC, are essential for efficient plasmid transfer, and also contribute substantially to both biofilm formation and *C. elegans* infection. Further studies supplied evidence that: i) PrgA contributes to processing of PrgB and ii) synthesis of only one or two of the three Prg proteins disrupts various surface-associated phenomena whereas deletion of genes for all three surface proteins restores conjugation proficiency of donor cells. Taken together with earlier findings, we propose that the pCF10-encoded Prg proteins interact to form a fibrous mesh that surrounds the cell periphery and contributes to attachment of donor cells to both biotic and abiotic surfaces as well as to infection. However, removal of this fibrous mesh through genetic ablation effectively unmasks a chromosomally-encoded surface adhesin(s) which can then functionally substitute for the Prg surface complex. The pheromone-dependent plasmids of *E. faecalis* appear to have retained genes for a Prg-like surface complex over evolutionary time through selective pressures that are unrelated to plasmid dissemination.

**S3:3**

**MOLECULAR ANALYSIS OF THE PIP501 TYPE IV CONJUGATION SYSTEM**

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Conjugative transfer is the most important means of spreading antibiotic resistance and virulence factors among bacteria. The key vehicles of this horizontal gene transfer
are conjugative plasmids. They contain as
minimum instrumentation an origin of transfer
(oriT), DNA processing factors (a relaxase and
accessory proteins) as well as proteins
that constitute the trans-envelope transport
channel, the so-called mating pair formation
(Mpf) proteins. On the Inc18 Enterococcus
plasmid pIP501, all these protein factors are
encoded by one transfer (tra) operon. They
form together the conjugative type IV secre-
tion system (T4SS). Three of the pIP501 T4SS
proteins, TraK, TraM and TraN, have been
recently crystallized and their structure has
been solved.
The structure of the N-terminally truncated
construct TraKΔ was determined to 3.0 Å
resolution and exhibits a novel fold. The
protein localizes to the cell wall, but does not
exhibit surface accessibility. Weak inter-
action with single-stranded, as well as double-
stranded DNA was observed, suggesting a role
in the transport of DNA. Sequence-based and
structural relatives were exclusively found in
closely related T4SSs, marking TraK as an
exclusive, specialized member of the pIP501
T4SS (Goessweiner-Mohr et al., submitted).
TraM localizes to the cell wall and was demon-
strated to be surface accessible. The crystal
structure of the C-terminal, surface-accessible
domain of TraM was determined to 2.5 Å
resolution, biophysical and biochemical data
indicated that a TraM trimer acts as the bi-
ological unit. Despite lacking sequence-based
similarity, TraM displays a fold similar to the
T4SS VirB8 proteins from Agrobacterium
tumefaciens and Brucella suis and to the T4SS
protein TcpC from Clostridium perfringens
plasmid pCW3.
TraN is a cytoplasmic DNA binding protein.
The specific DNA binding site was identified
upstream of the pIP501 oriT by a novel foot-
printing technique. The structure
of TraN was determined to 1.35 Å resolution.
It reveals an internal dimer fold with anti-par-
allel beta sheets in the center and a helix-turn-
helix motif on both ends. Based on structural
similarities we speculate that TraN is involved
in early steps of DNA transfer, possibly in trig-
gering pIP501 T4SS gene expression.
Finally a new secondary structure homology-
based classification system for T4SS protein
families will be presented.

**S3:4**

**POST-TRANSCRIPTIONAL MECHANISMS
AMPLIFY THE SMALL DIRECT EFFECT OF
SEX PHEROMONES ON CONJUGATION**

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University of Minnesota, Minneapolis, MN.

**S3:5**

**BACTERIOPHAGES, INTESTINAL
COLONIZATION, AND THE HOST INNATE
IMMUNE RESPONSE**

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Research over the past decade has provided
insight into the bacterial communities that col-
onize the mammalian intestine and has led to
a detailed understanding of how they interact
with their hosts. More recently, it has been re-
vealed that the intestinal microbiota is also rich
in viruses. Many of these viruses infect bacte-
ria and are known as bacteriophages (phages).
A large proportion of intestinal phages exist as
lysogenic prophages that are stably inte-
grated into bacterial chromosomes. In vitro,
prophages undergo lytic induction to produce
infectious phage particles when bacterial cells
are stressed or when they are stimulated by
nutrients. We have recently discovered that the
intestinal commensal bacterium Enterococcus
faecalis induces prophages when growing in
the mouse intestine and that the release of lytic
phage particles allows E. faecalis to compete
with genetically related enterococci vying for
similar intestinal resources. These studies have
raised major questions about how phage preda-
tion of susceptible bacteria in the intestine
influences host-bacterial interactions, inflam-
mation, and the immune response. To begin
to explore this idea we have isolated lytic phages from wastewater that efficiently kill *E. faecalis*. Using these phages in vitro we have discovered that 1) *E. faecalis* phage DNA is a potent activator of mammalian antiviral immunity and 2) that phage predation of *E. faecalis* enhances the expression of proinflammatory cytokines during bacterial infection. Enhancement of the inflammatory response requires phage susceptible bacteria as *E. faecalis* clones that have evolved resistance to phage attack stimulate proinflammatory cytokines to a lesser extent in the presence of phage particles. This work provides important initial insights into how phages may impact the mammalian innate immune response and is a first step toward understanding the role of phages in shaping host health and physiology during inflammatory diseases. Furthermore, these studies may yield novel phage-based therapeutic strategies for combating intestinal bacterial infections and inflammation. Studies are now underway using mouse models of infection and intestinal colonization to understand how phage-bacteria predator-prey interactions can impact host innate immunity in vivo.

**S3:6**

**ANALYSIS OF HIGHLY CONJUGATIVE PMG1-LIKE PLASMIDS**

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pMG1 (65.1 kbp) is a highly conjugative, pheromone-independent plasmid isolated from an *E. faecium* clinical strain obtained in our university hospital (Maebashi, Gunma). pMG1 carries a Tn4001-like transposon encoding gentamicin-resistance and transfers at a high frequency around 10⁻³ to 10⁻⁴ per donor cell during broth mating in both *E. faecium* and *E. faecalis*. The transfer is not induced by the sex pheromones of plasmid-free *E. faecalis*. During the mating, recipient cells and donor cells harboring pMG1 form mating aggregates, which can be observed by microscopy. In Southern hybridization analysis, pMG1 does not hybridize to other conjugative plasmids in Gram-positive bacteria including pheromone-responsive plasmids, indicating that pMG1 has a unique conjugation system. A transfer-related gene, *traA* is identified as whose transcript was up-regulated during the mating. The *traA* gene product associated with the formation or stabilization of mating aggregates during broth mating. The complete nucleotide sequence (65,029 bp) of the pMG1 plasmid revealed 73 ORFs lying in the same transcription orientation. Of 73 ORFs, 22 ORFs showed homology with ORFs present on the pXO2 plasmid (96.2 kb), which is the virulence plasmid essential for capsular formation by *Bacillus anthracis*. Analyses of the transposon insertion mutants and transcripts indicated that ORFs 15-49, lying in the 31.7 kb region were related transfer. A 5.9-kb HindIII fragment that replicates autonomously in *E. faecalis* was cloned and analysis of this fragment by deletion and in vitro insertion mutations showed that ORF10 (rep) and the inverted repeat sequence in the noncoding region between ORF8 and ORF9 were necessary for pMG1 replication. VanA-type vancomycin resistance pMG1-like highly conjugative pHT plasmids (α, β, and γ) were isolated from the outbreak strains of VanA-type vancomycin-resistant *E. faecium* and *E. avium* in a Japanese hospital. The complete DNA sequences of pHTβ plasmid showed a high degree of similarity to pMG1 plasmid. A functional oriT region and a deduced nickase gene *traI* of pHT plasmid were identified. The determinants of the mating-aggregates were also identified and composed of five ORFs. A transfer-related gene *traB* which positively regulates the expression of the genes including mating-aggregation and plasmid-transfer was identified in pHTβ. Recently, we identified two key regulatory genes designated...
as \textit{traD} and \textit{traF}. \textit{traD} was crucial for plasmid transfer and \textit{traF} was the transcriptional negative regulator for \textit{traD}, respectively.

The pMG1-like plasmids were found in the vancomycin-resistant \textit{E. faecium} outbreak strains isolated from Michigan (United State), Taiwan and Spain, respectively. The basic structures of these pMG1-like plasmids were almost identical and all of the ORFs of pMG1 were conserved in the plasmids except for the mobile elements including transposons, ISs, and group II introns.

\section*{S3:7}
\textbf{EVIDENCE FOR SIMULTANEOUS TRANSFER OF THE ESP-CONTAINING PATHOGENICITY ISLAND AND THE VANB RESISTANCE LOCUS IN ENTEROCOCCUS FAECIUM}

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Vancomycin-resistant enterococci (VRE) pose a serious concern to health care facilities worldwide and are detected at worrisome numbers over the last few years. Vancomycin resistance development in hospital-associated strains of \textit{E. faecium} is mainly due to an acquisition of mobile vanA- and vanB-type gene clusters, whereas their prevalence and trends vary across countries and continents. Independent acquisition of the vanB-encoding Tn1547 transposon was reported to occur at high frequency, thus leading to the emergence of clonally distinct VRE populations. As vanB-positive strains of sequence type ST192 are on the rise in Germany in recent years we set out to characterize several clinical isolates on a molecular level. Selected strains were sequenced by means of Illumina technology, in order to elucidate whether the emergence of vanB-positive ST192 strains in Germany is congruent with studies from around the globe. Further, we analyzed a subset of vanB-expressing \textit{E. faecium}, including those of various sequence types, to determine the insertion site of the respective transposon. As up to 70\% of all \textit{E. faecium} clinical isolates encode for the esp-containing pathogenicity island (PAI) ICEEfm1, unsurprisingly, all strains investigated not only exhibit the vanB locus, but the PAI as well. Investigation of vanB transferability by filter mating experiments into enterococci of various genetic backgrounds revealed the simultaneous acquisition of both, the vanB locus and the PAI. Subsequent whole genome sequencing analysis disclosed the chromosomal arrangement of selected recipients and their respective transconjugants (TCs). Of all TCs analyzed, vanB or/and concomitant integration of the PAI occurred at two distinct sites, one of which was previously declared a hot spot for insertion of foreign DNA. Integration of both pathogenicity markers occurred in close proximity throughout the experiments, hence suggesting a simultaneous excision and/or integration event of the two loci. Notably, the PAI itself does not encode a respective transfer system and thus most likely hijacks the Type IV secretion machinery provided by the neighboring vanB transposon. Moreover, insertion of vanB was uniformly associated with a certain cluster of non-virulence genes in a way that they were either present in the recipient bacteria or were carried along during the processes of transfer and site-specific integration. Due to the fact that a pathogenicity island co-transferred under selective pressure for the prominent resistance marker vanB, it is feasible to anticipate the spread of virulence-associated genes and hence, the generation of a bacterial population with enhanced pathogenic potential under antibiotic treatment.
S3:8
CHARACTERIZATION OF YAJC: A TRANSMEMBRANE PROTEIN INVOLVED IN CELL WALL STABILITY AND BIOFILM FORMATION IN ENTEROCOCCUS FAECIUM

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The multi-drug resistant nosocomial pathogen Enterococcus faecium (EFm) is the third most frequent pathogen causing nosocomial bactere-mia and biofilm (BF) associated infections. BF formation is a critical step in these difficult-to-treat infections, and increased understanding of the role of virulence factors in this process may lead to more effective targeted drugs. In this study we used Microarray-based Transposon Mapping (M-TraM) to identify genes essential for BF. Mutants affected in BF were selected from the transposon-mutant by repetitively subculturing of the planktonic cells from a semi-static BF model. Using microarrays, the composition of the mutant library recovered from the planktonic phase after four rounds of subculturing was compared to the entire transposon mutant library. This revealed 26 genes that were significantly enriched in the planktonic phase. A markerless double crossover mutant of yajC, identified in the screen as most essential in BF formation, was constructed to confirm the phenotype and to perform additional functional assays. The yajC gene is conserved in several gram negative and gram positive bacteria and it is predicted to encode a transmembrane protein. YajC deficient mutant showed a significant decrease in BF formation compared to the wild-type in a semi-static model and was attenuated in a rat endocarditis model, confirming the relevance of this gene in vivo. In addition, initial adherence of the mutant was reduced (in polystyrene assays) and several LPxTG surface proteins (i.e. pili, Esp, von Willebrand factor type protein) were detached from the cell wall of the mutant after exposure to limited external stress, unlike the WT. Mass spectrometry analysis on the detached proteins revealed apart from several surface proteins and transporters, mainly intracellular proteins. Moreover, the mutant was more sensitive to β-lactams antibiotics and lysozyme; however susceptibility to lysis by triton X100 remained unchanged and growth rate was not different from wild-type. Based on these results, we hypothesised that YajC plays a role in the cell wall stability and anchoring of surface proteins in E. faecium, and therefore, is a promising candidate for targeted-drug development against E. faecium infections.

S4:1
EVOLUTION AND DISSEMINATION OF VANCOMYCIN RESISTANCE

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Glycopeptides, vancomycin and teicoplanin, inhibit peptidoglycan synthesis by binding to the C-terminal D-alanyl-D-alanine of pentapeptide precursors, preventing transglycosylation and transpeptidation in cell wall assembly. Based on this mode of action it was predicted that resistance to these drugs could not occur. However, we reported in 1986 resistance to vancomycin in enterococci after more than 30 years of use. Inducible high level resistance is due to a sophisticated mechanism that combines synthesis of peptidoglycan precursors with low affinity for glycopeptides and elimination of the normal target precursors. This mechanism involves seven genes organized in two transposon-borne operons, one responsible for resistance, the other for its inducible expression by a two-component system. Reversible acquisition of higher levels or of broader resistance is secondary to mutations in the regulatory system, by the host becoming vancomycin dependent, by suppression of transcription termination between the two operons, or by combination of these genetic events.
Although this dual mechanism is predicted to have a high fitness cost, resistant enterococci have disseminated world-wide. Evaluation of the biological cost of VanB-type resistance due to acquisition of conjugative transposon Tn1549 in *E. faecium* and *E. faecalis* indicated that, both in vitro and in vivo, carriage of inactivated or inducible Tn1549 had no cost for the host in the absence of induction by vancomycin. In contrast, induced or constitutive resistant strains not only had reduced fitness but were severely impaired in colonization ability and dissemination among mice. Tight regulation of resistance expression thus drastically reduces the biological cost associated with vancomycin resistance in *Enterococcus* spp. and accounts for the widespread dissemination of these strains. These findings are in agreement with the observation that regulation of expression is common in horizontally acquired resistance and represents an efficient evolutionary pathway for resistance determinants to become selectively neutral.

**S4:2**

**CFR AND OXAZOLIDINONE RESISTANCE**

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**S4:3**

**FROM SINGLE VANX, VANY TO BI-FUNCTIONAL VANXY RESISTANCE ENZYMES**

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Vancomycin resistance in Gram-positive bacteria is due to production of cell wall precursors ending in D-Ala-D-Lac or D-Ala-D-Ser to which vancomycin exhibits low binding affinities and to the elimination of the high-affinity precursors ending in D-Ala-D-Ala. Depletion of the high-affinity precursors is catalyzed by the zinc-dependent D,D-peptidases VanX and VanY acting on dipeptide (D-Ala-D-Ala) or pentapeptide (UDP-MurNac-L-Ala-D-γ-Glu-L-Lys-D-Ala-D-Ala), respectively. Some of the vancomycin resistance operons encode VanXY D,D-peptidase, which hydrolyzes both di- and penta-peptide. The evolution and the molecular basis for the diverse specificity of Van D,D-peptidases remains unknown. We obtained the crystal structures of VanXyc and VanXYG in apo and transition state analog-bound forms and of VanXYC in complex with its D-Ala-D-Ala substrate and D-Ala product. Structural and biochemical analysis identified a mobile cap over the catalytic site as the key structural element involved in the switch between di- and penta-peptide hydrolysis. The structure of the BaLdcB VanY-like enzyme from *Bacillus anthracis* showed that the active site of VanY peptidases lacks this element providing a molecular architecture fitting with larger substrates. The structures also highlight the molecular basis for selection of D-Ala ending precursors over the modified resistant targets. This study illustrates the remarkable adaptability of the D,D-peptidase fold in response to antibiotic pressure which confirms the elegance and sophistication of the mechanism of resistance to glycopeptides.

**S4:4**

**STRATEGIES TO INTERRUPT GASTROINTESTINAL COLONIZATION BY GLYCOPEPTIDE RESISTANT ENTEROCOCCI**

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**S5:1**

**NOVEL PEPTIDOGLYCAN ASSEMBLY PATHWAYS IN THE ENTEROCOCCI: CONTRIBUTIONS OF GENE ACQUISITION AND MODIFICATION OF PREEXISTING REGULATORY CIRCUITS.**

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Resistance to antibiotics results from gene acquisition by horizontal transfer and mutational
alteration of the drug target. The latter applies to acquisition of β-lactam resistance in clinical isolates of Enterococcus faecium that harbor amino acid substitutions in the peptidoglycan transpeptidase domain of a low-affinity penicillin-binding protein (PBP5). An unusual mechanism for acquisition of drug resistance was identified in in vitro-selected E. faecium mutants that resist ampicillin by a by-pass mechanism. In these mutants, peptidoglycan cross-linking relies on an L,D-transpeptidase (Ldtₕₐᵣ), which is not inhibited by ampicillin. Activation of the L,D-transpeptidation pathway leading to ampicillin resistance was found to result from modifications of two signal transduction systems. The first one is a classical two-component regulatory system, composed of a sensor kinase/phosphatase (DdcS) and its cognate response regulator (DdcR). In wild type strains, the DdcRS system negatively controlled the activity of Ldtₕₐᵣ at the substrate level. In the ampicillin-resistant mutants, amino acid substitutions in DdcS led to impaired phosphatase activity, activation of transcriptional activator DdcR by phosphorylation, and transcription of a cryptic gene encoding a D,D-carboxypeptidase (DdcY). This metallo-enzyme cleaved the C-terminal residue of cytoplasmic peptidoglycan UDP-MurNAc-pentapeptide to form a tetrapeptide. The modification redirects the peptidoglycan assembly pathway since classical PBPs and Ldtₕₐᵣ exclusively function with pentapeptide- and tetrapeptide-containing substrates, respectively. The second system is a eukaryotic-like Ser/Thr protein kinase (Stk) and its cognate phosphatase (StpA). The latter enzyme was found to negatively control the level of protein phosphorylation both by direct dephosphorylation of target proteins and by dephosphorylation of kinase Stk. In the ampicillin resistant mutants, amino acid substitutions in essential residues of StpA resulted in impaired phosphatase activity and hyper protein phosphorylation. Impaired phosphatase activity of DdcS altered production of a single protein essential for resistance (DdcY) whereas phosphorylation of multiple proteins was affected by impaired StpA phosphatase activity. Activation of the L,D-transpeptidation pathway is an unprecedented mechanism of emergence of a new metabolic pathway since it involved recruitment of preexisting functions following modifications of regulatory circuits.

**S5:2**

**NOVEL ANTIMICROBIAL COMBINATIONS FOR THE TREATMENT OF ENTEROCOCCAL INFECTIONS**

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**S5:3**

**ADAPTIVE EVOLUTION OF ANTIBIOTIC RESISTANCE IN ENTEROCOCCI**

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Hospital acquired infections by Enterococci remain a challenge to health care systems worldwide. To date, surveillance for antibiotic resistance within clinical populations and genomic studies of clinical strain pairs have provided a wealth of information about the alleles associated with adaptation to antibiotics, but they do not provide essential information about relative importance of genomic changes, their order of appearance, or potential epistatic relationships between adaptive changes. We have used quantitative experimental evolution of a single polymorphic population in continuous culture to study daptomycin and tigecycline resistance in vancomycin resistant Enterococcus faecalis S613. Importantly, we sustain both planktonic and non-planktonic (e.g. biofilm) populations in co-culture as the concentration of antibiotic is raised, facilitating the development of more ecological complexity than is typically observed in laboratory evolution. Quantitative experimental evolution revealed a clear order and hierarchy of genetic changes leading to resistance, the signaling and metabolic pathways responsible, and
the relative importance of these mutations to the evolution resistance. Despite the relative simplicity of this ex vivo approach compared to the ecological complexity of the human body, we show that experimental evolution allowed for rapid identification of clinically relevant adaptive molecular pathways. Drugs targeted at the pathways responsible for the development of antibiotic resistance could be administered to increase usable lifespan of current antibiotics. In its fullest application, quantitative experimental evolution allows us to move from reactive post hoc understanding of resistance to a strategy that can anticipate resistance in a predictive manner.

**S5:4**

**ENTEROCOCCAL VACCINES**

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Enterococci are among the most difficult to treat bacteria causing nosocomial infections especially in immunocompromised patients. Especially in vancomycin-resistant *Enterococcus faecium* strains, resistance to almost all clinically available antibiotics has been observed, but also some infections due to *Enterococcus faecalis* (especially foreign-body infections and endocarditis) can lead to prolonged treatment and considerable attributable mortality. Vaccination against enterococci may not only help to avoid the development and spread of antibiotic resistances but may also be prophylactically given to prevent enterococcal infections in susceptible patient populations. Several protein and carbohydrate antigens have been identified in *E. faecalis* and *E. faecium* that are targets of opsonic and protective antibodies. In addition, we will present new results on synthetic carbohydrate antigens and recombinant human monoclonal antibodies able to prevent and/or treat multiresistant enterococcal infections.

**S5:5**

**ENTEROCOCCAL CELL MEMBRANE AND RESISTANCE TO ANTIMICROBIAL PEPTIDES**

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The bacterial cell membrane (CM) is an important component that controls and regulates the interaction of the cell with the environment. Some antibiotics used in clinical practice and naturally-occurring antimicrobial peptides target the CM and disrupt its architecture and function resulting in severe damage that usually leads to bacterial cell death. Our studies in the mechanisms of resistance to the lipopeptide daptomycin (DAP), a key front-line antibiotic commonly used against vancomycin-resistant enterococci (VRE), have provided novel insights into the enterococcal CM response to the attack by antimicrobials. Both *E. faecalis* and *E. faecium* contain four major CM phospholipids (PLs), phosphatidylglycerol (PG), cardiolipin (CL), lysyl-phosphatidylglycerol and glycerolphospho-diglycodiacylglycerol (GP-DGDAG). In addition, *E. faecalis* CMs (but not *E. faecium*) also contained two other unknown species of amino-containing PLs. Development of DAP resistance in both enterococcal species is associated with a significant decrease in PG content and a concomitant increase in GP-DGDAG. However, the most striking adaptive CM response upon exposure to DAP appears to be related to the remodeling of CM CL arrays. Using the fluorescent dye 10-N-nonyl-acridine orange (NAO), which interacts with CL, and bodipy-labelled DAP (a fluorescent derivative of DAP), we showed that development of DAP resistance in *E. faecalis* is associated with redistribution of CL microdomains away from the division septum (the principle target of DAP) likely to result in diversion of the antibiotic from this vital structure. Surprisingly, PL remodeling was not associated with mutations in genes encoding enzymes involved in PL metabolism. Instead, it was the result of a single amino acid dele-
tion within the transmembrane protein LiaF, a member of the three-component regulatory system LiaFSR predicted to function in cell membrane homeostasis. Genomic studies of a variety of *E. faecalis* and *E. faecium* have revealed that mutations in liaFSR are frequently found among DAP-resistant isolates and those with MICs close to the clinical breakpoint (4 µg/ml). Moreover, a single liaF mutation was sufficient to abolish in vitro DAP killing of *E. faecalis* and deletion of liaR restored DAP susceptibility and markedly increased the activity of a cadre of antimicrobial peptides with varied and distinct mechanisms of action. Our results suggest that LiaFSR system is the “master” regulator of the enterococcal CM adaptive response to the antimicrobial attack.

**S5:6**

FOCAL VIRULENCE FACTOR ASSEMBLY IN ENTEROCOCCUS FAECALIS

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The Gram-positive Enterococci are responsible for a wide variety of diseases including endocarditis, bacteremia, meningitis, wound infections, and urinary tract infections. Conserved among Enterococcus and other Gram-positive species is the utilization of membrane-associated sortase enzymes to catalyze the attachment of virulence proteins to the cell wall. In *Enterococcus faecalis*, sortases are involved in the attachment of surface proteins and in the biogenesis of endocarditis and biofilm-associated pili. Sortase enzymes localize with the Sec secretion apparatus at distinct foci at the cell membrane and this localization is crucial for normal pilus secretion and assembly. Sortase focal localization is dependent upon its highly cationic cytoplasmic tail, which we now show to be necessary and sufficient for localization.

We have tested the hypothesis that cationic antimicrobial peptides similarly interact with *E. faecalis* in a focal manner to target sites of virulence factor secretion, sorting, and assembly. We show that human beta defensins interact with *E. faecalis* at discrete foci, target sites of secretion and sorting, and disrupt their localization and function. We present data for genetic determinants which alters focal secretion, virulence factor assembly, and focal interaction of human beta defensins with *E. faecalis*. Together these findings suggest a novel model for targeted interaction between antimicrobial peptides and Gram-positive bacteria.

**S6:1**

ETHANOLAMINE UTILIZATION IN *E. FAECALIS*: ROLES AND REGULATION

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The ability to breakdown ethanolamine, a source carbon and nitrogen, can affect the infectivity of certain human bacterial pathogens including *Enterococcus faecalis*. The long-term goal of our work is to define the mechanisms that control the expression of the ethanolamine (EA)-utilization genes (eut) in *E. faecalis*. It is known that the substrate, EA, and the enzymatic co-factor adenosylcobalamine (AdoCbl) are both required for expression of the eut genes. Much progress has recently been made in understanding how EA regulates the system. EA is sensed by a two-component system comprising a histidine kinase (EutW) that in response to ethanolamine (EA), autophosphorylates, and then transfers the phosphate to its cognate response regulator, EutV. EutV has an RNA-binding output domain called AmiR and NasR Transcriptional Antiterminator Regulator (ANTAR) and utilizes antitermination to control gene expression at the post-initiation level. EutV acts on terminators in the 5’ untranslated regions (UTRs) of four eut genes. Antitermination occurs by EutV binding RNA structures consisting of dual hairpins that overlap the terminators, and this post-initiation
mechanism contributes to the induction of gene expression. How AdoCbl regulates the system is less understood and more controversial. An AdoCbl-binding riboswitch is present in an intergenic region of the eut locus and is required for expression. Riboswitches are generally cis-acting regulatory components located in untranslated regions of mRNAs. They comprise an aptamer domain that directly interacts with various metabolites and an expression platform, which undergoes structural changes upon metabolite binding to influence gene expression. In this presentation, a model for how the eut AdoCbl-binding riboswitch regulates gene expression will be presented, representing a novel mechanism of riboswitch action.

**S6:2**

**PEPTIDE TRANSPORTERS CONTRIBUTE TO SIGNALING IN ENTEROCOCCUS FAECALIS BIOFILM DEVELOPMENT**

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Enterococci are a leading cause of hospital-acquired infections throughout the world and display an ever increasing resistance to commonly used antibiotics. One of the hallmarks of enterococcal biology is the efficient exchange of genetic information through the process of conjugation. Genetic exchange between a donor cell harboring a pheromone responsive plasmid and a recipient cell is initiated by the recipient’s production of peptide sex pheromones to which the donor cell responds. The pheromones are generally derived from the signal sequence of bacterial lipoproteins that undergo proteolytic processing to yield the 7-8 amino acid peptide pheromone. Using a novel bacterial killing assay dependent on the presence of sex pheromones, we screened a transposon mutant library for additional factors that contributed to the production of pheromones. Here we describe a mutant that is significantly altered in its ability to kill *E. faecalis* indicator cells. In addition, this mutant showed a broader defect in its ability to mediate conjugation with 3 unique pheromone responsive plasmids, suggesting a global role in the secretion of peptide pheromones. Furthermore, this mutant displayed aberrant biofilm formation, consistent with a role for peptide pheromones as cell-signaling molecules in the development of enterococcal biofilms. As additional proof of peptide signaling being important in biofilm development, we examined the contribution of the oligopeptide permease to biofilm formation, and discovered that *E. faecalis* possesses two oligopeptide permease complexes and the deletion of both complexes is required to affect a strong phenotype on biofilm development.

**S6:3**

**(P)PPGPP METABOLISM IN ENTEROCOCCUS FAECALIS: BEYOND THE STRINGENT RESPONSE**.

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The stringent response (SR), mediated by the alarmone (p)ppGpp, is a conserved bacterial adaptation system controlling broad metabolic alterations necessary for survival during adverse conditions. In *Enterococcus faecalis*, production of (p)ppGpp is controlled by the bifunctional synthetase/hydrolase Rsh and by the monofunctional synthetase RelQ. Previous characterization of strains lacking *rsh*, *relQ*, or both in *E. faecalis* revealed that Rsh is responsible for activation of the SR and that alterations in (p)ppGpp production negatively impact bacterial stress survival and virulence. Despite its well characterized role as the effector of the SR, the significance of (p) ppGpp during balanced growth remains poorly understood. Microarrays of *E. faecalis* strains producing different basal amounts of (p) ppGpp identified several genes and pathways regulated by modest changes in (p)ppGpp. In particular, the transcriptional profile of the (p) ppGpp⁰ (*ΔrshΔrelQ*) strain and physiological
assays revealed that a complete absence of (p)ppGpp leads to severe dysregulation of energy-generating pathways and guanosine homeostasis. Surprisingly, the Δrsh strain, which also lacks the ability to mount the SR but produces approximately 4-fold higher basal levels of ppGpp compared to the wild-type strain, was able to maintain cell homeostasis. Thus, while both rsh and ΔrshΔrelQ strains cannot use (p)ppGpp to respond to stresses, fitness of the (p)ppGpp0 strain appears to be further impaired by an unbalanced metabolism. In fact, we showed that the previously described association of (p)ppGpp with antibiotic tolerance occurs at levels that are well below those needed to trigger the SR. These results highlight the critical but still underappreciated role of basal (p)ppGpp pools in cell homeostasis and stress tolerance.

**S6:4**

**ALLEVIATION OF CODY REGULATION BY (P)PPGPP IS CENTRAL FOR THE ACTIVATION OF A COMPLEX STRESS RESPONSE NETWORK IN ENTEROCOCCUS FAECALIS**

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In Gram-positive bacteria, the CodY protein and the (p)ppGpp alarmone, the effector molecule of the stringent response, are global transcriptional regulators that coordinate the expression of a large number of genes involved in stress survival and virulence. Regulation by CodY is stimulated through association with its co-effectors GTP and branched chain amino acids (BCAA). The production of (p)ppGpp during the stringent response depletes intracellular GTP, both directly and indirectly, and therefore negatively affects CodY activity. Global transcriptional analysis of the stringent response in *E. faecalis* revealed that a subset of stress- and virulence-associated genes under (p)ppGpp control contain putative CodY-binding motifs, suggesting that part of the regulatory effects of (p)ppGpp in gene transcription is mediated by CodY. Among the (p)ppGpp-regulated genes with CodY-binding motifs were genes encoding for the carbon catabolite repression regulatory protein CcpA, the regulator of the ClpP proteolytic complex CtsR, and the oxidative stress regulator Spx. Electrophoretic mobility shift assays revealed that CodY specifically interacted with the binding sequences upstream of *ccpA*, *ctsR* and *spx*. Previously, we showed that a complete lack of (p)ppGpp ((p)ppGpp0 strain) in *E. faecalis* leads to partial BCAA auxotrophy, reduced intracellular survival in murine macrophage, and attenuated virulence in the *Galleria mellonella* invertebrate model. We hypothesize that these phenotypes result from constitutive CodY control of stress survival and virulence-related traits. When compared to the wild-type ((p)pp-Gpp+) strain, deletion of *codY* in the (p)ppGpp0 strain alleviated BCAA auxotrophy, partially restored intracellular survival in macrophages, and fully restored virulence in *G. mellonella*. These results clearly demonstrate that CodY is intimately associated with (p)ppGpp-mediated gene regulation in *E. faecalis*. Together our *in silico* and gel shift data integrate (p)ppGpp, through CodY, into a complex stress response network with other prominent stress regulators that is crucial for *E. faecalis* to achieve its full pathogenic potential.

**S6:5**

**SYNTHETIC LTA-PROTEIN CONJUGATES ELICIT OPSONIC AND PROTECTIVE ANTIBODIES**

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Lipoteichoic acid (LTA) is amphiphilic polymer that is an important constituent of the cell wall of almost all gram-positive bacteria (e.g. staphylococci, streptococci, bacilli, clostridia, corynebacteria, listeria). The chemical structure of LTA varies among organisms, albeit in the majority of gram-positive bacteria the LTAs express a shared poly-1,3-(glycerolphosphate) (GP) backbone. We demonstrated previously the specificity of opsonic antibodies for this backbone present in S.epidermis, S.aureus and group B streptococci suggesting that this minimal structure may be sufficient for vaccine development against some gram-positive species. The immunogenicity of this antigen could be improved by conjugating this epitope to protein antigens.

The aim of the present work is to study a well-defined synthetic fragments (mimetic) of LTA as a promising vaccine candidates for active or passive immunotherapy. A library of synthetic LTA structures consisting of repeating glycerol phosphate backbone with different decorations was prepared by automated solid-phase synthesis and subsequently evaluated by opsonophagocytic inhibition assay (OPIA) to establish their antigenic activity. The inhibition of this compound together with serum raised against E. faecalis LTA was proportional to the length of the fragments. Single glucosyl substituents on the GP significantly enhanced inhibition of killing. The most promising candidate compound was conjugated to BSA used to immunize a rabbit. The opsonic activity and specificity of the immune rabbit serum showed killing at high serum dilutions in an opsonophagocytic assay (OPA) mediating killing of E. faecalis 12030, E. faecium E1162 and S. aureus MW2. Opsonic killing was inhibited by 96% and 68% in E. faecalis 12030 and E. faecium E1162, respectively using the purified synthetic compound. These results confirm that the antigenic backbone structure is shared in different gram-positive strains and that it is a potent target for vaccine development. The protective efficacy of the serum raised against synthetic LTA was evaluated in an E. faecalis rat endocarditis model, showing statistically significant reduction of endocarditis in the animals that were passively immunized with the immune serum compared to normal rabbit serum. We are currently evaluating additional novel synthetic compounds with distinct decorations to better understand structure-activity relationships.
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The Firmicutes bacterial phylum participates extensively in virulence and pathological processes. Enterococcus faecalis, an important member of this phylum, plays a dualist role, since it is a human gastrointestinal commensal microorganism as well as a pathogenic bacterium mainly related to nosocomial infections of immune compromised patients. Iron-sulfur [Fe-S] clusters are inorganic prosthetic groups of several proteins and, in bacteria are involved in different biological processes as pathogenicity and biofilm formation. In vivo [Fe-S] cluster biogenesis in Enterococci requires specific machineries coded by suf genes. This study aimed to investigate whether suf operon in E. faecalis is up- or down-regulated under oxidative stress and iron depletion conditions. Real-time quantitative-PCR (RT-qPCR) was performed with SYBR green. Expression of sufC, sufD, sufS, sufU, sufB, and oxyR was analyzed to confirm the system’s response to specific stress conditions. In order to characterize cellular responses to oxidative stress and iron depletion, the expression patterns of responsive genes kat and fur were also measured. Transcriptional levels of 23SrRNA, tuf, rpoB, and gyrB were tested as reference controls for data normalization, and all expression assays were normalized to the most stable standards (gyrB and rpoB) using the comparative critical threshold ($\Delta\Delta$Ct) method. RT-qPCR results were analyzed by One-Way ANOVA using the SAS software package, with p<0.05 being considered statistically significant. The iron depletion responsive gene fur was up-regulated in all 2,2’-Bipyridyl (DIP) concentrations tested, which confirmed stress induction. The sufCDSUB operon showed differential expression patterns according to DIP concentrations used: lower concentrations promoted operon inhibition, while doses equal or higher than 0.6 mM increased its transcription. These data suggest the presence of a specific mechanism for the regulation of iron inside cells. Expression of the oxidative stress responsive gene kat was enhanced upon exposure to all H$_2$O$_2$ concentrations tested. For the first time, it was verified that Gram-positive bacteria possess an oxidative stress responsive OxyR component, well described in Escherichia coli models. Low concentrations of H$_2$O$_2$ were already capable of increasing sufCDSUB expression. Since even low concentrations of oxygen free radicals are highly damaging to [Fe-S] clusters, this might enhance sufCDSUB transcription to promote cluster replacement. Our work describes innovative and important data on the operon expression of genes responsible for [Fe-S] cluster biogenesis in E. faecalis, which are up-regulated during iron depletion and oxidative stress.

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Introduction. Enterococcus faecalis is a “two-faced” organism. Although some strains coexist with humans as part of the gastrointestinal (GIT) microbiota, the opportunistic
nature combined with increased incidence of antibiotic resistant *E. faecalis* nosocomial infections prompts the need to understand what separates commensal and pathogenic behavior. In the effort to unravel the involvement of genetic traits contributing to the development of infection, Bioluminescence Imaging (BLI) has been employed in both Gram-negative and Gram-positive bacteria as a cost effective imaging method that allows tracking the expression of selected genes in intact animals in real-time. **Aim.** This study was carried out to develop a luxABCDE-based reporter system to allow noninvasive localization and differential gene expression of *E. faecalis* during in vitro growth and the progression of infection within animal model systems. **Methods.** The vector pSL101 and its derivatives, conferring a genetically encoded bioluminescent phenotype on *E. faecalis*, were constructed by combining the luxABCDE operon from pPL2lux and the pREG696 broad-host-range replicon and axe-txe toxin-antitoxin cassette. A synthetic *E. faecalis* 16SRNA P1 promoter, the gelatinase promoter (PgelE) and the cytolysin promoter and its cognate regulatory genes (Pcyl-cylR1R2) were cloned in front of the luxABCDE cassette and plasmids introduced in *E. faecalis* MMH594. Recombinant strains were employed to determine the in vitro promoter activity, to visualize the bacterial burden during colonization of the murine GIT and explore the expression profiles of the two prominent *E. faecalis* virulence traits, cytolysin and gelatinase, in vivo during systemic infection of Galleria mellonella and mice. Moreover, persistence of the nosocomial blood isolate MMH594 in the murine large and small intestines was compared to that of the human commensal EF62. **Results.** These reporters provided high-level bioluminescence in vitro, were stably maintained in *E. faecalis* in the absence of antibiotic selection and emitted light in a manner consistent with the modulation of gene expression at different stages of culture growth. Systemic infection of G. mellonella with *E. faecalis* MMH594 revealed temporal regulation of both gelatinase and cytolysin promoters. Gavage of mice pretreated perorally with antibiotics resulted in efficient colonization of the murine GIT in a strain-dependent manner, where the commensal baby isolate EF62 was more persistent than the nosocomial isolate MMH594. In the murine intravenous infection model, bioluminescent MMH594 were found in the spleen and heart and preferentially accumulated in the liver and kidneys. Cytolysin expression was readily detected in the liver, kidneys, and bladder. **Conclusion.** The presented system is established as the first substrate addition-independent reporter developed for BLI of *E. faecalis*.

**3A**

**CONTRIBUTION OF EMPA PILUS SUBUNIT OF ENTEROCoccus FAECium TX82 TO PILUS ARCHITECTURE AND BIOFILm FORMATION.**

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**Background.** The ability of *Enterococcus faecium* to form biofilm is considered important to its virulence potential. Our group previously showed that the genome of *E. faecium* TX16 (DO) encodes four predicted pilus-encoding gene clusters, and demonstrated that in the endocarditis-derived *E. faecium* strain TX82, deletion of one of these operons, the empABC pilus-encoding operon, affected biofilm formation and the resulting mutant was also significantly attenuated in an experimental model of urinary tract infection. **Methods.** We created unmarked, non-polar single deletion mutants of the pilus subunits EmpA, EmpB and EmpC in *E. faecium* TX82, and we used western blotting and immunoelectron microscopy to study pilus architecture. In addition, we evaluated the effect of the single deletions on the ability of the strains to form biofilms. **Results.** We showed that EmpA localizes at the tip of the pili and that deletion of this subunit is as-
associated with increased length of the pili. The biofilm density assay revealed that deletion of EmpA (ΔempA) reduced biofilm formation to the same level as observed with the deletion of the operon (ΔempABC) (P <0.001), while deletion of EmpB (ΔempB) and EmpC (ΔempC) had modest and minimal effect in biofilm production, respectively. Conclusion. Our results indicate that EmpA acts as the tip pilin, and that this subunit is important for determining wild-type length of the pilus fiber. Furthermore, we demonstrated that EmpA is the component of the Emp pili that mediates biofilm formation. 

4B

A THERMO-REGULATED PROLINE-RICH SURFACE PROTEIN OF ENTEROCoccus FAEcium BINDS TO FIBRINoGEN, FIBRONEcTIN AND PLATELETS AND CONTRIBUTES TO BIOFILM FORMATION

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Enterococcus faecium is a commensal of the mammalian gastrointestinal tract but in the last decades, it has become an important nosocomial pathogen, causing infections that are difficult to treat due to the organism’s intrinsic and acquired antibiotic resistance and ability to form biofilms. However, little is known about the mechanisms of E. faecium involved in colonization and infection of the mammalian host. We hypothesized that genes involved in colonization or infection might exhibit temperature-regulated control of expression, and we therefore performed a transcriptome analysis of E. faecium E1162 during mid-exponential growth at 25°C and 37°C. Thirty-three genes showed significantly higher expression at 37°C than at 25°C. One of the most highly upregulated genes (4.4-fold; 37°C versus 25°C) encodes a 48-kDa surface protein with an LPxTG-type anchor. The N-terminal domain is unique to E. faecium and closely related enterococci (E. hirae and E. durans). The C-terminal domain contains multiple proline-rich repeat regions and we consequently named this protein PrpA for proline-rich protein A. Confocal and electron microscopy revealed that PrpA is found exclusively at the poles of the cells. We also showed by flow cytometry, that surface-exposed levels of PrpA are highest in exponentially growing cells at 37°C compared to stationary phase cultures or when grown at 25°C. The thermo-regulated production of PrpA on the surface of E1162 was also observed in other E. faecium strains. Furthermore, specific antibodies against PrpA were observed in patients that have previously suffered from an E. faecium bacteremia, indicating that PrpA is immunogenic. The heterologously expressed and purified N-terminal domain of PrpA was able to bind to fibrinogen, fibronectin and platelets. In addition, a prpA deletion mutant is defective in the early stages of biofilm formation compared to the wild type strain. Our data indicate that PrpA may contribute to pathogenesis of E. faecium-mediated infections.

5A

SYSTEMATIC ANALYSIS OF SURFACE PROTEINS IN ENTEROcoccI: DISCOVERY OF POTENTIAL TARGETS FOR VACCINE DEVELOPMENT

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Infections by opportunistic bacteria have significant contributions to morbidity and mortality of patients and also generate high costs for medical care. A major clinical problem is caused by Gram-positive bacteria such as Enterococci. These bacteria are
considered as emerging nosocomial pathogens with a high degree of resistance to a wide range of antibiotics. The two more common species Enterococcus faecalis and E. faecium are responsible for over 90% of enterococcal infections. Due to this problem, the objective of this work was to extract, purify, identify and characterize some of the immunogenic cell wall associated proteins present in E. faecium strains associated with hospital outbreaks of vancomycin-resistant enterococci. We compared three different extraction methods to obtain the surface proteins: trypsin shaving (TS), biotinylation (BT) and alkaline pH extraction (AE). After extraction, the identification and crude purification was carried out by SDS-PAGE gel separation and subsequent analysis by nanoLC-MS/MS. The number of proteins identified with the different methods were 401 (trypsin shaving), 329 (extraction at high pH) and 45 (biotinylation-based approach). Among all the proteins identified, 63 (16%), 63 (19%) and 33 (73%) polypeptides were predicted as cell wall associated proteins for the trypsin shaving, the extraction at high pH and for the biotinylation approach, respectively. Among the methods tested we found that the biotinylation-based approach seems to be the best strategy to isolate surface proteins from E. faecium. This technique is more reproducible than the other methods and leads to significantly less contamination with obviously intracellular polypeptides. By using all these methods we were able to identify approximately 70 putative surface proteins. A comparison between the three extraction methods allowed us to identify seven protein vaccine candidates: a protease, a penicillin-binding protein, a lipoprotein, a peptidoglycan-binding protein, a carboxypeptidase, an isomerase and a glycolytic enzyme. These candidate proteins were overexpressed and purified, and rabbit polyclonal antibodies were raised against them. Currently, the opsonic killing activity of these sera is being tested as well as different immunological assays (ELISA, Western blot), to get more information about the nature of these antigen protein targets.

6B
THE FUNCTIONS OF HTRA IN ENTEROCOCCUS FAECALIS

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Bacterial pathogens carry factors that contribute to virulence via direct or indirect interactions with the host. We have shown in Enterococcus faecalis that sortase-assembled virulence factors are secreted and processed at 1-2 distinct foci near the septum of the cell. Sortase enzymes, nearly ubiquitous in Gram-positive bacteria, act on substrates bearing distinct C-terminal sorting signals to mediate substrate biogenesis and attachment to the cell wall. In E. faecalis OG1RF, sortase A (SrtA) catalyzes the attachment of substrates to the cell wall, whereas sortase C (SrtC) is essential in pilus polymerization. Both enzymes, along with SecA are focally localized near the division plane of the cell. In S. pyogenes, secretion is similarly localized to discrete domains, and processing of the focally secreted protease SpeB to its mature form is mediated by the co-localized protease HtrA. HtrA and SecA are also spatially restricted at the septa in S. pneumoniae. Highly conserved across all domains of life, HtrA is typically involved in various aspects of protein quality control, including cellular response to protein-folding stress and degradation of misfolded and mis-localized cell envelope proteins. In this study, we investigated the role of HtrA in E. faecalis and its contribution to localized virulence factor secretion and assembly. To study HtrA in E. faecalis, we have constructed an in-frame deletion of htra and have also complemented the deletion with a wild type copy of htra expressed from a plasmid. To examine the role of E. faecalis HtrA in monitoring the bacterium’s response to cellular stress, we performed kinetic growth assays in the presence of various environmental stressors including, temperature, acid, pH and osmotic shock. Wild type E. faecalis and the Δhtra mutant strain grow similarly in acid, pH or osmotic stress condi-
tions, whereas ∆htrA grows less well than wild type at temperatures >50°C. To investigate if there is any difference in expression levels of HtrA at various temperatures, we carried out immuno blots using an antibody raised against E. faecalis HtrA. We show that HtrA expression does not vary with increasing temperature. We next investigated whether HtrA is focally localized at the septa in E. faecalis using immunofluorescence microscopy. Whereas HtrA was homogenously distributed around the cell periphery at 37°C, we observed an increase in septal focal localization of HtrA at higher temperatures. SrtA and SrtC focal localization were not altered in the absence of HtrA, nor was pilus polymerization, at 37°C. Together, these data suggest that HtrA may be active in protein quality control under heat stress, but not acid, pH or osmotic stress conditions. Although HtrA does not affect SrtA or SrtC localization at physiologic temperatures, HtrA may be involved in monitoring heat sensitive cell cycle-related events or septally localized processes under heat stress conditions.

7A

GENOTYPIC CHARACTERIZATION AND IDENTIFICATION OF VIRULENCE FACTORS IN ENTEROCoccus SPP. ISOLATED FROM RAW BUFFALO MILK IN SOUTHERN REGION OF BRAZIL.

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The buffalo’s milk consumption and derivatives have increased significantly in the last year due to the growing demand for healthy foods. Enterococci are natural component of foods, representing important role in ripening and flavor enhancement of cheese and sausage. They have been used as probiotics to improve the microbial balance in humans and animals. On the other hand, they are not considered “generally recognized as safe”, due to its use as an indicator of fecal contamination, and the frequent association with food-borne illnesses. The aim of this study was characterized genotypically and screened for the presence of virulence factors in enterococcus isolated from raw buffalo mam in southern region of Brazil. Seventy-nine enterococcus, previously identified to species level by conventional biochemical methods were submitted to identification by genotypic methods using genus-specific primers (tuf gene). The species identifications were confirmed by multiplex PCR. Isolates were also evaluated for their ability to form biofilm and production of gelatinase enzyme. The presence or absence of virulence genes (agg, ace, gelE) was determined by PCR. Of the 79 enterococci tested, only three showed discordant results between the phenotypic and genotypic identification. More than 60% of E. faecalis were classified as strong and 20% moderates bifilme producers, and for E. faecium, 13.8% were strong biofilm formers and 10.3% moderates. Genes encoding the virulence factors were more frequently in E. faecalis than in E.faecium, ace (96% vs. 10.34%), and gelE (96% vs. 17.24%), while the agg gene was found only in E. faecalis strains (26%). The presence of gelE and the activity of gelatinase were not fully concordant. Emergence of enterococci harbouring virulent factors in bufalos milk suggest a situation of risk for community, since Enterococcus spp. are opportunist pathogens and their research, it is necessary to ensure safety and quality of food we consume.
A GENOMICS APPROACH TO UNDERSTANDING THE SUCCESS OF ST203 VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECIUM

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A significant rise in the rates of vancomycin-resistant Enterococcus faecium (VREfm) bacteremia had been observed at the Austin Hospital in Melbourne, Australia over a 12-year time frame leading up to 2009. Multilocus sequence typing of the strains isolated in this period revealed the replacement of the previously dominant ST17 VREfm strains with a recently emerged ST203 clone. In efforts to understand the emergence and increase in incidence of ST203 VREfm infections, comparative genomic studies were employed to investigate the genetic differences that distinguish the two clone types. Sequence data for a representative ST17 and ST203 isolate were obtained using 454 GS FLX and Illumina GAIIx instruments. The complete genomes were derived using a process of gap closure that involved PCR and primer walking, and the sequencing of selected clones from large-insert E. coli bacterial artificial chromosome E. faecium clone libraries. Assembly accuracy was confirmed by reference to optical maps, and the finished genomes were annotated. Draft genome sequence data for a collection of additional ST17 and ST203 strains were also derived using Illumina sequencing. Comparisons between the two representative genomes revealed significant differences in genome architecture and 502kb of Aus0085-unique genome content. These Aus0085-unique regions encompassed three other plasmids and genes that encode for resistances against other antimicrobials as well as additional metabolic functions. A notable difference in the length of the vancomycin resistance-conferring Tn1549 transposon and insertion sites of the transposon was also observed between the two strains. Further analysis extending to a larger collection of 12 ST17 and 14 ST203 genomes revealed only 41kb of the 502kb, spanning 6 chromosome regions, present in all the ST203 strains and wholly absent from the ST17 collection. The genes within these regions are predicted to play a role in metabolism, and could help explain previously observed enhanced growth phenotypes. Our comparative studies have revealed key genomic differences that distinguish the ST203 strains from the ST17, and have provided further insights into the underlying factors that are likely to have contributed to the success of the ST203 VREfm strains in the clinical environment.

ENTEROCOCCUS FAECIUM; DOES IT HAVE A NEW FACE?

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Introduction: Enterococcus faecium has emerged as a nosocomial pathogen worldwide. Vancomycin resistant E. faecium (VREfm) is of concern as it leads to limitations in treatment options. There are eight different van genes coding for glycopeptide resistance; vanA and vanB are the most important in E. faecium [1-3]. VanA causes resistance to vancomycin and teicoplanin, vanB causes resistance to vancomycin only. E. faecium strains susceptible to teicoplanin have minimum inhibitory concentration (MIC) values between 0.5 and 1.0 mg/L. We identified E. faecium strains which were less susceptible to teicoplanin (MIC 2.0-4.0), though still susceptible to vancomycin. We performed phenotypic and genotypic investigations to gain insight into
this phenomenon. **Methods:** Since 2012, we identified 44 patients carrying *E. faecium* isolates less susceptible to teicoplanin. The majority were identified in rectal samples, mainly of haematological patients. Strains were tested for antibiotic susceptibility using Vitek2®. Strains were typed by Multiple-Locus Variable number tandem repeat Analysis (MLVA). A selection was made to perform additional Etests to confirm Vitek results and to perform PCR for *vanA/B* genes. Seven strains were selected to perform whole genome sequencing (WGS) and were compared with another 19 *E. faecium* strains. Electron microscopy (EM) was performed on one representative *E. faecium* strain less susceptible to teicoplanin and an *E. faecium* strain with normal susceptibilities to glycopeptides.

**Results:** Vitek2® results showed MIC values for teicoplanin of 2.0–4.0 and vancomycin MIC values of 0.5–1.0 mg/L. MLVA typing showed different MLVA types (MT) over time. In 2012 we found predominantly MT-432 and some MT-1 strains, since 2013 predominantly MT-12. Etests showed the same MIC patterns (teicoplanin MIC 2.0–6.0, vancomycin MIC 0.75–1.5). PCR results on *vanA/B* genes were negative. WGS showed that *E. faecium* isolates less susceptible to teicoplanin belonged to different phylogenetic clusters and are evolving independently. The resistome created for these strains showed no common resistance genes. EM showed a thicker glycocalyx in the *E. faecium* strain less susceptible to teicoplanin.

**Conclusion:** We identified a new phenotype of *E. faecium* which is characterized by a reduced susceptibility to teicoplanin while vancomycin susceptibility remains unaltered. The phenotype is associated with different MLVA types. Moreover, it seems to evolve in different clonal background *E. faecium*. By PCR and sequencing no responsible resistance genes are identified yet that might cause this phenomenon. The exact mechanism responsible for this phenotype is still unknown, but might relate to components of the cell wall and/or glycocalyx. Correlation between teicoplanin use and these strains is likely since they were obtained from haematological patients which often get teicoplanin in our hospital.

**10B**

**A NEW BURST OF BACTEREMIA CAUSED BY BAPS 2.1 (ST117, ST80, ST203) ENTEROCoccus FaECium THAT ARE SUPERIMPOSED (2006-2012) BUT DO NOT REPLACE THOSE CAUSED BY BAPS 3.3 (ST18, ST17 AND ST16) (1995-2012)**

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**Objective:** *Enterococcus faecium* (Efm) is one of the most common causes of nosocomial infections. Multi-drug resistant Efm is endemic in healthcare institutions worldwide, with some human adapted lineages causing most clinical infections (ST17, ST18, ST78) belonging to an specialized population. We assessed the evolution of population structure of Efm blood isolates in our institution (1995-2012); during this period the rate of bloodstream infections caused by Efm increased dramatically in the absence of MDR outbreaks. **Material and Methods:** We analyzed 324 blood Efm isolates collected from different patients hospitalized at HRyC in the period 1995-2012. Identification was performed using WIDER; MICROSCAN and MALDI-TOF. Antibiotic (AB) susceptibility testing was performed by standard CLSI methods. Clonal relationship was established by SmaI-PFGE, Multilocus sequence typing (MLST) and Bayesian Analysis Population Structure (BAPS) clustering. Known putative virulence factors (esp, and hyl) were investigated by PCR as described. **Results:** An increasing trend in Efm bloodstream infections was observed in our institution during the period analyzed. This trend was accompanied
by the increase of Efm isolates resistant to ampicillin, levofloxacin, and streptomycin (40%, 30% and 30% in 1995 vs 100%, 94% and 97% in 2012, respectively). The 324 Efm isolates corresponded to 141 PFGE-types and 47 STs grouped in 4 phylogenomic groups inferred by BAPS, namely subgroups 3.3 (56%), 2.1 (32%), 1 (3%) and 6 (0.6%). BAPS 3.3 isolates (56%, 180/324) grouped in 81 PFGE-types, mostly belonging to ST17 (44%, 79/180), ST18 (41%, 74/180) and ST16 (9%, 16/180), with prevalent ST18, ST17 and ST16 PFGE-types recovered throughout the years. BAPS2.1 (32%, 102/324) isolates, grouped in 22 PFGE-types, mostly belonged to ST117 (50%, 51/102), ST203 (25%, 25/102) and ST80 (17%, 16/102). Prevalent clones of ST117-AREF-25 (49), ST80-AREF-68 (12) and ST16-AREF-9 (16), were enriched in putative virulence factors (esp - 67% and 53%; hyl - 23% and 57%, mobile genetic elements). BAPS 1 isolates (3%, 10/324) were associated with community based patients and AB susceptible. Through the study an increase in the number of BAPS 2.1 isolates (10% in 1995 vs 65% in 2012) and a decrease of isolates belonging to less frequent BAPS groups, such as BAPS1 was observed, the number of hospital associated BAPS 3.3 isolates was stably maintained (40% vs 32%). Within BAPS2.1 and 3.3, major human lineages, successive waves of epidemic clones were observed, reflecting constant evolution and adaptation to human populations. Conclusions: The sharp increase in the number of Efm bloodstream infections seems to be associated with the introduction in HRyC of major BAPS 2.1 clones enriched in colonization factors, which did not replace the constant presence of BAPS 3.3 in bacteremia, which suggest a different virulence space for both major phylogroups.

11A

THE DEVELOPMENT OF AN ORTHOGONAL POOLING APPROACH FOR THE RAPID AND AFFORDABLE MAPPING OF E. FAECALIS INSERTIONAL MUTANTS VIA DEEP SEQUENCING.

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Targeted gene knockout via the insertion of transposable elements is one of the most powerful tools of reverse genetics, and its utility has been enhanced with the advent of next-generation sequencing (NGS) technology. Creation of an arrayed, genome-wide library of thousands of disruption mutants, using an insertional mutagen such as the mariner Himar1 transposon (Tn), provides a tremendous resource for functional genomic analysis. The mapping of Tn insertions in viable clones provides de facto identification of non-essential genes. More importantly, the resulting clones enable targeted functional reverse genetics, as well as high-throughput genome-wide screens using approaches such as Tn-Seq. Unfortunately, the high-throughput mapping of tens of thousands of Tn insertion sites from single-colony isolates remains an expensive proposition using traditional methods (inverse PCR and classic Sanger sequencing of individual clones). In order to overcome practical obstacles to the mapping of arrayed insertional mutant libraries, we have combined a) three-dimensional orthogonal pooling of bacterial cultures (row-wise, column-wise, plate-wise), b) bulk amplification of mariner-tagged insertion sites into NGS-ready libraries, c) bulk NGS-based sequencing of pools for identification of insertion sites, and d) simple informatic tools for parsing of NGS data and deductive identification of Tn insertion sites in original wells of arrayed plates. We have successfully applied these tools to the mapping of Himar1 insertions in over 17,000 OG1RF clones, achieving a reduction in effort and cost of roughly one order of magnitude.
12B
CHARACTERIZATION OF VANCOMYCIN-RESISTANT ENTEROCoccus FAECium (VREFm) ISOLATED FROM NOSOCOMIAL BACTEREMIA

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Introduction: Nosocomial infections (NI) are a major public health problem worldwide. The selective pressure caused by the indiscriminate use of antimicrobials, as well as an increase of immunocompromised patients within hospitals have caused multiresistant microorganisms, to emerge in, such as vancomycin-resistant Enterococcus faecium (VREfm). The VREfm genome present a high plasticity, facilitating acquisition of mobile genetic elements encoding antibiotic resistance and virulence factor genes. Since 1980’s, It has been reported an increased number of infections caused by VREfm in the U.S.A and Europe In Mexico, the first outbreak caused by VREfm occurred in 2008. Objectives: Determine the metabolic and molecular profile of VREfm strains isolated from a tertiary Hospital in Mexico. Methodology: A total of 35 VREfm clinical strains PCR identity confirmed, were analyzed for substrate use pattern and antimicrobial resistance profile. Using Vitek compact-2 automated equipment. Molecular characterization was performed by pulsed field gel electrophoresis (PFGE) and plasmid profile determined by lysis in horizontal agarose gel (Eckhardt). Results: The identification of strains showed that all the strains included in the study are E. faecium and that 34 of them have the VanA resistance genotype. Analysis of metabolic profile showed that 80% of strains differ in exact use of substrates, these differences suggest a high variability among the VREfm strains. Although, higher than 90% of strains are resistant to ampicillin, trimethoprim-sulfamethoxazole, quinolones, vancomycin and teicoplanin on the antimicrobial resistance profile analysis. Interestingly, the molecular PFGE analysis allowed to identify at least 4 genetic clusters among VREfm strains, while, Eckhardt analysis showed that 90% of strains have a unique plasmid profile with the presence of 5 to 9 plasmids by strain and all strains have a common plasmid of around 100 kb. Conclusions: VREfm strains isolated from nosocomial bacteremia in a tertiary hospital in Mexico, present a high variability in their metabolic and molecular profile, which suggests a high level of adaptation to their environment. Molecular analysis help us to identify a common plasmid in all strains, which may be related to vancomycin resistance.

13A
FIRST REPORT OF DAPTOMYcin-NONSUSCEPTIBLE ENTEROCoccus FAECium ISOLATED IN BRAZIL

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Vancomycin-resistant Enterococcus faecium (VREfm) is a pathogen frequently associated to a multiresistant phenotype, leading to challenges considering therapeutical choices. The lipopeptide antibiotic daptomycin became an alternative therapy for VRE infections, however, cases of daptomycin-nonsusceptible enterococci had been reported, and little is known about the mechanisms of resistance of this microorganism to this antimicrobial. The objective of this work was to determine the susceptibility profile of VREfm to antimicrobial agents and the genetic relationship among them. From September 2012 to August 2013, consecutive Enterococci recovered from infections or surveillance sites were obtained from patients attended at general Hospital in Porto Alegre, Brazil, as part of an epidemiological surveillance study. The species identification and the presence of vanA gene were determined by PCR. For antibiotic susceptibility testing it was used the disk diffusion method and Minimum Inhibitory Concentration. Pulsed-field gel
Electrophoresis (PFGE) was performed to analyze the clonal diversity. During the study period, 441 Enterococci were consecutively recovered and among these isolates, 27 (6.1%) were characterized as VREfm. All twenty-seven isolates exhibited vancomycin MICs higher than 256 µg/mL and resistance to teicoplanin (all carrying vanA gene), as well as, all isolates were resistant to ampicillin, ciprofloxacin, and susceptible to linezolid and quinupristin-dalfopristin. High-level Gentamicin Resistance (HLGR) was detected in 14.8% (4) of the isolates. Thirteen isolates (48.1%) were daptomycin-nonsusceptible. PFGE profile analysis enabled us to characterize a main clone referred as clone A, which presented 24 (88.9%) isolates. Among them, 11 (84.6%) isolates were daptomycin-nonsusceptible. By comparing the clonal relationship of these isolates with a VRE recovered in 2011 in a different hospital in Porto Alegre, we observed a close genetic relationship, suggesting a clonal dissemination of VREfm in our city. Our study firstly reported cases of E. faecium daptomycin-nonsusceptible in Brazil, demonstrating the importance of epidemiological surveillance researches.

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### 14B

**DETECTION OF CLASS 1 AND 2 INTEGRASES (INTI-1, INTI-2) AND GENETIC CASSETTES BLAOXA AND BLATEM AMONG MULTIDRUG RESISTANT SHIGELLA ISOLATES IN COSTA RICA**

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**Introduction:** Shigella is a virulent bacterium and a major cause of dysentery throughout the world. The emergence of antibiotics resistance in Shigella is related to molecular plataforms, called integrons. Recent studies worldwide have detected class 1 and class 2 integrons among multidrug-resistance Shigella spp. However, there are no studies related to the molecular detection of integrons among Shigella isolates in Costa Rica. **Results:** Thirty Shigella isolates (S. sonnei 17% and S. flexneri 83%) were obtained from stools of individuals with diarrhea. 97% (29/30) were resistant to at least one antibiotic. Most of them were resistant to amoxicillin (83%) and ampicillin (73%), followed by tetracycline (70%), cotrimoxazole (40%), chloramphenicol (40%) and gentamicin (7%). Class 1 integrase (intI1) was detected in 100% and class 2 integrase in 90% of isolates, the blaOXA genetic cassette in 87% and blaTEM in one isolate (7%). **Conclusion:** We are reporting for the first time the presence of class 1 and class 2 integrons and beta-lactamase genetic cassettes, blaOXA and blaTEM, among multidrug resistance Shigella isolates in Costa Rica. The importance of integrons in the acquisition of resistance genes, and therefore emergence of multidrug resistance strains, is a global problem that requires urgent actions.

### 15A

**EFFECTS OF COPPER SUPPLEMENTATION ON THE PREVALENCE OF COPPER (TCRB), MACROLIDE [ERM(B)] AND TETRACYCLINE [TET(M)] RESISTANCE GENES AMONG FECAL ENTEROCOCCI OF CATTLE AND SWINE**

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Copper, as copper sulfate, is often used as an alternative to in-feed antibiotics for growth promotion in both swine and cattle diets. Because of the potential genetic link between acquired copper (tcRB), tetracycline [tet(M)] and macrolide [erm(B)] resistance in Enterococcus spp., we hypothesized that copper supplementation may exert selection pressure for enterococci to become resistant to macrolides and tetracyclines, and possibly to other antibiotics. We conducted two studies in
cattle and swine to investigate the relationship between copper supplementation and the fecal prevalence of tcrB-positive enterococci, as well as its potential co-selection for macrolide and tetracycline resistance. The swine study consisted of 240 weaned piglets, which were assigned randomly to 6 treatment groups. The pens were assigned in an incomplete 2×2×2 factorial arrangement with treatments of copper, tylosin, and chlortetracycline. The cattle study consisted of 80 crossbred yearling heifers, which were assigned randomly to a 2×2 factorial arrangement of supplemental copper (10 or 100 mg/kg of feed) and tylosin (0 or 10 mg/kg of feed) in the diet. Enterococci were isolated from the fecal samples. Overall, prevalences of tcrB-positive enterococci were 14.5% (372/2592) and 4.6% (37/800) in swine and cattle, respectively (P < 0.05). The tcrB-positive isolates belonged to either Enterococcus faecalis or E. faecium; the majority were E. faecium. All tcrB-positive isolates also contained both erm(B) and tet(M) genes; however, none of them harbored the vanA gene. The median MICs of copper for tcrB-negative and tcrB-positive enterococci were 4 and 20 mM, respectively (P < 0.0001). The overall prevalence of erm(B) and tet(M) genes among enterococcal isolates of cattle were 46.8% and 57.5%, respectively; in contrast, fully 100% of the swine isolates tested were positive for both erm(B) and tet(M) genes. The potential associations between copper resistance and resistance to other antibiotics were investigated among an equal number of tcrB-positive and tcrB-negative isolates in cattle and swine. A large majority of the isolates - 95% (95/100) and 83% (64/77) in swine and cattle, respectively - were resistant to three or more antibiotics, and therefore classified as multidrug resistant (MDR). Our results suggest that the epidemiology of antibiotic and metal resistance differs somewhat between swine and cattle enterococcal isolates. The higher occurrence of tcrB-positive enterococci in cattle and pigs fed elevated copper confirms that supplementation of copper in both cattle and swine diets selected for resistant strains. The genetic link between copper resistance and other antibiotic resistance determinants indicates the potential importance of elevated copper supplementation in exacerbating the spread and persistence of antibiotic resistance.

16B

INCREASED FREQUENCY OF LINEZOLID RESISTANCE AMONG CLINICAL ENTEROCOCCUS FAECIUM ISOLATES FROM GERMAN HOSPITAL PATIENTS

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Objective: Linezolid (LNZ) is an antibiotic of last resort to treat infections with multi- and vancomycin-resistant Enterococci (VRE). Here we report the increasing prevalence of LNZ resistance among clinical E. faecium strains and analyze their resistance mechanisms.

Methods: Totally 5224 clinical E. faecium isolates from German patients (2002-2013) were sent to our National Reference Centre and tested by broth microdilution for their susceptibilities to several antibiotics including linezolid. Strains originated from more than 200 private microbiological and hospital laboratories. LNZ-resistant isolates from 2007-2013 were screened for 23S rDNA resistance mutations and the presence of the cfr gene. cfr-positive strains were analyzed by different methods including whole genome (WGS) and plasmid sequencing. Results: The following resistance frequencies to linezolid were determined: 1.04% in 2002, 0.53% in 2003, 2.77% in 2004, 2.96% in 2005, 1.13% in 2006, 2.13% in 2007, 0.62% in 2008, 0.84% in 2009, 2.98% in 2010, 4.92% in 2011, 4.05% in
2012, and 8.88% in 2013 (Jan 1st - Nov 21st). Altogether 181 linezolid-resistant *E. faecium* strains including 50 positive for vanA (27.6%) and 21 for vanB (11.6 %) from 46 different laboratories were analyzed. All but 4 isolates contained the G2576T 23S rDNA mutation and showed a mix of wildtype and mutated alleles per genome. Four LNZ-resistant isolates exhibited wildtype 23S rDNA alleles only suggesting other unknown resistance mechanisms. Long term growth experiments over several weeks under non-selective conditions revealed a stable LNZ MIC in six investigated LNZ-resistant strains. Two of 181 LNZ-resistant isolates were cfr-positive and possessed G2576T mutations. The sequenced cfr gene was 75% identical to the cfr gene of staphylococci. Preliminary analysis indicated a chromosomally-borne cfr gene in one of the isolates and a plasmid-borne cfr gene in the other. We are currently performing WGS analysis of both strains to determine the chromosomal localization and the cfr plasmid type. For a few hospitals we observed outbreaks with linezolid-resistant *E. faecium* isolates. **Conclusions:** LNZ resistance in clinical *E. faecium* isolates increased in recent years and first outbreaks with LNZ resistant VRE strains in German hospitals were reported. The majority of LNZ-resistant isolates investigated in this study was vancomycin-susceptible. Almost all LNZ-resistant *E. faecium* isolates showed the characteristic G2575T mutation in 23S rDNA. cfr in clinical *E. faecium* isolates is rare and its relevance for LNZ resistance expression remains to be elucidated.

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**17A**

**GENOME-WIDE SCREENING FOR GENETIC DETERMINANTS INVOLVED IN DECREASED SUSCEPTIBILITY TO CHLORHEXIDINE IN ENTEROCoccus faecium**

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*Enterococcus faecium* is a natural inhabitant of the gastrointestinal track of humans. However over the last few decades it has emerged as one of the primary causes of nosocomial infections. Its ability to acquire resistance determinants to almost all antibiotics has severely limited treatment options. Although understanding the mechanisms of antibiotic resistance may lead to the discovery of novel drug targets and eventually to the development of novel therapeutics, it is equally important to prevent the spread of multidrug resistant pathogens (MDR) in the nosocomial environment. Antiseptics such as chlorhexidine (CHX), have been commonly and effectively used for this purpose, in surgical scrubs and surface disinfectants for decades. Worryingly, decreased susceptibilities in MDR pathogens to disinfectants are currently also being reported. In this study, we used a high-density transposon mutant library constructed in E1162, a sequenced *E. faecium* clinical isolate, to perform a genome-wide screening for genetic determinants involved in decreased susceptibility to CHX. Screening of the transposon mutant library was done using Microarray-based Transposon Mapping (M-TraM) and led to the identification of candidate genes that could be involved in loss of susceptibility to this antiseptic. Among the genes identified, we found a two-component system (2CS), composed of a sensor histidine kinase and its DNA-binding response regulator. In order to validate the M-TraM results, we constructed mutants in the genes encoding the response regulator and the histidine kinase. We have shown that interfering with individual components of this 2CS, is sufficient to abolish growth of *E. faecium* E1162 in the presence of CHX. This result confirms the role of this two-component system in the loss of susceptibility of *E. faecium* to chlorhexidine. The genes that are regulated by this 2CS are currently being identified and their role in chlorhexidine resistance will be investigated.
Expression Analysis of the ftsW-psr-pbp5 Operon in Enterococcus hirae.

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In enterococci, their intrinsic β-lactam resistance is associated with production of low affinity penicillin binding protein PBP5. In Enterococcus hirae and Enterococcus faecium, the pbp5 gene is part of the ftsW-psr-pbp5 operon, with identical genetic organization. It has been shown that the overproduction of PBP5 in both species is due to overexpression of a polycistronic mRNA including ftsW-psr-pbp5. In E. faecium, mRNA hybridization revealed four transcripts: monocistronic ftsW; bicistronic psr-pbp5; monocistronic pbp5; and finally polycistronic ftsW-psr-pbp5 (Rice et al., 2001). However, in E. hirae only the longest polycistronic mRNA was observed. With the aim of verifying this result and to highlight putative promoters in E. hirae ATCC9790 ftsW-psr-pbp5 operon, the efficiency of putative promoters was evaluated in order to understand their implications in the expression of β-lactam resistance. Plasmids were constructed in which the promoterless gene for firefly luciferase was placed under the control of the sequence upstream each one of the gene of the ftsW-psr-pbp5 operon. For each construction the production of light by the reporter gene in the E. coli DH5α strain and the E. hirae ATCC9790 genetic environment was followed by luminometry. The results obtained in E. coli differ from those obtained in E. hirae suggesting a trans regulation probably related to the enterococcal context. To further investigate these observations, electrophoretic mobility shift assays were performed using putative promoter sequences and cytoplasmic extracts. Band shifts were observed mainly for a palindromic sequence and for another highly conserved short sequence both located upstream ftsW. All this results suggest that the expression of this operon is complex and probably involving specific enterococcal regulatory proteins. These studies shed new light on the molecular mechanism involved in the expression of β-lactam resistance in enterococci.

Enterococcus faecalis Genetic Determinants Responsible for Biofilm-Induced Antibiotic Resistance and Conjugal Transfer of PCF10

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Inherent and acquired antibiotic resistance is an attribute of the commensal bacterium Enterococcus faecalis that contributes to its prevalence as a nosocomial opportunistic pathogen. There is intense interest in the genetic basis for antibiotic resistance in E. faecalis since enterococci exhibit the ability to acquire resistance to most antibiotics encountered. The limited deployment of new classes of antibiotics also makes the identification of specific resistance determinants imperative. E. faecalis readily forms biofilms that are innately more resistant to the effects of antibiotics and has a propensity to acquire antibiotic resistance determinants via conjugation. We employed genetic approaches to identify E. faecalis chromosomal determinants responsible for biofilm-induced antibiotic resistance, and conjugal exchange of an antibiotic-marked plasmid. Using an arrayed library of transposon (Tn) mutants and two separate screening approaches, we identified E. faecalis chromosomal genes implicated in biofilm-induced antibiotic resistance and conjugal transfer of plasmid DNA. The genetic determinants responsible for biofilm-induced antibiotic resistance include the quorum sensing response regulator fsrA and glycosyltransferase-associated genes. Some of these genes have been previously identified as genes affecting biofilm formation in E. faecalis; however, here we show that the respective Tn mutants have minimal impact on biofilm production in the absence of antibiotic. Instead, the Tn mutants exhibit enhanced
susceptibility to sub-inhibitory concentrations of antibiotics when cultured as a biofilm. Two genetic determinants were identified for their involvement in efficient conjugal transfer of the pheromone-inducible plasmid pCF10. Tn disruption of epal, a group-2 glycosyl transferase, reduced pCF10 transfer during early and late liquid mating by approximately 100- to 1000-fold. Disruption of the heat shock chaperone dnaK resulted in significantly decreased mating efficiency only during prolonged liquid matings when the cells were within stationary phase. These results suggest that epal provides display of appropriate surface components for mating pair formation while dnaK allows newly generated transconjugants to adapt to stresses associated with pCF10 acquisition in stationary phase. We are currently elucidating the role of each gene in regard to E. faecalis conjugal exchange of plasmid DNA and biofilm-induced antibiotic resistance.

20B

MUTATIONS IN LIASR OF ENTEROCoccus faecium ARE NOT ASSOCIATED WITH REMODELING OF CELL MEMBRANE PHOSPHOLIPIDS

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Background: In Enterococcus faecalis, daptomycin (DAP)-resistance (DAP-R) has been associated with mutations in liaFSR, a three-component regulatory system that controls the cell envelope response to stress. We recently described that changes in LiaFSR redistribute phospholipids of the membrane and divert DAP away from its main target, the septum of E. faecalis. In E. faecium, the mechanism of resistance is not fully elucidated, but changes in liaSR have been seen in strains whose DAP MICs are in the higher range. More importantly, substitutions in LiaSR were sufficient to abolish DAP bactericidal activity. In this study we evaluated the impact of substitutions in LiaSR on the distribution of phospholipids and DAP binding to the cell membrane of E. faecium. Methods: We studied 2 strains of E. faecium whose genomes have been sequenced: HOU503, a clinical isolate (DAP MIC 3 µg/mL) harboring substitutions in LiaS [T120A] and LiaR [W73C], and TX16, a clinical strain (DAP MIC 2 µg/mL) without substitutions in LiaSR or any other mutation related to DAP-R. We evaluated the binding of DAP using Boron-dipyrromethene (BODIPY)-labeled DAP (BDP-DAP) at 4 µg/ml and 32 µg/ml. We quantitated the fluorescence intensity after treatment with BDP-DAP, normalized to protein content of the sample. In addition, we visualized CL-rich domains of HOU503 by fluorescence microscopy using the fluorescent dye 10-N-nonyl acridine orange (NAO).

Results: At concentrations of 4 µg/ml and 32 µg/ml, no differences in fluorescence intensities were observed (P>0.05), suggesting that the total amount of bound antibiotic was similar in these 2 strains. Furthermore, visualization of CL-rich domains by NAO in HOU503 revealed no redistribution and an intense linear staining at the bacterial septum, similar to the pattern previously seen in DAP-susceptible E. faecalis.

Conclusions: Substitutions in LiaSR of E. faecium did not appear to affect the binding of BDP-DAP to the cell membrane. Unlike E. faecalis, the alterations in LiaS and LiaR studied here were not associated with remodeling of cell membrane CL domains in E. faecium.
CFR-MEDIATED LINEZOLID RESISTANCE (LNZ-R) IN CLINICAL ENTEROCOCCUS FAECALIS FROM LATIN AMERICA

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Background: LNR-R mechanisms of resistance include i) mutations in the 23S rRNA, ii) mutations in the genes encoding ribosomal proteins L3 and L4, and iii) the presence of the ribosomal methyltransferase Cfr (encoded by cfr). The cfr gene is particularly problematic since it confers resistance to several antimicrobials and is potentially transferable. Cfr has been well described in S. aureus but was only recently reported in two clinical isolates of E. faecalis from Thailand and Canada. We describe for the first time cfr-mediated LNZ-R in three E. faecalis clinical isolates from Latin America. Methods: We studied three LNZ-R E. faecalis isolates, from Mexico (two) and Panama (one). We confirmed the antimicrobial susceptibilities by agar dilution and PFGE was used to assess for genetic relatedness. The cfr gene was detected by PCR and was sequenced using flanking primers (based on sequences available in NCBI). Genes encoding domain V region of 23S rRNA and ribosomal proteins L3 and L4 were also sequenced. Results: The linezolid MICs of the two isolates from Mexico were 8 µg/mL and 16 µg/mL for the strain from Panama. The Mexican isolates exhibited identical PFGE patterns and were not genetically related to the one from Panama. No mutations in domain V of 23S rRNA or the genes encoding ribosomal proteins L3 and L4 were found. The presence of cfr was confirmed in all three isolates. The cfr sequences in the Mexican strains were identical to those described in both staphylococci and enterococci. We were not able to obtain the entire cfr sequence from the isolate from Panama. Conclusions: We report for the first time the presence of cfr in E. faecalis clinical strains from Latin America. The fact that we could not sequence cfr in the isolate from Panama suggests cfr in this strain has a different genetic environment. Our findings highlight the success of dissemination of the multi resistance gene cfr among relevant human pathogens in different geographical regions.

LINEZOLID RESISTANCE DUE TO G2576T MUTATION IS HIGHLY STABLE UNDER NO SELECTIVE ANTIBIOTIC PRESSURE IN THE VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECALIS ST526 AND ENTEROCOCCUS FAECIUM ST412 CLONES

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Background: Linezolid has been one of the most active agents tested against enterococci, including vancomycin-resistant enterococci (VRE). Linezolid resistance in VRE clinical isolates has been rarely reported and it is mainly mediated by G2576T mutation in the central loop of domain V of the 23S rRNA gene. In this study, we examined the stability of the mutant rRNA copies in linezolid and vancomycin-resistant Enterococcus faecalis and Enterococcus faecium (LRVRE) strains belonged to different sequence types (ST). These strains were isolated from blood and
urine cultures from different patients admitted to a tertiary care hospital in São Paulo, Brazil and it had no other mechanisms for linezolid resistance, such as an adenosine modification catalyzed by Cfr at position 2503 of 23S rRNA or mutations in the ribosomal proteins L3 and L4. Respecting glycopeptide resistance, all strains had the vanA genotype. Methods: From August 2009 to December 2011, 2 LRVR E. faecalis (ST525 and ST526) and 1 LRVR E. faecium (ST412) strains exhibiting high level resistance both to vancomycin (MIC, >256 mg/L) and linezolid (MIC, 8-32 mg/L) were selected for evaluation of stability of linezolid resistance phenotype. These strains were subcultured 100 times on free antibiotic MH agar. The domain V fragment was digested with the NheI restriction enzyme on the first and last day of the experiments and the linezolid MICs were determined by CLSI broth dilution method.

Results: No significant reduction in the linezolid MIC was observed for the LRVR E. faecium ST412 (MIC, 32 µg/mL) and LRVR E. faecalis ST526 (MIC, 16 µg/mL) strains, whereas the linezolid MIC of the LRVR E. faecalis strain ST525 decreased from 8 to 4 µg/mL and from 4 to 2 µg/mL, respectively, after 20 and 25 subcultivates. The incomplete digestion of domain V with NheI indicated the presence of fragments with both G2576T mutant and wild-type sequences in all strains on the first and last day of the experiments.

Conclusions: The linezolid-resistant phenotype due to G2576T was highly stable after 100 serial passages under no selective antibiotic pressure in the LRVR E. faecium ST412 and LRVR E. faecalis ST526 clones. Our data showed the persistence of mutant rRNA copies in the absence of antibiotic pressure, even in the derivative linezolid-susceptible LRVR E. faecalis ST525 clone. Keywords: linezolid; G2576T mutation; Enterococcus spp.

ALTERATIONS IN THE LIAFSR SYSTEM IMPACT SUSCEPTIBILITY TO CATIONIC ANTIMICROBIAL PEPTIDES (CAPS) IN ENTEROCOCCUS FAECALIS

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Background: LiaFSR orchestrates cell-envelope response to stress in Gram-positive bacteria. Alterations in this system are involved in the development of daptomycin resistance (DAP-R) in enterococci. In S. aureus, DAP-R has been correlated with cross-resistance to several host defense CAPs. We evaluated in vitro activity of different CAPs against E. faecalis harboring liaFSR mutations. Methods: We assessed the in vitro susceptibilities to three CAPs: LL37 (PMN and epithelial-derived), nisin (bacterial-derived), and RP-1 (synthetic platelet CAP congener) at concentrations of 20, 0.8 and 10 µg/ml respectively. We studied four strains: i) a DAP-susceptible strain S613 (DAP MIC 0.5 µg/ml), ii) a laboratory derived DAP-R mutant carrying a codon deletion in liaF, and changes in the phospholipid metabolism genes cls and gdpD, S613ΔliaF177gdpgdpD170cls61 (DAP MIC 6.0 µg/ml) iii) a liaR deletion mutant constructed in the background of the above DAP-R mutant, S613ΔliaF177gdpgdpD170cls61ΔliaR (DAP MIC 0.094 µg/ml) and iv) the complemented strain of this liaR deletion mutant, S613ΔliaF177gdpgdpD170cls61ΔliaR::liaR (DAP MIC 8.0 µg/ml). After 2 hr CAP exposures, the mean percent survivals (±SD) were calculated and statistically compared. Results: As compared to S613, S613ΔliaF177gdpgdpD170cls61ΔliaR showed significant reduction in killing by all CAPs (P<0.05). In contrast, the ΔliaR mutant was highly susceptible to killing by all CAPs tested (P<0.05 for PR-1). The DAP-R...
phenotype was restored with reintroduction of liaR (ΔliaR::liaR), which was coupled with significant reduction in CAPs killing (P<0.05 for LL-37 and Nisin). **Conclusions:** Our results show that DAP-R in *E. faecalis* correlates with a reduced killing phenotype with a variety of CAPs. Importantly, we identified LiaR as a key factor associated with both DAP-R (as presented in another abstract) and CAP-R, and a potential target to combat such resistances.

**24B**

**AMPICILLIN (AMP) FAILED TO ENHANCE DAPTOMYCIN (DAP) ACTIVITY AGAINST AN ENTEROCOCCUS FAECIUM WHOSE DAP RESISTANCE DEVELOPES VIA AN LIAFSR-INDEPENDENT PATHWAY**

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**Background:** The addition of β-lactams has been shown to enhance the activity of DAP against *S. aureus* and *E. faecium*. Specifically, AMP restored the bactericidal activity of DAP in enterococci, including strains harboring mutations in the LiaFSR system, which has been associated with DAP-R and tolerance. We previously reported a clinical strain-pair of *E. faecium* that developed DAP-R through a LiaFSR-independent pathway. Of the 8 genes with changes one resulted in a substitution in the putative histidine kinase of the YycFG. Similar to LiaFSR, YycFG has been involved in cell envelope homeostasis and DAP-R. We postulated that the addition of AMP would restore the bactericidal activity of DAP against a DAP-R *E. faecium* harboring a mutation in yycG. **Methods:** We included 2 strains of *E. faecium* (whose genomes have been sequenced): S447, an AMP and vancomycin-resistant clinical isolate (DAP MIC 3 µg/ml) and R446, a DAP-R derivative of S447 isolated from the same patient (DAP MIC 16 µg/ml) with mutations in 8 genes, including yycG. Time-kill assays were performed with an initial bacterial inoculum of 107 CFU/ml in Mueller Hinton broth (supplemented with Ca²⁺ 50 mg/L) using DAP concentration of 5X MIC in the presence and absence of AMP at 64 µg/ml. Bacterial burden was evaluated at 0, 6, and 24 h. Bactericidal activity was defined as a decrease of ≥ 3 log10 CFU/ml at 24 h. **Results:** DAP at 5X MIC produced bactericidal activity against S447 after 24 h (4.6±0.17 log10 CFU/ml decrease) in the presence and absence of AMP. Conversely, DAP at 5X MIC failed to produce bactericidal activity against R446 after 24 h (1.3±0.35 log10 CFU/ml decrease). Interestingly, the addition of AMP at 64 µg/ml did not produce additional killing against R446 (1.6±0.87 log10 CFU/ml decrease). **Conclusion:** Our results showed lack of synergism of the combination of DAP and AMP against a clinical strain of *E. faecium* with a LiaFSR-independent DAP-R pathway. These results further suggest a genotype-specific response of combination therapy of DAP and AMP in *E. faecium*.

**25A**

**DELETION OF THE RESPONSE REGULATOR OF LIAFSR REVERTS DAP RESISTANCE AND CARDIOLIPIN (CL) DOMAIN REDISTRIBUTION IN THE CELL MEMBRANE OF *E. FAECALIS***

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**Background:** Daptomycin (DAP) attacks the cell membrane with affinity for the septum. LiaFSR is a three-component regulatory system that has been involved in the development of DAP resistance (DAP-R) in *E. faecalis*. We
showed that the decrease in DAP susceptibility of *E. faecalis* due to changes in LiaFSR is coupled with redistribution of CL-enriched domains (normally localized in septal and polar regions) away from the septa. In this work, using a deletion mutant of liaR, the response regulator of LiaFSR, we further studied the role of this system in the redistribution of CL domains and its contribution to DAP-R. **Methods:** We included i) a laboratory derived DAP-R mutant carrying a codon deletion in liaF, and changes in the phospholipid metabolism genes cls and gdpD (S613ΔliaF177gdpD170cls61), ii) a liaR deletion mutant constructed in the above DAP-R mutant (S613ΔliaF177gdpD170cls61ΔliaR) and iii) the complemented strain of this liaR deletion mutant (S613ΔliaF177gdpD170cls61ΔliaR::liaR). DAP MICs were performed by Etest. Visualization of CL-rich domains in cell membranes was performed using the fluorescent dye 10-N-nonyl-acridine orange (NAO).

**Results:** Microscopic examination with NAO of S613∆liaF177gdpD170cls61 revealed markedly reduced fluorescence at sites of division septum, as previously described. Deletion of liaR from this strain decreased the DAP MIC from 8 to 0.094 µg/ml. Remarkably, NAO staining of this ΔliaR mutant revealed localization of CL-domains at septum and poles, similar to DAP-S *E. faecalis*. Significant CL redistribution away from the septum was seen in the restored ΔliaR::liaR strain, together with an increase in the DAP MIC to 8 µg/ml. **Conclusion:** Deletion of liaR from the resistant strain tested produced a DAP hyper-susceptible phenotype, as presented in another abstract. This observation was coupled with a reversion of the redistribution of CL-domains in the cell membrane. These results further confirm the link between remodeling of CL domains and DAP-R and corroborate the importance of the LiaFSR system in this process.

**26B**

**EFFECT OF LIAFSR ON THE SUSCEPTIBILITY TO DAPTOMYCIN (DAP) AND CEPHALOSPORINS OF ENTEROCOCCUS FAECALIS**


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**Background:** Although not fully elucidated, the addition of β-lactams (BLs) has been shown to enhance the activity of DAP. Data suggest that the synergism seen with this combination may be affected by changes in LiaFSR, a regulatory system implicated in DAP resistance (DAP-R) in *E. faecalis*. Also, recent data showed that the penicillin-binding protein targeted by the BLs could also play a role. We recently found no difference in the MIC of ceftriaxone (CRO) in a DAP-susceptible (DAP-S) clinical isolate of *E. faecalis* and its DAP-R derivative (harboring liaFSR mutation). However, the latter exhibited a dramatic growth defect in the presence of sub inhibitory concentrations of CRO. In order to further understand the implications of changes in LiaFSR, we tested different cephalosporins against mutants with altered in LiaF and in LiaR, the response regulator of the system. **Methods:** We determined MICs of 5 cephalosporins targeting a diverse range of PBPs: cephalothin (CET), cefotetan (CTT), CRO, cefepime (FEP) and ceftaroline (CPT) using Etest. Isolates in the study include i) S613 (DAP MIC 1 µg/ml), a DAP-S strain, ii) a laboratory mutant derived from S613 harboring a codon deletion in liaF, (DAP MIC 4 µg/ml) iii) a DAP-R mutant from S613 carrying a codon deletion in liaF and changes in the phospholipid metabolism genes cls and gdpD (S613ΔliaF177gdpD170cls61) (DAP MIC 8 µg/ml), iv) a liaR deletion mutant constructed in the background of the above DAP-R mutant (S613ΔliaF177gdpD170cls61ΔliaR).
(DAP MIC 0.098 µg/ml) and \( v \) the comple-mented strain of this liaR deletion mutant (S613\(\Delta liaF177\Delta liaR::liaR\)) (DAP MIC 8 µg/ml). **Results:** No changes in the MIC were observed for any of the isolates when tested for CTT, CRO and CEP. Interestingly, S613\(\Delta liaF177\Delta liaR::liaR\) showed lower MICs to CET and CPT as compared to S613 (12 vs 256 and 0.25 vs 3µg/mL, respectively). Similarly, introduction of the liaF resistant allele decreased the MIC to CET (32 µg/mL) and CPT (0.75 µg/mL). On the other hand, deletion of liaR (S613\(\Delta liaR::liaR\)) increased the CET and CPT MICs to levels reaching those of S613. These findings were reverted in the liaR complemented strain. **Conclusions:** As presented in another abstract, deletion of liaR and, to a lesser extent changes in liaF, decreased the MIC to DAP-R and also resulted in a DAP hyper-susceptible isolate. Interestingly, we observed a seesaw effect, in which MICs to CPT and CET decreased as DAP MICs increased. This change was not observed with CRO, CTT or CEP. Our results suggest that LiaFSR system impact BL susceptibility and that this interaction is compound specific.

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**27A IDENTIFICATION OF MUTATIONS RELATED WITH LEVOFLOXACIN RESISTANCE IN CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA**

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**Introduction:** Pseudomonas aeruginosa is an opportunistic gram-negative bacillus with significative incidende in patients with noso-comial infections, especially respiratory, been Cystic Fibrosis (CF) patients frequent carriers of multirresistant strains, and thereby P. Aeruginosa being the cause of many of deaths from this pathology. Quinolones are drugs frequently used for the treatment of patients with this kind of infections, inhibiting the bacterial DNA synthesis, impeding in gram-negative the action of DNA gyrase (Topoisomerase II). Unfortunately the emergence of Levofoxacin resistance P. aeruginosa strains hinder the treatment of our patients, justifying our work. **Objective:** Identify mutations in the quinolone resistance-determining region (QRDR) in the gyrA gene which codifes for the subunit A of DNA gyrase in clinical isolates from patients with CF and other pathologies (non CF). **Methods:** In total 60 clinic isolates of P. aeruginosa were studied, 30 from CF patients, and the other 30 from patients with other pathologies (non CF). Susceptibility assays with Levofloxacin were done using the micro broth method. PCR amplification of QRDR region in the gyrA gene was performed and the PCR products were sequenced and analized using MEGA software for indentifying mutations. Statistical analysis was performed using R software using univariate analysis, X² test and logistic regression, with \( p\leq 0.05 \). **Results:** The susceptibility assays with Levofloxacin showed that 48.3% of the isolates were resistant, 45% susceptible and 6.7% intermediate. From the resistant strains 45% belonged to CF patients and 55% to non CF without statistical significance (\( p=0.26 \)). After sequenciation only 44 isolates were analized and mutations Met101Leu, Gln106Lys, and Gln106Arg were identified with frequencies of 100%, 22.7% y 31.8% respectively; without statistical significance by comparing mutations versus resistence to levofloxacin. **Conclusions:** Gram-negative resistance like P. aeruginosa to quinolones as levofloxacin is a reality more frequent in patients with healthcare associated infections, our findings suggest the existence of alternative mechanisms of resistance to this drug different from the QRDR region in the clinical isolates analized. **Key Words:** Pseudomonas aeruginosa, resistance, quinolones, DNA gyrase, mutations, Cystic Fibrosis.
FUNCTIONAL ANALYSIS OF BACTERIOCIN BAC41 PRODUCED BY ENTEROCOCCUS FAECALIS.

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Enterococcus faecalis is Gram-positive commensal bacteria in human or animal intestinal tract. It is also an opportunistic pathogen causing endocarditis, urinary tract infection, and the other infectious diseases. Enterococcal virulence associated factors including cytolyisin/hemolysin, adherence factors, resistances to ultraviolet and multiple antimicrobial agents, and bacteriocins have been found in E. faecalis clinical strains. Bacteriocin 41 (Bac41) showing specific bactericidal activity against E. faecalis is produced by many of the E. faecalis clinical isolates, suggesting that Bac41 is involved in the E. faecalis infection. Previous genetic analysis revealed that Bac41 determinant is encoded on the pheromone-responsive conjugative plasmid and consists of four genes, bacL1, bacL2, bacA, and bacI. Products of bacL1 and bacA genes, BacL1 and BacA, respectively, are responsible for the bactericidal activity against E. faecalis. BacL1 is a 595 amino acid protein (64.5 kDa) consisting of two domains located in the 1-151 and 160-309 amino acid regions of the amino acid sequence. The domains show homology to the bacteriophage-type peptidoglycan hydrolase and the NlpC/P60 family peptidoglycan hydrolase, respectively. A C-terminal, three-repeat structure located in the 318-577 amino acid region of BacL1 shows homology with the bacterial Src homology 3 (SH3) domain that is reported to bind to the bacterial cell wall. BacA is a 726 amino acid protein (79.1 kDa) and has a putative peptidoglycan-binding domain and a domain similar to the GH25 family peptidoglycan hydrolase in the 40-140 and 208-491 amino acid regions, respectively. In this study, we analyzed the functions of BacL1 and BacA to reveal the bacterial killing mechanism of Bac41 (BacL1/BacA). The coincidental treatment with the recombinant BacL1 and BacA resulted in the complete bacteriolytic activity against E. faecalis, but neither BacL1 nor BacA protein alone showed the activity. Interestingly, BacL1 alone was sufficient to degrade the cell wall of E. faecalis even in the absence of BacA. Furthermore, MALDI-TOF MS analysis revealed that BacL1 has a peptidoglycan D-isoglutamyl-L-lysine endopeptidase activity via a NlpC/P60 homology domain located in 163-315 a.a. region of BacL1. Meanwhile, BacA did not show peptidoglycan degrading activity. Furthermore, the fluorescence or immune-electron microscopy analysis showed that BacL1 localizes septum or equatorial region on the cell surface of target bacteria. Indeed, the activity of BacL1 and BacA did not kill bacteria under the growth arrest. These results suggest that BacL1 acts as a peptidoglycan hydrolase against under the bacterial cells during growth, and, when BacA coexists, leads viable E. faecalis cell to be lysed.

INTESTINAL COLONIZATION BY ENTEROCOCCUS IN HOSPITALIZED AND AMBULATORY PATIENTS OF DIFFERENT AGE GROUPS

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Objective: The increase in enterococcal infections is linked to the spread of multi-drug-resistant (MDR) Enterococcus faecium (Efm) and Enterococcus faecalis (Efc) pathogenic clonal lineages, but much less is known about
the lineages of commensal strains. The population structure of commensal enterococci recovered from the gut of hospitalized and ambulatory patients of different age groups from our institution (HRyC) was studied.

**Material and Methods:** 306 fecal isolates from 167 ambulatory (AMB) and 139 hospital patients (HOSP) were collected at HRyC and its ambulatory area (2009-2011). Patients were classified in 3 age groups (YP:0-19y, AD:20-59y and EL:>60y). Pre-enriched and non-enriched samples were inoculated on m-Enterococcus agar with and without antibiotics (AB) (ampicillin 10µg/mL or vancomycin 6µg/mL) and incubated for 48h at 37ºC. Identification was performed by PCR (ddlEfc, aac(6')-IiEfm) and MALDI-TOF. AB susceptibility testing was performed by standard methods (CLSI). Clonal relationship was established by SmaI-PFGE, Multilocus sequence typing (MLST) and Bayesian Analysis of Population Structure (BAPS) clustering. **Results:** Enterococci were recovered from HOSP and AMB patients (78%/79%) of different ages (ranging 75%-83%). Similar colonization rates for Efc and Efm were found in this study (59%/54%). Efc colonization rates were similar among age groups, while Efm colonizes YP less frequently (41 %) than AD (68%) and EL (67%). Conversely, the recovery of other enterococcal species was more frequent in YP (33% YP, 7% AD, 15% EL). Efm isolates (n=150, 95 PFGE-types, 83 STs) grouped in BAPS group 2 (39%, mostly BAPS 2.1 -69%), group 3 (39%, mostly BAPS 3.3 - 66%) and group 1 (21%). BAPS 2.1 mainly comprised AREfm clinical isolates of the ST117 clone, nowadays widespread among Spanish hospitals. BAPS 3.3 comprises both HOSP and AMB isolates, ASEfm/AMB CC102 clone being predominant. Both ST117 and ST102 are known causes of bacteremia in HRyC. BAPS 1 was significantly associated with AMB and ASEfm isolates. Most Efc isolates (n=130, 73 PFGE, 58 STs/30 CCs) corresponded to CC16 (14%), CC40 (13%) and CC2 (12%). BAPS analysis revealed that all Efc isolates were clustered into three phylogenomic groups, arbitrarily designated as groups 1, 2 and 3, which represent 37%, 40% and 23% of the analyzed Efc. Isolates of these groups were equally distributed among HOSP and AMB. Group 1, include isolates identical to the globally spread Mid-Atlantic ST6 clone (n=16), including all our VRE isolates (n=5). Group 3 includes two predominant PFGE-types belonging to CC16, mostly associated with AMB isolates. **Conclusions:** Differences in colonization rates by different enterococci reflect the possibility of “commensal epidemics” of successful Efm and Efc clones. The success in colonization might influence further evolution of enterococci towards pathogenicity and MDR.

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**30B DETECTION AND IDENTIFICATION OF THE ENTEROCOCCI BY CAPILLARY ELECTROPHORETIC TECHNIQUES**

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**Background:** Lactic acid bacteria including enterococci represent a major part of the commensal microbial flora of the human gastrointestinal tract and are frequently used as probiotics and in the dairy industry to produce fermented milk products. Detection and quantification of these microorganisms in foods is an important step in the quality control of these products. Unfortunately, the most common used detection and identification techniques based on cultivation are time-consuming. The new diagnostic techniques applicable to this purpose, e.g. PCR and matrix assisted laser desorption/ionization (MALDI) mass spectrometry, are rather expensive and relatively laborious. So, new methods and procedures are necessary to search for this purpose. **Methods:** In this work, we suggest Capillary electrophoretic techniques (CE) for the separation and
Identification of enterococci. Probiotic enterococci (E. durans, E. faecalis, E. faecium) were examined by Capillary Zone Electrophoresis (CZE) with UV detection and by Capillary Isoelectric Focusing (CIEF) in the pH gradient range of 2.0-4.0 and. The pH gradient in CIEF was traced by the low-molecular-weight pI markers. The isoelectric points of examined bacteria were calculated via the comparison of their migration times with the migration times of pI markers. **Results:** All examined enterococci were focused reproducibly according to their surface characteristic, particularly according to their isoelectric points (pI), in distinct zones. Isoelectric point of tested E. durans strain was found as 2.8. The pIs for two other enterococci were found below 2. Under this pH value we cannot ensure linearity of pH gradient. So the value of *E. faecalis* pI was in the rage 1.8-2.0 and *E. faecium* focused in the rage 1.7-1.8 of pH gradient. Tested strains were separated also from cow’s milk spiked with these bacteria. The minimum detectable number of cells was 2 10^6 in one millilitre. Moreover, the quantification of bacteria is possible according to the peak area in the electrophoreogram. **Conclusions:** The capillary electromigration techniques seem to be suitable for the detection and separation of the probiotic species. This work was supported by the grant VG20112015021 and MSMT INGO LG13011.

**31A**

**EFFECT OF PREBIOTIC ON ADHERENCE ABILITIES OF ENTEROCOCCUS STRAINS**

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Adherence ability is crucial feature which allows bacteria to survive in various abiotic or biotic environment. Enterococci belong to the lactic acid bacteria (LAB) and have been used for many years as probiotic for humans or animals, often in combination with prebiotic.

One of the probiotic criteria is good adherence to intestinal epithelial cells. We have previously shown that effect of prebiotic is strictly strain-individual. We have tested influence of commercially available prebiotics (Orafti GR, Orafti P95, Orafti Synergy and Vivinal) and three simple saccharides (glucose, galactose and lactose) on the adherence abilities of *Enterococcus* durans CCDM 922, *Enterococcus* durans CCDM 500T and *Enterococcus faecium* CCDM 945. Adherence of bacteria after mixing with prebiotic was tested in the microtiter plate coated with co-culture of the cell lines Caco2 and HT-29-MXT (1:9). Non-adherent bacteria were washed out by double rinsing, sonification was used to remove remaining bacteria from cell tissue and adherence was evaluated by measurement of colony forming units (CFU). Under conditions of this study by *Enterococcus* durans CCDM 500T after addition of the prebiotic P95 or Vivinal, increase of adherence abilities was shown, but effect of other probiotic and combination of remaining strains with prebiotic was negative. this work was support by MSMT grant LG13011 and MZe ČR - QI 91B274

**32B**

**FINDINGS ON SELF-REPORT ADVERSE DRUG REACTIONS RELATED WITH THE USE OF SYSTEMIC BETA-LACTAMS BY A PHARMACOVIGILANCE PROGRAM**

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Pharmacovigilance (PV) is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem. Despite surveillance efforts, unexpected and serious adverse drug reactions (ADRs) repeatedly occur. This surveillance depends on the self-report that lack basic information of critical aspects of quality and under report. The aim of this work has the intention to show a description of the reports of the ADRs related
to the use of systemic antibiotics in terms of quality of the information reported and the type of ADRs reported to the producer. As a part of PV national program leaded by the National Regulatory Agency INVIMA the health care professionals are committed to report any case of ADRs to INVIMA through a self-report format or at INVIMA web-site and the pharmaceutical producer. The PV program receives these reports and analyzes each case and provides a conclusion based on the information for each case. Between October 2011 - October 2013 twenty nine reports from six cities were submitted and classified by type of ADRs 22 reports (75.8%) unexpected failure of therapy, 6 reports (20.7%) Non dose-related ARDs and 1 (3.5%) of these present a serious anaphylactic reaction which requires ICU admission and ventilator support with non-fatal resolution in a patient with a history of penicillin allergy treated with cephalozolin. The unexpected failure of therapy 15 reports lack crucial information for analysis (time of use, diagnosis, number of doses, other medicines used, reports of cultures and antibiograms); based on the information we conclude wrong use were detected in 7 reports (2 cases of MRSA bacteremia treated with meropenem, 1 case of H1N1 pneumonia treated with piperacillin tazobactam, 1 case of S. maltophilia and 1 case of B. cepaciae with decrease susceptibility treated meropenem and 2 cases treated with meropenem caused by carbapenemases strains producers. The self-report information is biased by under-report practice; here we show the wrong use of antibiotics is self-reported (31.8%) when the clinician suspect a failure instead that a wrong use of antibiotic. The rationale use of antimicrobials and effective surveillance programs should contribute to reduce the misuse of antimicrobials and should pursues stops the negative deep impact of antimicrobial resistance, as a standard goal of the national health system, that urges for improve equity in the quality of health services.

**33A**

**POPULATION STRUCTURE OF ENTEROCOCCUS FAECALIS FROM WILD AND MIGRATORY BIRDS**

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**Objectives:** Enterococcus faecalis (Efc) is a gut commensal of mammals, reptiles, insects and birds. Efc clonal structure and extrachromosomal elements in non-mammal strains remains scarcely studied despite its contribution to the transmission of antibiotic resistance (AbR) genes and the nutritional upgrading in different animals. This study highlights the diversity of Efc from the gut of wild and migratory birds. **Methods:** Efc isolates (n=100) from wild birds (orders Acipitriformes, Paseriformes, Falconiforme, Strigiformes, Gruiformes, Ciconiiformes) attended at two Spanish Veterinarian Centers of Nature Conservation were studied for clonal relatedness (PFGE, MLST), and antibiotic susceptibility (CLSI). The presence of antibiotic and virulence genes, conjugative transposons (CTns) and plasmids was inferred by PCR, hybridization and sequencing. Plasmid analysis included identification of replication initiation proteins (Rep); relaxases (Rel); and toxin-antitoxin systems, (TA). CTns were screened for integrases, excisionases and relaxases. **Results:** Eight major PFGE clusters (75% similarity) comprising isolates with diverse PFGE (19 main subgroups of 2-5 strains each, 85-95% similarity) and MLST types (most STs being singletons firstly described here, but also STs of CCs detected in the gut of mammals as CC58, ST245). Strains were resistant to tetracycline (67%), chloramphenicol (42%) erythromycin (28%) and high levels
of streptomycin (26%), kanamycin (19%) and gentamicin (5%). Most Efc contain 0-3 plasmids per cell (3-90kb) belonging to RepA_N (pheromone plasmids containing rep9, 73%; rep9ApAD1-like, 23%; rep9BpBEE99-like, 30%), Inc18 (rep1pRE25, 12%; rep2pVEF1, 5%), Rep_trans-RCR (rep7pS194-like, 44%; rep17pRUM, 16% and rep14/ orf1pEFNP1, 5%) and Rep3_small theta (rep18pEF418, 5%; repCIZ2, 5%; rep6pAMα1/pS86, 33%) families. Only MOBC (Orf57pAD1, 44%) and MOBP (PcfGpCF10/pBEE99, 56%) relaxases were detected; 44% of the strains contained both MOBC and MOBP. TA systems were fstpAD1 (23%) and ε-ζInc18(7%). All but pheromone-related plasmid sequences were highly homologous (>99%) to those from mammals. CTn916-like elements (Tn916, Tn5801, Tn5397, Tn6000, Tn917) were detected. Virulence genes gel, asa, esp, and cyl were present in 100%, 84%, 61%, and 35% of the isolates respectively. Conclusions: Clonal diversity among Efc from the gut of wild and migratory birds reflects high levels of recombination, which might influence the host-range. While the ubiquitous presence and diversity of pheromone plasmids of Efc from mammals, farm animals and birds reflects that these plasmids co-evolved with Efc populations of different hosts, the presence of RCR, small tetha and Inc18 plasmids identical to other reported among Staphylococci, Macroccoci, Lactobacilli and Enterococcus faecium indicates frequent transfer events among Firmicutes of animals and humans.

34B

ACTIVE SURVEILLANCE OF VRE IN PREOPERATIVE SURGERY PATIENTS AND TO FORMULATE A MANAGEMENT POLICY TO PREVENT NOSOCOMIAL INFECTION.

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Introduction: Healthcare associated infections (HAI) remain a major cause of mortality and morbidity. Indiscriminate use of Antimicrobials; prolonged hospital stay; severe Immunosuppressive state and malignancy are found to be the important risk factors for the emergence of these infections. One of the important risk factors in the VRE infection is extensive use of 3rd generation cephalosporin which should be used only when indicated and not otherwise. Due to prolonged hospital stay, additional diagnostic tests and treatment adds an excess healthcare cost to the healthcare system and patients’ family. Active surveillance for VRE will facilitate their early detection and thereby restrict the transmission of infection to other patients. Based on the surveillance data, the healthcare organization can formulate an appropriate infection prevention and management strategies. Results: 1. 50 preoperative patients from the General Surgery dept. were studied. Among these patients the maximum number of cases were in the age group >60yrs (42%). Out of which 85.71% were males and 14.29% were females. The minimum individuals were in the age group <20 yrs (4%) who were exclusively males. 2. Prevalence of VRE was 14% in preoperative patients of General surgery. 3. Prevalence of VRE (14%) was higher in the age group of >40yrs (10%) than in age group of <40yrs (4%). 4. Prevalence of VRE was higher in males with a prevalence of 14%. 5. Prevalence of VRE among cancerous patients was 4%. Cancerous patients were having 2 times more risk of developing VRE as compared to non-cancerous patients. Conclusion: The infections by VRE are difficult to treat and are associated with increased mortality. Of all the available antimicrobials agents, very few drugs such as Linezolid and Teicoplanin are the most active and reliable treatment options for the infections caused by VRE. In order to reduce the transmission of these multidrug resistant organisms from one patient to another, the active screening of VRE should be done in every preoperative patient and proper hospital infection control interventions should be implemented and followed routinely. Implications: 1. The results of this
study will be helpful in training the health care professionals regarding the appropriate use of antibiotics in the preoperative patients. 2. This study will also serve as a base for the larger community based trials so as to overcome the scarcity of data in this field. 3. In the long run this study will help to curtail the morbidity and mortality VRE and thus helps to reduce the financial burden on Healthcare system and patients’ family.

35A

DIVERSITY AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ENTEROCOCCI ISOLATED FROM FECAL SAMPLES OF FUR SEAL (OTARIIDAE: ARCTOCEPHALUS SPP.) FROM THE NORTH COAST OF RIO GRANDE DO SUL, BRAZIL.

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Enterococcus species are commensally the gastrointestinal tract of mammals, birds, insects and reptiles and, in the last 70 years, this microorganism has showed remarkable ability in acquiring and expressing genes of microbial resistance. The aim of this study is to evaluate the diversity and antibiotic susceptibility of enterococcal isolated from fecal samples obtained from fur seals (OTARIIDAE: Arctocephalus spp.) from the north coast of Rio Grande do Sul, Brazil. Samples were obtained from 10 fur seals and 1 gram of each fecal sample was inoculated in 10 mL of saline solution 0.85% (35°C, 18h, 100 rpm). Then, 1 mL was inoculated in 9 mL of Broth Azide media (35°C, 18h, 100 rpm) and, afterwards, 100 µL was inoculated on agar plates with Brain Heart Infusion supplemented with 6.5% NaCl and incubated as described above. Phenotypic criteria such as size/volume, shape, color, Gram staining, catalase production, and esculin hydrolysis tests were used to separate the enterococci and the non-enterococcal strains. DNA was extracted by boiling method, and all isolates were submitted to polymerase chain reaction (PCR) using genus-specific primer of tuf gene. The multiplex-PCR was used to identify the E. faecalis, E. faecium, E. casseliflavus and E. gallinarum species. In case of a negative multiplex PCR result, standard biochemical testing for species identification was performed. Antimicrobial susceptibility was determined using the disk diffusion, according to the recommendation of NCCLS. Ten antibiotics commonly used in the treatment of clinical infection and agricultural procedures were tested (concentrations are expressed in µg mL-1): ampicillin (10), vancomycin (30), erythromycin (15), tetracycline (30), ciprofloxacin (5), norfloxacin (10), nitrofurantoin (300), cloramphenicol (30), gentamycin (120) and streptomycin (300). One hundred and sixty samples (80%) from a total of 200 tested were enterococci. The most predominant species identified were E. faecalis (50.6%), E. hirae (18.75%), E. casseliflavus (11.3%), E. gallinarum (3.12%), E. asini (2.5%), and E. faecium (0.6%). Twenty-one strains could not be identified as specie and were classified as Enterococcus spp. No resistant strains to ampicillin, vancomycin, cloramphenicol, gentamycin and streptomycin were found. The most frequent resistance properties were to erythromycin (20.62%), nitrofurantoin (15.62%), tetracycline (6.25%), norfloxacin (3.12%) and ciprofloxacin (2.5%). The sensitivity of the strains to almost all antibiotics tested could possibly be explained by a lack of contact of these animals with antibiotics. However, the presence of resistant strains could be associated with food chain or with the environmental resistome. Further studies are being addressed to determine the presence of virulence and resistant genes and also the relatedness among enterococci isolated from other sources.
THE PATHOLOGY OF ANTIBIOTIC-INDUCED INTESTINAL OUTGROWTH BY MULTI-DRUG RESISTANT ENTEROCOCCUS FAECIUM

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Background. Enterococcus faecium was considered a commensal of the microbiota within the large intestine of the human gastrointestinal tract, but is now recognized as a multi-drug resistant (MDR) pathogen. In hospitals, patients are typically administered antibiotics that perturb and reduce the intestinal commensal microbiota, leading to outgrowth of MDR-E. faecium, subsequent spread, infections, and hospital outbreaks. However, intestinal ‘blooming’ by MDR-E. faecium has not been thoroughly studied. Therefore, the objective was to analyze the pathology of antibiotic-induced outgrowth of MDR-E. faecium within the large intestine.

Methods. Four BALB/c mice were treated for 2 days with ceftriaxon prior inoculation with 1x10^10 CFU’s of MDR-E. faecium E1162 (Group A), and left on cefoxitin antibiotics during the experiment. Group B is similar as Group A, but animals did not receive antibiotics. Three mice were left untreated (Group C). Colonization of E1162 was monitored by enumeration of CFU’s from faeces. After 10 days intestinal parts were: (1) formalin fixed, paraffine embedded (FFPE), thin sectioned and subjected to Gram-, Hematoxylin and Eosin- (H&E), and Periodic acid-Schiff (PAS)-staining followed by light microscopy (LM); and (2) fixed in 2% glutaraldehyde, serially dehydrated, and analyzed by scanning electron microscopy (SEM).

Results. Enumerating CFU’s demonstrated that Group A mice were highly colonized with E1162 (10x10^10 CFU/gram faeces), while Group B mice were low-level colonized (1x10^6 CFU/gram faeces), and Group C revealed no enterococci. SEM on cecum and colon tissue confirmed the presence of only cocci on the epithelial cells (Group A) in high levels, and Group B +C revealed dense microbiota consisting of various rod-shaped bacteria, cocci and spirochetes. In addition, LM on H&E-, Gram- and PAS-stained FFPE cecum and colon sections of Group A mice showed absence of microbiota and enrichment of enterococci aligned onto the thin mucus layer and surrounding the undigested faecal material. In the colon, enterococci were predominantly present in areas of mucus secreting Goblet cells and at the apical side of columnar epithelium. Group B + C mice had a very diverse Gram-negative and -positive microbiota separated by a thick mucus layer from the epithelium. Conclusion. Antibiotics reduce the intestinal microbiota and mucus layer and allow MDR-E. faecium to bloom in the gut. The finding that enterococci predominate at the apical side of the epithelium and surround faeces likely enables the bacteria to interact with host receptors and to spread after defecation.

ISOLATION OF A NOVEL ENTEROCOCCUS SPECIES FOUND IN THE FECAL MATERIAL OF THE ENDANGERED TIMBER RATTLESNAKE

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The Timber Rattlesnake is a venomous species of pit viper and was listed as a threatened species in the state of Minnesota in 1996. To better understand disease transmission between snakes and humans, knowledge of microorganisms associated with these reptiles is essential. Enterococcus species are found in a wide range of environments, such as in food, water, soils and the gastrointestinal tract of humans and animals. To characterize bacteria present in the fecal material of the snake, a culture dependent approach was used. Bacteria were
inoculated onto a blood agar plate containing 10 µg/ml rifampicin and incubated aerobically at 37°C for 48 h. Small, convex, circular and off-white colonies were visualized. A pure culture was obtained and to identify the bacterium the 16 rRNA gene was sequenced. Pairwise sequence comparisons to sequences deposited in the GenBank nucleotide database using the BLAST algorithm indicated that the sequence generated showed 99.7% identity to *E. silesiacus* and *E. caceae* 16S rRNA gene, partial sequences. A draft genome was then constructed and an approximate 1300-bp portion of the RNA polymerase beta subunit (*rpoB*) gene was used for comparison. A 96.8% and 96.0% identity to *E. caceae* and *E. silesiacus*, respectively was shown. In conclusion, using the greater than 97% criterion as indicative of species-level relatedness, the *Enterococcus* species isolated in this study is novel.

**38B**

**ENTEROCOCCUS FAECALIS ISOLATED FROM PIG FECES: ANTIMICROBIAL RESISTANCE WITH PUBLIC HEALTH IMPACT**

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Intensive breeding of animals, e.g. pigs, represents a large reservoir of antimicrobial resistant-bacteria. Use of antimicrobials in feed as growth promoters in a prophylactic and therapeutic fashion is a regular practice. Widespread selection of antimicrobial resistant bacteria in animals’ gut facilitates spread of multi-resistant strains to humans through environment and food chain. Multiple antimicrobial resistance among enterococci is an important Public Health issue. One of the main reasons for the rapid increase of resistance in enterococci is based on their ability to acquire and spread determinants of antimicrobial resistance. *Enterococcus faecalis* can cause invasive infections with high morbidity and mortality in immunocompromised patients and / or with associated co-morbidity factors. The aim of this research was to investigate antimicrobial resistance with Public Health impact in *E. faecalis* isolated from pig feces. Random sampling from pigs of different categories was done. During three months (March-May, 2013), *E. faecalis* isolates were recovered from fresh pig fecal samples from two intensive breeding system farms at South-East region of Buenos Aires Province (Argentina); with a wide range use of antimicrobials (gentamicin, amoxicillin, ceftiofur and tetracycline). Fecal samples (ca. 20 g in a plastic vial) were enriched in buffer peptone water (1/10) for 16 h at 35°C and 0.1 mL aliquot was inoculated onto Bile Esculin Azide Agar supplemented with 125 mg/L, gentamicin; 1,000 mg/L, streptomycin; 6 mg/L, vancomycin or 16 mg/L, ampicillin. For genus and specie identification the *tuf* and sodA genes, respectively, were amplified by PCR. In isolates recovered from selective media, Minimum Inhibitory Concentration (MIC) was performed by broth dilution method following CLSI specifications (2013). Detection of genes conferring resistance to vancomycin (vanA, vanB) and aminoglycosides (aac (6’)-Ie-aph (2’’)-Ia, aph (2’’)-Ib, aph (2’’)-Ic, aph (2’’)-Id) was done by gene amplification (PCR). The following resistant *E. faecalis* isolates were recovered in different selective media: gentamicin, 10 (MIC= 1,000-2,000 mg/L); ampicillin, 2 (MIC= 16 mg/L); vancomycin, 1 (MIC= 512 mg/L). *E. faecalis* isolates were not recovered from streptomycin selective agar. vanA gene was detected in one isolate and aac (6’)-Ie-aph (2’’)-Ia in all isolates with high-level gentamicin resistance. One isolate was resistant to ampicillin and gentamicin. Pig feces have a potential role in the transmission of *E. faecalis* with antimicrobial resistance with Public Health impact. Emergence of resistance to antimicrobials of broad clinical utilization implies the need for continued surveillance in pig farms and designing politics of rational use of antimicrobials in these breeding systems.
Iron, an essential nutrient for microbial growth, plays a key role in biofilm development. The correlation between bacterial iron acquisition efficiency and virulence has been well established, with systems that mediate this process ranging from siderophore and heme to free-iron uptake. Bacterial infection can develop to a chronic stage through the formation of biofilm. These are interactive microbial communities attached to surfaces, which promote bacterial resistance to host immune responses and antimicrobial chemotherapy. They represent a significant proportion of nosocomial infections, an issue compounded by the continuing emergence of antimicrobial resistant pathogens. Mutualism and competition with respect to iron acquisition is likely to be a key biofilm determinant. Iron acquisition systems are therefore an attractive antimicrobial target, which would act to prevent biofilm formation (virulence) and growth. This study evaluates biofilm-associated community-mediated mechanisms for Uropathogenic Escherichia coli (UPEC) UTI89 survival under iron limitation through interaction with Enterococcus faecalis OG1RF. Together these species represent the most frequent causes of urinary tract infection and are often co-isolated from infected individuals. Using biofilm models, we have examined the influence of iron limitation on monomicrobial versus polymicrobial populations. We show that while UPEC survival is strongly influenced by the levels of iron, E. faecalis is highly tolerant to iron limitation. Intriguingly, in polymicrobial populations where E. faecalis is present, UPEC has a greater capacity to survive iron limitation than in monomicrobial macrocolonies. UPEC survival is not affected during planktonic growth indicating that the polymicrobial-mediated iron dependent growth effects are biofilm dependent.
Poster Abstracts

Effect. Additionally, there was a difference of 1-log in the net bactericidal effect at the maximal dose studied between both models (-4.38 ± 0.10 vs. -3.04 ± 0.14 log CFU/g for optimized and standard models, respectively) corresponding to a fAUC/MIC of 62. For bacteriostasis, a two-fold higher exposure to MOX was required in the optimized versus standard model (fAUC/MIC = 40 vs. 20, respectively).

Conclusions. Compared with other bacteria like pneumococci, higher fAUC/MIC against enterococci are required with MOX for both endpoints (bacteriostasis and killing). Stasis would be clinically achievable with strains with MIC up to 0.5 mg/L. Additional data will be presented at the meeting.

41A

MATRIX ASSISTED LASER DESORPTION/IONIZATION TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS) AS A TOOL TO CHARACTERIZE ENTEROCOCCAL SPECIES

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Enterococci are important nosocomial pathogens. Accurate identification at the species level is important for appropriate antimicrobial therapy and effective surveillance. Enterococcus faecalis and Enterococcus faecium are usually the predominant species isolated from clinical sources, although other enterococcal species have increasingly been reported. However, identification of less commonly found enterococcal species by routine standard methods is not always reliable. In the present study, we evaluated the ability of MALDI-TOF MS to identify 135 enterococcal isolates, including reference strains and isolates from human clinical sources. The isolates were identified by conventional biochemical testing and by one or more molecular tests (whole-cell protein profiling after SDS-PAGE, species-specific PCR and 16S rRNA gene sequencing), and included Enterococcus avium, Enterococcus casseliflavus, Enterococcus durans, E. faecalis, E. faecium, Enterococcus gallinarum, Enterococcus hirae and Enterococcus raffinosus. For MALDI-TOF analysis, fresh colonies were both directly applied to the target plate and submitted to an extraction step using formic acid with or without acetonitrile before covering with CHCA matrix solution. Measurements were performed with a Microflex LT mass spectrometer (MALDI Biotyper, Bruker Daltonics), using a dedicated software, whose database contains 70 reference spectra comprising 34 different enterococcal species. All tested isolates were unambiguously identified to the species level, with scores >2.0, even for difficult to distinguish strains presenting atypical phenotypic characteristics. Scores ranged from 2.112 (E. durans) to 2.577 (E. faecalis) for reference strains, and from 2.003 (E. raffinosus) to 2.574 (E. faecalis) for clinical isolates. The lowest scores were observed for E. durans and E. raffinosus, which are underrepresented in the Bruker database, having respectively only 1 and 2 reference spectra deposited. Only E. faecalis isolates generated significantly higher scores (p=0.0083) when an extraction step was performed. In addition, analysis of spectra with the BioNumerics software, using a reference strain of Vagococcus fluvialis as outgroup, clustered the enterococcal isolates according to the species. Even more, unique peaks in the range of 3000 to 10000 Da (m/z) could be assigned with statistical significance (p<0.05) to each species evaluated, except for E. gallinarum, that shared one of its most significant peaks (6313.93 Da) with E. casseliflavus. Our results indicate that MALDI-TOF MS is an accurate method to identify enterococcal isolates recovered from human sources, highlighting its usefulness for rapid and cost-effectiveness routine application in clinical laboratories.

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ASM Conferences
**42B**

**IDENTIFICATION OF THE ENTEROCOCCUS FAECALIS SOS REGULON**

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**Introduction:** The SOS response is a cellular mechanism which permits the survival of bacteria to DNA damages. Briefly, two proteins are involved: RecA is activated by single-stranded DNA (product of DNA damage) and this active form favoured the autoproteolysis of a second protein, LexA, which is a transcriptional repressor of several genes involved in DNA repair and other survival processes. These genes, derepressed by the induction of the SOS response, form the SOS regulon. In *Enterococcus faecalis*, second cause of nosocomial infections after *Staphylococcus aureus*, the SOS mechanism has not been studied to date. To characterize the *E. faecalis* SOS regulon, a *lexA* mutant, leading to a constitutive-induced SOS response, was constructed. In silico analysis and microarray experiments permitted to identify 54 genes taking part of the *E. faecalis* SOS regulon.

**43A**

**POPULATION DYNAMICS OF ENTEROCOCCI IN A METROPOLITAN RIVER AFTER AN EXTREME FLOOD EVENT**

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Enterococci are commonly found in the gut microflora of many mammals and birds, and as such they serve as a predictor of faecal contamination in polluted surface waters. However, enterococci are also used as an indicator of pathogen loading and/or risk assessment monitoring strategies. Although largely commensal they are also opportunistic pathogens and are known to cause infections in human and animals. Their numbers spike significantly during wet weather events and potentially pose serious public health risks, however to our knowledge there have been limited studies that have assessed their presence and persistence after such an event, and not to the extent of this study. The aim of this study was to investigate the temporal and spatial enterococci population changes after an extreme weather event in south each Queensland, Australia between December 2010 and January 2011. This resulted in a state wide flood and overflow of several sewage treatment plants (STPs) along the Brisbane River and inundation of part of the Brisbane downtown area. The lower Brisbane River Catchment is a highly modified and urbanised catchment that supports > 1.8 million inhabitants. Water samples were collected from 24 sites along this system at four, six and eight weeks after the flooding event. Enterococci were enumerated and isolated from all water samples, identified to the spices level and typed using a high resolution biochemical fingerprinting method (Phene-Plate-RF, AB, Stockholm, Sweden). Strains with similarity above the identity level of the system were regarded as having identical biochemical fingerprints and assigned to the same biochemical phenotypes (BPTs). BPTs with more than one isolate were termed common (C) and those with a single isolate were termed single (S) BPTs. Twenty-three C-BPTs were found across all samples, and representative strains from each type were further assessed for their pathogenic properties. Results indicated significantly (*P* >0.01) higher numbers during the first round of sampling when compared to subsequent rounds suggesting a die off within the species. However certain C-BPTs were found to persist in the river in all sampling rounds. These C-BPTs normally possessed similar virulence gene (VG) and antibiotic resistance profiles when compared across the sampling rounds. Conclusion: Our data suggest that certain clonal groups of enterococci can survive in water after major floods. These strains normally carry several VGs and can be
44B

CHARACTERIZATION OF THE ADAPTIVE IMMUNE RESPONSE TO E. FAECIUM

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Background: Enterococcus faecium (E. faecium), one of the main causative agents of nosocomial infections, possesses several factors associated with membrane and cell wall (e.g. glycoconjugates, capsular polysaccharide) that protect it against phagocytosis. These factors can be used as targets for the development of an alternative treatment through monoclonal antibodies (MAbs) in order to reduce the frequency of invasive infections. Objective: Generate and characterize monoclonal antibodies against E. faecium E155. Methodology: An inoculum of membrane and cell wall of E. faecium E155 was used to immunize a group of female BALB/c mice. The mouse with the highest antibody titer was selected for MAb clones generation; their lymphocytes were removed and fused with myeloma cells. The antibodies with an enhanced reactivity against the strain were selected for characterization by opsonophagocytic assay and carbohydrate structures recognition assessed. Results: The MAbs generated promote bacterial kill of E. faecium E155 by opsonophagocytosis, also were capable of recognition of polysaccharide structures associated to cell wall in E. faecium E155 and cross-reactivity was shown against other enterococci. Conclusions: We have MAbs against E. faecium E155 that promote opsonophagocytosis of the bacteria and recognize polysaccharide structures in cell wall of different enterococci.

45A

THE GENETIC CONTEXT OF VAN A IN ENTEROCOCCAL ISOLATES FROM AUSTRALIA

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Since the emergence of vancomycin-resistant enterococci (VRE) in Australia in the early 1990s, VRE have placed an increased burden on the national healthcare system. In North America and Europe, high-level vancomycin resistance has been associated with Tn1546 (and its structural variants) harbouring the vanA determinant. However, in Australia where the vanB determinant has been predominant, vanA-positive E. faecium isolates have recently emerged in hospitals nationwide. To genetically characterise these emerging isolates, whole genome sequencing (WGS) was performed on a selection of vanA-positive E. faecium, isolated from hospitals in four Australian capital cities, using the Ion Torrent Personal Genome Machine (Life Technologies, Carlsbad CA). Assembly of WGS reads revealed diverse structural variants of Tn1546 mediated by different insertion sequences interspersed within the transposon. Despite the different Tn1546 structural variants having similar neighbouring genetic regions, they were further located on different plasmid backbones indicating that homologous recombination has played an important role in the diversity of the genetic context observed. In silico multi-locus sequence typing also identified a wide range of sequence types. The diversity of genetic context and background of vanA-positive E. faecium suggests that the emergence of vanA in Australia is not likely
the result of the dissemination of a ‘dominant’ sequence type or vanA-harbouering plasmid, but was rather introduced multiple times over recent years.

### 46B

**ANALYSIS OF HIGHLY CONJUGATIVE PMG1-LIKE PLASMIDS**

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pMG1 (65.1 kbp) is a highly conjugative, pheromone-independent plasmid isolated from an *E. faecium* clinical strain obtained in our university hospital (Maebashi, Gunma). pMG1 carries a Tn4001-like transposon encoding gentamicin-resistance and transfers at a high frequency around 10⁻³ to 10⁻⁴ per donor cell during broth mating in both *E. faecium* and *E. faecalis*. The transfer is not induced by the sex phenornones of plasmid-free *E. faecalis*. During the mating, recipient cells and donor cells harboring pMG1 form mating aggregates, which can he observed by microscopy. In Southern hybridization analysis, pMG1 does not hybridize to other conjugative plasmids in Gram-positive bacteria including pheromone-responsive plasmids, indicating that pMG1 has a unique conjugation system. A transfer-related gene, traA is identified as whose transcript was up-regulated during the mating. The traA gene product associated with the formation or stabilization of mating aggregates during broth mating. The complete nucleotide sequence (65,029 bp) of the pMG1 plasmid revealed 73 ORFs lying in the same transcription orientation. Of 73 ORFs, 22 ORFs showed homology with ORFs present on the pXO2 plasmid (96.2 kb), which is the virulence plasmid essential for capsular formation by *Bacillus anthracis*. Analyses of the transposon insertion mutants and transcripts indicated that ORFs 15-49, lying in the 31.7 kb region were related transfer. A 5.9-kb HindIII fragment that replicates autonomously in *E. faecalis* was cloned and analysis of this fragment by deletion and in vitro insertion mutations showed that ORF10 (rep) and the inverted repeat sequence in the noncoding region between ORF8 and ORF9 were necessary for pMG1 replication. VanA-type vancomycin resistance pMG1-like highly conjugative pHT plasmids (α, β, and γ) were isolated from the outbreak strains of VanA-type vancomycin-resistant *E. faecium* and *E. avium* in a Japanese hospital. The complete DNA sequences of pHTβ plasmid showed a high degree of similarity to pMG1 plasmid. A functional oriT region and a deduced nickase gene traI of pHT plasmid were identified. The determinants of the mating-aggregates were also identified and composed of five ORFs. A transfer-related gene traB which positively regulates the expression of the genes including mating-aggregation and plasmid-transfer was identified in pHTβ. Recently, we identified two key regulatory genes designated as traD and traF. traD was crucial for plasmid transfer and traF was the transcriptional negative regulator for traD, respectively. The pMG1-like plasmids were found in the vancomycin-resistant *E. faecium* outbreak strains isolated from Michigan (United State), Taiwan and Spain, respectively. The basic structures of these pMG1-like plasmids were almost identical and all of the ORFs of pMG1 were conserved in the plasmids except for the mobile elements including transposons, ISs, and group II introns.

### 47A

**FITNESS COST OF PLASMIDS CARRYING TN1546-VANA IN DIVERSE ENTEROCOCCUS FAECIUM CLONAL CONTEXTS**

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**Objectives:** Vancomycin resistance [Tn1546 (vanA)] among enterococci has increasingly been reported worldwide since its 1st description, being endemic in many areas. Tn1546 is
associated with a specialized Enterococcus faecium (EfM) population designated clade “A”, overrepresented by clinical isolates enriched in plasmids (Pl) of families RepA_N and Inc18. This study aims to determine the intrinsic fitness of a collection of EfM susceptible and resistant clones, and the fitness cost imposed by the acquisition of Pl carrying different Tn1546 variants. Material and Methods: Sixty-five isolates of EfM major clades “A” (27 STs) and “B” (5 STs), associated with populations of hospital and community isolates (Lebreton et al, mBio2013), respectively, were studied. Globally spread Tn1546 mosaic Pl containing replication modules (rep) of families RepA_N (3 pRUM, 2 pLG1) and/or Inc18 (n=4) and/or pMG1 (n=1) were analyzed. Prototype pRUM and Inc18 (pRE25, pIP501) Pl were used as controls. Pl transferability and fitness cost were assessed using EfM (GE1, 64/3) and Efc (JH2-2, FA202, UV202) as receptor strains. Growth curves were performed using Bioscreen C and fitness cost of Tn1546 and Tn1546-Pl was determined by calculating Relative Growth Rates (RGR) in presence/absence of vancomycin. Pl stability was analyzed in evolved strains after serial passages (300 generations). Results: Isolates of group “B” presents a better RGR (using GE1 as reference strain) than group “A” (1.2540±0.1187 vs 1.0277±0.1626, average RGR=1.0482±0.1704). Significant differences in fitness were observed between AmpS and AmpR isolates, but not among VRE and VSE (AmpS/VanS 1.1616±0.1503; AmpS/VanR 1.1089±0.1207, AmpR/VanS 0.9443±0.1352, AmpR/VanR 0.9286±0.1002). While all mosaic Tn1546, Inc18 and pRUM prototype Pls were transferred at high frequencies into different EfM phylogenomic backgrounds, only mosaic Pl carrying replInc18 and Inc18 prototypes were transferred into Efc which occurred at low and high frequencies, respectively. Mosaic Tn1546 Pl and prototype Inc18 Pls slightly improve or reduce GE1 and 64/3 fitness (-2%-8%) but, pRUM lacking Tn1546 imposed a highest cost (up to 16%). Fitness cost of Tn1546 expression varies according with the Tn variant and clonal background (5%-49%). Stability of Tn1546 Pls in GE1 and 64/3 after 300 generation was verified in all cases, sometimes with loss of phenotypic resistance. In the same conditions, prototype pRUM was also highly stable but Inc18 plasmids were lost at variable rates (pRE25 up to 20%) and pIP501 (up to 95%). Conclusions: EfM of clade “B” (community isolates) exhibited a better relative fitness than those of “clade A” (hospital infections). The presence of multireplicons seems to increase host range. Tn1546 Pls have little to no fitness cost and are very stable in the EfM backgrounds analyzed what might explain the high dissemination of these plasmids worldwide.

SPREAD OF CTN5801 AMONG ENTEROCOCCAL SPECIES FROM DIFFERENT ORIGINS

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Objectives: A diversity of CTns encoding resistance to tetracycline has been detected among enterococci (CTn916, CTn6000, CTn5397). They display common synteny but differ in the integrase/excisionase sequences and the specificity for the insertion site as well as the host range. CTn5801 was originally described in Staphylococcus aureus Mu50, and have been sporadically identified in Clostridium perfringens (as CW459tet(M)) and Streptococcus agalactiae. We describe the presence and diversity of CTn5801 among tetracycline resistant (tetM) enterococci from different origins. Methods: Isolates from humans, animals and environmental samples collected in different countries (1996-2010) were searched for the presence of tetM-CTns. Detection of CTn5801 was inferred by iden-
tifying integrase-intCTn5801 and tetM genes using a PCR CTn typing scheme. Genomic location was established by ICeu-I-PFGE hybridization. Characterization of CTn5801 backbone was accomplished by long-PCR mapping based on known elements (GeneBank No. BA000017.4) and sequencing. Clonality of *E. faecalis* (Efc) and *E. faecium* (Efm) isolates was established by PFGE and MLST. Transferability was analyzed by filter mating assays. Sequences obtained were compared in silico with other elements in the available database.

**Results:** The intCTn5801 was detected among different clonal backgrounds of Efc (n=12, ST9-CC9, ST30-CC30, ST55-CC55, ST159, ST318, ST445) Efm (n=38; ST16, ST17, ST18, ST50, ST64, ST80, ST182 and ST202) and Enterococcus spp (n=7) of humans, animals and environmental samples in South and North America, Europe, Australia and Asia. We identified 9 CTn5801 variants (I-IX), seven detected in enterococci, three of them newly identified in isolates of this study. They were clustered in two main groups designated as "A" (25kb, types I-V) and “B” (20kb, types VI-IX), types within each group differing by indels and mutations in the three functional regions of CTn5801. Group A comprises Tn5801 variants of *S. aureus*, Clostridium, Efm and Efc including that annotated as Tn6086. The early isolate in this group corresponds to an Efm collected in the USA in 1992. Group B comprises variants of *E. faecalis* (2000) and *Streptococcus agalactiae* (2010). All Tn5801 variants were chromosomally located but they were transferred by conjugation using Efc JH2-2 as recipient. Transconjugants harboring an extra band of 49kb and 90kb were identified.

**Conclusions:** The presence of CTn5801 among enterococcal species of different geographical locations and hosts since 1992 suggests a successful dissemination of this element among different gene exchange communities. The diversity of CTn5801 highlights the plasticity of CTns and enterococcal genomes although the interplay of other elements in the transferability of CTn5801 remains to be established.

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**49A**

**IN VITRO EXPRESSION OF VIRULENCE FACTORS GENES IN CLINICAL VRE STRAIN UNDER VANCOMYCIN STRESS.**

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Glycopeptides antibiotics are effective against Gram-positive bacteria. Although, Vancomycin Resistant Enterococci (VRE) has emerged as important nosocomial pathogens worldwide. Studies have made possible the advanced of knowledge about the vancomycin resistance and practically nothing is known about the importance of van and other genes in VRE under stress conditions. In this study, we used the vancomycin to experimentally simulate the antibiotic stress conditions in order to evaluate the modulation of virulence-related genes expression in VRE isolate from Urinary Tract Infection (UTI), using a relative quantitative expression (qRT-PCR). Five VRE strains, named UTI-1854, UTI-1950, UTI-1953, UTI-2319 and UTI-2389, were confirmed by PCR with specie-specific primers for ddlE.faecalis gene. A DNA fingerprint of each isolate was generated by REP-PCR analysis to determine clonality of VRE strains. The 2xYT broth was supplemented with 10 different gradient of sterile urine (100, 50, 25, 15, 10, 8, 6, 4, 2 and 0%). The gene expression was evaluated on 2xYT broth supplemented with 10% of sterile urine (2xYT-U) in the presence (64 µg/ml) and absence of vancomycin. The total RNA was extracted using TRizol® method and it was previously treated with DNase I, RNase-free. Complementary DNA (cDNA) was synthesized by using random. The vancomycin resistance (vanA), maltose phosphorylase (bopA), beta-phosphoglucomutase (bopB), LacI family sugar-binding transcriptional aldose epimerase (bopC), regulator (bopD),
collagen adhesin protein (ace), OxyR family transcriptional regulator (oxyR), FUR family transcriptional regulator (fur) and aggregation substance (asc10) genes were analyzed. The qPCR was performed using EcoTM Real Time PCR System (Illumina®). The NormFinder analysis was used to estimate the stability of the 23S and tuf genes. Differential expression was calculated by the Pfaffl method and t-test was performed in Excel. REP-PCR analysis placed these 5 strains into a single clonal type. All strains were able to grow in urine concentration from 50-0%; however, the UTI-2389 strain showed a high mean OD value when compared with the other isolates. Based on these results, we selected the UTI-2389 strain and 10% of urine for qPCR assay. Among the genes studied, bopB, bopD, ace and oxyR genes showed a lower relative gene expression during the stress antimicrobial. On the other hand, we observed an increase in the transcript abundance of bopA, bopC, fur and asc10 genes. The significant increment of vanA expression detected in 2xYT-U supplemented with vancomycin (p ≤ 0.05), previously described as vancomycin-inducible, confirmed the up- and downregulation of all the selected genes. In conclusion, the vancomycin may alter the expression of virulence genes in VRE strains of E. faecalis and lead to worse clinical outcomes.

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COMPARISON OF VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECALIS ISOLATED 2 YEARS APART IN A BRAZILIAN HOSPITAL REVEALS THE EMERGENCE OF ST6 AND TIGECYCLINE-RESISTANT ST103

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Enterococci are Gram-positive cocci belonging to the intestinal tract of a variety of hosts, but have been proved to be important nosocomial pathogens and the emergence of vancomycin-resistant enterococci (VRE) significantly reduced the treatment options. The aim of this study was to identify and to compare a set of 43 vancomycin-resistant E. faecalis (VREfs) isolated from patients in 2009 and 2011 during a surveillance program in a hospital in Belo Horizonte, Brazil. The confirmation of vanA genotype and the detection of elrA, cylLL, esp and gelE genes was performed by PCR. Long-PCR was used for the characterization of Tn1546. The minimum inhibitory concentration (MIC) for vancomycin, linezolid, daptomycin and tigecycline was determined according to CLSI (2013). Typing was performed by PFGE and MLST. Finally, the families of plasmids present on the isolates were determined according to Jensen et al. (2010). The vanA genotype was found in all samples. Except for one, all isolates presented an intact Tn1546. Plasmids of rep9 family were detected in all VREfs. In 2009 all isolates were also positive for rep1 and some for rep2. The 14 VREfs from 2009 were ST103, belonged to pulsotype A and their prevalent virulence profile was elrA+gelE+. Only one presented cytolisin gene in addition. Eleven out 29 isolates from 2011 were ST103 with the same characteristics described for those of 2009, except for the fact that there was an emergence of tigecycline resistance in 2011, with 10 resistant isolates. One 2011 isolate showed also intermediate resistance to linezolid. The remaining 18 VREfs from 2011 were all ST6 belonging to pulsotypes B, C and D and were susceptible to tigecycline. In 2011, the prevalent virulence profile was cyl+esp+elrA+gelE+, and the 12 isolates with this profile belonged to pulsotypes B and C. Six 2011 isolates have profile cyl+elrA+gelE+ and are of pulsotypes B and D. All of them were susceptible to daptomycin and resistant to high levels of vancomycin, with MICs greater than 256 mg/L. It was concluded that there were changes in the profile of VREfs in this hospital over the two year period. The emergence of ST6, a multiresistant clone disseminated in hospitals in Europe and the United States, was observed in this hospital in 2011. In the same year, the emergence of resistance to tigecycline was observed in the ST103, which is of concern specially because
this was shortly after the hospital began using this drug. The Hospital Infection Control Committee was notified so that measures can be taken.

**51A**

**THE MOLECULAR EPIDEMIOLOGY OF ENTEROCOCCAL BACTERAEMIA IN AUSTRALIA**

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Enterococci are a major cause of healthcare-associated infections and globally account for approximately 10% of all bacteraemias. In 2011 29 institutions across Australia participated in the Australian Enterococcal Sepsis Outcome Programme (AESOP). From the 1st January to 31st December 2011 1,079 unique episodes of bacteraemia were investigated of which 95.8% were either *Enterococcus faecalis* (61.0%) or *E. faecium* (34.8%). The majority of bacteraemias were healthcare-associated and approximately one third polymicrobial. 90.4% of *E. faecium* were ampicillin resistant. Ampicillin resistance was not detected in *E. faecalis*. Vancomycin non-susceptibility was reported in 0.6% and 36.5% of *E. faecalis* and *E. faecium* respectively. Unlike Europe and USA where vancomycin resistance in *E. faecium* is predominately due to the acquisition of the vanA operon, 98.4% of *E. faecium* isolates harbouring van genes carried the vanB operon. 16.1% of the vanB *E. faecium* had vancomycin MICs at or below the CLSI susceptible breakpoint. Although molecular typing identified 126 *E. faecalis* pulsed-field gel electrophoresis pulsotypes over 50% belonged to two pulso-

types, which were isolated across Australia. *E. faecium* consisted of 73 pulsotypes from which 43 multilocus sequence types were identified. Almost 90% of *E. faecium* were identified as CC17 clones of which approximately half were characterised as ST203, which was isolated Australia wide. In conclusion the AESOP study has shown although polyclonal, enterococcal bacteraemias in Australia are frequently caused by ampicillin resistant vanB *E. faecium*. Further studies on the enterococcal genome will contribute to our understanding of the evolution of enterococci in the hospital environment.

**52B**

**VANCOMYCIN RESISTANT ENTEROCOCCI WITH VAN A GENE IN MUNICIPAL WASTEWATER TREATMENT PLANT EFFLUENTS IN BRNO, CZECH REPUBLIC**

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The emergence and spread of vancomycin resistant enterococci (VRE) have become a substantial clinical and epidemiological concern since they cause hospital outbreaks worldwide. The global occurrence of resistant bacteria in wastewater treatment plant (WWTP) is also an increasing concern. WWTPs are ideal locations for transfer of resistance genes and spread of the resistance because of frequent interaction of bacteria and good environmental conditions. In addition, the presence of antibiotics and their metabolites in sewage may promote selection of resistant strains and also horizontal transfer of antibiotic resistance genes. The samples were taken using the Moore swabs, which were exposed to the
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DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI (VRE) IN INPUT AND OUTPUT SAMPLES OF BIOGAS PLANTS

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The application of antibiotics in livestock husbandry led to an increasing prevalence of antibiotic resistant bacteria. Among those are potential human pathogens as Vancomycin resistant Enterococci (VRE). Beside the direct application in agriculture, manure is also used as substrate for biogas plants, but the resulting output material is then often spread on fields as well. Hence antibiotic resistant bacteria can be released via this path into the environment. The aim of our study was to investigate the abundance of VRE in input and output samples of biogas plants to investigate whether or not biogas plants act as a biotechnological barrier eliminating potentially pathogenic and antibiotic resistant bacteria such as VRE. In the first step of the study we used the commercially available CHROMagar™ VRE (Mast Diagnostica, Reinfeld, Germany) to determine the concentration of VRE by counting colony forming units (CFUs) of colonies represent potentially VRE (pink to mauve or blue colonies) after cultivation of the CHROMagar. CFU data indicated a decrease or even an elimination of potential VRE after the transfer through biogas plants. Spiking experiment of manure from cows not treated with antibiotics with 100 cells of vanA and vanB containing E. faecium and E. faecalis strains per g manure showed however a specific re-detection of those strains after cultivation on VRE CHROMagar. But, in the investigated biogas plant samples, growth of fungi and non-target bacteria identified belonging to the Caulobacteriaceae, Brucellaceae, Lactobacillaceae, Sphingobacteriaceae, Acetobacteraceae, Leuconostocaceae, and Flavobacteriaceae (partially showing the same colony colors as VRE) were observed, showing the restricted specificity of the CHROMagar medium when used directly for VRE detection by direct cultivation. A VRE specific pre-enrichment before cultivation on the CHROMagar enabled a higher detection efficiency of VRE from manure and output samples of biogas plant. The detected VRE containing either a vanA, vanB and/or vanC1 genes were cultured from input and output samples of biogas plants. The obtained isolated were phy-

WWTP effluents for 7 days. In total, we collected 37 wastewater samples between March and December 2012 on weekly basis. We also collected 284 samples of cloacal swabs from black-headed gull nestlings in the colony located about 30 km downstream from WWTP, to detect whether VRE strains can be spread by the effluents flowing into river water. Samples were cultured on Slanetz-Bartley agar with 32 mg/l vancomycin and incubated at 37°C for 48 hours. Individual colonies with enterococcal morphology were selected, identified and screened for vanA gene. The vanA-carrying isolates were tested for minimal inhibitory concentration of vancomycin, susceptibility to other antibiotics by disk diffusion method and occurrence of genes responsible for resistance and virulence genes by PCR. Pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) were used to examine the genotypic diversity of vanA-containing VRE. VRE with the vanA gene were found in 32 (86%) wastewater samples, among them we obtained 49 isolates of Enterococcus faecium (44), E. gallinarum (2), E. casseliflavus (2) and E. raffinosus (1) species. All E. faecium isolates were multi-resistant with resistance to five and more antibiotics, the resistances were encoded by vanA, ermB, tetM and tetL genes. A total of 35 (71%) isolates carried virulence genes hyl and/or esp. All samples from gulls were negative for VRE. Clinically important enterococci with the vanA gene occurred in the outflow from WWTP and could be a risk of the environmental contamination. Spreading of VRE to the gulls was not proved.
logenetically closely related to E. gallinarum, E. viikkiiensis E. lemanii, and E. casseliflavus with 16S rRNA gene sequence similarities of 99.3 to 100%, but E. faecium or E. faecalis were not detected. Our data indicate that the direct application of VRE CHROMagar for the detection of Enterococcaeae is problematic but the application of VRE specific pre-enrichment procedures can increase the detection efficiency of VRE. Vancomycin-resistant Enterococcus spp. could be detected in input and output samples, but E. faecium or E. faecalis were not found. Our data indicate that Vancomycin resistant Enterococcus spp. were not eliminated by the biogas plant process and thereby will be released into the environment with the application of biogas plant output material on fields.

54B

VANCOMYCIN RESISTANT ENTEROCOCCI IN CORVIDS IN EUROPE AND NORTH AMERICA

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We studied vanA-carrying vancomycin resistant enterococci (VRE) isolated from American crows (Corvus brachyrhynchos), ravens (Corvus corax) and rooks (Corvus frugilegus) wintering in various European countries, USA and Canada. Samples of bird feces were collected from roosting places of corvids during winters 2010-2013. The samples were cultivated selectively for VRE and tested for minimum inhibitory concentration (MIC) for vancomycin, susceptibility to other antibiotics by disk diffusion method and occurrence of genes responsible for resistance and virulence. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were used to examine epidemiologic relationships of vanA-containing VRE. The vanA-carrying VRE were tested in vitro for horizontal transfer of the vancomycin resistance trait. In Europe, VRE with vanA gene were found in 8 (0.7%) of 1073 rook samples originated from the Czech Republic and Germany. All 8 isolates obtained from Europe were determined as Enterococcus faecium which belonged to ST (sequence type) 92 (6 isolates), ST121 (1), ST671 (1). Seven out of eight isolates from Europe were able to transfer the vanA gene with a low transfer rate ~10-7T/D (transconjugants per donor). In USA and Canada, VRE with vanA gene were found in 15 (2.5%) of 590 and one of 400 crow samples (0.3%), respectively. We obtained 22 different isolates of VRE with the vanA gene from morphologically different colonies in the USA samples. Among these 22 VRE, 14 were determined as E. faecium and 8 as E. faecalis. E. faecium isolates from the USA belonged to ST18 (6 isolates), ST555 (2) and novel types ST749 (1), ST750 (3), ST751 (1), ST752 (1). An isolate from Canada belonged to ST448. E. faecalis isolates belonged to ST6 (1), ST16 (3) and ST179 (4). All isolates from the USA were able to transfer the vancomycin resistance trait via filter mating with a high transfer rate ~10-3 T/D. Clinically important enterococci with the vanA gene occurred in omnivorous corvids in both Europe and North America reflecting probably the local epidemiological situation.
and may pose a risk of environmental contamination. The role of the aggregative migratory corvids in dissemination of VRE needs further investigation.

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EVALUATION OF THE CEPHEID XPERT VAN A/VAN B ASSAY IN COMBINING IT WITH ENRICHED INOCULATED BROTHS FOR THE DIRECT DETECTION OF CARRIERSHIP WITH VAN B-TYPE VRE.

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Introduction: Rapid and accurate detection of VRE (vancomycin resistant enterococci) is needed for an adequate treatment and infection control. Previous studies using Cepheid’s Xpert vanA/vanB assay showed good results for vanA, however many false positive values were found for vanB [1-3]. This could partially be explained by the fact that non-enterococcal vanB genes can be found in the gut [4, 5]. We evaluated the use of Xpert vanA/vanB assay on enriched inoculated broths instead of using direct rectal specimens. Methods: In the first evaluation, a total of 235 E-swabs were tested in parallel with enriched inoculated broths. First, 100 µL E-swab medium was evaluated directly with the Xpert vanA/vanB assay. Further, E-swab medium was used to inoculate Brain Heart infusion (BHI) broth containing amoxicillin 16mg/L amphotericin B 20 mg/L, aztreonam 20 mg/L and colistin 20 mg/L. Broths were incubated and again evaluated with the Xpert vanA/vanB assay. Broths were subcultured on VRE Brilliance agars (Oxoid®). Growth of blue colonies was suspected for enterococci and identification was performed using MALDI-TOF Mass Spectrometry (Bruker). Confirmed growth of enterococci on the VRE agars was again evaluated with the Xpert vanA/vanB assay and these results were considered as “golden standard” for VRE negativity or positivity. After the first evaluation, we evaluated 112 enriched inoculated broths to test our new algorithm. Results: In the first evaluation 157 E-swabs as well as broths were negative (CT value 0 or >36). For the remaining 78 E-swabs, 32 had a VRE vanB positive culture, no vanA was found. Using the cut-off value of the GeneXpert™ system (≤36 for positivity) on the E-swabs, true positive and negative results were correct. However, false positive results were high (51.5%), which resulted in sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) of 100%, 83.3%, 48.5% and 100%, respectively. Therefore, we defined an own cut-off value for PCR on broth (≤25 for positivity). Now no false positive results were found, although 1 true positive VRE was missed (CT-value 25.9). This resulted in sensitivity, specificity, PPV and NPV of 96.9%, 100%, 100% and 99.5%, respectively. In the prospective 112 tested enriched inoculated broths, we again found no false positive results, one true-positive VRE was missed (CT-value 28.4). Conclusion: This study shows the usability of Cepheid’s Xpert vanA/vanB assay for vanB VRE in combination with enriched inoculated broths. Patients’ samples with CT-values ≤ 25 can be considered as true positive. In addition, samples with CT-values between >25-30 (2.6% of all samples), should be confirmed to be negative by culture. Using the cut-off value of ≤25 for positivity strongly improved the PPV compared to that found in previous studies in which the Xpert vanA/vanB assay was used on direct rectal swab specimens.
**56B**

**VANCOMYCIN-RESISTANT ENTEROCOCCI WITH VAN A GENE IN HOSPITAL INFECTION UNIT PATIENTS AND LOCAL ENVIRONMENTAL RESERVOIRS**

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Vancomycin resistant enterococci (VRE) cause serious nosocomial infections in many hospitals, particularly in immunosuppressed neutropenic patients, who are at high risk for bacteremia with organisms from their gastrointestinal tract. Aim of our study was to reveal epidemiology of the vanA-carrying VRE colonization of patients treated for Clostridium difficile infection at Department of Infectious Diseases, University Hospital Brno, Czech Republic. During the period from June to October 2013 we collected 49 and 11 fecal samples from hospitalized patients (some patients were sampled repeatedly) and from staff (one sample per person), respectively and 23 smears from various hospital settings. Samples were cultured selectively for VRE on Slanetz-Bartley agar supplemented with 32 mg/l vancomycin and incubated at 37°C for 48 hours. Morphologically different colonies were selected, identified and screened by PCR for vanA gene. The vanA-carrying isolates were tested for susceptibility to other antibiotics by disk diffusion method and for occurrence of genes responsible for resistance and virulence. Clinical and environmental VRE isolates with vanA gene were typed by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). VRE with the vanA gene were found in 26 (72%) of 36 tested patients. Some of the patients were colonized by more than one isolate, hence we obtained 40 vanA-carrying VRE. VRE belonged to Enterococcus faecium (35 isolates), E. faecalis (4) and E. raffinosus (1). All isolates were multi-resistant with resistance to six and more antibiotics. Resistance to erythromycin was encoded by ermB gene. All E. faecalis isolates carried gelE, cylA and asa1 virulence genes. Virulence of 31 (89%) E. faecium isolates was encoded by esp and/or hyl gene. We found VRE at different hospital settings including bed, floor, utility cart, support walker, handles of commode chair toilet, and also bed trapeze in the empty room after disinfection. Despite of using disposable protective equipment, five of the patients, who had not been colonized on admission to the hospital, became colonized with VRE after few days in hospital. Hospital personnel haven’t been identified to be colonized by VRE. Despite of low VRE prevalence in European hospitals, including Czech ones, we have found frequent VRE colonization of patients treated for Clostridium difficile infections at the Department of Infectious Diseases. Colonized patients can serve as sources for secondary transmission of VRE.

**57A**

**NOSOCOMIAL INFECTIONS CAUSED BY VANCOMYCIN-SUSCEPTIBLE MULTIDRUG-RESISTANT ENTEROCOCCUS FAECALIS OVER A LONG TERM IN A UNIVERSITY HOSPITAL IN JAPAN**

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**Background:** Vancomycin-resistant enterococci (VRE) are not widespread in clinical environments in Japan compared to other advanced nations. There have been no VRE outbreaks, and a few VRE strains were only sporadically isolated in our university hospital in Gunma, Japan. To examine the drug susceptibility of Enterococcus faecalis and nosocomial infections caused by non-VRE strains, a retrospective surveillance in our university
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hospital was conducted. **Methods:** Molecular epidemiological analyses were performed on 1,720 *E. faecalis* clinical isolates collected in our hospital over a six-year period (1998-2003). **Results:** Of 1,720 *E. faecalis* isolates, 1,462 (85.0%) were antibiotic resistant and 1,015 (59.0%) were resistant to two or more drugs. The major group of multidrug resistant *E. faecalis* (MDR-Ef) strains consisted of 298 (17.3%) isolates resistant to the five drug combination TC/EM/SM/KM/GM. The incidence of MDR-Ef isolates in the intensive care unit (ICU) increased after enlargement and restructuring of the hospital. Pulsed-field gel electrophoresis (PFGE) analysis of the major MDR-Ef isolates showed that nosocomial infections have been caused by MDR-Ef over a long term (more than three years). Multilocus sequence typing (MLST) showed that those strains were mainly grouped into ST16 (CC58) or ST64 (CC8). Mating experiments suggested that the drug resistances were encoded on two conjugative transposons (integrative conjugative element: ICE), one encoded TC-resistance and another encoded EM/SM/KM/GM-resistances. **Discussion:** The present results demonstrate that nosocomial infections caused by vancomycin-susceptible MDR-Ef strains have continuously occurred. The data suggest that our hospital has been jeopardized by a risk of nosocomial VRE infections for a long time. Strict infection control measures are needed to prevent outbreaks caused by multidrug resistant bacteria, including VRE, and to control bacterial nosocomial infections, especially in the ICU of every university hospital.

References:

### 58B

**VANN-TYPE VANCOMYCIN -RESISTANT ENTEROCoccus FAECium STRAINS IN JAPAN**

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**Background:** The first VanN-type vancomycin-resistant *E. faecium* strain (UCN71: ST240) have been isolated from a patient in France in 2008 and reported in 2011. We have also reported the identification of VanN-type VRE strain (*E. faecium* GU121: ST669) from a domestic chicken meat sample in Japan in 2012. In 2009, unidentified-type *E. faecium* strains showing the low-level resistance to vancomycin have been isolated from the domestic chicken meat samples in Japan. In this study, we retrospectively examined the unidentified VRE isolates. **Methods:** Totally 364 meat samples (90 domestic chicken swabs, 47 domestic pork swabs, 107 imported minced chickens, and 120 imported minced pork meats) were examined. **Results:** Nineteen low-level vancomycin-resistant *E. faecium* strains (MIC: 6 - 12 mg/L) were isolated from eight domestic chicken meat samples. PCR analysis revealed all of the nineteen isolates carried the *vanN* ligase gene, which confirmed all isolates were VanN-type VRE. PFGE analysis showed that the nineteen isolates from eight samples were categorized into two distinct groups (fourteen isolates from six samples, five isolates from two samples, respectively).
One of the group (fourteen isolates from eight samples) showed the same PFGE profile to our previously reported VanN-type VRE strain GU121. Each of two representative isolates (one strain from GU121-like group and AA-22 from another group) was further examined by MLST analysis. The GU121-like group isolate was categorized into ST669 identical to GU121 and another group AA-22 was categorized into a new ST. The new ST was the single locus variant of ST240, suggesting that AA-22 was genetically related with the first reported VanN-type VRE in France. S1 nuclease-treated PFGE and Southern blotting analysis showed that the vanN gene was encoded on a plasmid. Discussion: All of the nineteen VRE isolates from eight domestic chicken meats were VanN-type VRE and grouped into two clones. One clone was identical to the Japanese VanN-type VRE GU121 (ST699), another clone was closely related to the French VanN-type VRE UCN71 (ST240). The results indicate that VanN-type VRE strains are already spread in the clinical fields and environments in global.

References:


SENSITIVITY AND RESISTANCE OF THE GENUS ENTEROCoccus ANTImICROBIAL OF RESERVE IN THE SOUTH OF LIMA, PERU.

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The objective of this work was to study the prevalence of resistance to different antibiotics of reservation, at 93 clinical isolates of enterococcus isolated consecutively during eleven months of microbiological surveillance from January to November of 2013 in the National Hospital Maria Auxiliadora hospital referential to the South of Lima, in inpatient, outpatients and emergency services. The isolates were identified in regard to species by a semi-automatic system for the study of sensitivity of diffusion method was used in disco-placa. The percentage of Enterococcus sp were 61% for vancomycin (VRE), 61% to gentamicin, 52% for tetracycline, 51% for nitrofurantoin, 50% for meropenem and teicoplanina, 49% for rifampicin. Being sensitive to streptomycin cefoperazona/ sulbactam, 33.3% 40%, 25% for azithromycin and 21% for levofloxacin. The global distribution of species does not coincide with the majority of consulted series, being highest for our hospital in up to 20% compared to hospitals in Europe and North America. Our isolates are resistant to antimicrobial drugs of first choice and high resistance to antimicrobials reservation requiring is strengthen the prudent use of antimicrobials of reserve program.
Background: Until now, nine types of vancomycin-resistances including VanA, VanB, VanC, VanD, VanE, VanG, VanL, VanM, and VanN are reported. Of these types, VanA-, VanB-, VanD-, and VanM-type VRE strains usually show the high-level resistances to vancomycin. In this study, we report the vancomycin-susceptible vanB2-containing VRE (low-MIC VRE) clinical isolates in Japan.

Methods: Nine vancomycin-susceptible E. faecium clinical isolates carrying vanB2 gene cluster (low-MIC VRE) were isolated from the stool samples from nine inpatients in a hospital during February to March 2010 in Japan. The nine low-MIC VRE isolates were characterized and the DNA sequence of vanB2 gene cluster was examined.

Results: All of the nine isolates harboring the vanB2 gene cluster showed the vancomycin-susceptible phenotypes (MIC: 2 - 4 mg/L) by CLSI standard methodology or the improved method using oxgall. PFGE analysis showed that eight strains of nine E. faecium isolates were identical and remaining one strain was similar to other strains. The data suggested those nine low-MIC VRE isolates had a same origin (clonal), and nosocomially transmitted in the hospital. Southern blotting analysis indicated that the vanB2 gene clusters were encoded and located on the plasmids. The DNA sequence analysis of a representative isolate revealed that there were several point mutations in the vanB2 gene cluster comparing to the reported high-level vancomycin-resistant VanB-type VRE in Japan.

Discussion: The DNA sequence data implied that the vancomycin-susceptible phenotype of the VanB-type VRE might be caused by the amino acid substitutions found in VanSb (sensor protein), VanB (D-Ala:D-Ala ligase), VanHb (dehydrogenase), and VanW (unknown function). However, we could not determine the responsible mutation(s) for low-MIC VRE in this study, and also could not exclude any host factors affecting the vancomycin-susceptibility.

References:

GLOBAL TRANSCRIPTOMIC ANALYSIS (RNA-SEQ) OF ENTEROCOCCUS FAECIUM IN RESPONSE TO DAPTOMYCIN
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Despite their low virulence, enterococci are responsible for many community- and hospital-acquired infections mainly due to Enterococcus faecalis and Enterococcus faecium species. Adapted to harsh conditions and doted of a high genomic plasticity, E. faecium strains multiply resistant to antibiotics have largely disseminated worldwide, becoming a serious problem in clinical practice. Importantly, there is a global spread of vancomycin-resistant E. faecium (VREF) isolates belonging to a specific clonal complex (CC17), which is well adapted to the hospital environment. Sub-inhibitory concentrations (SICs) of antibiotics do not visually influence the bacterial growth kinetics but they can significantly alter expression of major genes involved in cell physiology, virulence and antimicrobial resistance. Noteworthy, such concentrations may be observed in vivo, e.g. when the antibiotic weakly diffuse at the site of infection or during the antibiotic exposure on bacterial flora of the gastrointestinal tract. The aim of the study was to determine the impact of SICs of daptomycin, a novel cyclic lipopeptid used for the treatment of VREF infections, on the expression of genes of E. faecium by a global transcriptomic approach. A VREF member of the CC17, the vanB-positive E. faecium
Aus0004 entirely sequenced and annotated, was used. After determination of both SIC and MIC values, daptomycin was tested at a concentration of 0.5 mg/L (with a calcium concentration adjusted to 50 mg/L). Transcriptomic analysis was performed by strand-specific RNA-seq (HiSeq 2500, Illumina) in the absence or presence of daptomycin. The reliability of RNA-seq was further validated by qRT-PCR experiments. In the presence of 0.5 mg/L of daptomycin, 55 genes were significantly induced (expression fold-change >4) whereas 112 were significantly repressed (expression fold-change <-4). Most of those genes encoded hypothetical proteins or proteins with unknown function. Among up-regulated genes, four coded for transcriptional regulators (including one of the MerR family) and one was the acm gene coding for a collagen adhesin, a well-known virulence factor in E. faecium. Down-regulated genes were mainly genes involved in intermediary energy metabolism. This study has revealed the importance of interesting candidates involved in the response to daptomycin stress. Further investigations are in progress: the confirmation of the antibiotic-mediated acm induction potentially responsible for higher adhesion capabilities, and the full characterization of the MerR family transcriptional regulator assumed to be important in the antibiotic response. This project represents the first global study of the response of E. faecium to low concentrations of antimicrobial agents, its results providing elements for a better understanding of the mechanisms of adaptation and resistance to daptomycin.

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THE GENOMIC VARIABILITY OF VANM-TYPE ENTEROCOCCUS FAECIUM ISOLATES FROM AN INFECTIVE ENDOCARDITIS PATIENT

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Background: A 68-year-old woman, who underwent coronary artery bypass grafting and biological mitral valve replacement 7 months ago, complained fever for 40 days. After been admitted, the echocardiography detected vegetation on the biological mitral valve. With 5 continuous blood cultures Enterococcus faecium positive, the patient was diagnosed infective endocarditis. The patient was treated with daptomycin for two weeks. But the patient still had fever and the echocardiography showed the vegetation enlarged. Then therapy was changed to linezolid, together with rifampin and fosfomycin. Two weeks later, the patient recovered, her echocardiography showed the vegetation calcified. While the positive blood culture broths were transferred to blood agar plates, we found the colony size of some isolates was bigger than the other. The reason was unknown. Methods: The 8 clinical isolates from blood cultures were identified using VITEK 2 System and the differences of biochemical reactions among them were compared. The minimal inhibitory concentrations (MICs) were determined by agar dilution method. Vancomycin resistance gene and multilocus sequence typing (MLST) were determined by PCR amplification and sequencing. PFGE was used to analyse genome evolution. Results: All these 8 clinical isolates were identified as the Enterococcus faecium, but the identification system indicated that 1-3 biochemical reactions were different. Seven isolates were resistant to vancomycin; one isolate was susceptible to vancomycin according to the antibiotic susceptibility test. All 8 isolates carried esp gene and seven vancomycin resistant Enterococci were vanM type based on the PCR amplification and sequencing results. All 8 isolates were MLST ST78 type. In the PFGE analysis, the earlier 5 strains were same, the later strains were different, which had 1 to 3 bands difference. Conclusion: It is still to be clarified that the phenomenon was caused by an Enterococcal mixed infection, or a DNA recombination occurred during the process of the infection and treatment. As all the strains were MST ST78 type and the PFGE pattern of the first 5 strains was different from the others, we can infer that DNA recombination probably
EMERGENCE OF CC17 ENTEROCOCCUS FAECIUM STRAINS AND FIRST REPORT OF VANCOMYCIN-RESISTANT ENTEROCoccus AVIUM AND ENTEROCoccus RAFFINOSUS ISOLATES AMONG PATIENTS IN A TEACHING HOSPITAL IN BRAZIL

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The enterococci are important agents of healthcare-associated infections frequently associated with resistance to multiple antimicrobials. The occurrence of vancomycin-resistant enterococci (VRE) is of peculiar importance due to their high ability for dissemination and association with therapeutic challenges. In the present study, we evaluate the occurrence and describe the phenotypic and genotypic characteristics of VRE isolates recovered from patients admitted to a teaching hospital located in Rio de Janeiro state, Brazil, during a period of 3 years. Thirty-one VRE isolates were obtained. They were identified as Enterococcus faecium (VREfm; 27 isolates), Enterococcus avium and Enterococcus raffinosus (two isolates each). Most (26) of the isolates were recovered from surveillance cultures for monitoring VRE intestinal colonization, while five of the VREfm isolates were obtained from patients with enterococcal infections. All the isolates were shown to carry the vanA gene and expressed multidrug resistance profiles, associated with the presence of a variety of other antimicrobial resistance genes, such as the ermB, aac(6)-I-aph(2)-Ia, aph(3')-IIIa, ant(6)-I, tetM and vatD genes. The esp gene was largely present among the VRE isolates tested. Other virulence-associated genes, including gelE, hyl, asa1 and cylA, were not detected. Analysis by PFGE revealed high levels of similarity among isolates of each enterococcal species identified, suggesting intra-hospital dissemination and cross-contamination. By using MLVA and MLST, VREfm isolates were identified as belonging to the high-risk worldwide disseminated clonal complex CC17. The data illustrate the national scenario on the occurrence and characteristics of VRE circulating in Brazil, including aspects related to rarely occurring VRE species. Overall, the results emphasize the importance of continued surveillance programs in order to better understand the epidemiology of VRE, and are useful to delineate control strategies to prevent the spread and, consequently, the occurrence of infections associated with these microorganisms. Moreover, this is the first report on the isolation of VRE belonging to the species E. avium and E. raffinosus in Brazil.

PREVALENCE OF VANCOMYCIN-RESISTANT ENTEROCOCCI IN A TEACHING HOSPITAL OF SHANGHAI, CHINA

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Background: Vancomycin-resistant enterococci (VRE) are common nosocomial pathogens in many countries. Before 2003, however, nosocomial infections caused by VRE were rare in Mainland China. In recent years, VRE strains have emerged and been increasingly isolated in Beijing, Hangzhou, and Shanghai. In this study, we investigated the prevalence of VRE in a teaching hospital. Methods: Between October 2010 and September 2013, 369 consecutive and non-duplicate Enterococ-
Enterococcus spp. strains were isolated from clinical specimens in a 1600-bed teaching hospital in Shanghai. The van genotype was determined by PCR. And the similarity of the VRE strains was determined by multilocus sequence typing (MLST). The antimicrobial susceptibility was measured by disc diffusion or agar dilution method with results interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Filter-mating experiments were carried out with Enterococcus faecium BM4105RF (Fus', Rif') as the recipient. Results: Among 369 Enterococcus spp. isolates, 17 strains harbored van gene (12 E. faecium isolates containing vanM, 4 E. faecium isolates containing vanA, and one E. gallinarum isolate containing vanC1). The 16 E. faecium isolates containing vanA or vanM were highly resistant to vancomycin with minimal inhibitory concentrations (MICs) ≥256 μg/ml. The MIC range for teicoplanin was 0.25-64 μg/ml. For the E. gallinarum isolates containing vanC1, the vancomycin and teicoplanin MICs were 8 μg/ml and 0.25 μg/ml, respectively. All isolates of VRE were resistant to levofloxacin and ampicillin, and 16 isolates were resistant to erythromycin, but none was resistant to linezolid. The glycopeptide resistance of 15 VRE strains could be transferred to E. faecium BM4105 RF by conjugation, but the resistance to other antibiotics could not. MLST analysis of E. faecium isolates identified 3 STs, including ST 78 (9 VanM-type strains and 3 VanA-type strain), ST 262 (1 VanM-type strain), and ST 555 (3 VanM-type strains and 1 VanA-type strain). Conclusion: All VRE strains are multidrug-resistant. VanM-type is the most common genotype of VRE. MLST 78 is the most predominant clone. Several years ago, VanM-type VREs were reported in another hospital. The present finding may indicate there could be an inter-hospital spread of VanM-type VREs in Shanghai. Keywords: VRE, MLST, Inter-hospital spread

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A SYSTEM BIOLOGY APPROACH TO IDENTIFY PUTATIVE NEW TARGETS INVOLVED IN ANTIBIOTIC RESISTANCE IN ENTEROCOCCUS FAECALIS.

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Background. The highly ability showed by E. faecalis to tolerate different antimicrobial agents, constantly keeps the interest for identifying new targets involved in this resistance process. Recently, Abranches et al. published a complete set of microarrays where E. faecalis was exposed to four different antibiotics (ampicillin, bacitracin, cephalotin and vancomycin) during 30 and 60 min. In order to identify new targets involved in antibiotic resistance, we combine this information with an E. faecalis global transcriptional gene regulatory network (GTRN), strategy that allowed us to describe a complete new sub-network of genes activated by these four antibiotics putative controlled by different transcriptional factors.

Methods. Microarray E. faecalis OG1RF antibiotics data were obtained from Geo-DataSet Id N°GSE45306. The GTRN of E. faecalis were generated by Consensus/Patser and MotifSampler/Scanner algorithms using bacterial transcriptional factors binding sites probabilistic weight matrix (GTRN published in Latorre et al. Metallomics, 2013). Microarray data and GTRN were merged and analyzed by Cytoscape software.

Results. The resulting antibiotic activated sub-network is composed by 306 activated genes (163 operons) highly connected by 14 putative transcriptional factor families. Into the regulators identified, highlights members for the LysR, ArgR, MalI and CRP-FNR families.
controlling the expression of genes induced by the four antibiotics. Particularly, these two last transcription factors are likely controlling the expression of two genes (EF1533 and EF3245) which encode for proteins directly involved in the survival of E. faecalis against bacitracin and vancomycin.

Conclusions. Our System Biology approach allowed to identify a new set of transcriptional factors putative involved in the regulation of antibiotic resistance determinants, positioning these regulators as possible new targets for antimicrobial drug discovery. Finally, this strategy could potentially be used to identify new networks activated by others antibiotics.

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THREE COMPONENT REGULATORY SYSTEM INVOLVED IN VANG-TYPE VANCOMYCIN RESISTANCE FROM ENTEROCOCCUS

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Three component regulatory system involved in VanG-type vancomycin resistance from Enterococcus

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Glycopeptide resistance results from 1) the production of modified peptidoglycan precursors ending in D-Ala-D-Lac or in D-Ala-D-Ser to which vancomycin and teicoplanin bind with low affinity and 2) the elimination of the high affinity D-Ala-D-Ala ending precursors. The 3’ end of the chromosomal vanG cluster encodes VanG, a D-Ala:D-Ser ligase, VanXY, a bifunctional D,D-peptidase, and VanTG, a serine racemase. In contrast to the other van operons, the 5’ end contains vanUG, vanRG, and vanSG, which are co-transcribed constitutively from the PUG regulatory promoter, whereas transcription of the resistance genes is inducible from the PYG resistance promoter. Additional vanUG encodes a predicted transcriptional activator which role remains unknown. The entire VanUG, VanR, and the cytosolic domain of VanS have been purified. VanSG autophosphorylated, transferred its phosphate to VanR, but not to VanUG. Gel mobility shift and DNaseI footprinting with VanUG and VanR phosphorylated or not showed that VanUG bound to the PYG promoter whereas transcription of the resistance genes is inducible from the PYG resistance promoter. Additional vanUG encodes a predicted transcriptional activator which role remains unknown. The entire VanUG, VanR, and the cytosolic domain of VanS have been purified. VanSG autophosphorylated, transferred its phosphate to VanR, but not to VanUG. Gel mobility shift and DNaseI footprinting with VanUG and VanR phosphorylated or not showed that VanUG bound to the PUG and PYG promoter regions whereas VanR bound only to PYG but protected the same DNA region as VanUG. Genes vanUG, vanRG, and vanSG were insertionally inactivated individually leading to PUG, PUG, and PUG and the vanTG gene was used as a reporter. The level of expression of the regulatory genes in the absence or in the presence of vanUG was determined by qRT-PCR. The vanURSG genes were cloned into E. coli under the control of an inducible promoter upstream from the PUG regulatory or PYG resistance promoters and a chloramphenicol acetyltransferase reporter gene. Every gene was inactivated to study its regulatory role. The first results indicated that VanUG acts as a repressor which was confirmed by the analysis of its 3D-structure. Moreover VanUG did not allow full expression of the resistance genes in the presence of low vancomycin concentrations. Thus, expression of the resistance genes was dependent on the concentration of vancomycin due to the control by vanUG as opposed to modulation of VanR activity by VanS in the other van operons.

The interactions between VanUG, VanRG, and VanSG by SPR and analytical ultracentrifugation indicate that only VanRG and VanSG were able to interact.
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**CONTRIBUTION OF ADE RND EFFLUX SYSTEMS TO ANTIBIOTIC RESISTANCE IN ACINETOBACTER BAUMANNII**

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Acinetobacter baumannii, responsible for epidemics of nosocomial infections, is a health problem due to its propensity to develop multidrug resistance (MDR). We have shown that increased expression of chromosomal broad substrate range RND efflux systems AdeABC, AdeFGH, and AdeIJK plays a major role in MDR. Increased expression of the adeABC operon, tightly regulated by AdeRS, is due to mutations in the two-component system. AdeFGH, controlled by LysR-type AdeL, confers MDR when overexpressed but its prevalence is low. AdeIJK, regulated by TetR-like AdeN, is responsible for intrinsic resistance. However, the contribution of each pump to resistance of the host yielded contradictory results, mainly because most studies dealt with MDR clinical isolates encoding multiple resistance mechanisms. We have derived, by antibiotic selection, from susceptible A. baumannii clinical isolate BM4587 resistant mutants overexpressing each pump following a point mutation in their respective regulator, as verified by whole genome sequencing. The corresponding adeB, adeG, and adeJ deletant mutants were obtained by allelic replacement. Expression of the pumps was measured by qRT-PCR and the MICs determined by microdilution. The complete genome of A. baumannii BM4587 consisted of a 3.8-Mb chromosome and an 8-kb plasmid. Antibiotic resistance genes were only found in the chromosome: ampC and blaOXA-104 for intrinsic β-lactamases; MFS efflux pumps tetA and craA, RND adeT, and MATE abeM; acquired aadA1 and catB. There were no resistance islands and a single partial IS66-like copy. Ticarcillin and aztreonam were substrates for AdeIJK. Among carbapenems, imipenem but mostly meropenem and doripe-}

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**FITNESS COST OF AMINOGLYCOSIDE RESISTANCE 16S METHYLTRANSFERASES**

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In Gram-negative bacteria, acquired 16S rRNA methylases ArmA and NpmA confer high-level resistance to all aminoglycosides that could be used to treat systemic infections by modifying, respectively, G1405 and A1408 in the A site. These enzymes must co-exist with several endogenous methylases which are essential for fine-tuning of the decoding center, such as RsmH and RsmI in Escherichia coli which methylate C1402 and RsmF C1407. The resistance methylases have a contrasting distribution, ArmA has spread worldwide whereas a single clinical isolate producing NpmA has been reported. The rate of dissemination of resistance depends on the fitness cost associated with the acquisition of these enzymes.
with its expression. We have compared ArmA and NpmA in isogenic E. coli harboring the corresponding structural genes and their inactive point mutants cloned under the control of their native constitutive promoter in the stable plasmid pGB2. Growth rate determination and competition experiments showed that ArmA had a fitness cost due to methylation of G1405 whereas NpmA conferred only a slight disadvantage to the host due to production of the enzyme. MALDI Mass Spectrometry indicated that ArmA impeded one of the methylations at C1402 by RsmI, and not at C1407 as previously proposed, whereas NpmA blocked the activity of RsmF at C1407. A dual luciferase assay showed that methylation at G1405 and A1408 and lack of methylation at C1407 affect translation accuracy. These results indicate that resistance methylases impair endogenous methylation with different consequences on cell fitness. Biological cost affects the fate of resistance but associated mobile genetic elements also play an important role in its persistence and dissemination.

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DO ALCOHOL BASED HAND RUBS HAVE EFFICACY AGAINST ANTIBIOTIC RESISTANT BACTERIA?

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Background: Multidrug-Resistant Organisms (MRO) including Vancomycin-Resistant Enterococci (VRE) are often passed from person to person by the contaminated hands of caregivers. Hands become contaminated by contact with patients infected with MRO or contaminated surfaces. Cleaning hands with soap and water (particularly when visibly soiled) or use alcohol-based hand rubs (ABHR) are the main recommendation of WHO for preventing the spread of MRO. However, the efficacy of hand hygiene products against MRO is not well documented.

Objective: Determine the efficacy of several hand hygiene products including hand washes and ABHR against different antibiotic resistant and sensitive strains of VRE, MRSA, and Klebsiella.

Experimental Methods: Twenty commercial hand hygiene products including 6 hand washes, 3 sanitizing wipes and 11 alcohol-based hand rubs ranging from 62% to 80% were evaluated using a 15 second \textit{in vitro} Time-Kill (ASTM E 2315) against 15 strains of antibiotic susceptible and resistant bacteria. Additionally, ABHR with 62% ethanol, antimicrobial hand wash with 4% chlorhexidine gluconate (CHG), and antimicrobial foaming hand wash with 0.3% triclosan (TCS) were evaluated by an \textit{in vivo} hand wash method (ASTM E 2755-10) against MRSA (strain ATCC #33591).

Results: By \textit{in vitro} testing, ABHR containing ethanol gel or foam from 62- 80% reduced all bacteria strains tested by ≥6 log10 (≥ 99.9999%) in 15 seconds. Reduction of MRSA by non-alcohol products was variable, and was both product and strain dependent. By \textit{in vivo} testing, the 62% ethanol ABHR and TCS hand wash produced LR of 2.05 and 1.93, respectively and were statistically equivalent. The CHG hand wash produced a 1.53 LR and was statistically inferior to the alcohol sanitizer and TCS hand wash.

Conclusions: No differences in susceptibility to alcohol were shown by antibiotic resistant bacteria when compared to the sensitive strains. Alcohol 60% or higher showed the same level of efficacy. These results support WHO recommendations for use of ABHR by healthcare personnel for preventing the spread of MRO. Non-alcohol products should be chosen carefully due to the variable susceptibility showed by MRSA strains tested, and the formulation specific performance of these biocides.
70B

**EVOLUTION OF MULTI-DRUG RESISTANT E. FAECA LIS DURING AN EXTENDED HOSPITAL OUTBREAK**

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High-level gentamicin resistant *Enterococcus faecalis* first appeared in the United States in the mid-1980s. An early outbreak of high-level gentamicin resistant *E. faecalis* bacteremia at the University of Wisconsin Hospital and Clinics began in 1985 and was found to be due to an apparent sequence type 6 clone, with one of the first isolated strains being MMH594. To understand how this strain evolved over the 32-month duration of this outbreak, we generated high quality draft genome sequences for 65 isolates spanning the course of the outbreak, as well as 28 strains isolated before the beginning of the outbreak. Illumina® and Pacific Biosciences® sequencing technologies were used to completely sequence the chromosome and two plasmids of the reference strain MMH594. Preliminary results indicate that single nucleotide polymorphisms (SNPs) occurred throughout the course of the outbreak, resulting in an accumulation of mutations at a rate of approximately one SNP every 40 days. So far, the majority of SNPs examined are singletons that did not become fixed in the population (i.e. they are present in only one outbreak isolate). This finding supports the expected mode of transmission, whereby patient-to-patient spread occurred first by colonization of the intestinal tract, followed by incidence of the microbe in the bloodstream, with the latter being the site from which the microbe was isolated. However, a few SNPs appeared to become progressively fixed in the population, implying their involvement in the adaptation of this lineage to cycles of colonization and re-infection. These differences are beginning to be studied to determine the exact consequence of these genetic changes. In preliminary studies, differences in growth, cytolyis, and *in vitro* susceptibility to both ampicillin and carbenicillin were observed among the isolates. This study highlights the power of genomics for understanding the emergence of new hospital pathogens.

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**THE GENETIC BASIS OF MULTIDRUG RESISTANCE IN BETA-LACTAMASE (BLA+) POSITIVE ENTEROCOCCI**

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In the effort to study, at the molecular level, different strains of *bla* positive enterococci already described (Sarti et al., JCM 2012), we studied in depth the multidrug resistance phenotype of two strains of *E. faecium* (G40 and G90) and one strain of *E. faecalis* (SMT). All strains showed high level resistance to macrolides, aminoglycosides (HLSR-HLGR) and tetracyclines but only *E. faecium* strains showed to be resistant to ampicillin; surprisingly, the strain of *E. faecalis*, despite possessing the *bla* gene, was defined as susceptible by in vitro susceptibility testings. The aim of this study was the genetic characterization of elements carrying resistance genes and their possible association. For the first time, the complete sequence of the *bla* element was obtained. All strains, showed the presence of three main *orf*s: *blaZ* (coding for a type A staphylococcal beta-lactamase), *blal* (putative transmembrane antirepressor) and *blar1* (putative repressor); all genes exhibiting 98% nucleotide identity and the same genetic organization with the corresponding genetic elements of *Staphylococcus* spp. Furthermore, it is interesting to note that the G90 strain possessed one IS256

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integrated into the \textit{bla}R gene: this integration could determine the alteration of the enzyme expression. Further experiments are still ongoing. Moreover, all strains harbored \textit{erm}B, \textit{aad}E, \textit{tet}M, \textit{aph}A3, \textit{sat}4, \textit{aac}A-\textit{aph}D genes and, only the G90 isolate, the \textit{van}B gene. To evaluate the possible resistance gene association, we performed long PCR assays using primers located on \textit{erm}B and \textit{aph}A3 or \textit{aac}A-\textit{aph}D genes. We found that all strains possess the MAS-like elements in which the \textit{erm}B-\textit{aad}E-\textit{sat}4-\textit{aph}A3 produced one amplicon of 6 kb and, only in the G40, linked also with \textit{aac}A-\textit{aph}D gene, producing one amplicon of approximately 10 kb in size. The conjugal transfer of the \textit{bla} and MAS (macrolides-aminoglycosides-streptothricin) elements was performed by filter mating experiments using OG1SS (Spec\textsuperscript{R}) \textit{E. faecalis} as recipient. While the \textit{bla} element could not be transferred from donors (G40, G90 and SMT) to the recipients, the MAS element was transferred by conjugation from the \textit{E. faecalis} SMT to OG1SS at a frequency of $11 \times 10^2$ transconjugants per donor. All transconjugants exhibited the macrolide, aminoglycosides and spectinomycin resistance and carried \textit{erm}B, \textit{aad}E, and \textit{aph}A3. The conjugation experiments in two \textit{E. faecium} are ongoing. In conclusion, in our study, the sequences of two “unusual” genetic elements in Enterococci were reported, i.e. the \textit{bla} operon and the MAS element, both are responsible for the dissemination of antibiotic resistance determinants intra and inter-species.

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\textbf{MOLECULAR CHARACTERIZATION OF VANCOMYCIN RESISTANT ENTEROCOCCI FROM LAGOS, NIGERIA}

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Background: Since the late 1980’s there have been increasing reports of vancomycin resistance among enterococcal isolates. These strains have emerged as significant nosocomial pathogens responsible for a wide variety of infections and posing great challenge for therapy. Although these organisms have been isolated from clinically significant infections, the great majority have been colonizers. In Nigeria, earlier reports indicated that vancomycin resistance among enterococci had not emerged; however, more recently, vancomycin resistance has been reported among enterococcal isolates.

Objective: To determine the prevalence of vancomycin resistant enterococci (VRE) among patients on prolonged hospitalization in Lagos University Teaching Hospital.

Methods: Rectal swabs were collected from patients hospitalized for seven days and above and screened for vancomycin resistant enterococci. The isolates were identified by MALDI-TOF spectroscopy, glycopeptide resistance was determined by E-test and vancomycin resistance genotype by PCR (using primers described by Biavasco et al.) and confirmed by GeneXpert RT PCR.

Results: 14 VRE were isolated from 363 patients screened, 1 vanA, 2 vanB and 11 vanC. Both patients colonized with vanB VRE were on admission on the same ward.

Conclusion: this first report of VRE genotypes in Nigeria shows that all major genotypes of VRE are circulating in the country hence the need for increased surveillance.
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