admitted to our hospital after two weeks of malaise and fever. Three weeks earlier, she had undergone pacemaker replacement surgery. Blood cultures drawn on admission yielded growth of *Enterococcus faecalis*, and a subsequently performed transesophageal echo (TEE) showed vegetations on her mitral valve. Due to technical difficulties, the valve could not be replaced, and antibiotic treatment with amoxycillin 6dd 2000 mg iv and gentamicin 3 mg/kg iv was started. After five days, gentamicin was discontinued due to nephrotoxicity. Kidney function recovered after cessation of gentamicin, but four weeks later a TEE showed an increase in vegetation size on the mitral valve. Gentamicin was again added to the amoxycillin, after which her kidney function deteriorated again. Therapy was switched from amoxycillin and gentamicin to daptomycin 6 mg/kg iv, 3 weeks after reintroduction of gentamicin. This regimen was continued for another 9 weeks, until the patient was discharged in good health and without any clinical or laboratory signs of infection. However, two weeks after discharge she was readmitted with fever and blood cultures yielded a daptomycin resistant *E. faecalis*. In this study we investigated the nature of the *E. faecalis* strains isolated from blood cultures from this patient.

Methods: Isolation and antibiotic susceptibility testing of *E. faecalis* strains was performed using routine microbiological diagnostic methods and subsequently stored at -80°C. Molecular fingerprinting was performed using Multi Locus Sequence Typing (MLST) and Amplified Fragment Length Polymorphism (AFLP). These experiments were performed at UMC (Utrecht) and VUmc (Amsterdam), respectively. Attachment and biofilm formation were analysed using a microtiter plate assay, whereby attached cells were quantified using 0.5% (w/v) crystal violet upon 1 (attachment) and 24 hours (biofilm formation) of static culture in *Luria-Bertani media*, respectively. Data shown are from three independent biological experiments.

Results: Antibiotic susceptibility testing of the *E. faecalis* strains isolated from the blood cultures showed that the initial isolates were susceptible to daptomycin (MIC of 2 g/ml), whereas the *E. faecalis* strains isolated upon readmission were resistant to daptomycin (MIC of 12 g/ml). In order to differentiate relapse from reinfection, molecular fingerprinting of the strains was performed using both MLST and AFLP. MLST analysis showed that all strains belong to MLST sequence-type 21, and AFLP analysis confirmed that the daptomycin susceptible and resistant strains were identical. These findings demonstrate that a relapse occurred, whereby the *E. faecalis* strain acquired daptomycin resistance during daptomycin therapy. As these isolates were assumed to cause the endocarditis, we investigated the biofilm forming ability of both strains. The daptomycin resistant isolate showed a reduced biofilm formation compared to the susceptible strain in both early attachment and biofilm formation.

Conclusion: *E. faecalis* acquired daptomycin resistance during daptomycin therapy, which resulted in a relapse *E. faecalis* mitral valve endocarditis. Current work focuses on the identification of the daptomycin resistance mechanism and the link between this resistance mechanism and the reduced biofilm formation phenotype observed.

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Effect of simultaneous exposure of pigs to *Streptococcus suis* serotypes 2 and 9 on colonization and transmission of these serotypes, and on mortality

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Introduction: *Streptococcus suis* (*S. suis*) is a major pathogen in pigs worldwide, causing meningitis, septicemia, arthritis, endocarditis, and mortality. *S. suis* in humans is considered as an emerging life-threatening infection, especially in Asia. Main risk factor for human infection is direct contact with infected pigs or their products.

In pigs, various serotypes of *S. suis* have been identified as cause of clinical infection. Comparable to e.g. *Streptococcus pneumoniae* in human, the presence of *S. suis* serotypes in pigs, however, differs between geographical areas, and varies over time. In several European countries, including The Netherlands, there has been a shift in predominant serotype from serotype 2 towards serotype 9 in the last two decades. We hypothesize a relation with one serotype affecting the other in colonization, transmission and invasion. The aim of this study was to evaluate whether simultaneous exposure of pigs to serotypes 2 and 9 affects the colonization and transmission of each serotype, and affects mortality.

Methods: Thirty-six caesarean-derived/colostrum-deprived piglets were randomly assigned to three groups, and there housed pair-wise. At 6 weeks-of-age one pig per pair was inoculated intranasally with either one (serotype 2 or 9; mono-group') or two serotypes simultaneously (dual-group'). Pigs in the mono-groups received $1x10^9$ CFU serotype 2 or 9, and in the dual-group a mixture of $1x10^9$ CFU serotype 2 and $1x10^9$ CFU serotype 9 (i.e. $2x10^9$ CFU *S. suis*/pig). The other pig of each pair was contact-exposed. Tonsillar brushing samples were collected from all pigs during three weeks post inoculation. Bacterial loads in the samples were quantified using multiplex real-time PCR. Transmission rates of the serotypes among pigs were estimated using a mathematical SI-model.

Results: The transmission rates for serotype 9 were 67/day (95%CI: 0-8) and 4.1/day (95%CI: 1.6-10.6), for the mono- and dual-group, respectively (p = 0.99). The transmission rates for serotype 2 were estimated at 29.4/day (95%CI: 0-8) in the mono-group, and 2.9/day (95%CI: 1.2-6.9) in the

dual-group (p = 0.99).Bacterial loads did not differ significantly between serotypes (p = 0.99). In the dual-group the average serotype 2 load in tonsillar samples from contact pigs was 1.4-1.8 ^{10}LogCFU/sample (i.e. 25-40 fold) reduced on days 1 to 4 and 6, in comparison to the mono-group (p < 0.01).Simultaneous exposure to the serotypes reduced the mortality hazard 6.3 times (95%CI: 2.0-19.8) compared to exposure to serotype 2 only, and increased it 6.6 times (95%CI: 1.4-30.9) compared to exposure to serotype 9 only.
Conclusions: Transmission rates for serotype 2 did neither differ significantly between serotypes, nor for a single serotype between the mono- and dual-group. This implies that simultaneous exposure to serotypes 2 and 9 does not affect the relative transmission rates of each serotype.Natural contact exposure to serotypes 2 and 9 simultaneously affects the clinical outcome of an infection of a particular serotype in a population, possibly by affecting the mucosal load. This might have contributed to the observed shift in distribution of clinical isolates in the field from serotype 2 to 9.

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Biological upgrading of syngas to fuels and chemicals

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Microbes can be used as biocatalysts to obtain added-value products from renewable sources. The big challenge of today is to efficiently convert lignocellulosic materials and wastes into fuels and other commodity chemicals, and a promising route for this is the combination of thermal and biological treatments. Presently cost-effective gasification technology exists to generate syngas (composed of CO, CO_2 and H_2) from biomass and wastes, and syngas fermentation to ethanol is performed at pilot and industrial scale. However, biological conversion of syngas has far more potential than this current application and innovative processes need to be explored. Here, we summarize our work on syngas conversion by anaerobic mixed microbial communities. We also addressed gas-liquid mass transfer limitations during syngas conversion by the utilization of a novel bioreactor configuration.
Stable thermophilic enrichments converting syngas (60% CO, 10% CO_2 and 30% H_2) and/or CO at 55C were obtained by incubation of a thermophilic anaerobic suspended sludge with these substrates. Cultures were successively transferred on syngas (cultures T-SYN) or pure CO (cultures T-CO). Initial CO partial pressure was increased from 0.09 to 0.88 bar over the duration of the enrichment experiment. T-SYN cultures produced mainly acetate, while hydrogen was the main product formed in T-CO cultures. *Desulfotomaculum* and *Caloribacterium* species were predominant in T-SYN cultures, while bacteria assigned to *Thermincola* and *Thermoanaerobacter* genera were abundant in T-CO cultures. A novel CO-tolerant bacterium, strain PCO, was isolated from the T-SYN culture. This bacterium is closest related to *Thermoanaerobacter thermohydrosulfuricus* (97% 16S rRNA gene identity). Although strain PCO does not utilize CO, it is able to grow in the presence of high CO concentrations (pCO = 1.7 bar). A new thermophilic hydrogenogenic carboxydotrophic bacterium, *Moorella stamsii*, could be isolated from the T-CO enrichment. This bacterium is able to utilize CO coupled to the production of hydrogen.
Mesophilic granular sludge was shown to efficiently convert syngas (60% CO, 10% CO_2 and 30% H_2) to methane. In batch assays, complete consumption of CO and H_2 was achieved in less than 72 h with syngas supplemented at 1 atm, and within 240 h for higher syngas pressures (up to 2.5 atm). Superior results were obtained using a novel multi-orifice baffled bioreactor (MOBB). A 10 L MOBB was fed with syngas and operated under oscillatory flow mixing, which is recognised to generate strong radial mixing and increased residence time of the gas-phase. Continuous syngas injection in the MOBB showed up to 15-fold enhancement of the CH production rate compared to the batch incubations. The overall performance of the novel MOBB in respect to syngas fermentation demonstrates its relevant impact on the anaerobic syngas fermentation process, opening perspectives for future technological development and industrial applications.
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Decrease of the frequency of the mosaic penA gene in *Neisseria gonorrhoeae* between 2010 and 2012 was associated with less resistance to third generation cephalosporins

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Introduction: Resistance of *Neisseria gonorrhoeae* against third generation cephalosporin is considered a major threat for public health. Increased MICs against cephalosporins are related to changes in the *penA* gene. A major determinant is the presence of a mosaic *penA* gene in