



POSTER ABSTRACT BOOK

Extracellular electron transfer capability of oral pathogens *Aggregatibacter* actinomycetemcomitans and *Porphyromonas gingivalis*

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Abstract

Polymicrobial oral biofilms, which consist of fermentative-bacteria, are associated with periodontitis, gingivitis and cause systemic diseases1. Unlike aerobic-respiration, fermentation does not require electron acceptors like O₂; and redox-cycling of biological electron-carriers, like NADH, drives the intracellular oxidation and reduction of organic-substrates². Thus, the energy gain is potentially lower than that of respiratory metabolism; however, the high pathogenic activity in anaerobic conditions remained ambiguous³. A few studies have shown that fermentative gut microbes are capable of reducing external electron acceptors via extracellular electron transfer (EET)⁴⁻⁷. EET, a phenomenon initially found in environmental-bacteria, where metabolically generated electrons are transferred to external electron-acceptors through an outer-membrane redox protein complex⁸⁻⁹. Thus, the pathogens colonization in the human microbiome may be supported by their EET capability and is important to explore such potentiality. Here, we electrochemically characterized oral-biofilm pathogens Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis, to examine their EET ability with lactate/glucose. Both strains showed current production on an electrode surface, associated with consumption of substrate¹⁰. The addition of antibiotics that suppress the biosynthesis of membrane or protein showed a significant current decrease, demonstrating that current production reflects the cellular-activity. Further, transmission-electron-microscopy of 3,3'-diaminobenzidine (DAB) stained cells revealed the presence of redox-enzymes on the cell-membrane suggesting a potential EET mechanism via membrane proteins9. These results could be a basis to reevaluate human oral pathogens from an electroactive point of view. The identified EET activity of the two strains can be utilized for an effective test for assessing the impact of antibacterial compounds on the pathogen cellular-activity on an electrode¹¹.

Disruptions in the intestinal microbial ecology associated with C. perfringens

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Abstract

Clostridium perfringens epsilon toxin (ETX) produces a combination of brain and intestine alterations which play a key role in the pathogenesis of enterotoxaemia in ruminants. Although many reports describe gut microbiota changes in relation to neurological or intestinal diseases, no reports describe how neurodegenerative effects of ETX can shape intestinal environment and the resident microbial populations.

In the present study, groups of mice were challenged with sub-lethal ETX doses. After ETX challenge, mice were sacrificed and cecal contents were collected. DNA was extracted and the V3–V4 region of the 16S rRNA gene was amplified. HTS was performed in the Illumina MiSeq platform. Bioinformatics analysis was done with QIIME2. In addition, brains sections were analysed to confirm ETX neurotoxic actions.

In the ETX challenged mice, microbial diversity was reduced and changes in the relative abundance of some taxonomic groups were observed. At the phylum level, a significant increase were observed in the Bacteroidetes while Firmicutes were reduced (p<0.01). Significant reductions were observed in the proportion of Lactobacillus (p<0.05) and Roseburia (p<0.01) genus, while Bacteroides were significantly increased (p<0.01). Neurodegenerative alterations compatible with ETX effects were observed in brain slides.

Observed changes suggest that ETX exposure can result in significant disruptions of gut microbiota, and these alterations can be related with increase biological fitness of C. perfringens in the intestinal environment.

Optimising biofilm production of *Clostridioides difficile* for downstream applications: potential for methodological bias

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Abstract

The CDC named *Clostridioides difficile* an 'Urgent threat' in 2019 as this pathogen causes the most common healthcare-associated infection in the USA. Our lab used ClosTron to disrupt the *dnaK* gene encoding a key molecular chaperone responsible for protein folding and correct heat stress response. The *dnaK* mutant exhibited 50% cell elongation, increased hydrophobicity, and greatly increased biofilm production, measured by crystal violet staining in microtiter plates. Here we investigated the biofilm forming ability of *C. difficile* 630, 630D*erm*, and the *dnaK* mutant using a number of different assay systems.

The three strains were grown individually in 24-well microtiter plates, on glass coverslips, and on semi-permeable mixed cellulose ester membranes. Biofilm properties on each substrate were investigated using crystal violet assays to measure biomass, Triphenyl tetrazolium chloride assays to measure metabolic activity, viable cell counts to count viable cells and *via* the BacLight staining system to measure both live and dead cells within the biofilm where appropriate.

As previously reported, the $630\Delta erm:dnaK$ mutant strain produced significantly more biofilm at 72 h on both plastic and glass substrates compared to the parent Derm and WT 630 strains. In contrast, using a colony biofilm assay method on semi permeable membranes revealed no significant increase in metabolic activity, cell number or biomass in the $630\Delta erm:dnaK$ mutant biofilm compared to the parent and WT strains. Therefore, we conclude that the colony biofilm method is a better representation of an undisrupted, mature biofilm compared to systems that include planktonic cells in liquid media.

Variability in oxygen tolerance among bacterial strains associated with the normal intestinal microbiota

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Abstract

Anaerobic incubation methods are widely used to cultivate pathogenic anaerobes, but recent years have seen increased interest in potentially therapeutic species originating from the normal intestinal microbiota. We compared the abilities of selected anaerobic pathogens, "normal microbiota" and more recently characterized potentially therapeutic strains to grow on agar at 37°C in the presence of increasing oxygen concentrations, using a variable atmosphere workstation to control oxygen concentration in increments of 0.1%. In initial screening with a high inoculum of 10^5 to 10^6 cfu on streak plates, Bacteroides fragilis and Clostridioides difficile strains grew in the presence of up to 2.4% v/v oxygen. Bifidobacterium, Fusobacterium and Finegoldia strains tolerated 0.5 – 1.0% and Eggerthella lenta tolerated 0.1%. Strains of Roseburia, Alistipes, Blautia and Faecalibacterium grew only in strictly anaerobic conditions (<0.01% oxygen) and not in 0.1% oxygen. For strains tolerating >0.1% oxygen in initial experiments, percentage recoveries of smaller inocula (100 – 300 cfu on surface spread plates) were determined in atmospheric oxygen concentrations increasing by 0.1% increments, in comparison with strictly anaerobic colony counts. In 2.0% v/v oxygen, inoculum recovery for $2 \times B$. fragilis and $1 \times C$. difficile, was >90%, while recovery of a second C. difficile strain was 25%. For F. magna, inoculum recovery ranged from approximately 100% in 0.1% oxygen to <1% in 0.5% oxygen. These findings demonstrate the variable oxygen tolerance of obligately anaerobic bacteria and emphasize the need for stringent anaerobiosis when culturing the more recently characterized strains currently being developed as live biotherapeutic products.

Efficacy of UV-C disinfection upon Clostridioides difficile spores

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Abstract

Clostridioides difficile is the causative agent of antibiotic-associated diarrhoea in susceptible patients. The spore form of the organism is able to withstand microbicidal insult and exhibit increased adherence to clinical surfaces within healthcare environments. Its spores are usually transmitted via the faecal to oral route from contaminated surfaces; thus appropriate disinfection of these surfaces is critical. Recent studies have highlighted that use of chlorine-releasing agents at UK government recommended concentrations is not optimal for effective disinfection of *C. difficile* spores on these surfaces; however, germicidal UV-C light may present a viable alternative to disinfection with biocides. This could limit transmission in healthcare environments. The effectiveness of a UV-C emitting cabinet (253.7 nm) was determined against spores from strains R20291 (027 PCR Ribotype) and CD630 (012 Ribotype) at a range of concentrations between $1x10^5 - 1x10^9$ spores/ml on a range of clinically-relevant surfaces. Sporicidal activity against spores suspended in inert liquid was also determined. Spore survival was determined via anaerobic agar culture and a plate transfer method after exposure at a range of times. Our results demonstrate a maximum 4-log reduction in spore viability after UV-C exposure across several clinical surfaces. However, spores deposited on surgical scrubs were able to survive UV-C disinfection, showing limitations of the technology. This study demonstrates the feasibility of using UV-C technology as a potential alternative to biocides to disinfect clinical surfaces.

An indolent presentation of brain abscess due to a slow growing organism

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Abstract

Propionibacterium propionicum is an anaerobic gram positive bacteria. It's rarely described as to cause brain abscess. We report the isolation of this organism from a brain abscess and emphasise the problems associated with it's diagnosis. A 62 year old lady with a history of acromegaly underwent emergency surgery for recurrent pituitary lesion felt to be apoplexy. Four weeks later she presented with headache and confusion. Comorbidities included autoimmune diseases on long term steroids and previous use of immunosuppressants. Clinical examination showed neck stiffness. Brain MRI demonstrated an enhancing lesion in the fourth ventricle causing secondary obstructive hydrocephalus requiring endoscopic third ventriculostomy. Blood tests, CSF analysis and whole body PET scan were unable to differentiate between infection and malignancy. Excision of the lesion was felt very high risk as deep in the tectal area. A sporadic trial of antibiotics, high dose steroids and immunotherapy was tried. Patient developed worsening headache with progression of the lesion. Underwent craniotomy and pus was found which isolated P. propionicum sensitive to penicillin and betalactams. Treated with intravenous meropenem for a year. There was significant reduction in the size of the abscess and hydrocephalus on brain MRI at 8 months post-diagnosis. P. propionicum has been isolated and associated with lesions of the lacrimal glands, lung and abdomen. We could find only two case reports of brain abscess due to P.propionicum. Identification of P.propionicum in the microbiology laboratory can be difficult requiring anaerobic growth conditions and extended cultures. Treatment is prolonged antimicrobial therapy.

Prediction and Assessment of Potential Microbial Substrate Utilisation Among More Recently Identified Health-Associated Gut Taxa

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Abstract

The contribution of the gut microbiota to health and disease is becoming ever more apparent in the last number of years, due to developments in DNA sequencing technology and more well-defined cultivation techniques. This has resulted in the identification of health-promoting bacteria. Until recently, prebiotics, non-digestible food substrates which are selectively utilised by beneficial bacteria, were employed with a view to increasing the growth of well-established health promoting bacteria, namely *Lactobacillus* and *Bifidobacterium*. However, other beneficial bacteria recently revealed may also be targeted to enhance their growth as they establish themselves as the next generation of health-promoting microbes. These include anaerobes such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* and *Eubacterium rectale*. Identification of growth substrates/bioactives through the analysis of genome sequence data can aid in elucidating which substrates may best enhance the growth of these microbes which are often difficult to grow.

The phenotypic microbial trait analyser, Traitar, can predict 67 phenotypes based on the genome sequence inputted. Some of these traits include substrates that could potentially be utilised by the bacteria. Another tool, CarveMe, which has been created with the aim of making metabolic modelling more user-friendly, was also used with the same genomes. A select number of substrates identified in both tools have been chosen to be evaluated *in vitro* in order to establish the accuracy of these predictive tools as well as giving an indication as to how these beneficial microbes can be modulated through dietary components.

In-Vitro Vitamin B12 Production by Human Gut Microbial Strains

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Abstract

Background: Recent omics research has shown that the human gut microbiota can act as a B-vitamins biosynthetic factory. Despite this, little is known about the actual range in which vitamin-B12 is produced by gut bacteria.

Methods: Based on *in silico* prediction, we investigated the *in vitro* intra- and extracellular vitamin-B12 (as cyanocobalamin) production capabilities of three human gut vitamin-B12 producers: *Anaerostipes caccae* DSM 14662, *Blautia hydrogenotrophica* DSM 10507, *M*arvinbryantia *formatexigens* DSM 14469, and a non-producer strain, *Akkermansia muciniphila* DSM 22959. Strains, in biological triplicates, were grown in their respective cultivation medium without vitamin B12 for 48 hours. Vitamin-B12 was quantified using a UHPLC/UV based method coupled with a single quadrupole mass spectrometer.

Results: No extracellular vitamin-B12 was detected. As expected, all tested vitamin-B12 producing strains produced intracellular vitamin-B12, with M. formatexigens exhibiting the highest production (110 \pm 10 ng/mL), and A. caccae the lowest (75 \pm 2.1 ng/mL). No vitamin-B12 was detected for A. muciniphila. In addition, a detailed mass spectroscopy analysis revealed that B. hydrogenotrophic is capable of synthesizing an undefined B12 vitamer.

Conclusion and perspective: Our findings suggest that human gut microbial strains can produce vitamin-B12 but retain it intracellularly. Further screening of more gut microbes and their B12 production kinetics will provide a representative picture of potential bacterial vitamin-B12 production and trophism in the gut.

Patient risk factors, blood variables and bacterial ribotypes predict disease outcome in *Clostridioides difficile infection*

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Abstract

Background: *Clostridioides difficile* is the leading cause of nosocomial and antibiotic-associated diarrhoea worldwide. There is a high relapse rate of CDI, with current data indicating that 15-35% of patients relapse with a second CDI infection within two months post-treatment, which puts them at greater risk of further relapse (65%), with poor prognosis and increased mortality.

Methods: In this longitudinal CDI study, we assessed 535 subjects to determine disease progression of patients with nosocomial diarrhoea. Here we compare CDI associated diarrhoea with healthy controls and non-CDI associated diarrhoea groups to identify markers of clinical outcomes of CDI patients using medical history, routine blood tests, treatment regimen, laboratory detection and PCR-ribotyping of *C. difficile* isolates. We analysed patient metadata to determine the impact of advanced age, sex, BMI, use of PPIs and antibiotics on the risk of *C. difficile* infections and prognosis.

Results: We stratified the CDI group into CDI cleared, relapse, re-infected, continuously colonised and dead groups. The most significant patient risk factors for CDI patients were prior exposure to medium-and-high-risk antibiotics, proton pump inhibitors and advanced age (>75 years). Unexpectedly, we also identified a group of 18 to 44 year-old patients with increased risk of CDI. However, 80% of these patients had underlaying comorbidities, including cancer, gastrointestinal diseases or infections.

Conclusion: Patients that died from CDI-related complications had an overactive inflammatory response, manifesting as leucocytosis, hypoalbuminemia and elevated CRP levels. This suggests that the inflammatory process, not renal or hepatic dysfunction, could be utilized to predict the patient's clinical prognosis.

Antimicrobial Susceptibility Patterns of Anaerobic Bacteria at an Irish University Hospital over the past Decade

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Abstract

Background

Various studies have demonstrated poor outcomes in infections caused by anaerobic bacteria due to inappropriate therapy, directly due to emergence of resistant strains. To date there is a paucity of available data on antimicrobial resistance trends of anaerobic bacteria among the Irish population, and our study aims to determine such patterns among isolates processed at our institution over the previous decade.

Methodology

Selected anaerobic bacteria isolated from clinical specimens processed at our laboratory from January 2010 to January 2020 inclusive were collected and studied. Bacteria were identified using MALDI-TOF, with VITEK and E-tests for antimicrobial susceptibility testing. Data was processed through WHONET.

Results

A total of 2098 clinically significant anaerobic bacterial isolates were reviewed during the study period; with the majority of isolates being *Bacteroides spp* (32.79%, n=688) and *Clostridium spp* (18.68%, n=392). *Bacteroides spp* and *Prevotella spp* were unsurprisingly highly penicillin-resistant at 98.8% (n=680) and 77% (n=114) respectively. Resistance rates against metronidazole were expectedly high amongst *Propionibacterium spp* (100%, n=341), *Actinomyces spp* (98.8%, n=79), *Lactobacillus spp* (59.7%, n=95) and *Bifidobacterium spp* (53.8%, n=7); whilst remaining anaerobes were largely susceptibility. All isolates were particularly susceptible to co-amoxiclav, piperacillin-tazobactam and meropenem. Clindamycin exhibited a more variable susceptibility pattern, from 6.7% resistance in *Staphylococcus saccharolyticus* to 81.1% resistance in *Prevotella spp*.

Conclusion

Metronidazole and beta-lactams remain highly efficacious against the majority of anaerobic isolates reviewed, and remain the backbone of empiric therapy in suspected infections. However, it remains important for periodic surveillance of resistance trends.

Global discovery of Hfq-associated sRNA-target networks in C. difficile

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Abstract

Small regulatory RNAs are well-known regulators of virulence pathways in many pathogenic bacteria. However, little is known about sRNA-based gene regulation in the gram-positive human pathogen *Clostridioides difficile*.

To test whether the RNA-binding protein Hfq exerts a global post-transcriptional control similar to that in gram-negative bacteria, we performed Hfq-3×FLAG immunoprecipitation followed by RNA sequencing (RIP-seq). We further performed rifampicin assays in a WT and Δhfq strain to asses if Hfq affects transcript stabilities of Hfq-associated sRNAs. Finally we characterized the target spectrum of the Hfq-bound sRNA CDIF630nc085 by pulse-expression, in-line probing and *in silico* prediction of target mRNAs.

RIP-seq identified 39 ncRNA bound by Hfq in addition to a vast number of mRNAs (124 5'UTRs, 179 CDSs and 157 3'UTRs), encoding genes involved in amino acid biosynthesis, membrane transport, signal transduction, sporulation, motility and adhesion. Rifampicin treatment resulted in decreased sRNA half-lives and, in some cases, steady-state levels when comparing C. difficile WT and Δhfq , providing evidence that Hfq affects sRNA stability. Finally, characterization of CDIF630nc085-target interactions by pulse-expression revealed a regulation of ethanolamine utilization. In-line probing of CDIF630nc_085 with the ethanolamine-specific two-component response regulator eutV verified a direct interaction. Furthermore, when grown in minimal medium supplemented with ethanolamine, both Δ CDIF630nc085 and Δhfq exhibited accelerated growth when compared to the WT.

In summary, we provide the first example of extensive Hfq-dependent post-transcriptional regulation in a gram-positive bacterium and identify a regulatory function of CDIF630nc085 in ethanolamine utilization, an abundant intestinal carbon and nitrogen source known to impact *C. difficile* pathogenicity.

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Gut microbiota composition in health-care facility-and community-onset diarrheic patients under *Clostridioides difficile* infection

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Abstract

Background: The role of gut microbiota in the establishment and development of *Clostridioides difficile* infection (CDI) has been widely discussed. Studies showed the impact of CDI on bacterial communities and the importance of some genera and species in recovering from and preventing infection. However, most studies have overlooked important components of the intestinal ecosystem, such as eukaryotes and archaea.

Methods: We investigated the bacterial, archaea, and eukaryotic intestinal microbiota of patients with health-care-facility- or community-onset (HCFO and CO, respectively) diarrhea who were positive or negative for CDI, using 16S and 18S rRNA deep sequencing

Results: The CDI-positive groups (CO/+, HCFO/+) showed an increase in microorganisms belonging to Bacteroidetes, Firmicutes, Proteobacteria, Ascomycota, and Opalinata compared with the CDI-negative groups (CO/-, HCFO/-). Patients with intrahospital-acquired diarrhea (HCFO/+, HCFO/-) showed a marked decrease in bacteria beneficial to the intestine, and there was evidence of increased Archaea and *Candida* and *Malassezia* species compared with the CO groups (CO/+, CO/-). Characteristic microbiota biomarkers were established for each group. Finally, correlations between bacteria and eukaryotes indicated interactions among the different kingdoms making up the intestinal ecosystem.

Conclusions: We showed the impact of CDI on microbiota and how it varies with where the infection is acquired, being intrahospital-acquired diarrhea one of the most influential factors in the modulation of bacterial, archaea, and eukaryotic populations. We also highlight interactions between the different kingdoms of the intestinal ecosystem, which need to be evaluated to improve our understanding of CDI pathophysiology

eGUT: A Predictive Tool to Understand Gut Microbiota and Host Interactions

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Abstract

Background

Current research reveals numerous links between the gut microbiota and human health. The majority of microbiota research is performed *in vivo* in rodents, which can be costly, requires ethical considerations and may not always be a reliable analogue of human guts.

Methods

We are building an *in silico* platform for gut modelling – eGUT. eGUT can simulate the activities and interactions of microbes in the gut and the mucosa, which can be connected to a 'rest of the body' compartment. Individual-based modelling allows us to investigate how the behaviours of and interactions between individual cells lead to observed phenomena. eGUT is a highly flexible system that allows users to combine different behaviours and traits in different ways and model a wide variety of organisms.

Results

We have carried out various numerical tests to assess the accuracy of eGUT's reaction-diffusion system and used linear discriminant analysis to identify parameter space in which mucus behaves as a sticky, spreading fluid that coats the epithelium. Validation with simple two-species test cases is underway using both HuMiX and SHIME *in vitro* models. Later *in vivo* validation based on published results is also planned.

Outcome

eGUT allows users to model a wide range of species and metabolic activities in the gut environment without requiring extensive understanding of programming languages. It will be open-source and freely available upon release. Ongoing validation, as well as engagement with potential users, will be used to improve the platform. Researchers interested in using eGUT are encouraged to enquire.

Exploring the mechanisms of immunomodulation by a commensal Clostridium species

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Abstract

The microbiome is intricately linked to human health and, when dysregulated, can cause disease. It has been shown that specific members of the intestinal microbiota regulate immunity, a finding that offers an approach for treating autoimmune disease through microbiome engineering. In previously published work, we identified Clostridium immunis, a new human-derived commensal bacterial species that protects mice against colitis. To understand the host immunological response to C. immunis, we performed comprehensive flow cytometric analysis of the colonic immune system of C. immunistreated mice. We observed a significant decrease in the number of group 3 innate lymphoid cells (ILC3s), a rare but critical tissue-resident immune cell population that has been implicated in autoimmune diseases. We hypothesize that C. immunis produces a bioactive molecule that downregulates ILC3s. To identify the genetic determinants of ILC3 downregulation by C. immunis, we compared the ability of closely related clostridia to modulate ILC3-produced cytokines in a colonic explant model. By excluding genes encoded by two inactive clostridia strains, we managed to identify 40 genes that are associated with ILC3 modulation. We similarly observed that C. immunis culture supernatant contained activity. By excluding genes that have predicted intracellular products, we further narrowed this list to 8 candidate genes. Critically, we have recently developed a method to perform targeted genetic knockouts in C. immunis, and are currently generating mutants for these genes. This work will therefore identify the gene product required for ILC3 modulation and potentially identify novel classes of bacterially derived molecules to treat ILC3-driven disease.

The resistance to beta-lactams in the isolates belonging to *Prevotella* genus recovered from patients with periodontitis

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Abstract

The genus Prevotella is one of the most important anaerobic gram-negative bacteria in oral cavity and may cause various infections. Here, we aimed determine the rate of resistance to these antibiotics and antibiotic resistance genes. A total of 129 periodontal samples were collected from patients with periodontitis. The samples were cultured on KVLB and Brucella blood agar. Molecular identification was performed by 16S rRNA PCR- sequencing. Antibiotic susceptibility of Prevotella isolates was determined by MIC Test Strips. Beta-lactamase-producing isolates were identified using a nitrocefin disk. The presence of bla_{TEM}, cfxA, and mobA antibiotic resistance genes was detected by PCR and CfxA variants were identified based on amino acid and nucleotide substitution differences. A total of 80 isolates were recovered, which were confirmed by phenotypic tests and identified by 16S rRNA gene sequencing at the species level. Two isolates were identified as P. oris and P. nigrescens. Prevotella isolates were sensitive to the antibiotics tested. The frequency of cfxA gene among Prevotella isolates and other isolates recovered from periodontitis cases were (12.5%). The bla_{TEM} gene was not detected in any of the isolates and the mobA sequence was detected in 13.75% of the isolates. The highest frequency was observed in CfxA2 variant, followed by CfxA3. The isolates of the genus *Prevotella* were sensitive to the beta-lactams of ampicillin, amoxicillin, amoxicillin / clavulanic acid, ampicillin / sulbactam, and cefxime. However, more than one-tenth of isolates from periodontitis carried the antibiotic resistance genes which might be transferable to other bacterial community of mouth.

Amoxicillin Inhibits Methanogenesis and Increases Total Methane Production

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Abstract

Antibiotics are widely used in the UK and have enabled our modern way of life. But even after use, some of the antibiotic remains in our urine. As a result, they are regularly observed in the influent/effluent of traditional urban wastewater treatment plants (WWTPs). Antibiotics are present at low concentrations due to dilution effects such as stormwater runoff and other waste within large catchments. In rural areas decentralised biological waste treatment systems, such as septic tanks (which treat waste through anaerobic digestion and settlement) are common. The presence of antibiotics in WWTP effluent is strictly regulated, however decentralised waste treatment, particularly septic tanks, are rarely monitored. Yet, effluents from these systems are discharged directly to the environment. It is therefore not known how the presence of harmful organic content, resistance genes, or the parent antibiotic compound is in the effluent and it's potential to negatively impact surrounding groundwater and subterranean ecosystems.

This poster summarises ongoing research on the impact of exposure to amoxicillin (the UKs most commonly prescribed antibiotic) on an anaerobic microbial community. Lab-scale systems were exposed to amoxicillin at varying concentrations as might be found on a typical septic tank for changes in biogas production, the presence of antimicrobial genes in the population, and the efficiency of organics removal in the effluent. The impact of prescribed antibiotic concentrations on treatment efficiency, including the removal efficiency of amoxicillin, the microbial community performance and the development of amoxicillin resistant bacteria was then determined.

