ANAEROBE 2023

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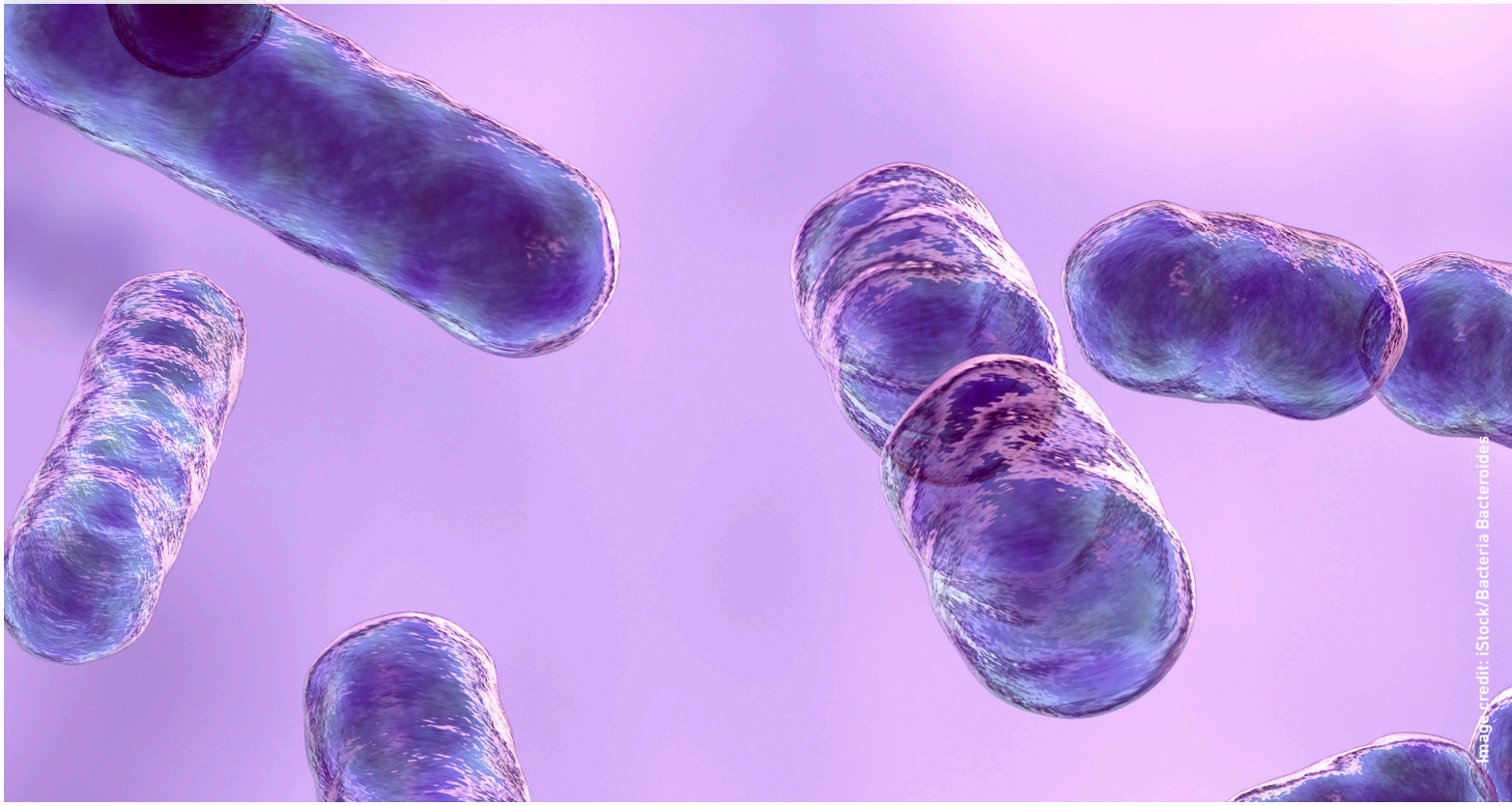
ABSTRACT BOOK



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FOCUSED MEETING 2023
Leonardo Hotel Cardiff

MICROBIOLOGY SOCIETY

Offered Talks

Furious *Fusobacterium*

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Abstract

Fusobacterium necrophorum is an opportunistic anaerobe which colonises the human oral cavity and respiratory tract. *F. necrophorum* is particularly associated with the dreaded Lemierre's syndrome, but also tonsillitis and peritonsillar abscesses, sinusitis, otitis media and brain abscesses. Here we present the case of an 18-year old male who presented with significant bilateral periorbital cellulitis, having been generally unwell with “flu-like” symptoms over the preceding week. A periorbital washout and drainage was performed and intra-operative samples as well as blood cultures yielded *F. necrophorum*. Follow-up radiological imaging reported maxillary, ethmoid and sphenoid pyogenic sinusitis with multiple enhancing collections along the temporalis musculature, which warranted further multiple surgical interventions. Of note, this gentleman had no notable background history and no common cause of immunocompromise was elucidated. Antimicrobial therapy was rationalised to benzylpenicillin and metronidazole, guided by antimicrobial susceptibility testing. A follow-up Magnetic Resonance Imaging revealed new osteomyelitis of the skull vault and worsening radiological signs of meningitis; which was particularly troubling as the patient remained clinically stable throughout with clinical resolution of all symptoms and normal biochemical markers. It was presumed then that these findings were radiological progression of the original infection, as opposed to new pathology and he completed a 6-week course of intravenous antimicrobial therapy, facilitated through Outpatient Parental Antibiotic Therapy. Our case highlights the often insidious nature of infections caused by *F. necrophorum* and its potential sequalae. Noteworthy is also the evident disparity in clinical or physical characteristics and the extent of disease.

Anaerobic surveillance in the Netherlands: the antimicrobial susceptibility profile of Dutch clinical *Bacteroides* and *Prevotella* isolates

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Abstract

Background

Recently, five multidrug resistant *Bacteroides fragilis* isolates were found at different Dutch hospitals, emphasizing the need for surveillance of antimicrobial resistance among clinical anaerobic bacteria in the Netherlands.

Method

From March-June 2021, 8 clinical microbiology laboratories across the Netherlands collected 20-25 *Bacteroides* and 10-15 *Prevotella* isolates per center. Isolates were sent to the UMCG and identification was confirmed using MALDI-TOF MS. Antimicrobial susceptibility testing was performed using agar dilution and when needed supplemented with Etest. MIC values were interpreted according to EUCAST v11.0 (2021) breakpoints, when not available CLSI breakpoints were used.

Results

In total, 165 *Bacteroides* and 96 *Prevotella* isolates were collected. *Bacteroides thetaiotaomicron/faecis* was the most resistant species, with high resistance to piperacillin-tazobactam (41.9%), amoxicillin-clavulanic acid (29.0%) and clindamycin (41.9%). Resistance among *B. fragilis* was lower, 1.6%, 6.5%, and 17.7%, respectively. Among the *Bacteroides* isolates, resistance to meropenem and imipenem was 3.0% and 5.5%, respectively. Among the *Prevotella* species, *Prevotella buccae* had the highest resistance to

amoxicillin-clavulanic acid (7.1%), while all *Prevotella bivia* isolates were susceptible. *P. bivia* had the highest resistance to amoxicillin (77.0%), and clindamycin (33.3%). None of the *Prevotella* isolates were resistant to piperacillin-tazobactam or a carbapenem. One *B. thetaiotaomicron/faecis* isolate was resistant to metronidazole and two *Prevotella* isolates. Differences in resistance rates between the different centers were observed.

Conclusion

Resistance to carbapenems and metronidazole was low, with 3.2% and 1.1%, respectively. Antimicrobial susceptibility patterns differed per location. Regular surveillance is needed to monitor trends in resistance and to improve empirical treatment.

Analysis of the pangenome of *Fusobacterium nucleatum* subspecies *polymorphum* reveals variation in adhesin repertoire and recombination between subspecies.

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Abstract

Fusobacterium nucleatum is an anaerobic commensal of the oral cavity, associated with periodontitis and extra-oral diseases, including colorectal cancer. Previously, our studies have shown an increased relative abundance of this bacterium on potentially malignant oral leukoplakia (OLK).

Using direct culture, we found that 79% of *Fusobacterium* species isolated from OLK patients were *F. nucleatum* subspecies *polymorphum*. To characterise these isolates, we carried out whole genome sequencing (Illumina and MinION) and pangenome analysis with Panaroo. 76 isolate genomes, including 60 genomes sequenced in this study and 16 genomes recovered from GenBank, were included.

Analysis of the pangenome has shown that these isolates possess a relatively small core genome, compared to the larger accessory genome. A phylogenetic tree based on the alignment of the core genome shows that isolates from healthy and OLK sites of the same patient are genetically closely related and cluster together on the tree. A large repertoire of adhesins was identified and copy number of major adhesins, including FadA and Fap2, was shown to vary greatly between isolates. fastGEAR was used to investigate recombination at adhesin encoding loci and we detected evidence of recombination not only between strains of *F. nucleatum* subspecies *polymorphum*, but also between subspecies *nucleatum*, *animalis* and *vincentii* genomes. This heterogeneity among isolates in genotype is also seen in phenotypic assays such as hemagglutination, serum resistance and adhesion to different cell lines.

Overall, it appears that *F. nucleatum* subspecies *polymorphum* isolates exhibit great variability in adhesin gene repertoire, shaped by gene exchange and inter-strain recombination.

Diversity of *Coriobacteriia* within the environment

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Abstract

The class *Coriobacteriia*, which belongs to the phylum *Actinomycetota,* represents bacteria prevalent in the gut microbiota of animals but their diversity is poorly understood. Over 200 novel species of the class have been identified through culturomics studies and recovery of metagenome-assembled genomes (MAGs). However, the taxonomy of the *Coriobacteriia* is still unclear due to lack of sensitivity of 16S rRNA gene-based analysis. Because of this, some species are misclassified and there are also inaccurate descriptions of species in the literature. This study aims to define the taxonomy of the class *Coriobacteriia* using publicly available sequence (NCBI, GTDB) data, including 319 (210 MAGs and 109 isolates) genomes of *Coriobacteriia* and, where available, associated 16S rRNA gene sequences. Only 177 genomes were of high quality (>90 % completeness, <5 % contamination). Phylogenetic and functional (eggNOG-mapper) analyses were undertaken, and average nucleotide identity (ANI), average amino acid identity (AAI) and percentage of conserved-protein were determined (PCOP). Phylogenetic trees based on 16S rRNA gene sequences or whole-genome sequences showed '*Olegusella massiliensis*' and '*Candidatus* Coprovicinus avistercoris' belong to family *Atopobiacaeae,* and the novel family UMGS124 clusters with the family *Coriobacteriaceae*. Furthermore, the ANI showed there were 16 species with >95 % ANI and high values in both AAI and POCP. This suggested they might be the same species. Principal component analysis of COG profiles confirmed the affiliation of UMGS124 with *Eggerthellaceae*. In conclusion, greater care needs to be taken when incorporating novel species derived from culturomics studies or MAGs into existing taxonomic frameworks.

Suppression of butyric acid levels following multiple antibiotic treatments in an in vitro gut model of gut dysbiosis

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Abstract

Background

Butyric acid (BA) is important for intestinal barrier maintenance and function, and the microbiota community. The intestinal microbiome plays an important role in its production, with recent studies indicating a potential role for BA as a marker for dysbiosis. We investigated the metabolomic changes occurring within an in vitro gut model (GM) of antimicrobial-mediated dysbiosis, with reference to BA.

Methods

In-vitro GMs (n=4) were seeded with healthy donor faeces and run to establish steady-state bacterial populations (SS). GMs were sequentially instilled with amoxicillin (2.4mg 3x daily/5days), ciprofloxacin (39mg 2x daily/5 days) and piperacillin-tazobactam 83mg 1x daily/ 5days). At 3 weeks post-antibiotic challenge, GMs were challenged with Clostridioides difficile (CD)and KPC-producing strain of Klebsiella pneumoniae (KPC) to test for colonisation resistance (CR). Samples were taken for culture, CD toxin, shotgun whole-genome sequencing, and metabolic profiling with High-Performance liquid chromatography/Mass Spectrometry (HPLC).

Results

Marked changes in bacterial abundance and composition were observed following multiple antibiotic instillations. CD and KPC were able to colonise the GMs, indicating loss of CR. At experiment end, microbial abundance and composition to a genus level had returned to that seen prior to antibiotic instillation. Metabolic profiling showed significant reductions in the relative abundance of BA (-2.75 log2-fold change (P-value 2.93e-3)), concurrent with CD and KPC colonisation and CD Toxin production. BA failed to return to pre-antibiotic levels.

Discussion

While bacterial abundance and diversity recovered after multiple antibiotic instillations, metabolic changes, specifically BA levels, did not.

Reduced BA may be a potential marker for dysbiosis.

Bile salt hydrolase aminoacyltransferase activity from gut anaerobes expands the human bile acid pool

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Abstract

Bile acids (BAs), the dominant chemicals in bile, play key roles in nutrient absorption, cell signaling, and microbiome regulation. Host derived BAs cholate (CA) and chenodeoxycholate (CDCA) are conjugated with glycine or taurine in the liver prior to undergoing enterohepatic circulation. Approximately 5% of BAs are not reabsorbed, instead entering the colon and undergoing metabolism by our gut microbiota. Deconjugation, the "gateway reaction" of BA metabolism, is performed by bile salt hydrolase (BSH) as it liberates glycine or taurine from host-conjugated BAs. Here, we show that BSH from *Clostridium perfringens* and other gut bacteria can also ligate amino acids to BAs, expanding the diversity of the BA pool. Interestingly, different gut bacteria produce highly varied amino acid conjugation profiles. Comparisons between BSH protein sequences suggested that Asn82 plays a role in dictating amino acid ligand diversity and overall rates of reconjugation. Indeed, site-directed mutation and expression of bsh^{N82Y} in *E. coli* resulted in reduced overall MCBA production with enrichment of glycine conjugates compared to WT. We sought to determine if a BA-enzyme intermediate forms at residue Cys2, similar to deconjugation, and expression of bsh^{C2A} results in complete ablation of production. Further analysis suggests an optimal pH of 5.3 for reconjugation, slightly more basic than the pH 4.7 optimum for deconjugation. Together, our findings suggest that BSH, one of the most highly studied enzymes involved in microbial BA metabolism, plays a much broader role than previously thought in shaping the human bile acid pool and affecting gut or liver function.

Invasion and attachment dynamics of Fusobacterium nucleatum subspecies into colorectal cancer cells

Onalenna Neo

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Abstract

A growing body of research has shown an association of certain oral bacteria with colorectal cancer (CRC). Of particular interest is the oral anaerobic bacterium Fusobacterium nucleatum, which has been found by several studies to be enriched in CRC tissues compared to controls. This study here investigated the adhesion and invasion of colorectal cancer (CaCo-2) cells by three subspecies of F. nucleatum: ssp. animalis, ssp. nucleatum and ssp. polymorphum through adhesion and invasion assays.

F. nucleatum subspecies showed different rates of adhesion to CaCo-2 cells with the ssp. polymorphum showing a statistically significant increase in adhesion compared to the others. Furthermore, the role of tetraspanin 6 (Tspan 6), a protein expressed by eukaryotic cells and known to be involved in adhesion was determined. The subspecies showed differential adhesion to Tspan 6 expressing cells. Using deletion mutants, two major F. nucleatum adhesins Fap2 and FadA were found to be important for adhesion and invasion to Caco-2 cells. Finally, adhesion/invasion experiments revealed that rate of adhesion was bacterial growth phase dependent. Transcriptomics analysis is being performed to investigate this further.

Understanding the differences between F. nucleatum subspecies in invasion/adherence of/to CRC cells can uncover specific mechanisms which are key to their role in CRC, and this can inform diagnostic and therapeutic interventions for CRC.

A comparative study of the vaginal microbiome of women with *Trichomonas vaginalis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Abstract

Chlamydia trachomatis (CT), *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis* (TV) are three most common curable genital sexually transmitted infections (STIs).

Materials/methods: A total of 327 vaginal swabs specimens were originally collected from women with vaginitis who visited 8 hospitals in Northern Taiwan. There were 53 STI-positive cases among the vaginitis patients. The vaginal bacterial composition of three groups, TV-infected women (n = 11), CT-infected women (n = 38) and NG -infected women(n = 4) were characterized by deep sequencing of barcoded 16S rRNA gene fragments (V3–4) using Illumina MiSeq.

Results: The results revealed that both TV, CT and NG infection can increase vaginal bacterial microbial richness and diversity. Bacterial α-diversity, by both Chao1 and Shannon's indices, was significantly higher, and respectively, among women with either TV than among CT or NG (mean α-diversity TV > CT > NG-infected). At the species level, women infected with TV had a significantly higher abundance of *Sneathia sanguinegens* (11.16%), *Gemella asaccharolytica* (6.04 %) and *Prevotella bivia* (5.67%) compared to both NG and CT-infected women, whereas women infected with CT had a significantly higher abundance of *Gardnerella vaginalis* (12.46%) and *Prevotella amnii* (1.20%), and NG-infected women had a significantly higher abundance of *Lactobacillus iners* (33.55%).

Conclusions: The vaginal microbiomes of TV, CT and NG-infected women were markedly different from each other. Understanding how these bacterial species increase a woman's risk of STIs acquisition could help to guide the development of novel strategies to reduce women's risk of STIs.

Microvascular endothelial cells display distinct inflammatory responses to different periodontal-associated oral anaerobes

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Abstract

Periodontitis is a chronic inflammatory disease of the gingival mucosa, where tissue damage is caused by a combination of bacterial virulence factors and an inappropriate host inflammatory response. Anaerobic oral bacteria can invade the bloodstream at diseased sites and enter the circulation. DNA of these anaerobes has been found in atherosclerotic plaques and several clinical studies have associated periodontitis with cardiovascular disease. However, there is limited data on how different oral anaerobes interact with endothelial cells and if this interaction favours a proinflammatory plaque forming environment.

Human microvascular endothelial cells (HMEC-1) were incubated with the oral anaerobes *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Fusobacterium nucleatum ssp nucleatum* (Fnn) or *polymorphum* (Fnp) at increasing MOI (0.1 - 1000) for 4 hours, followed by conditioned medium collection and RNA isolation. Reverse transcription-qPCR was used to measure fold-change in cytokine gene expression, while ELISA was used to measure protein secretion levels.

Fnp and Fnn caused a significant ($p<0.05$) MOI-dependent fold-change increase in gene expression and protein release of proinflammatory cytokines CXCL8, CCL2 and IL-6, compared to uninfected controls, at MOI 100 and 1000. Tf also caused a significant increase in gene expression of CXCL8 and CCL2 at MOI 1000. In contrast, Pg did not cause any significant differences.

These data indicate that endothelial cells have different inflammatory responses to different oral anaerobes, suggesting that periodontal-induced cardiovascular disease may be dependent on specific bacterial species. Future work using multispecies biofilm infections may identify bacterial combinations important in the disease process.

Immunogenicity of *Fusobacterium nucleatum* subspecies towards human neutrophils

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Abstract

Introduction:

Periodontitis is caused by dysregulated host immune-inflammatory responses to pathogens within dysbiotic oral biofilms. The most abundant immune cells in periodontal tissues, neutrophils, perform key antimicrobial functions, but can also cause collateral tissue damage, when exposed to such pathogens. *Fusobacterium nucleatum*, an anaerobic opportunistic pathogen, is a key structural bacterium in periodontal biofilms. Five subspecies have been described: *animalis, fusiforme, nucleatum, polymorphum* and *vincentii*. This study aimed to investigate differential responses of human neutrophils to *F. nucleatum* subspecies.

Methodology:

Human neutrophils isolated from peripheral blood were stimulated with planktonic and biofilm-grown *F. nucleatum* subspecies. Generation of intra- and extracellular reactive oxygen species (ROS) was quantified, as well as neutrophil extracellular trap (NET) formation. Secretion of cytokines, neutrophil elastase and matrix metalloproteinase-9 was quantified by enzyme-linked immunosorbent assay (ELISA).

Results:

At a species level, biofilm-grown *F. nucleatum* stimulated more robust and rapid responses by neutrophils when compared to planktonic bacteria. Furthermore, significant subspecies-specific differences were observed: planktonically-grown *F. nucleatum* ssp. *polymorphum* elicited the highest mean ROS generation as well as NET formation, whilst planktonic ssp. *vincentii* triggered the lowest response. Interestingly, ssp. *polymorphum* stimulated the lowest and ssp. *vincentii* the highest neutrophil cytokine release.

Conclusion:

F. nucleatum subspecies elicit differential responses from neutrophils in terms of ROS generation, NET formation and cytokine release. Understanding subspecies-specific immunogenicity and pathogenicity of *F. nucleatum* may help to identify novel bacterial and/or biofilm targets for antimicrobial therapies, as

well as neutrophil targets to abrogate tissue damage in periodontitis and *F. nucleatum*-related systemic diseases.

POSTERS

P001

“Probiotic Property of Lactobacillus spp Isolated from Different Food Sample”

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Abstract

Lactic acid bacteria (LAB) are a group of Gram positive, non-sporing cocci or rod shaped bacteria, which produce lactic acid as the major end products of the fermentation of carbohydrates and a significant group of probiotic organism. Probiotics are live microorganism, associated with various beneficial effects in human and animal health. This study was executed with the objective to isolate, screen and identify lactic acid bacteria from indigenous fermented food products and analyze its probiotic characteristics. A total of 20 food samples [fermented leafy vegetables (Gundruk), yoghurt (Dahi), bamboo shoot (Tama), pickle (Achar) and fresh cabbage] were selected for the isolation of lactic acid bacteria. Lactobacillus spp. were isolated and identified following Gram’s reaction and various biochemical tests. The isolated LAB were screened for their ability to show probiotic nature by detecting resistant to gastric pH 3.5 and tolerance to bile salts of concentration (0.3%) under in vitro conditions. Their susceptibility to selected antibiotics was determined by modified Kirby Bauer disc diffusion method. Similarly, antibacterial property of isolated LAB was tested against some pathogenic bacteria. Fifteen LAB were isolated from fermented food products and among them two exhibited promising probiotic properties. The finding of the study indicates indigenous fermented food products harbor Lactobacillus species having potential probiotic properties.

P002

Effect of Fluoride on the Growth of Probiotic and non-probiotic microorganisms

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Abstract

Fluoride can prevent dental caries by inhibiting demineralisation and promoting remineralisation of teeth. Topical fluoride can also affect the physiology of oral microbiota, inhibiting cellular enzymes. However, the effect of systemic fluoride on gut microbiota is unknown.

Probiotic and non-probiotic strains were selected based on their importance in the human gut to evaluate fluoride's impact on their viability and growth. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and growth dynamics were assessed on selected probiotics strains (*Lactobacillus fermentum, L. sakie, L. plantarum, L. buchneri, L. brevis, L. rhamnosus, L. paracasie, Bifidobacterium longum and B. breve*) and non-probiotic strain (*Escherichia coli*) in the presence of different fluoride concentrations (ranging from 4500 to 0.45 ppm) to determine the effect of high and low doses of fluoride on their growth at different time points in vitro. Growth was correlated with control cells grown in MRS broth in the absence of sodium fluoride.

Lactobacilli and *Bifidobacterium* strains were more susceptible to fluoride at higher concentrations, where the chronic lethal concentration was 2250 ppm. *E. coli* was found to be more tolerant to fluoride in comparison to probiotic strains. A positive correlation was found between MIC and MBC values. Growth densities of all the probiotic organisms ($P \leq 0.01$) and *E. coli* ($P \leq 0.05$) were reduced significantly on chronic systemic exposure.

This study enhances our understanding of the impact of systemic fluoride on probiotic and non-probiotic strains which could have important implications for investigating the two-way interaction between fluoride, and the gut microbiome.

P003

The ENRIA2 project

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Abstract

Background

During the ENRIA project, European laboratories worked together to optimize the Bruker MALDI-TOF MS database for the identification of the most common anaerobic species. In the ENRIA2 project, the team at the UMCG continued working on updating the database by adding reference spectra of anaerobic bacteria that could not yet be identified by MALDI-TOF MS, for different reasons.

Method

Anaerobic isolates from the clinical bacteriology laboratory at the UMCG that could not be identified using MALDI-TOF MS (Bruker) were collected and identified using 16S rRNA gene sequencing. From anaerobic species not represented in the database or under-represented (<5 strains), main spectral profiles (MSPs) were created from at least 20 spectra of good quality.

Results

A total of 146 MSPs were created, covering 47 different genera and 71 different species. 52 MSPs represented 33 different species which were under-represented in the database (v2022; 11897 MSPs). Furthermore, 66 MSPs were added of 38 different species that were not present in the database. 50 MSPs were of 29 different valid species, including *Catonella morbi*, *Jonquetella anthropi*, *Porhpyromonas pasteri* and *Scardovia wiggsia*. 16 MSPs were of 9 different non-valid species, including *Actinomyces lingnae*, *Peptoniphilus urinimassiliensis* and *Sutterella massiliensis*. 27 MSPs were created of novel species and novel genera, among others 11 novel *Anaerococcus* species, 3 novel *Actinomyces* species and 2 novel *Fusobacterium* species.

Conclusion

The MALDI-TOF MS database needs further optimization in order to identify less common anaerobic species, aiding our comprehension of their clinical relevance.

P004

RT-qPCR detection of the expression of 18 selected genes of *Bacteroides fragilis* strains with or without *nim* genes and various metronidazole MICs

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Abstract

Background: *Nim* genes are regarded as mediators of metronidazole resistance in *B. fragilis* group strains. However, some data request that additional or independent factors are also needed to understand this resistance mechanism. E.g., *nim* gene expression is determined by the upstream insertion sequence elements but the metronidazole MICs are independent from that, and without haemin supplementation metronidazole resistance of *B. fragilis* strains is abolished.

Methods: Based on differential RNASeq and proteomic data, *nim*-positive (n=8) and negative (n=7) *B. fragilis* strains we examined for the expression of 18 selected genes from various cellular pathways by RT-qPCR. We recorded metronidazole MICs, analysed the data by bioinformatic methods and also drew gene interaction networks.

Results: In the *nim*-positive group the expressions of *cytB* (cytochrome), *mdh* (malate dehydrogenase), *pgk* (phospoho-glycerate kinase), *relA* (stringent response regulator) were lower and *nanH* (sialidase) expression was higher compared to *nim*-negative ones by means of variance analysis. By correlating the expression of the studied genes with metronidazole MICs the same set of genes was obtained; *cytB*, *mdh*, *pgk* and *relA* correlation was negative, while *nanH* correlated positively with the metronidazole MICs. For the *nim*-positive and negative subset of strains, however, we could not detect significant correlations, may-be, because of low number of strains. We could also detect gene correlation networks since the cross-correlations of the studied genes were sometimes very high ($r>0.7$, $p<0.001$).

Conclusion: We disclose that *cytB*, *mdh*, *pgk*, *nanH*, and *relA* should be considered as interacting partners or participants of interacting pathways in *nim*-mediated metronidazole resistance.

P005

Antimicrobial susceptibility of *Bacteroides* and *Parabacteroides* spp. for beta-lactam antibiotics in combination with beta-lactamase inhibitors

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Abstract

Background:

Antibiotic resistance among anaerobic bacteria has risen sharply in the last thirty years. Slovenian data about antimicrobial resistance of anaerobic bacteria from 2015 showed that resistance of *B. fragilis* group to amoxicillin/clavulanic acid was 8%, and the resistance to imipenem was 1%, but there were no data available for the other beta-lactam antibiotics.

Methods:

A total of 120 prospectively collected clinically significant isolates belonging to the genus *Bacteroides* (n=104) and *Parabacteroides* (n=16), obtained in 2021, and 87 selected isolates (77 *Bacteroides* and 10 *Parabacteroides* spp.) from the institutional archive collection with known resistance to amoxicillin/clavulanic acid from the same two genera were tested for susceptibility to beta-lactam antibiotics in combination with beta-lactamase inhibitors using gradient diffusion method and interpreted according to EUCAST breakpoints v11.0.

Results:

In total, 17.3% of prospectively collected *Bacteroides* and 31.3% of *Parabacteroides* spp. isolates were resistant to amoxicillin/clavulanic acid. The resistance to piperacillin/tazobactam was 11.5% in *Bacteroides*, and as high as 43.8% in *Parabacteroides* spp. isolates. In a group of selected isolates with known resistance to amoxicillin/clavulanic acid, the resistance to piperacillin/tazobactam was 58.4% in *Bacteroides* and 50% in *Parabacteroides* spp. As there are no EUCAST breakpoints defined for ceftolozane/tazobactam, we only determined MIC distribution. For prospectively collected isolates, the MIC_{50} for both genera was 16 µg/mL, and MIC_{90} was 256 µg/mL.

Conclusion:

Resistance to beta-lactam/beta-lactamase inhibitors in gram-negative anaerobic bacteria is relatively high. Antimicrobial susceptibility testing is crucial for monitoring resistance trends.

P006

Elucidating the Role of Glycosylation at the Oral Host-Pathogen Interface

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[1]University of Sheffield, United Kingdom. [2]Ludger, United Kingdom

Abstract

Periodontitis affects 700 million people globally. Oral microbiome dysbiosis, characterised by the expansion of the red complex which includes, Tannerella forsythia (Tf), has been heavily correlated with poor oral health. Human-derived sialic acids (Sias) can be found on host cell surfaces as terminal residues. They play important roles in various physiological and pathological processes, including immune response, development, and infection. The aim of this project is to study the effect of Sias on host-Tf interaction. Infection assays were used to determine the invasion abilities of Tf. Bacterial strains were incubated with H357's and gingival keratinocytes (GKs). One-third of bacteria were incubated with metronidazole and another third without host cells providing invasion and adhesion statics of bacterial strains. Additionally, the surface N-glycan landscape was analysed after bacterial incubation using hydrophilic-interaction liquid chromatography coupled with ultra performance liquid chromatography. Infection assays demonstrate that NanS, the auxiliary sialate-O-acetyl esterase of NanH, is required for effective invasion of Tf into H357's. NanS removes 9-O acetyl groups from Neu5,9Ac enabling terminal sialic acid to be removed, revealing previously hidden adhesion epitopes. H357s and Gks were incubated with and without Tf for 90 minutes, and surface n-glycans analysed. Incubation of Tf changed the surface n-glycosylation landscape of both H357's and gingival keratinocytes. Significantly, removal of terminal sialic acid residues was seen indicating the importance of the initial interaction between Tf and sialic acids. Our data demonstrates the importance of Sias for Tf invasion, the initial stage of pathogenesis for H357's and Gks.

P007

Improving Methods for Isolating Anaerobes from Human Milk

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Abstract

Human milk (HM) provides a source of microorganisms for both infants' and life-long health. However, only approximately 38% of over 500 microbes, detected by culture-independent methods, have been isolated in vitro. Moreover, exposure to oxygen during sampling become a major impediment to successfully culture anaerobes from HM. These have reduced our ability to study them under experimental conditions. Therefore, robust methods to collect, transport, and culture samples are vital to isolate anaerobes from low-biomass HM. This study sought to develop a novel workflow from sample collection to culture to improve the isolation of anaerobes from fresh HM. An anaerobic pouch was tested for at-home sample collection and transportation anaerobically from participants to the laboratory. A culturomics workflow was designed to maximally mimic the natural habitat of HM for fastidious anaerobes, including broad sample dilution, diverse liquid enrichment culture, and diverse culture media, etc. The combination of different conditions was implemented into 96-well microplates for liquid and solid agar culture. The anaerobic pouch with gas generating sachet can successfully maintain an anaerobic atmosphere for at least 72 hours – as shown by the successful recovery of Bifidobacterium longum from ambiently stored samples and anaerobic indicators. The culturomics methodology was developed on glycerol stocks of frozen HM before being deployed for fresh HM, generating 50 isolates, and acquiring six anaerobes by MALDI-TOF, including both facultative and aerotolerant species. The application of the anaerobic pouch and the high-resolution culturomics in low-biomass microbial samples is believed to maximally isolate anaerobes from fresh HM.

P008

Human botulism in Belgium: two atypical cases in 2022

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Abstract

Botulism is a paralyzing and potentially fatal disease caused by botulinum neurotoxins (BoNTs) , most often produced by the *Clostridium botulinum* bacterium. In 2022, three cases of human botulism have been confirmed by the Belgian National Reference Center (NRC) for *C. botulinum, C. perfringens* and *C. tetani*. Because of their very distinct progression, two of these cases will be presented.

Patient 1 was admitted to the Emergency Department with typical symptoms after consuming a 2-day old fish dish. Botulism was suspected rather quickly which is why antitoxin could be administered very fast. Multiple samples were analyzed by the NRC at 2 time points and were positive for BoNT E and/or *C. botulinum* type E.

Patient 2 was hospitalized after a fall and showing very diverse symptoms. Only after 7 weeks of hospitalization, botulism could be confirmed by the NRC. BoNT was detected in the patients stool using the *in vivo* method. In this case, the less obvious symptoms combined with the fact that botulism is very rare in Belgium were probably the reason for the late diagnosis.

Botulism is likely underdiagnosed in Belgium precisely because it is so rare. It is important that botulism is suspected at an early stage so that antitoxin can be administered as soon as possible to prevent further progression of the disease. For laboratory confirmation of the diagnosis, both a serum and stool sample should be taken as soon as possible and before administering antitoxin.

P009

Antimicrobial activity of microparticles containing morin, against periodontal pathogens

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Abstract

Morin offers potential for use in the management of periodontitis. To improve morin's solubility and stability microparticles (MPs) containing morin were developed by spray drying. This study aims to evaluate the antimicrobial activity of MPs containing morin, against periodontal pathogens. The minimum inhibitory concentration (MIC) of MPs (containing morin) and morin on its own was determined against *Porphyromonas gingivalis* (W83) and *Fusobacterium nucleatum* ATCC 23726 (Fn/23). *P. gingivalis* (W83) and *F. nucleatum* (Fn/23), were grown for 24 hours as a planktonic culture, anaerobically at 37°C. Multi-species biofilms (*Streptococcus oralis*, *F. nucleatum* and *P. gingivalis*) were grown on coverslips for 7 days, anaerobically at 37°C. Bacterial cultures were exposed to MPs (containing morin – 1mg/mL), morin (1mg/mL), blank (MPs without morin), DMSO and a control (only bacteria). For planktonic culture, the treatment was added at the beginning of the experiment, for the biofilms it was replenished once a day for 7 days. The microbial viability was determined by counting colony forming units (CFU/mL). The biofilm biomass was determined using a crystal violet assay. The MIC of morin was 117.188 µg/mL (*F. nucleatum* and *P. gingivalis*). The MIC of MPs' containing morin was 78.125 µg/mL for *F. nucleatum* and 39.063 µg/mL for *P. gingivalis* respectively. Microbial viability (Log CFU/mL - planktonic culture and mature biofilm) and the biofilm biomass reduced after treatment with morin and MPs containing morin compared with the control group. The MPs containing morin can inhibit two key periodontal pathogens and hence offers therapeutic potential.

P010

I still haven't found what I'm looking for - optimising methods for metabolomic investigations of the microbiome.

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Abstract

Background

Gut dysbiosis, commonly as result of antibiotic action on the intestinal microflora affects both diversity and metabolic function. Investigations of the functional changes in gut dysbiosis are necessary to understand how to combat them. High Performance liquid chromatography / Mass Spectrometry (HPLC) has potential for intestinal metabolomic analysis. We improved initial on-line compound annotation by profiling against chemical compound libraries.

Methods

Two commercially available Mass Spectrometry libraries of gut microbiome metabolites, Bile Acids, Carnitines and Sterols (IROA technologies GUTMLS & BACSMLS) were analysed with a C18 LCMS column (Thermo Fisher Hypersil gold) and Orbitrap Exploris 240 Mass Spectrometer (Thermo Fisher), evaluating it's potential to resolve and identify 281 metabolites (96 BACMLS + 185 GUTMLS) associated with the intestinal microbiome.

Results

Of the 281 metabolites analysed we were able to reliably identify 170 compounds.

The GUTMLS library contained 185 compounds; 132 were resolved and reliably identified using the C18 LCMS column method. Of the compound classes contained within, this method was particularly suited to identifying carboxylic acids and derivatives (26/27 compounds Identified) and bile acids (21/33 compounds). It identified fewer Fatty Acyls (24/41 compounds), carnitines (21/33) or sterols (7/31).

Conclusions

Optimising identification of gut metabolic compounds is complex, likely due to the high variability observed in the chemical composition of metabolites.

Single column methods may be appropriate for targeted metabolomic investigations of amphiphilic compounds.

Untargeted screening may require multiple column chemistries. Increased compound identification must be balanced with the higher costs and processing times associated with additional column chemistries.