MICROBIOLOGY SOCIETY

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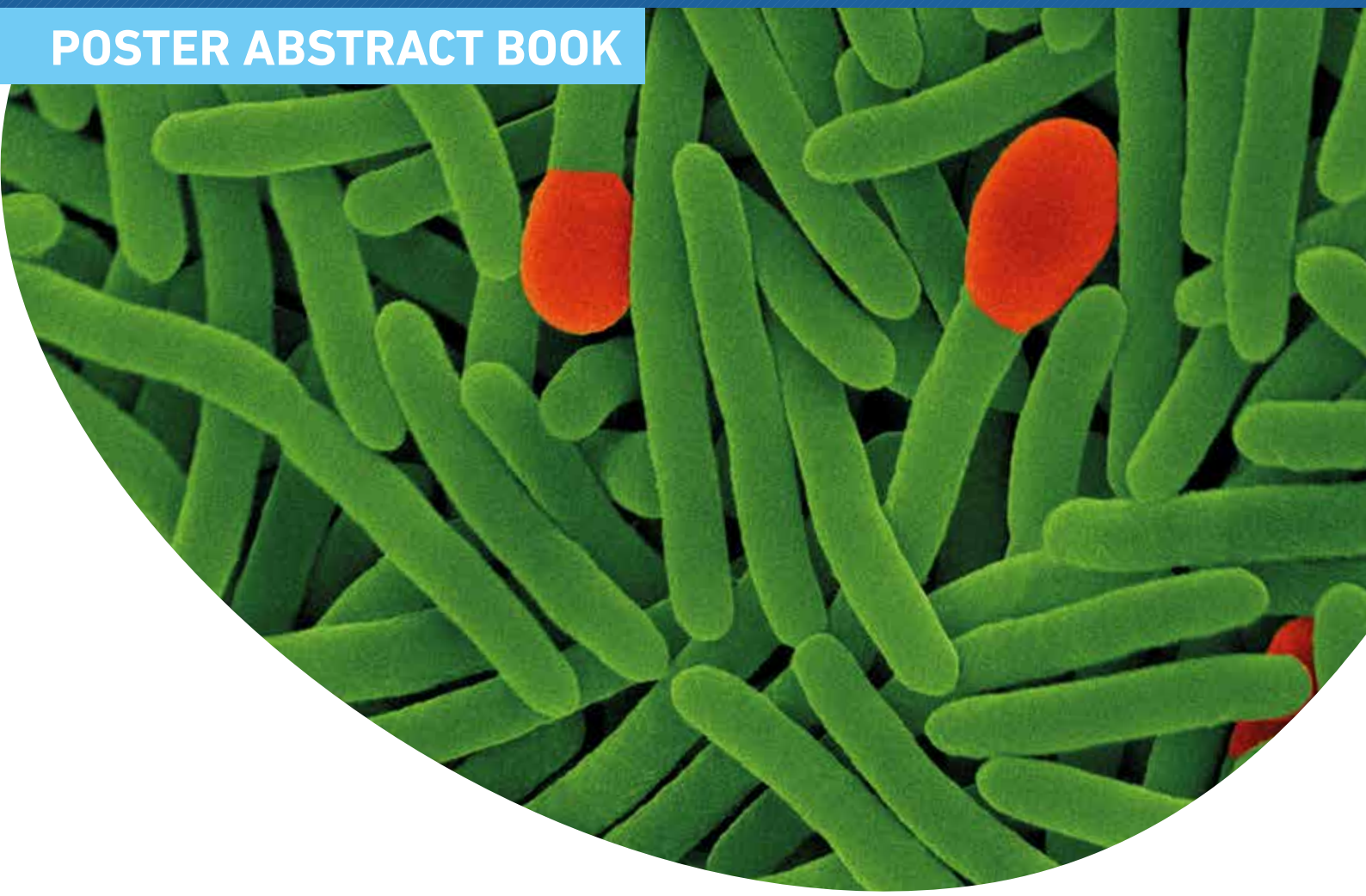
Anaerobe 2019:

Changing perceptions of anaerobic bacteria; from pathogen to the normal microbiota and back

13–14 June 2019 Jurys Inn, Cardiff, UK

POSTER ABSTRACT BOOK



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Poster Numbers

Poster Number	Presenting Author	Abstract Title	Page Number
01	David Nygren	A nationwide retrospective study of invasive infections with *Fusobacterium necrophorum* in Sweden 2010-2017	3
02	Dafydd Thomas	Improving the surveillance of antimicrobial resistance trends amongst anaerobic oral pathogens	4
03	Kathleen Boiten	Prevalence of resistance genes among less known gram-negative anaerobic bacteria, isolated from clinical human infections	5
04	Kathleen Boiten	Discovering a novel resistance gene for carbapenem resistance in Parabacteroides timonensis using Whole Genome Sequencing	6
05	Rupa Rai	Authenticating Anaerobes – Use of MALDI-TOF MS to identify anaerobes in the NCTC Collection	7
06	Maria Carlos Martins	Survival strategies of Clostridium difficile to fluctuating concentrations of oxygen	8
07	Filipe Folgosa	The role of metalloenzymes for the survival of the anaerobe Clostridium difficile during infection	9
08	Lesley Hoyles	Diversity of the class Coriobacteriia within different ecosystems	10
09	Andrew McDowell	Multiplex PCR phylotyping of Propionibacterium (Cutibacterium) acnes: characterisation of PCR negative and untypable isolates.	11
10	Carmen McLeod	Poo and Puns: The representation of Faecal Microbiota Transplants in English-language print media (2003 – 2017)	12
11	Alida Veloo	Conjugative transposons and other mobile genetic elements in human clinical Prevotella bivia strains. How multi-drug resistance strains are created.	13
12	Michael Perry	The Sensitivity of PCR combined with the Specificity of Toxin Enzyme Immunoassay: Could an Ultra-sensitive Single Molecule Counting Technology Offer a Standalone Solution for Diagnosis of Clostridioides difficile Infection?	14
13	Michael Perry	Using PCR in place of GDH as the first line assay in a two-step CDI testing algorithm – evidence of help not hindrance from samples processed at clinical microbiology laboratories in Wales	15
14	Miriam Cordovana	Evaluation of MICROANAUT-S Anaerobes MIC	

Poster Number:01

A nationwide retrospective study of invasive infections with *Fusobacterium necrophorum* in Sweden 2010-2017

Background:

Fusobacterium necrophorum primarily causes throat infections, mainly among teenagers and young adults. Invasive infections, including Lemierre´s syndrome, are rare but a few studies have shown a potential increase. To gain further knowledge on incidence and clinical presentation, we performed a nationwide study in Sweden.

Methods:

Data from 2010-2017 on blood cultures and cultures or PCR from sterile sites positive for *F. necrophorum* were acquired from all 23 microbiological laboratories in Sweden. Medical records were reviewed. Incidence, seasonality, age and gender distribution, clinical presentation, care and outcomes were analyzed.

Results:

From 2010-2017 300 cases of invasive infection with *F. necrophorum* were diagnosed in Sweden. Cases increased from 2.9 to 5.0 per million per year in 2010-2013 vs. 2014-2017 ($p<0.01$). 61,4% of patients were male. No seasonal distribution was found. Of 300 cases, 203 originated from head and neck infections, where 102 developed Lemierre´s syndrome. 47 patients had abdominal foci. Median age was 26 years, 45% of cases occurred in patients aged 15-25 years and 29% in patients aged >50 years. Patients with head and neck infection were younger than patients with other foci ($p<0.01$). 55,6% of all patients had sepsis on admission, 20,7% were admitted to intensive care units and 8,3% developed septic shock. Mortality rates at 1 and 6 months were 5,3 and 8,3% respectively.

Conclusion:

Prior findings that *F. necrophorum* primarily affects adolescents and young adults and causes potentially life-threatening infections are confirmed. Furthermore, diagnoses of invasive infections with *F. necrophorum* in Sweden have increased significantly.

David Nygren, Karin Holm

Department of Clinical Sciences Lund, Medical Faculty, Lund University, Lund, Sweden

Poster Number:02

Improving the surveillance of antimicrobial resistance trends amongst anaerobic oral pathogens

Background:

Orofacial infections when treated by dental practitioners are not routinely sampled for culture and susceptibility testing. Consequently, antimicrobial resistant rates amongst the causative bacteria are unknown, hindering efforts to appropriately manage these infections and undertake surveillance of resistant rates. As part of the Cardiff health board leadership in patient safety quality improvement programme a multidisciplinary group was tasked with the aim of improving sampling practices.

Methods:

Dental professionals' limited knowledge regarding the role of diagnostic support and an associated lack of confidence in sampling procedures was addressed by the creation and distribution of training aids. Sampling and transportation kits were developed and transport routes identified enabling dentists within the community setting to send samples to the specialist microbiology services based at Cardiff University Dental Hospital [UDH]. All significant anaerobes were identified and antibiotic susceptibility testing undertaken via agar dilution through referral of isolates to the UK Anaerobic Reference Unit.

Results**:**

A 10% increase was seen in the proportion of pus aspirates being received in comparison to pus swabs. For the first time, pus aspirate samples have been received from community dental clinics. A vast diversity of anaerob species and susceptibility profiles have been identified. Increasing rates of resistance amongst *Prevotella* species to amoxicillin and clindamycin is of concern.

Conclusion**:**

This project has shown that through educational interventions and support the barriers to the sampling of orofacial infections can be overcome. The ultimate aim is for all dentists in Wales to have access to diagnostic support.

Dafydd Thomas[1], Selina Scotford[2], James Gillespie[3], James Crossland[3], Grace Kelly[4], Trefor Morris[2], Michael Lewis[4], Melanie Wilson[4]

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Poster Number:03

Prevalence of resistance genes among less known gram-negative anaerobic bacteria, isolated from clinical human infections

The past years there has been an increase in antibiotic resistance among anaerobic bacteria. Among these anaerobes, the best known and most studied group is the *Bacteroides* group. Other well studied genera are *Clostridium* spp. and *Prevotella* spp. Other less known genera are not studied. Therefore, lack of information exists about their antimicrobial susceptibility profile and the presence of resistance genes. In this study we aim to assess the prevalence of known resistance genes in these less common species.

From the years 2015-2017 a number of anaerobic gram-negative rods, consecutively isolated, were studied. All isolates were identified using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). MIC values were determined using E-test (Biomerieux, Marcy-l'Étoile, France) and interpreted according to the EUCAST guidelines. A targeted PCR, using specific primers, will be used to detect known resistance genes. Genera that will be studied are: *Alistipes* spp., *Bilophila* spp., *Dialister* spp., *Fusobacterium* spp. and *Sutterella* spp.

So far, one *Dialister microaerophilus* strain was resistant to metronidazole, 4 *Sutterella wadsworthensis* strains were resistant to clindamycin and 3 *S. wadsworthensis* strains were resistant to metronidazole. None of these strains harbored any resistance genes. One *Alistipes finegoldii* isolate did harbor a *tetQ* gene, but was sensitive for tetracycline.

The results obtained so far suggest that an unknown resistance mechanism might be present in the tested strains. Also, some of the observed resistance might be intrinsic. Further studies on this subject is warranted and more strains will be tested. The results will be presented.

Kathleen Boiten, Willemtje Baas, Pauline Buijs, Alida Veloo

University Medical Center Groningen, Groningen, Netherlands

Poster Number:04

Discovering a novel resistance gene for carbapenem resistance in Parabacteroides timonensis using Whole Genome Sequencing

Up to 95% of the human microbiome consists of anaerobic bacteria. However, they are not as well studied as the aerobic bacteria. Among all anaerobes, the *Bacteroides* group is the best known and most studied. There has been an increase in carbapenem resistance in Bacteroidales strains without a known cause.

We isolated a *Parabacteroides timonensis* strain, related to *Bacteroides*, resistant for amoxicillin, clindamycin and meropenem.

The strain was sequenced using the Miseq (Illumina, San Diego, CA), followed by a *de novo* assembly using CLC workbench to create a draft genome. Resistance genes were detected with ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/). Genome annotation was performed by RAST (http://rast.nmpdr.org/). Protein modeling was performed, on genes of interest, using Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) and 3DLigandSite (http://www.sbg.bio.ic.ac.uk/3dligandsite/). FatCat (http://fatcat.burnham.org/) was used to do a pairwise structure alignment to compare protein structures.

The draft genome had a size of 6,356,323 bp, present on 95 contigs. Using Resfinder 1 resistance gene was found, *tetQ*. A possible novel resistance gene for a metallo β-lactamase related to *cfiA* was found using RAST. The protein model showed similarities with the model for *cfiA* and includes several Zn2+ ions, used by metallo β-lactamase to hydrolyze carbapenems.

Our results indicate the presence of a not yet described metallo β-lactamase. There are also indications that a conjugative transposon is present, enabling horizontal gene transfer. No *erm* genes, encoding for clindamycin resistance, were detected in the draft genome.

Kathleen Boiten, Alida Veloo

University Medical Center Groningen, Groningen, Netherlands

Poster Number:05

Authenticating Anaerobes – Use of MALDI-TOF MS to identify anaerobes in the NCTC Collection

The National Collection of Type Cultures (NCTC) is the world's oldest repository of medically-relevant bacteria. NCTC contains 5,500 bacterial strains, 500 of which are anaerobes, with the collection regularly receiving new Type strains and recent clinical isolates.

Fastidious anaerobes pose a unique challenge during the authentication process. NCTC must ensure that the strains are free from contamination and that the organism survives the lyophilisation process. Species-level identification of anaerobic bacterial strains is achieved using a combination of the both MALDI-TOF MS and VITEK2 instruments.

This study evaluates the use of MALDI-TOF MS (Bruker) and VITEK2 (BioMerieux) to identify the anaerobic strains in the NCTC collection. 176 NCTC strains were tested on the MALDI-TOF platform and 60 strains were identified using VITEK2.

MALDI-TOF was able to identify 79% anaerobes to genus-level and 64% to species-level. In comparison the VITEK2 identified 68% to genus and 46% to species-level. The main limiting factor for both these platforms is the database. This may be due to novel anaerobe species NCTC receives not being present on the databases. In the event of no identification, 16S ribosomal RNA sequencing is employed. Furthermore, detection of specific characteristics is carried out by specialist reference laboratories in Cardiff and Colindale, ensuring a robust collection of anaerobic bacteria for use in research and as control strains.

Hannah McGregor, Dipali Pindoria, Rupa Rai, Sarah Alexander

National Collection of Type Cultures, Public Health England, Colindale , United Kingdom

Poster Number:06

Survival strategies of *Clostridium difficile* to fluctuating concentrations of oxygen

Oxygen and reactive oxygen species (ROS) can react with multiple cellular components, leading to the inactivation of a plethora of metabolic pathways. Therefore, organisms have systems to sense and eliminate O_2 and ROS, contributing to their survival in the adverse host environment.

Clostridium difficile P28 is an anaerobic pathogenic bacterium that contains in its genome a flavodiiron protein (FDP) and a rubrerythrin (Rbr), that are putatively involved in the detoxification pathways used by this organism.

Flavodiiron proteins are widespread in all life domains, with a crucial role in O_2 detoxification, through its reduction directly to water. FDPs are cytoplasmic enzymes with a minimal structural unit composed by two main domains, a metallo-β-lactamase domain, containing the catalytic diiron site, and a flavodoxin domain having a flavin mononucleotide. Rubrerythrins are generally considered to act as NADH-linked hydrogen peroxide reductases, thus eliminating this ROS, and are composed by two iron sites: a diiron center and a rubredoxin-like $FeCys_4$ center.

In this work with characterized biochemically, spectroscopically, structurally and kinetically the FDP and Rbr and their two putative redox partners, a High Molecular Weight Rubredoxin (HRb) and a Rubredoxin (Rd). We confirmed the existence of direct electron transfer between HRb, Rd and FDP and also between HRb, Rd and Rbr. In addition, we also established the reaction rates for the reduction, by FDP, of O_2 ($0.43s^{-1}$) and H_2O_2 ($0.06s^{-1}$) and for the reduction of H_2O_2 by Rbr ($1.53s^{-1}$). The enzymatic activity of Rbr towards O_2 was also investigated.

Romão, C.V., *et al*, 2016.JBIC., 21:39-52.

Martins, M.C., *et al.*, 2019. FRBM, in press.

Maria Carlos Martins, Bruno Salgueiro, Filipe Folgosa, Célia Romão, Carlos Frazão, Miguel Teixeira

ITQB- Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal

Poster Number:07

The role of metalloenzymes for the survival of the anaerobe *Clostridium difficile* during infection

Clostridium difficile is the most prevalent pathogen among all healthcare-associated infections. This anaerobic bacterium can colonize the human gut, typically following agents that disrupt the normal gut microbiota, like antibiotics. In the gut, *C. difficile* is subjected to oxygen, which it has to eliminate for survival. Its genome encodes for two flavodiiron proteins, capable to reduce oxygen to water. Besides, some FDPs also reduce NO to N_2O, an important feature as a resistance mechanism towards the human innate immune system[1,2]. Flavodiiron enzymes are constituted by a minimal core of two domains: a metallo-β-lactamase-like one, harboring the catalytic center, followed by a short-chain flavodoxin[1,2]. More complex FDPs exist, with multiple extra domains and redox centers[1,2]. *C. difficile* contains a "classical" FDP, and a very complex one, with an extra short-type rubredoxin domain followed by an NADH:rubredoxin oxidoreductase-like one[3]. The biochemical, redox and spectroscopic studies demonstrated that this enzyme receives electrons directly from NADH, reducing its substrates, precluding the need for extra partners. This FDP is selective for O_2 ($16s^{-1}$), almost 10x higher than with NO. The reactivity towards hydrogen peroxide, as an H_2O_2 reductase with formation of water, with a non-negligible turnover ($2s^{-1}$) is a novelty in the field of FDPs. Site directed mutants revealed that the rubredoxin-like center is essential for electron transfer from NADH to the catalytic center.

[1] Martins *et al*, Free Rad. Biol. Med., in press, 2019

[2] Romão *et al*, J. Biol.Inorg. Chem., 21,39-52, 2016

[3] Folgosa *et al*, Sci.Rep., 8, 10164, 2018

Filipe Folgosa, Catarina Alves, Maria C. Martins, Miguel Teixeira

ITQB-NOVA, Oeiras, Portugal

Poster Number:08

*Flash poster presentation

Diversity of the class *Coriobacteriia* within different ecosystems

Members of the class *Coriobacteriia* are little studied, important fastidious anaerobes within the human gut microbiota. *Collinsella aerofaciens* is a core member of the gut microbiota that can present several different fermentation profiles within individuals, while *Eggerthella lenta* is implicated in xenobiotic metabolism. Recent 'culturomics' studies have greatly increased the number of *Coriobacteriia* recovered from human-associated samples, with the names of nine novel genera comprising 12 species published to date. However, the ecological range and genomic diversity of the class *Coriobacteriia* are poorly understood. Taxonomic assignments within the class *Coriobacteriia* are unclear, limiting the ways in which data from 16S rRNA gene-based diversity studies in humans and rodent models are interpreted.

Whole-genome and 16S rRNA gene sequences of members of the class *Coriobacteriia* were analysed and assigned to the families *Atopobiaceae*, *Coriobacteriaceae* and *Eggerthellaceae*. Inconsistencies between 16S rRNA gene sequence and whole genome sequence data deposited in public databases were identified. Newly annotated data searched against 85,000 16S rRNA gene sequence datasets included in the IMNGS (Integrated Microbial Next Generation Sequencing) database demonstrates humans and rodents harbour distinct *Coriobacteriia* populations. Members of the family *Coriobacteriaceae* predominate in the human gut, while *Eggerthellaceae* are more representative of the rodent gut. Metabolic capabilities of the three families of *Coriobacteriia* vary greatly, with *Eggerthellaceae* asaccharolytic compared with *Coriobacteriaceae* and *Atopobiaceae*. Correct annotation of *Coriobacteriia* in 16S rRNA gene (and by extension shotgun metagenomic) datasets is required to determine the contributions of these increasingly important bacteria to different ecosystems.

Lesley Hoyles

Nottingham Trent University, Nottingham, United Kingdom

Poster Number:09

*Flash poster presentation

Multiplex PCR phylotyping of *Propionibacterium* (*Cutibacterium*) acnes: characterisation of PCR negative and untypable isolates.

We previously published a touchdown multiplex PCR for rapid species identification and phylotyping (types IA_1, IA_2, IB, IC, II, III) of *Propionibacterium acnes*. One recent study used the PCR assay to analyse 140 clinical *P. acnes* isolates (identified by MALDI-TOF MS), but three isolates were negative and nine were untypable due to atypical PCR banding profiles. We have now characterised eight of these isolates by broad-range 16S rDNA sequencing and single locus sequence typing (SLST). One isolate, which gave a negative multiplex PCR reaction, was not *P. acnes* but *P. humerisii* (SLST +ve). Three isolates, which generated only a single PCR band (pattern G) due to reaction with multiplex primers (PArA-1/PArA-2) that target the 16S rRNA gene of *P. acnes*, were identified as the newly described and closely related sister species *P. namnetense* (SLST –ve). One further isolate with pattern G was identified as a type IA_1 strain (SLST genotype D1). Three isolates which gave a novel three banded profile (pattern H) where confirmed as type II strains (SLST genotypes K1 and K2). These four *P. acnes* strains are now undergoing whole genome sequencing to determine the molecular/ phylogenetic basis of their atypical PCR profiles. In conclusion, this study has found that MALDI-TOF MS with VITEK MS v2.0 does not differentiate *P. humerisii* and *P. namnetense* from *P. acnes*. Negative or pattern G multiplex reactions with isolates identified as *P. acnes* by MALDI-TOF are suggestive of *P. humerisii* and *P. namnetense*, respectively pending confirmation by SLST and 16S rDNA sequencing.

Andrew McDowell[1], Joseph McLaughlin[1], Carey-Ann Burnham[2], Istvan Nagy[3]

[1]Northern Ireland Centre for Stratified Medicine, School of Biomedical Sciences, Ulster University, Londonderry, United Kingdom. [2]Department of Pathology & Immunology, Division of Laboratory and Genomic Medicine, Washington University School of Medicine, St Louis, USA. [3]Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Poster Number:10

*Flash poster presentation

Poo and Puns: The representation of Faecal Microbiota Transplants in English-language print media (2003 – 2017)

This study investigates how English-language news sources represented faecal microbiota transplants (FMT) between 2003 and 2017. In the context of this study, FMT is understood to be the process of transferring stool from a healthy donor to a recipient with a dysfunctional intestinal flora in order to repopulate their gut microbiome. A corpus of news articles on FMT, was produced by searching for 'fa(e)cal microbial', 'microbiota transplant' and 'stool transplant' on the Nexis® UK news database, generating a corpus suitable for qualitative analysis (n = 504 articles). In order to uncover emerging social representations, we investigated press coverage of stool transplants, as well as broader themes associated with health and the gut microbiome. Our findings show that print media focused particularly on creating novel, mainly hopeful, social representations of faeces through wordplay and punning, side-lining issues of risk and fear. We also identify changing metaphorical framings of microbes and bacteria from 'enemies' to 'friends'. Additionally, we found readers are familiarised with FMT through the depiction of the process as being both mundane and highly medicalised. We argue emerging media representations have the potential to shape more positive social representations of FMT in the general population, paving the way for FMT to become a more socially acceptable and effective medical procedure. Future research can build on this baseline in order to study how social representations circulate in the wider media and public sphere, and how they may change over time and differ between countries as research into FMT progresses.

Carmen McLeod, Brigitte Nerlich

University of Nottingham, Nottingham, United Kingdom

Poster Number:11

*Flash poster presentation

Conjugative transposons and other mobile genetic elements in human clinical *Prevotella bivia* strains. *How multi-drug resistance strains are created*

The best known anaerobic bacteria isolated from human clinical specimens are the members of the *Bacteroides* group. They belong to the Bacteroidetes phylum and can harbor a conjugative transposon (CTn), which transfers between strains in the presence of low concentrations of tetracycline. The best studied CTn is CTnDOT, which was encountered in *Bacteroides thetaiotaomicron*. Besides the CTn also the transfer of other mobile genetic elements (MGEs) present in the chromosome is triggered. In this study, we assessed the presence of CTns in *Prevotella bivia* strains, which belong to the same phylum as *Bacteroides*.

A draft genome of five *P. bivia* strains, selected on their antibiotic susceptibility profile, was obtained using Illumina sequencing. A *de novo* assembly was performed using the CLC Genomics workbench (Qiagen, Hilden, Germany). Genes were annotated using RAST (ww.rast.nmpdr.org) and manually checked using blast. Annotation and length of the gene was adjusted if necessary.

The presence of a CTn was observed in all five strains. Analysis showed that the five CTns could be divided in three different groups, which were all related to CTnDOT. Besides the CTns also other MGEs were encountered, harboring a selection of resistance genes.

As in *Bacteroides* strains, *P. bivia* strains can harbor a CTn related to the CTns encountered in *Bacteroides*. We will present what could happen if the transfer of the CTn in these *P. bivia* strains is triggered.

Alida Veloo, Heinrich Winter, John Rossen

University Medical Center Groningen, Groningen, Netherlands

Poster Number:12

*Flash poster presentation

The Sensitivity of PCR combined with the Specificity of Toxin Enzyme Immunoassay: Could an Ultra-sensitive Single Molecule Counting Technology Offer a Standalone Solution for Diagnosis of *Clostridioides difficile* Infection?

Background

Clostridioides difficile infection (CDI) continues to cause significant morbidity and avoidable mortality worldwide. Results from an ultra-sensitive toxin immunoassay (Singlulex Clarity C. diff toxins A/B assay) were compared with those of various other diagnostic and reference methods/algorithms for the detection of *C. difficile*.

Methods

293 residual clinical stool samples were tested using the Singulex assay. In total, 188 samples were tested by GDH and 239 were tested by PCR. All toxin B PCR (Serosep EntericBio C. difficile assay) positive samples (n=168) and prospectively tested GDH samples (n=97) were also tested using membrane-type toxin EIA (MT-EIA; Techlab Tox A/B Quik Chek^{Ò}). Culture (alcohol shock and Brazier's media; Oxoid) and ribotyping (capillary electrophoresis using Bidet *et al*. primers) information was available for 205 samples.

Results

The positive percent agreement (PPA) and negative percent agreement (NPA) of the Singulex Clarity C. diff toxins A/B assay compared with: GDH; toxin EIA; PCR; GDH/toxin EIA; GDH/toxin EIA/PCR; PCR/toxin EIA and culture were – 61% & 92%; 97% & 50%; 69% & 90%; 100% & 51%; 81% & 77%; 96% & 65%; and 69% AND 52% respectively.

Conclusions

The Singulex Clarity C. diff toxins A/B assay had high PPA compared to toxin EIA and multistep algorithms ending with toxin EIA, and high NPA compared to PCR and a multistep algorithm ending with PCR. The Singulex Clarity assay has the potential to be used as a standalone test for CDI diagnosis; additional clinical studies are required and will soon be underway.

Michael Perry[1], Lee Graham[1], Lauren Gilbert[1], Sweta Parida[2], Phoebe Katzenbach[3], Jose Baptista[3], Johanna Sandlund[3], Bethan Anderson[1], Sarah Copsey[4], Selina Scotford[4], Tefor Morris[1]

[1]Public Health Wales Microbiology, Cardiff, United Kingdom. [2]Cardiff and Vale University Health Board, Cardiff, United Kingdom. [3]Singulex Inc, Alameda, USA. [4]Public Health Wales Microbiology, Cardiff, USA

Poster Number:13

*Flash poster presentation

Using PCR in place of GDH as the first line assay in a two-step CDI testing algorithm – evidence of help not hindrance from samples processed at clinical microbiology laboratories in Wales

Background:

Guidance recommends the use of a two-stage algorithm for diagnosis of *Clostridioides difficile* infection (CDI) utilising either PCR or a glutamate dehydrogenase (GDH) assay as the first line screen. There is ongoing debate as to whether PCR is a hindrance due to fear of over-diagnosis.

Methods:

Between December 2017 and March 2019 over 65,000 tests for CDI were undertaken in Wales. PCR (Serosep EntericBio C. difficile assay) was employed as the first line test for 36% of samples with the remainder tested using a GDH assay (Techlab C. diff Chek^{TM} -60). Positive samples were tested using a toxin EIA (Techlab Tox A/B Quik Chek^{Ò}). Culture (alcohol shock & CCEY; Oxoid) and capillary electrophoresis PCR ribotyping were undertaken at the UK Anaerobe Reference Unit (UKARU).

Results:

The proportion of samples testing positive using PCR and GDH was 5.2% and 8.3% with a toxin EIA positive percentage of 30% and 25% respectively. Of these samples 73% (n=2543) of GDH and 80% (n=974) of PCR positive samples were referred for culture and ribotyping.
No *C. difficile* was isolated from 15% of GDH and 10.5% of PCR positive specimens. Non-toxigenic strains were isolated from 6.6% of GDH and 0.2% of PCR positive samples and approximately equal proportions of both GDH and PCR positive samples were ribotyped (60% vs. 63% of 2125 samples).

Conclusions:

There was no evidence of increased ascertainment of CDI using PCR. In fact, culture and ribotyping demonstrated an improved specificity for PCR that is helpful for accurate CDI diagnosis.

Michael Perry, Bethan Anderson, Selina Scotford, Lee graham, Lauren Gilbert, Alec Birchley, Sarah Copsey, Trefor Morris

Public Health Wales Microbiology, Cardiff, United Kingdom

Poster Number:14

*Flash poster presentation

Evaluation of MICROANAUT-S Anaerobes MIC broth microdilution panels for antibiotic susceptibility testing of anaerobes

Background:

Accurate and routine-friendly methods for MIC determination of anaerobes are demanded. Here, we evaluate the performance of a commercially available microdilution panel in comparison to a gradient MIC Strip test.

Methods:

N=163 anaerobes clinical isolates (60 species, 23 genera) were tested with the MICRONAUT-S Anaerobes MIC panel (MERLIN Diagnostika, Germany). The same bacterial suspension was used for the panel inoculum, and for testing by MIC Strips (Liofilchem, Italy). The panels were incubated at 37 °C in anaerobic conditions for ≥24 h, and read visually. In case of no bacterial growth for the growth control, incubation was prolonged to 48-72 h. Results were interpreted according to EUCAST guidelines. Comparison was performed in terms of essential agreement, category agreement and error rates.

Results:

Category agreement with MIC strips resulted overall > 95% (from 95.6% to 100%). Essential agreement resulted 91.1% for piperacillin/tazobactam, and > 95% for all the other antibiotics. Overall, n=15 minor errors, n=2 major errors and n=5 very major errors were observed. For doxycycline, tigecycline and moxifloxacin (for which no breakpoints are available), MICRONAUT results diverged for ≤1 dilution fold from MIC Strips results. For most isolates (117/163) the panels were readable after overnight incubation, in 42 cases after 48 h, in 4 cases after 72 h.

Conclusions:

The MICRONAUT-S Anaerobes MIC panels proved to be a reliable microdilution method for antibiotic susceptibility testing of anaerobes, providing results consistent with gradient methodology, with both fast and slow growing species. The ease-of-handling and -result interpretation makes this method suitable for routine implementation

Miriam Cordovana, Simone Ambretti

University Hospital Policlinico Sant'Orsola-Malpighi, Bologna, Italy

Poster Number:15

*Flash poster presentation

Descriptive epidemiological analysis of antimicrobial resistance in strict anaerobes in Scotland, 2013-2017

Background:

Antimicrobial resistance in anaerobic bacteria has been recognised to be increasing globally, with growing resistance to metronidazole and carbapenems being of particular concern. Health Protection Scotland receives clinical microbiology results, including susceptibility data from all Scottish diagnostic laboratories via the national database; Electronic Communication of Surveillance in Scotland (ECOSS).

Methods:

Data from 2013 to 2017 relating to strict anaerobes isolated from clinical samples was extracted from ECOSS (de-duplicated based on a 14 day episode definition). Predominant species, sample types and susceptibility to a variety of antibiotics was assessed. A literature review was conducted to compare resistance in Scottish isolates, to that reported globally.

Results:

The most commonly reported organisms were *Clostridium perfringens* (n = 1802), *Propionibacterium acnes* (n = 1784) and *Bacterioides fragilis* (n= 1750). The most frequently associated sample type varied by species. Of the clinically relevant antibiotics, susceptibility testing was performed most commonly for metronidazole (ranging from 58-89%, depending on species). Reported resistance varied by agent and species.

Conclusion:

Nationally, limited susceptibility testing is carried out for the majority of anaerobic bacteria. Where tested, resistance to most antibiotics including metronidazole was broadly comparable to that reported in the published literature, although comparatively higher resistance to clindamycin was observed for several species including *C. perfringens*. Work is ongoing in Scotland to improve identification of anaerobes and standardisation of susceptibility testing for isolates from sterile sites. It is anticipated that this will support more targeted treatments for individual patients and antimicrobial stewardship programmes.

Adriana Zalewska, Julie Wilson, Edward Mcardle, Michael Lockhart, William Malcolm

Health Protection Scotland, Glasgow, United Kingdom

Poster Number:16

*Flash poster presentation

Propionibacterium (*Cutibacterium*) acnes infection of the prostate gland as a risk-factor and biomarker of prostate cancer?

Prostate cancer (PCa) is the most common male cancer in the UK, killing approximately 11,000 men every year. There is now increasing interest in the role played by the anaerobic bacterium *Propionibacterium acnes* in the aetiology of this condition via chronic, intracellular and asymptomatic infection of the prostate leading to oncogenesis. To investigate this, we developed a novel Taqman® qPCR assay for retrospective detection of *P. acnes* in formalin-fixed paraffin-embedded tissue sections prepared from archived prostate biopsy samples. A total of 81 biopsy samples were examined from 53 patients with prostate carcinoma, versus 111 samples from 60 patients whose biopsies were histologically normal. Our assay revealed that 35% of men with PCa were positive for the presence of *P. acnes* in either one or both prostate lobes compared to only 8% of normal tissue (Fisher's exact test, 2-sided; $p<0.001$). This rate of detection is in keeping with previous culture-based studies, and equates to a 2-fold relative risk (95% CI: 1.45-2.75; $p=0.0001$) after adjustment for age; this level of risk approximates to having one first-degree relative (Father or brother) diagnosed with the condition. No statistical association between the presence or absence of infection/ colonization and age, blood prostate specific antigen (PSA) levels or Gleason score was observed. Our studies suggest *P. acnes* infection of the prostate gland may be a potential risk factor for PCa development. Furthermore, the presence of *P. acnes* in cancerous tissue is a highly specific biomarker for the condition versus serum PSA measurement (92% versus 65%).

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Poster Number:17

*Flash poster presentation

Investigating Gut Microbiota-Host Interactions in a Microaerobic Environment

The gut microbiota has an important role in maintaining intestinal health and protecting against enteric infections (colonisation resistance). Nevertheless the majority of these interactions haven't been explored, largely due to a lack of experimental model systems that can culture oxygen-sensitive commensals alongside intestinal cells. In this project, we have established a novel *in vitro* model system of the human intestinal epithelium (Vertical Diffusion Chamber, VDC) which also supports growth of strictly anaerobic bacteria. We have applied this system to investigate the interactions of gut symbiont *Ruminococcus gnavus* with a functioning mucus-producing epithelium, established using T84 and goblet-like LS174T cell lines, and its effect on infection with foodborne pathogen enteropathogenic *E. coli* (EPEC).

Initial work focused on identifying a culture medium that supports *R. gnavus* and EPEC growth whilst maintaining epithelial integrity and barrier function. This has been achieved by establishing bacterial growth curves in different media and assessing epithelial barrier function by transepithelial electrical resistance and immunofluorescence staining (IFS) of tight-junction protein occludin. Further IFS demonstrated that introduction of LS174T cells to the epithelium causes mucin secretion and facilitates colonisation by *R. gnavus*. Co-culture of EPEC with *R. gnavus* reduces numbers of viable and adherent EPEC, but only when LS174T are present.

These data indicate potential colonisation resistance activity by *R. gnavus*, discovered by utilising a model system that supports anaerobic culture and a functioning epithelium side-by-side. Future work will investigate colonisation resistance activity for a panel of *R. gnavus* strains and attempt to elucidate mechanisms behind this activity.

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Poster Number:18

*Flash poster presentation

Fastidious anaerobe agar (FAA) as a suitable medium for antimicrobial susceptibility testing (AST) of anaerobes by agar dilution

Background:

Agar dilution is the reference method for antimicrobial susceptibility testing (AST) of anaerobes, but currently the only verified and published method utilises supplemented brucella agar, which is not readily available in the UK.
As FAA is often the medium of choice for primary culture of anaerobes in clinical laboratories, the aim of this study was to investigate the suitability of this medium as an alternative to brucella agar for the reference method.

Methods

One hundred isolates submitted to the UKARU, all with pre-determined MICs to: clindamycin; meropenem; metronidazole; penicillin and doxycycline, were tested.
MICs were obtained by agar dilution using both in-house produced FAA, supplemented with 5% defibrinated horse blood, and supplemented brucella blood agar (BRU).
End points were selected according to CLSI guidance and interpreted using EUCAST clinical breakpoints for anaerobes (FDA guidance for doxycycline).

Results:

For the majority of isolates the correlation between FAA and BRU MICs was good.
Errors were found for each antimicrobial, with the highest numbers recorded for metronidazole and doxycycline. The majority of errors were within 1-2 dilutions, but spanned the clinical breakpoints. Others were single replicate errors, which were anomalous against a minimum of three additional results.
Several species such as *P. distasonis* and *C. ramosum* demonstrated raised numbers of errors/ variable results and will require further investigation.

Conclusions:

Overall the reproducibility of agar dilution testing on FAA compared to BRU was good, suggesting that FAA is a suitable media for AST of anaerobes. Further assessment of several species is required.

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Poster Number:19

*Flash poster presentation

Engineering a synthetic gut model to explore *Clostridioides difficile* infections

Our intestines play home to over one thousand bacterial species, often referred to as the gut microbiota. This microbiota profoundly affects our bodies, providing an array of benefits. Disturbances to this community are associated with pathogenic infection and diseases such as obesity, diabetes and inflammatory bowel disease. Our aim is to engineer a synthetic microbiota, with an emphasis on tracking individual species. Introducing this community into our *in vitro* gut epithelial culture system will provide insights into pathogen infection strategies, and mechanisms by which the microbiota generates resistance. This tool can be used for the screening of drug/probiotic candidates, potentially reducing the need for animals. Using PMA-qPCR we are able to track up to ten single species in a mixed biofilm. *Clostridium difficile* an opportunistic pathogen targets the gut during times of depleted microbiota. We have begun using our tracking technology to see how a representative microbiota reacts to a *C. difficile* invasion. Alongside this, we investigated the effect of the gut commensal - *Bacteroides dorei* on *C. difficile* utilizing classical quantification. In both a standard biofilm and in our *in vitro* model, we see a reduced number of *C. difficile* when in mixed culture.

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