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Emerging Zoonoses and AMR: A Global Threat

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Poster Number: 01

A novel approach to study transmission of AMR plasmids in complex microbial communities

Abstract

The study of antimicrobial resistance (AMR) transmitted by extrachromosomal genetic elements is currently limited by the absence of approaches able to link host-specific and plasmid-specific sequences within metagenomic datasets. To overcome this issue, we have developed a novel methodology that will allow the tracking of specific plasmids in complex microbial communities. Representative broad-host-range and narrow-host-range AMR plasmids were genetically modified to carry a rare bacterial methyltransferase. When expressed in the host bacterium, this enzyme methylates specific sites across the entire genome, including in species-specific genes useful for identifying organisms. Single-Molecule Real-Time sequencing technology can then detect the genomic methylation pattern based on polymerase kinetics. Thus far, we have verified methyltransferase activity in different bacterial hosts carrying the modified plasmids by using quantitative PCR to measure the extent of DNA protection after treatment with the cognate endonuclease, a restriction enzyme that digests unmethylated sites. Conjugative transfer, stability, and fitness of modified AMR plasmids was confirmed to be unaffected by the presence of the methyltransferase gene. As a proof of concept, *in vivo* assays using C57BL/6 mice infected with *Citrobacter rodentium* carrying the engineered plasmids are on-going. Metagenomic DNA isolated from faeces will be collected and sequenced to analyse the spread of these plasmids in the intestinal community. This approach will be useful to identify novel reservoirs and routes of plasmid dissemination in different environments, and test the effect of treatments to prevent AMR transmission.

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Poster Number: 02

Manuka honey inhibits the viability and virulence of *Staphylococcus pseudintermedius in vitro*

Abstract

Staphylococcus pseudintermedius is a common veterinary pathogen with significant zoonotic potential. The recent emergence of multidrug-resistant (MDR) *S. pseudintermedius* shows that novel therapeutic solutions are urgently required. Manuka honey inhibits many bacterial pathogens including methicillin resistant *Staphylococcus aureus*, and is used in both clinical and veterinary practice. It has also been demonstrated to act synergistically with antibiotics *in vitro*, increasing their potency and making it a promising addition to treatment regimens where MDR is predicted.

Here we determined the ability of manuka honey to influence *S. pseudintermedius* growth, biofilm formation and pathogenicity both alone and in combination with clinically relevant antibiotics. Low concentrations, ≥ 10% (w/v), of manuka honey were able to inhibit growth of 20 *S. pseudintermedius* isolates. Susceptibility to selected antibiotics (gentamicin, chloramphenicol, clindamycin, penicillin G, tetracycline) was significantly increased ($p = <0.05$) when combined with sub lethal concentrations of honey. Biofilm biomass was reduced by treatment with manuka honey, with live/dead staining and scanning electron microscopy showing inactivation of the cells within the biofilm and alterations in morphology. Finally, phenotypic expression of virulence was significantly reduced ($p = 0.05$) in the majority of isolates when treated with sub-lethal honey concentrations.

This study highlights the potential for manuka honey to be utilised in a veterinary setting, increasing the susceptibility of bacterial pathogens to antibiotics. With further *in vivo* testing this may offer an alternate therapy to those animals with infections that are not responding to conventional therapy, or when *S. pseudintermedius* causes opportunistic infections in humans.

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Poster Number: 03

Probiotics as an alternative disease mitigation in wildlife

Abstract

Bovine tuberculosis (bTB) is a chronic bacterial disease caused by Mycobacterium bovis that leads to significant economic losses worldwide. bTB has been eradicated from many European nations but it is still very prevalent in some countries where wildlife reservoirs of M. bovis have been confirmed. This is the case of wild boar and the European badger. Lactic acid bacteria (LAB) have been proposed as a new alternative for controlling bTB due to their probiotic properties, which include their ability to: (1) inhibit the growth of Mycobacterium species; and (2) trigger beneficial host immune responses. The main objective of this study was to evaluate the potentialas of LAB isolated from faeces of wild boar and badgers as a probiotic alternative against bTB. LAB have been isolated and identified as Pediococcus, Lactobacillus, Enterococcus and Weissella. Overall, the isolates have shown significant antimycobacterial activity and seem to be associated with innate immunomodulation. Some of the isolates have induced proinflammatory pathways, suggesting a potential role as vaccine adjuvants; whereas other isolates have showed potential anti-inflammatory properties. Further studies such as whole-genome sequencing and antibiotic resistance tests have confirmed the potential use of our LAB isolates as probiotics. Our data suggest that LAB could be used as vaccine adjuvants to reduce or prevent infection but also as a tool to reduce inflammation and the amount of viable excreted mycobacteria in highly infected animals. These measures could indeed lead to a long-term decrease in the prevalence of bTB and other infectious diseases in wildboar and badgers

Jorge Gutierrez-Merino

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Poster Number: 04

Development of a Nipah virus vaccine to eliminate porcine reservoirs and safeguard human health

Abstract

Nipah virus (NiV) poses a significant epidemic threat due to its broad host range and the widespread distribution of fruitbats which provide a natural reservoir. Human infection can occur following exposure to infected pigs resulting in severe and often fatal respiratory and neurological disease. Pig-to-human transmission was responsible for the first and most severe NiV outbreaks in Malaysia and Singapore. These outbreaks resulted in significant economic costs and long-term damage to local pig industries. Despite the importance of NiV as an emerging pathogen, no vaccines are currently approved for human or livestock use. We propose that an inexpensive, safe and efficacious vaccine could be developed to protect pigs against NiV infection and transmission, therefore reducing the risk to public health as well as pig industries in the endemic region. In this project, we are assessing the immunogenicity and efficacy of three NiV vaccine candidates: (1) a recombinant NiV G protein subunit vaccine; (2) a recombinant NiV F protein subunit vaccine and (3) a replication deficient adenoviral vector expressing NiV G protein. The most effective candidate will be further assessed to determine its ability to prevent NiV transmission, the durability of immunity and optimal immunisation regimen, followed by evaluation of safety and immunogenicity under field conditions in two high-risk countries. In addition to developing a porcine NiV vaccine, data generated through this study will directly inform efforts to develop a human NiV vaccine.

Rebecca McLean[1], Dalan Bailey[1], Nagendra Nath Barman[2], Li-Yen Chang[3], Keith Chappell[4], Sarah Gilbert[5], Teresa Lambe[5], Glenn Marsh[6], Miriam Pedrera[1], Rudiger Raue[7], Elma Tchilian[1], Nazia Thakur[1], Ooi Peck Toung[8], Daniel Watterson[4], Paul Young[4], Simon Graham[1]

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Poster Number: 05

Using a multi-disciplinary, one health, approach to understanding how emerging infectious diseases and antimicrobial resistance (AMR) can transfer from livestock to humans

Abstract

The European Joint Programme (EJP), is a co-fund action designed to support coordinated national research and innovation programmes. The EJP aims to pool a critical mass of national resources to work on the objectives and challenges of Horizon 2020. The University of Surrey, along with their partners across Europe, were successful in obtaining €90 million in order to investigate the emergence and transmission of infectious diseases, and Antimicrobial Resistance (AMR) throughout the food chain. Here we will present an overview of the project and its scope, highlighting the benefits of the one health, multidisciplinary approach applied.

The University of Surrey will focus primarily on two complementary research areas. The microbiome of key animal species (pigs and poultry) will be probed in order to determine if specific microbiota compositions are linked to the pathogen shedding status of the host. Probiotic species and nutraceuticals will then be identified and assessed for their ability to limit shedding, and so reduce spread of bacterial pathogens within herds and flocks. In parallel, the main drivers and agents involved in AMR transmission and dissemination across Europe will be interrogated. A particular focus of the studies will be on mobile genetic elements and their transfer potential between hosts. It is anticipated that the outputs of the studies will result in reduced zoonotic pathogens in the food chain and reduced antimicrobial usage and AMR. Emphasis will also be placed on training of scientists, in order to ensure the sustainability of the research area and collaborations.

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Poster Number: 06

Combating the rising global threat of antimicrobial resistance with clay minerals: A study on two major hospital superbugs.

Abstract

The global increase in antimicrobial resistance is placing increasing pressure on healthcare systems today. Historically, clay minerals have been used to treat intestinal ailments and mild skin conditions. More recently, research has demonstrated that specific clays may possess antimicrobial properties. With this in mind, we have focused on a new method to treat *Clostridium difficile* (*C. difficile*), the leading cause of infectious diarrhoea within hospitals, and Methicillin-resistant *Staphylococcus aureus* (MRSA), the number one cause of hospital skin infections worldwide. Using geochemical techniques, such as X-ray diffraction and Inductively-coupled plasma mass spectrometry, the physicochemistry of seven test clays was determined to assist in understanding the antimicrobial mechanism of the clay. To test the antimicrobial capability of the test clays, viability counts were used with hydrated clay minerals and both antibiotic-susceptible and antibiotic-resistant pathogenic bacteria to assess the feasibility of using clay minerals as therapeutic agents, ie. 'nutraceuticals'. The 'French green' clay, composed of 91% quartz, demonstrated complete sterilisation of both bacteria following overnight incubation; supporting previous research with other pathogenic organisms. To establish the use of clays as geo-medical therapeutics, further pharmacotoxicology using *in vitro* human tissue models (i.e. gut and skin) will be employed to elucidate the mechanisms of clay bioreactivity. This study will help to further the understanding of antimicrobial clays, potentially leading to alternative therapies to decrease the current over prescription of antibiotics and the rising emergence of antimicrobial resistance.

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Poster Number: 07

A survey of antibiotic resistance in enteric *Escherichia coli* isolated from ungulates at a zoological park

Abstract

Background: The antimicrobial resistance (AMR) profile of bacteria isolated from domesticated animals and free-ranging wildlife has been studied extensively. However, there are few studies describing the AMR profile of bacteria isolated from captive wild animals. This study investigated the presence of AMR phenotypes in commensal *Escherichia coli* and some of the environmental factors that might affect AMR dynamics in the target population.

Methods: In this study, freshly evacuated faecal samples were collected from 17 species of healthy ungulates at Marwell Zoo in Hampshire, England that yielded a total of 39 commensal *E. coli* isolates. Antibiotic sensitivity was investigated using agar disk diffusion methods.

Results: Seven out of 39 (18%) *E. coli* isolates were resistant to more than three antibiotic classes, the most common pattern of resistance was: penicillins, tetracyclines, aminoglycosides and sulphonamides. None of the isolates tested positive for extended-spectrum beta-lactamase (ESBL) or AmpC activity using a disk diffusion screening kit. The *E. coli* isolates were further analysed using multi-locus sequence typing (MLST) which identified four pairs of identical sequence type (ST) isolates and 27 diverse strains. Review of the medical records of individual animals showed previous use of penicillins, sulphonamides and tetracyclines. There was no apparent spatial clustering of the resistance profiles within the zoo suggesting that resistance genes were not being spread between enclosures.

Conclusion: Studying the resistance phenotypes of commensal bacteria is a useful indicator for monitoring the effects of antibiotic use and biosecurity measures in zoos.

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Poster Number: 08

Zoonotic antimicrobial resistant Candida and bacterial species isolated from companion animals.

Abstract

The increasing appearance of microorganisms showing resistance to a collective array of antimicrobial drugs presents a serious threat to public health safety. Prolonged infectious diseases such as pneumonia and candidiasis are becoming more difficult to treat, and often fatal, as therapeutics become less effective against deadly resistant pathogens. Furthermore, systemic and dermal fungal infections have become increasingly prevalent in companion animals where they represent a routine problem for veterinarians. Whereas, fungal infections in humans are common and often fatal, by end of the 1990s fungal infections had become the seventh leading cause of human morbidity resultant from infectious disease. The treatment and control of invasive fungal infections in animals traditionally relied on the use of amphotericin B with the azoles being introduced in the last decade. Drug resistance in fungal species such as *Candida* is an emerging problem with resistance to these antifungals a common occurrence in infected animals. Indeed, the prognosis for infected animals remains poor with mortality rates exceeding 80%. Studies conducted aimed to establish the type, frequency and level of resistance of isolated species from prolonged infections in companion animals. Samples of infection (swabs) were cultured and analysed for pathogenic species followed by antimicrobial susceptibility testing. Here we present a concise detailed summary of the types of pathogenic zoonotic species present in companion animals and their resistance to current therapeutic agents. Future work aims to develop novel disinfectant agents and therapeutics which may be used in the veterinary sector to prevent zoonotic transmission.

Keywords: Resistance, Zoonotic, Candida

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Poster Number: 09

Developing phage therapy for the treatment of *E.coli* infections in companion animals

Abstract

Background: Antibiotic resistance is a global issue threatening the future of medicine. The emergence of resistance to "antibiotics of last resort", such as colistin via the MCR-1 gene, highlights the urgent need for identification or development of alternative antimicrobial strategies. The possibility of using bacteriophages for treating multi-drug resistant infections in veterinary practice needs exploring. *Escherichia coli* is associated with a wide range of infections with increasing numbers of multi-drug resistant strains isolated in small animal practice.

Results: Ten strains of *E. coli* were isolated from dogs in a veterinary referral hospital, all of which were implicated in surgical wound infections or UTI's. *E. coli* strains were found to be distributed across 4 phylogroups, including groups B2 and D, which are the major phylogroups associated with extra-intestinal infections. Seven strains were multi-resistant, showing *in vitro* resistance to 3 or more classes of antibiotics by disk diffusion testing, and all 10 genomes contained genes associated with antibiotic resistance, such as penicillins, sulphonamides, aminoglycosides or tetracyclines. Canine faecal samples and soil were obtained to detect and extract lytic phages, using standard plaque assays against a control strain of *E. coli* and the 10 canine isolates. Two lytic phages have been isolated from the first faecal sample and are being tested against the panel of canine isolates.

Future work: This work is part of an ongoing project and is in the early stages of obtaining suitable phages for further testing and development.

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Poster Number: 10

Avian Pathogenic and canine, and human uropathogenic *Escherichia coli*; Reservoirs of antimicrobial resistance

Abstract

Background: AMR is a growing public health problem. The emergence of multidrug-resistance in extra-intestinal *Escherichia coli* (ExPEC) strains is of particular concern. In humans and dogs, a subgroup of ExPECs (UPECs) are an important cause of urogenital infections, while in poultry the APEC subgroup can cause high mortality. This study aimed to compare the APEC, canine and human UPEC subgroups using genotypic and phenotypic characteristics.

Methods: AMR profiles of 52 APEC, 98 human and 133 canine UPEC were determined using disc diffusion assays. MICs were determined for colistin. 167 strains (55 poultry, 64 canine and 48 human) were selected for genome sequencing and analysis.

Results: A range of AMR profiles was observed over the 283 strains tested. The profiles in human and canine UPEC were more similar to each other than to that of the APEC. Comparative genomics based on core genome SNPs and *in silico* phylogroup PCR showed that the majority (63.4%) of ExPEC strains sequenced were phylogroup B2. However, phylogroup distribution partly reflected isolation source, in that 70.8% human and 84.4% canine UPEC belonged to phylogroup B2 with <2% phylogroup C isolates, while 32.7% and 41.8% of APEC belonged to phylogroups B2, and C respectively. Interestingly canine and human UPEC of phylogroup B2 clustered together

Conclusion: The clustering of human and canine UPEC indicates a possible shared source or transmission route for UPEC, while sources of APEC are likely to be more diverse. Similarly AMR profiles indicate a closer relationship between human and canine UPEC than with APEC.

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Poster Number: 11

Complete Usutu virus genome sequences derived directly from clinical samples by using a multiplex PCR-based whole genome sequencing method.

Abstract

Usutu virus (USUV) is a zoonotic mosquito-borne flavivirus which emerged in Europe in 1996. Since then, USUV has been the causative agent of epizootics and smaller outbreaks among wild and/or captive birds in many countries. We have developed a protocol comprising of a novel multiplex PCR enrichment protocol, optimised library preparation methods for Illumina, and a bioinformatics pipeline for generating consensus genomes. It utilises multiplex PCR for targeted enrichment of viral genomes from samples containing as few as 50 genome copies per reaction. This method successfully recovered whole genomic sequences from organ samples recovered from birds that had naturally died from USUV infection during an outbreak in Austria and Hungary between 2010 and 2011, obtaining a sequence that was 99.4-99.7% identical to published sequences from the outbreak. During vector competence studies, native UK mosquitoes (*Culex pipiens*) were infected with an African strain of USUV (SAAR-1776) via a bloodmeal. We were able to obtain USUV whole genome sequences from the input virus and mosquito abdomen. Changes in the consensus level were identified between the input virus inoculum and the virus retrieved from the mosquito abdomen, leading to one synonymous and one non-synonymous amino acid change. This novel method provides a useful tool for characterising USUV infections during outbreaks, determining the virus origin as well as the chains of transmission within outbreaks.

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Poster Number: 12

Identification of lineage-specific genetic markers of *Listeria monocytogenes* by pangenome analysis

Abstract

Background: The rapid progress in DNA sequencing is revolutionising microbiology, and now allows for the analysis of large collections of pathogen genome sequences for evolutionary patterns, as well as the presence of markers for virulence, transmission and antimicrobial resistance. Here we have initiated an analysis of the pan-, core and accessory genomes of 966 isolates of the foodborne pathogen *Listeria monocytogenes*.

Objective: To identify *L. monocytogenes* genes associated with specific lineages and isolation sources.

Results: A total of 966 genomes were compared (Lineage I (510), Lineage II (421), Lineages III/IV (35)), with the isolates representing clinical isolates (281), food processing sites (341), food (225), animals (82) and environment (37). The pangenome was identified using Roary (80% identity with paralog clustering) and consisted of 2,494 core genes and 4,720 accessory genes. Genomes clustered strongly on their respective MLST-clonal complexes, with mobile genetic elements (prophages, plasmids, transposons) representing the major differences. Analysis of lineage CC6 confirmed the reported association between disinfectant resistance and clinical isolates, whereas in lineage CC2 isolates two mobile elements, both encoding Leucine-Rich Repeat (LRR) internalin-like proteins and regulatory proteins, were present in most clinical isolates, while absent in food-processing isolates.

Conclusion: Here we have analysed *L. monocytogenes* genome sequences to identify genes associated with clinical isolates in lineage CC2, although this association was absent in lineage CC6, highlighting the need to take genetic background into account when doing these comparative genomic studies. The LRR internalin-like genes identified here could represent candidates for future virulence studies.

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Poster Number: 13

The impact of host restriction of *Escherichia coli* on transmission dynamics and spread of antimicrobial resistance (HECTOR)

Abstract

The Joint Programming Initiative on Antimicrobial Resistance (JPI AMR) was formed in 2011 by 15 European Countries with the support of the European Commission, and now comprise 26 countries globally. It is funding €65 million of basic and exploratory research on new antibiotics, stewardship of existing antibiotics, and studies and control of the spread of antibiotic resistance between humans, animals, and the environment in a One Health perspective.

The prevalence of antimicrobial resistance (AMR) is increasing rapidly worldwide. The commensal flora of healthy humans and animals is a reservoir of AMR encoding genes, and *E. coli* in particular can carry multiple resistance factors that are easily mobilised. Host restriction may be an important determinant of the likelihood of transmission of AMR *E. coli* between different reservoirs, such as between animal and human hosts.

HECTOR is a multidisciplinary project that aims to combine the resources, infrastructures, and research strengths of multiple countries in order to identify genetic determinants of *E. coli* host restriction, and assess the associated impact of these on AMR transmission and prevalence. Multiple methods are being utilised, including whole genome sequencing of a large collection of *E. coli* isolates from human and animal sources from different geographical areas across Europe and Vietnam. Experimental models, such as continuous flow chicken gut bioreactor systems and *in vivo* animal models, will be employed to study the role of host restriction on AMR transmission and the influence of AMR on pathogen fitness.

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Poster Number: 14

Iodide Compositions for the Treatment of Bovine Mastitis

Abstract

Bovine mastitis is a common and costly disease affecting the dairy industry. It is an infection of the mammary gland caused by a plethora of organisms. Due to the shear number of causative organisms and their ubiquitous presence, mastitis eradication is unachievable. Therefore, control and treatment are the only feasible options. Treatment relies heavily on antibiotics. Economically, mastitis costs the EU €2 billion per annum; but at the individual farm level, mastitis leads to reduced milk quality, reduced milk production, high SCC, and culling.

At Westway Health a novel antimicrobial based on peroxidase-catalyzed systems has been developed, leaving no residues so it does not require a milk withdrawal and permits addition of milk to the bulk tank during treatment. The antimicrobial targets bacterial cells via the generation of the hypoiodite ion and experimental results demonstrates how this ion quickly eradicates any microbes present both *in vitro* and *in vivo*. *In vitro* experiments have determined the MIC to be comparable to antibiotics currently used to treat mastitis (6-15 mg/ml). Resistance was not induced after passaging test organisms in sublethal concentrations of the antimicrobial. In addition, the antimicrobial does not alter the pH of the milk.

This antimicrobial is currently being tested *in vivo,* showing that once it is administered, the clinical signs of mastitis are significantly reduced, no negative long-term effect has been seen, and it does not negatively affect the SCC. Overall, the results of this study show excellent potential for a non-antibiotic approach to treating bovine mastitis.

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Poster Number: 15

Antibiotic Resistance Patterns and Gene Interplay Between Animals, Humans and the Environment in a High-Density Livestock-Human Population in Western Kenya

Abstract

Antimicrobial resistance (AMR) is a global health problem. Antimicrobials are no long able to cure bacterial infections owing to the transfer of resistance genes between bacteria. The double-disc diffusion is the gold standard for determining AMR patterns, however, technologic advances in whole genome sequencing (WGS) has provided us with another method to investigate the diversity of antimicrobial resistance genes. This study determined resistance patterns of *E. coli* isolated from humans, animals, and the environment from multiple study sites in Western Kenya.

Faecal samples were collected from a representative number of farm animals, farmers, and their immediate environments. *E. coli* was isolated and purified from 680 samples. Phenotypic antimicrobial susceptibility was determined in all samples by double-disc diffusion (EUCAST), followed by WGS (Illumina, HiSeq) of a subset of 192 *E. coli* isolates. MLST was performed and resistance genes were identified using ResFinder.

High prevalence of AMR *E. coli* was found in farmer, animal, and environmental samples; resistance to tetracycline, trimethoprim, and sulfathiazole, was the most common. WGS analysis indicated that ST-196 was the most common ST-type. All ST-196 *E. coli* had the same serotype (O8:H7). Phylogenetic analysis (using SNPs) indicated that there was no specific cross-over over *E. coli* between humans, animals, and the environment within each farm.

Suitable bioinformatics methods which are rapid, accurate and easily interpretable are still lacking. The WGS work as part of this study will be compared to previous laboratory data to aid the validation of this method.

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Poster Number: 16

Anthrax and clay minerals: a novel solution.

Abstract

Bacillus anthracis, the cause of Anthrax, is endemic to large swathes of Africa and South America. Its endospores persist in the environment and are the primary cause of infection (to animals and humans). Multiple state actors and terrorism groups have utilised the spores as bioweapons. Current methods of decontamination, whilst effective, cause environmental decimation and are themselves environmentally toxic. Thus, it is desirable to consider novel methods for decontamination of affected environments.

Recently, some bio-reactive clays have demonstrated antimicrobial properties. Geochemical investigation by X-ray diffraction and Inductively-coupled plasma mass spectroscopy on a range of test clay minerals has been undertaken to help elucidate the mechanism of action.

Antimicrobial ability was assessed using viability counts of hydrated clay minerals on both vegetative cells and spores of Sterne strain *B. anthracis*. One clay ('French Green') has shown Log_{10}^{5} reduction of viable colonies in both vegetative cells and spores (0.15g/ml and 0.05g/ml, respectively) after 20-hour incubation. French Green clay is 91% quartz mineral and has been used as a topical treatment for cutaneous *Mycobacterium Ulcerans* infections. These results support previous studies on other organisms.

Further *in-vitro* toxicological studies will be employed to determine the activity of antimicrobial clay minerals on human tissue models; to ensure that deployment of the clay as an environmental decontaminant would not be hazardous to inhabitants.

This study will aid the understanding of the action mechanism of antimicrobial clays and help determine the possibility of clay mineral usage as an environmental decontamination solution for bacterial contaminants.

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