Biosciences

Risk Assessment for the growth and manipulation of *Enterococcus faecalis* and *E. faecium*

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Nature of revision:					

Risk Assessment for the growth and manipulation of *Enterococcus faecalis* and *E. faecium* strains

1. Responsibilities

1.1 Academic supervisors are responsible for authorising this procedure and ensuring that this procedure is implemented and complied with.
1.2 Individuals working with *E. faecalis* and *E. faecium* are responsible for ensuring that they read and understand the risk assessment and for complying with any duties or control measures.

2. Related documentation and other procedures

2.1 College of Life and Environmental Sciences (CLES) "Code of Practice: Microbiological Safety Policy"
2.2 Other relevant risk assessments including Biosciences "Risk Assessment for General Bacteriological Methods at CL2"

3. The Risk Assessment

3.1 Agent Specific Information

E. faecalis and *E. faecium* are commensal bacteria inhabiting the intestinal tract, female genital tract, and (less commonly) oral cavity. The organisms were formerly classified as part of the group D Streptococcus system (*Streptococcus faecalis and S. faecium*). Enterococci are opportunistic pathogens and can cause urinary tract and abdominal infections, wound and soft tissue infections, bacteremia and endocarditis (1). Individuals with underlying medical conditions and immunocompromised patients are most at risk of infection.

In recent years there have been increases in the incidence of infections due to vancomycin-resistant enterococci (VRE). Resistant enterococcal strains are the leading cause of endocarditis and are common pathogens in indwelling catheters. In addition, most VRE strains harbour resistance to multiple antibiotics besides vancomycin (e.g. aminoglyscoides and ampicillin) and this can pose a major challenge in the treatment of these infections. Genetic resistance to vancomycin involves 5 tandem genes that are easily transferred by plasmids and transposons and therefore resistance to vancomycin can rapidly spread. Vancomycin Resistant *Staphylococcus aureus* (VRSA) gained its vancomycin resistance from VRE in this way (2).

3.2 Identification and characteristics

Enterococci are facultatively anaerobic, catalase-negative Gram-positive cocci, arranged individually, in pairs, or short chains. The optimal temperature for growth is 35°C. They catabolize a variety of energy sources including glycerol, lactate, malate, citrate, arginine, agmatine, and many keto acids. Enterococci can survive harsh environments including extremely alkaline pH (9.6) and salt concentrations. They resist bile salts, detergents, heavy metals, ethanol, azide, and desiccation. They can grow in the range of 10 to 45°C and survive at temperatures of 60°C for 30 min.

3.3 Pathogenicity and virulence

Due to their ability to survive environmental challenges and their ability to acquire resistance to multiple antibiotics, enterococci have emerged as a major cause of hospital acquired infections. Several virulence factors are thought to contribute to *Enterocccus spp.* infections.

A plasmid-encoded hemolysin, called the cytolysin, is important for pathogenesis in animal models of infection, and the cytolysin in combination with high-level gentamicin resistance is associated with a five-fold increase in risk of death in human bacteremia patients (1).

3.4 Infectious Dose
Not known.

3.5 Host range
E. faecalis and *E. faecium* are found in the gastrointestinal tract of humans and animals including mammals, birds, insects, and reptiles. It is likely that transmission can occur from animals to humans.

3.6 Laboratory Acquired Infection
No recent cases have been reported.

3.7 Environmental Impact
Enterococci can grow and survive in harsh environments, and can persist almost anywhere including soil, plants, water, and food. When they are found in water it is typically due to faecal contamination. The organism can survive 5 days to 4 months on dry inanimate surfaces.

4. Process Descriptions
This risk assessment covers the growth of *E. faecalis* and *E. faecium* on agar plates, in broth in volumes of < 100ml. Cells harvested by centrifugation may be processed to isolate proteins or DNA for subsequent analysis.

The risk assessment covers the growth of *E. faecalis* and *E. faecium*. It does not cover the isolation and purification of toxins.

4.1 Isolation of bacteriophage with activity against *E. faecalis* and *E. faecium*

4.1.1 Phage enrichment
Samples such as river water, animal faeces and raw sewage will be collected from the environment and filtered through a 0.22 µm filter. Separate risk assessments are available for these processes (Working with animal faeces, Working with raw sewage). The filtrates will be incubated with *E. faecalis* or *E. faecium* in a 96 deep well plate. The plate will be covered with a plate seal and incubated at 30°C/37°C with shaking. Following this initial enrichment step, the deep well plate will be transferred to the biological safety cabinet (Class 2). This is to prevent any potential release of phage aerosols into the lab environment which could result in potential contamination of lab cultures. 200 µL from each well will be transferred into a 96 well filter plate on top of a regular 96 well plate. The plate stack will be placed in a safety centrifuge cup or sealed with parafilm and centrifuged (900 g, 4 minutes). A second enrichment step with *E. faecalis* or *E. faecium* will be carried out and the resulting filtrates used in spot assays. Filtrates containing potentially active phage will be handled in the safety cabinet.

4.1.2 Spot plaque assays
A lawn of *E. faecalis* or *E. faecium* will be created by mixing an overnight culture with molten top agar and pouring over the surface of bottom agar plates. Once the top agar has set, the plates will be transferred to the biological safety cabinet (Class 2) and the (phage) filtrates

will be spotted onto the surface. The lids will be left off of the plates to allow the drops to dry. Once dry, the lids will be replaced and the stack of plates taped together. The plates will be placed in a plastic storage box with holes in the lid and incubated at 37°C. Following incubation any resulting plaques will be picked using a pasteur pipette and placed into buffer. This will be carried out in the safety cabinet. Any plates containing phage that need to be transferred to the fridge will be sealed with parafilm.

Any spillages of *E. faecalis* or *E. faecium* will be decontaminated with 70% ethanol and the contaminated blue roll placed directly in the autoclave waste bin. Spent cultures will be autoclaved as described in section 10.1. Any spillages of (phage) filtrates will be decontaminated with 70% ethanol and the contaminated blue roll placed in a plastic bag prior to placing in the autoclave waste bin. Any plasticware containing (phage) filtrates which are no longer required will be placed in a plastic bag prior to placing in the autoclave waste bin.

5. Hazard, risk and control measures

Risk Rating	Action Required
Minimal	Controls Adequate (Unlikely harm would ever result from the activity)
Low	Review Controls, take action as necessary. (Harm would seldom result)
Medium	Action to be taken to reduce risk. (It is reasonably possible that harm could result)
High	Urgent action required. Consider halting activity/process. (It is certain or almost certain that could result)

Hazard	Exposure to *E. faecalis* and *E. faecium*. Any procedures which could result in ingestion or accidental inoculation through the skin pose the greatest risk. Medium Risk
Control measures	Handle under ACDP 2 conditions, by individuals who have been trained in microbiological practices and work according to the (CLES) Code of Practice: Microbiological Safety Policy. In the event of a spill swab with 70% ethanol. Gloves will be worn at all times in the laboratory to reduce the likelihood of hand to mouth transmission to a minimum.
Risk	**Low after measures described above are taken**

Hazard	Exposure to hazardous chemicals
Control measures	See “Risk Assessment for general bacteriological methods at CL2”
Risk	**Low**

5.1 Where and under what circumstances will the work be done.

All procedures detailed above will be carried out within a designated ACDP containment level (CL) 2 laboratory.

6. Who might be affected?

1. Staff competent to work at CL2 or those under their direct supervision.
2. Individuals not directly involved with the process/procedure/equipment etc but who may be affected; for example co-workers, students, contractors, cleaners etc.
3. Any individual who enters the laboratory whilst work is ongoing. Individuals working in close proximity.
4. Individuals working in the workplace who are likely to be at increased risk include:
    - Pregnant workers or those who are breast feeding
    - Young persons (i.e. < 18 years of age)
    - Persons on work experience or training schemes
    - Temporary employees
    - Contractors
    - Lone workers
    - Individuals with known hypersensitivity to antibiotics added to media
    - Individuals suffering from atopic dermatitis patients (individuals should seek professional medical advice before working with *E. faecalis* and *E. faecium*).
    - Immunosuppressed individuals (individuals should seek professional medical advice before working with *E. faecalis* and *E. faecium*).

7. Information, instruction and training

Individuals should be trained in the safe use of this procedure.

8. Health surveillance

Individuals suffering from atopic dermatitis and immunosuppressed individuals should seek professional medical advice before working with *E. faecalis* and *E. faecium*.

9. Emergency Procedures

9.1 Spillages

Any spillage of biological materials should be contained and covered with absorbent paper/cloth, which should subsequently be placed in an appropriate bin for autoclaving. The contaminated area should be washed exhaustively with 70% ethanol.

In normal use the risk to the environment is zero because working practices prevent the organisms from escaping.

9.2 Personal contamination

In the event of personal contamination with biological materials, remove any contaminated clothing as quickly as possible.

Eye contact: Wash exhaustively with the emergency eye bath.

Skin contact: Wash with soap and cold water.

Mouth contact: Flush mouth exhaustively with water. Avoid ingestion.

Puncture wounds: Encourage to bleed. Wash minor cuts and similar lesions with soap and water before applying a dressing as required.

Medical advice must be sought if there is a risk of infection.

10. Safe disposal

10.1 Autoclaving of contaminated waste

All waste containing biological material will be autoclaved using a destruct cycle at 130°C for at least 25 min, except where it contains formaldehyde or organic solvents. Records of the load will be kept, detailing the nature of the load and chart records of the temperature profile. The autoclave will be serviced and validated annually using thermocouples.

10.2 Contaminated waste which cannot be autoclaved

Procedures for disposal of biological waste containing organic solvents or formaldehyde are outlined below. These must be read in conjunction with procedure- and chemical-specific information contained within the relevant sections of the "Risk Assessment for general bacteriological methods at CL2".

10.2.1 Anything other than trace amounts of organic solvents must not be autoclaved and should be stored in a suitable waste container in a solvents cupboard for treatment with a biocide and subsequent incineration. Solvent waste which cannot be autoclaved should be treated with 10% v/v Teknon Biocleanse for at least 24 hr, and then removed from the laboratory for incineration.

10.2.2 Solutions containing formaldehyde should not be autoclaved. Waste formaldehyde should be stored in a suitable waste container in a solvents cupboard and sterility checked, before removal from the laboratory for incineration.

11. References

1. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection (2014). Gilmore MS, Clewell DB, Ike Y, et al., editors. Available from: https://www.ncbi.nlm.nih.gov/books/NBK190426/

2. O'Driscoll, T., & Crank, C. W. (2015). Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. *Infection and Drug Resistance*, *8*, 217–230. http://doi.org/10.2147/IDR.S54125