Biosciences

Risk Assessment for the growth and manipulation of *Staphylococcus aureus*

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Risk Assessment for the growth and manipulation of *Staphylococcus aureus* 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 *S. aureus* 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 School of Biosciences "Code of Safety Practice"

2.2 Other relevant risk assessments

3. The Risk Assessment

3.1 Agent Specific Information

S. aureus is an ACDP category 2 Gram-positive bacterium capable of causing infection in humans. *S. aureus* is commonly colonises human skin and mucosal surfaces. *S. aureus* can cause disease, particularly if there is an opportunity for the bacteria to enter the body. Illnesses such as skin and wound infections, urinary tract infections, pneumonia and bacteraemia may then develop. It can also cause food poisoning. Most strains are sensitive to antibiotics, and infections can be effectively treated. Some *S. aureus* bacteria are resistant to the antibiotic methicillin (methicillin-resistant *Staphylococcus aureus* or MRSA) which express a modified penicillin-binding protein encoded by *mecA* gene. MRSA strains producing the P-V leukocidin are especially virulent. Consequently, vancomycin became the primary antibiotic used to combat staphylococcus infection. In 1997 a strain of *S. aureus* resistant to vancomycin was isolated (VRSA).

3.2 Identification

S. aureus is a spherical bacterium. A Gram stain shows typical gram-positive cocci, which on microscopic examination appears in pairs, short chains, or bunched, grape-like clusters. Staphylococci are facultative anaerobes. They mainly grow by aerobic respiration, or fermentation that produces lactic acid.

S. aureus forms a fairly large yellow colony on rich medium, *S. epidermidis* has a relatively small white colony. *S. aureus* is often hemolytic on blood agar; *S. epidermidis* is non hemolytic. *S. aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent. Nearly all strains of *S. aureus* produce the enzyme coagulase: nearly all strains of *S. epidermidis* lack this enzyme. *S. aureus* should always be considered a potential pathogen.

For differentiation on the species level, catalase, coagulase (fibrin clot formation), DNAse , lipase and phosphatase tests are required. *S. aureus* is catalase catalase positive. A large percentage of *S. aureus* can be differentiated from other staphylococci by the coagulase test. *S. aureus* is primarily coagulase-positive while most other Staphylococcus species are coagulase-negative. However some *S. aureus* may be atypical in that they do not produce coagulase.

A range of commercial slide agglutination tests for *S. aureus* are available e.g. Masta-Staph (Mast Diagnostics), Staphylase-Test (Oxoid), Staphytect-Plus (Oxoid), Staphyloslide Latex (Becton Dickinson), Slidex Staph Plus (bioMerieux) and Dry Spot Staphytect Plus (Oxoid). Zschöck *et al*., 2005)

PCR based tests for *S. aureus* have been reported including species specific tests (Liu *et al*., 2007) and PCR tests for MRSA (Feil *et al*., 2008; Ornskov *et al*., 2008).

3.3 Pathogenicity & Virulence

S. aureus may cause various diseases including mild skin infections (boils, impetigo, folliculitis, etc.), invasive diseases (wound infections, osteomyelitis, endocarditis, bacteremia with metastatic complications, etc.), urinary tract infections and toxin mediated diseases (food poisoning, toxic shock syndrome or TSS, scaled skin syndrome, etc.). Infections are preceded by colonization. Common superficial infections include carbuncles, impetigo, cellulitis, folliculitis. Community-acquired infections include bacteremia, endocarditis, osteomylitis, pneumonia and wound infections are less common. *S. aureus* also causes economically important mastitis in cows, sheep and goats. Deeply situated *S. aureus* infections can be very severe. Prosthetic joints put a person at particular risk for septic arthritis, and staphylococcal endocarditis (infection of the heart valves) and pneumonia, which may be rapidly spread.

Staphylococcal food poisoning is normally dependent on the ingestion of food in which the bacterium has grown and produced the toxin. However, the toxins could be produced during growth in the laboratory, and ingestion of this toxin could cause food poisoning. A toxin dose of less than 1.0 microgram in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level is reached when *S. aureus* populations exceed 100,000 per gram. The toxin might also pose a risk if inhaled.

S. aureus expresses many potential virulence factors: (1) surface proteins that promote colonization of host tissues; (2) invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase); (3) surface factors that inhibit phagocytic engulfment (capsule, Protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production); (5) immunological disguises (Protein A, coagulase, clotting factor); and (6) membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (SEA-G, TSST, ET (8) inherent and acquired resistance to antimicrobial agents.

The toxins can be categorized into three groups. 1) Pyrogenic toxin superantigens (PTSAgs) have superantigen activities that induce toxic shock syndrome (TSS) 2) Exfoliative toxins are implicated in the disease staphylococcal scalded-skin syndrome (SSSS) 3) Staphylococccal toxins that act on cell membranes include α-toxin, β-toxin and δ-toxins, and several bicomponent toxins such as γ-toxin, Panton-Valentine (P-V) leukocidin and LukE-LukD leukocidin. PVL is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community-associated MRSA strains.

3.4 Infectious Dose

Not known

3.5 Host range

S. aureus causes disease in humans and it causes economically important mastitis in cows, sheep and goats. It can survive on domesticated animals such as dogs, cats and horses, and can cause bumblefoot in chickens.

3.6 Laboratory Acquired Infection

There are no reports of laboratory acquired infection on Medline or accessed via internet search engines. However in the community *S. aureus* infection is extremely prevalent in atopic dermatitis patients, who are less resistant to it than other people. It may also be problematic in immunosuppressed individuals.

3.7 Environmental Impact

S. aureus is widely disseminated in the environment and is a member of the normal bacterial flora in mammals including humans. It may occur as a commensal on human skin

(particularly the scalp, armpits, penis and vagina); it also occurs in the nose (in about 25% of the population and throat and less commonly, may be found in the colon and in urine. The occurrence of *S. aureus* under these circumstances does not always indicate infection and therefore does not always require treatment (indeed, treatment may be ineffective and re-colonisation may occur). It can survive on domesticated animals such as dogs, cats and horses, and can cause bumblefoot in chickens. *S. aureus* can survive for several dry environmental surfaces.

4 Process Descriptions

4.1 *S. aureus* Growth

This risk assessment covers the growth of *S. aureus* 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 *S. aureus*. It does not cover the isolation and purification of toxins.

4.2 Microscopy

4.2.1 Microscopy of fixed *S. aureus*-infected J774 & HL-60 (mammalian) cells

S. aureus infected-cell imaging should be carried out to ensure there is low risk to other microscope users. Cells infected with *S. aureus* mounted onto microscope slides should be sealed with a coverslip and a suitable fixing agent such as nail polish or VECTASHIELD®. Slides should be maintained in a sealed box during transportation to Bioimaging facility. Alternatively, cells may be grown on tissue culture plates then infected with *S. aureus*. Prior to transport to Bioimaging facility, the infected cells should be fixed using a suitable fixing agent such as VECTASHIELD® and the lid of the tissue plate sealed with parafilm. Tissue culture plates should be maintained in a sealed box during transportation to Bioimaging facility. After microscope analysis is completed the area should be decontaminated with 70% ethanol if necessary. All samples should be removed from the Bioimaging facility after microscopical observation. Glass slides should be discarded in suitable sharps bin and tissue culture plates disposed of according to protocols described in section 10.

4.2.2 Microscopy of live *S. aureus*-infected J774 & HL-60 (mammalian) cells

Cells will be grown on tissue culture plates then infected with *S. aureus*. Prior to transport to Bioimaging facility, the tissue culture's lid should be sealed with parafilm. Tissue culture plates should be maintained in a sealed box during transportation to Bioimaging facility. After microscope analysis is completed the area should be decontaminated with 70% ethanol if necessary. All live samples should be removed from the Bioimaging facility after microscopical observation and disposed of according to protocols described in section 10.

4.2.3 Microscopy of live biofilms of *S. aureus* on the surface of titanium and stainless steel coupons

Bacterial biofilms of *S. aureus* will be grown on the surface of stainless steel and titanium coupons. Prior to transport to the Bioimaging facility, plates will be sealed with parafilm and placed in a sealed box. After microscope analysis is completed the area should be decontaminated with 70% ethanol if necessary. All live samples must be removed from the Bioimaging facility after microscopical observation and disposed of according to protocols described in section 10.

4.3 Isolation of bacteriophage with activity against *S. aureus*

4.3.1 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 *S. aureus* 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 *S. aureus* will be carried out and the resulting filtrates used in spot assays. Filtrates containing potentially active phage will be handled in the safety cabinet.

4.3.2 Spot plaque assays

A lawn of *S. aureus* 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 *S. aureus* 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 *S. aureus*. 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 School of Biosciences "Code of Safety Practice". In the event of a spill swab with 70% ethanol. Gloves will be worn at all time 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 *S. aureus*).
   - immunosuppressed individuals (individuals should seek professional medical advice before working with *S. aureus*).

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 *S. aureus*.

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

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