



Biosciences

Risk Assessment for the growth and manipulation of *Klebsiella* spp.

Date reviewed:	May 2019 May 2021	Reviewer:	Julie Fletcher Julie Fletcher	Revision required?	May 2022
Nature of revision:	Addition of section 4.1 – Isolation of bacteriophage with activity against <i>K. pneumoniae</i> (JF)				

Risk Assessment for the growth and manipulation of *Klebsiella* spp. 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 *Klebsiella* spp. 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

The genus *Klebsiella* contains six species and three subspecies. There are four species related to humans and they include *K. pneumoniae subspecies pneumoniae*, *ozaenae*, and *rhinoscleromatis*; *K. oxytoca*; *K. granulomatis* and *K. variicola*.

Klebsiella spp. have been identified as important common pathogens for nosocomial pneumonia, septicaemia, urinary tract infection, wound infections, intensive care unit (ICU) infections and neonatal septicaemias. *Klebsiella* spp. can also cause bacteremias and hepatic infections, and have been isolated from a number of unusual infections, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis. They are also important opportunistic pathogens, particularly among the immunocompromised.

3.2 Identification and characteristics

Klebsiella spp. are Gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, belonging to the family Enterobacteriaceae. The genus consists of over 77 capsular antigens (K antigens), leading to different serogroups¹³. These well-developed polysaccharide capsules give the colonies their characteristic mucoid appearance. These bacteria produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test as well as indole and urease tests. They are generally facultatively anaerobic, and range from 0.3 - 1.0mm in width and 0.6 - 6.0mm in length. On MacConkey agar, the colonies typically appear large, mucoid, and red, with red pigment usually diffusing into the surrounding agar, indicating fermentation of lactose and acid production.

3.3 Pathogenicity and virulence

Pathogenicity factors of *Klebsiella* spp. include adhesins, siderophores, capsular polysaccharides (CPLs), cell surface lipopolysaccharides (LPSs), and toxins, each of which plays a specific role in the pathogenesis of these species. Depending on the type of infection and the mode of infectivity, cells of *Klebsiella* spp. may adhere and attack upper respiratory tract epithelial cells, cells in gastrointestinal tract, endothelial cells, or uroepithelial cells, followed by colonization of mucosal membranes. Common underlying conditions include alcoholism, diabetes mellitus, chronic liver disease (cirrhosis), chronic renal failure, cancer, transplants, burns, and/or use of catheters.

Klebsiella spp. are known to show resistance to penicillins, especially ampicillin and carbenicillin. Resistance of *Klebsiella* spp. to current antibiotics is increasing with more and more strains of *Klebsiella* spp. harbouring extended-spectrum beta-lactamases (ESBLs), cephalosporinases, and carbapenemases. Pan-resistant isolates have been identified in the Indian subcontinent.

3.4 Infectious Dose

Not known.

3.5 Host range

Humans, mammals (including horses, bovines, rhesus and squirrel monkeys, guinea pigs, muskrats, lemurs, and bats), aquatic animals (including elephant seals, California sea lions, and harbour seals), reptiles (including snakes, crocodiles, and American alligators), birds, insects, and plants (banana, rice sugar cane and maize).

3.6 Laboratory Acquired Infection

No recent cases have been reported.

3.7 Environmental Impact

Klebsiella spp. are widespread within the environment, including soil, water, sewerage and plants.

4. Process Descriptions

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

4.1 Isolation of bacteriophage with activity against *K. pneumoniae*

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 *K. pneumoniae* 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 *K. pneumoniae* 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 *K. pneumoniae* 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 *K. pneumoniae* 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 <i>Klebsiella</i> spp. 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 *Klebsiella* spp.)
 - Immunosuppressed individuals (individuals should seek professional medical advice before working with *Klebsiella* spp.)

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 *Klebsiella* spp.

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. Public Health England Standards for Microbiology Investigations.
SMI ID 16: Identification of Enterobacteriaceae