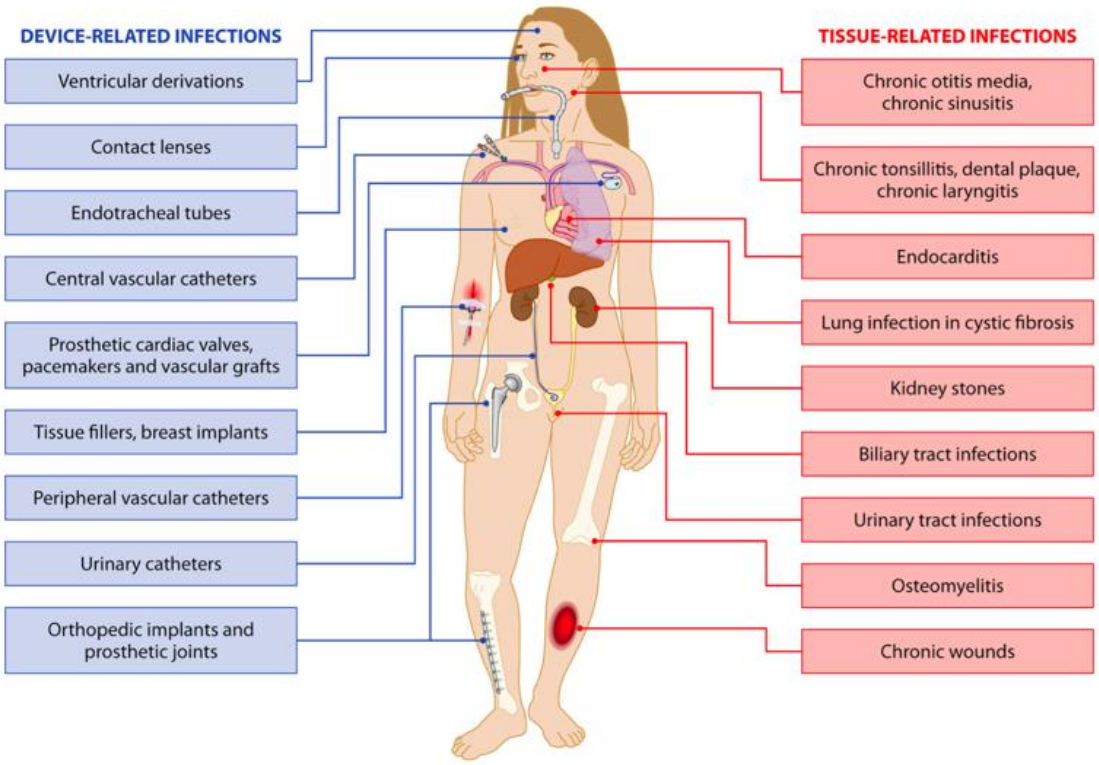
Pathogenic biofilms

Nurse: why don't antibiotics always work?



Medical Device and Tissue-Associated Infections (from Lebeaux *et al.*, 2014).

Kim Hardie and Paul Williams

Biodiscovery Institute, National Biofilms Innovation Centre and School of Life Sciences, University of Nottingham, UK

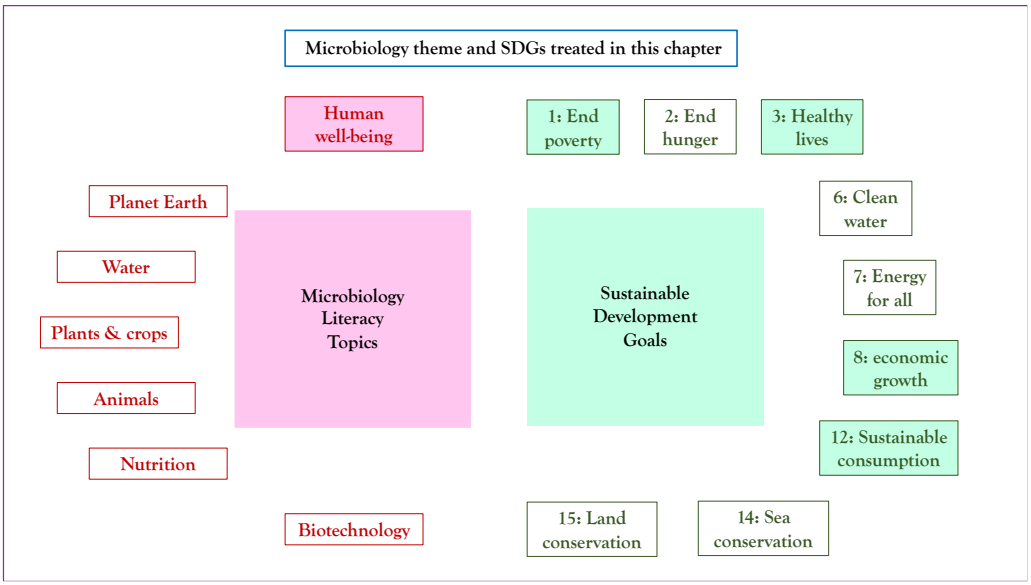
Pathogenic biofilms

Storyline

Populations of bacteria can live either as free-floating, individual cells or they can make **biofilms**, that are usually attached to a surface**.** Biofilms are communities of one or more bacterial species in which the individual bacterial cells produce and cover themselves with their own sticky 'slime'. Biofilms help bacteria to survive when food is scarce, the local environment is harsh and there are bacteria-eating predators about. Biofilms can be found naturally in damp environments such as on rocks in streams and pools where they are mostly harmless, but also in places where they cause serious health problems. These 'pathogenic' biofilms cause recurrent and long-term 'chronic' infections associated with implanted medical devices such as catheters and hip joint replacements. Medical devices are made out of materials which have the appropriate mechanical properties and do not by themselves cause local tissue inflammation and damage after implantation. Unfortunately, bacteria like to form biofilms on the plastics and metals used to make such devices. They also like forming biofilms in the lungs and on skin ulcers in people with chronic diseases such as **cystic fibrosis** and **diabetes**. By forming a biofilm, bacteria create an almost impregnable barrier that protects them from being killed by the human immune system as well as by antibiotics and disinfectants, making their treatment very difficult. Prevention of biofilm formation is usually much better than cure, especially for medical devices implanted deep within the body where their removal would have a major effect on patient health and well-being. Better hygiene and new drugs for killing or making biofilms antibiotic sensitive are needed, as well as making medical devices from smart materials that stop biofilms forming in the first place. Pathogenic biofilm infections have major sustainability issues for health and well-being, the economy and the environment - especially the disposal of millions of used plastic medical devices since they are not recycled.

The Microbiology and Societal Context

The microbiology: biofilms; immune responses to biofilms; antibiotic treatment failure; chronic infections; implanted medical devices; cystic fibrosis; diabetic foot ulcers. *Sustainability issues:* poverty; health; economy; pollution.



Pathogenic biofilms – the Microbiology

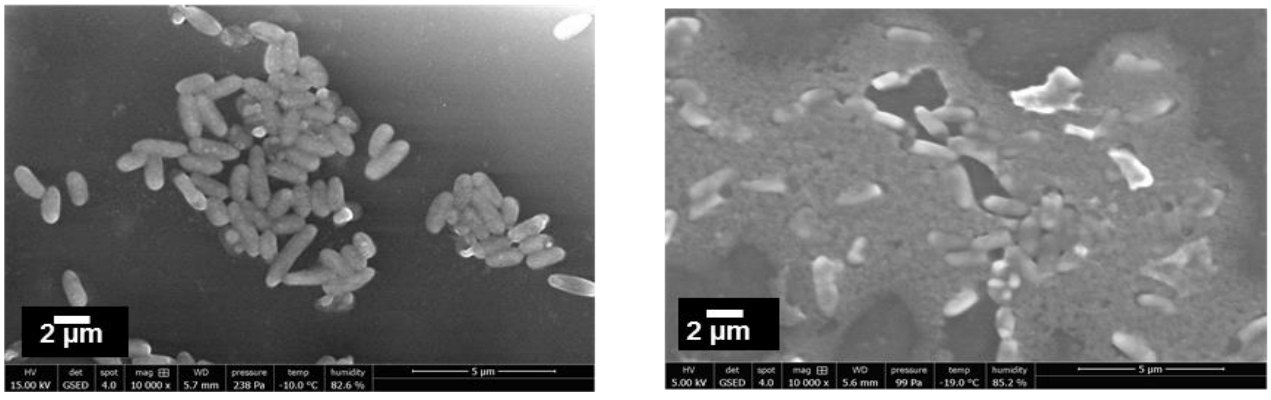
1. ***Pathogenic bacteria cause infections.*** Trillions and trillions of bacteria live everywhere on earth, in soil, water and air as well as on animals, plants and people. While most are harmless and some are beneficial, others cause infections that can make us ill. Infectious bacteria are called pathogens and infection occurs when they get inside a person's body (e.g. under the skin, into the lungs, blood, kidneys, brain or intestines). Pathogen entry can be via an accidental cut to the skin, from a surgical wound, by eating contaminated food or by breathing in harmful bacteria. Infection can be spread by coming into close contact with an infected person or with their body fluids.

The first signs that we have an infection might be because we have a fever, a sore throat, a cough or breathing difficulties. Once inside the body, pathogens reproduce and can cause damage to our vital organs, sometimes leading to the death of the infected person.

Fortunately, we are not defenceless and our immune defence systems are always on the lookout for intruder pathogens. When pathogenic bacteria get inside our bodies, one of the first lines of defence are predatory white blood cells called '**neutrophils**'. These patrol the bloodstream and internal organs and tissues looking for bacterial invaders which they capture and kill, either by using net-like traps or by engulfing and internalizing bacteria in a 'bubble-like' vesicle into which they release toxic **oxygen radicals** and **enzymes** to kill and then digest the bacterial cells. Neutrophils are usually very effective killing machines for individual bacteria and so play very important roles in eliminating pathogens.

If the immune defences fail to deal with an infection, then medical help usually in the form of treatment with an antibiotic is required. Antibiotics are medicines that either kill bacteria or prevent them from reproducing so that they can be cleared from the body by the immune system. However, pathogens can fight back against both antibiotics and the immune system by organizing themselves into protected communities: biofilms.

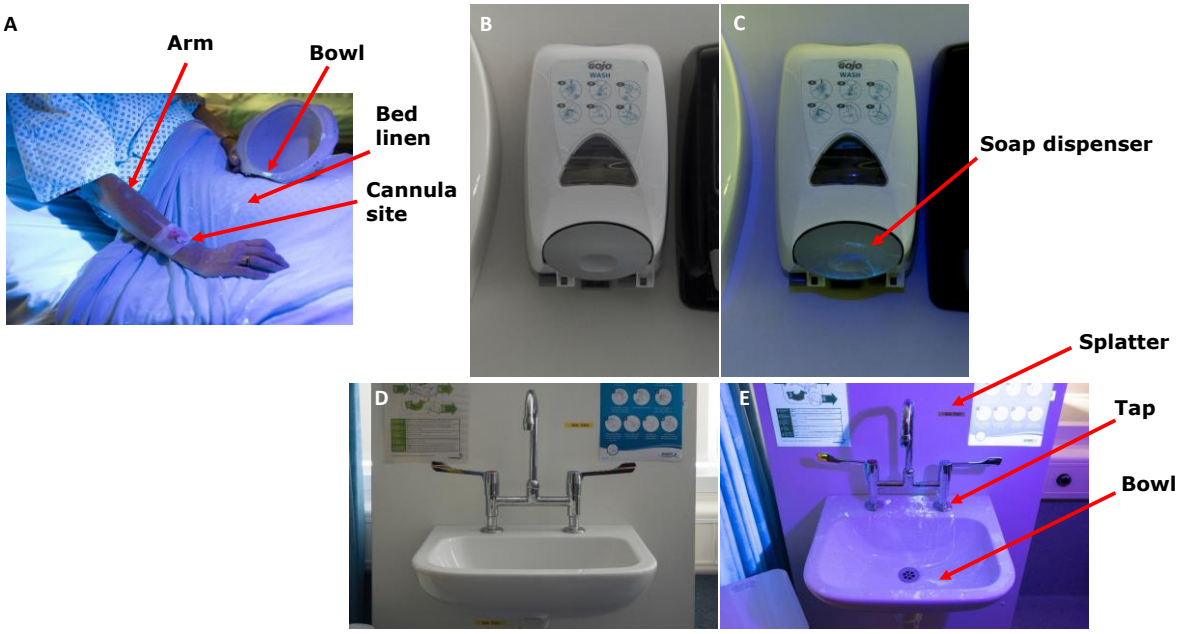
2. ***What are pathogenic biofilms and where are they found?*** In humans, pathogenic biofilms are 'slime cities' formed by pathogens in which the individual bacterial cells usually attach to a surface and produce and cover themselves with their own sticky protective 'slime'.



Scanning electron microscope images showing rod-shaped bacterial cells that have settled onto a surface (left) and after 24 hours (right) have embedded themselves into a sticky biofilm matrix.

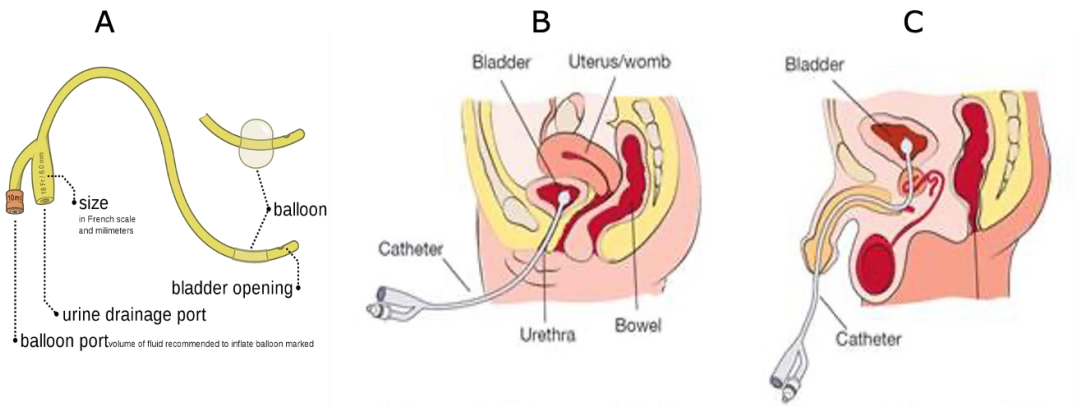
In biofilms, bacteria organize themselves into a new material with very different proprieties from the original, single bacterium. Biofilms can be formed by a single bacterial species or be 'polymicrobial', in which more than one bacterial species is present along with other micro-organisms such as fungi.

Although almost all bacteria can form biofilms, relatively few species are responsible for most pathogenic biofilm-associated infections. Those that do often live harmlessly on our skin surface (e.g. *Staphylococcus aureus* and *Staphylococcus epidermidis*) or come from the human gastro-intestinal tract (e.g. *Escherichia coli*) or the soil and water environment (e.g. *Pseudomonas aeruginosa*). Consequently, pathogenic biofilm-forming bacteria can come from the patient themselves, from the doctors, surgeons and nurses treating or operating on them, or from visitors. In hospital wards they may also be present in the air or on the surfaces of furniture, sinks, toilets, handbasins and in fact anywhere people have touched, coughed, sneezed, breathed, vomited or produced faecal (poo) material.



Contaminating bacteria are commonly found in hospital wards on surfaces touched by people such as the patient and the hand-washing area. These can be visualized by coating a healthcare worker's hands with a powder (to mimic bacteria) that glows when ultraviolet light is shone on it to show what has been touched**.** **(A) The patient.** Fluorescent areas can be seen on the patient's arm, cannula site, bed linen and washing bowl. **(B-E)** The soap dispenser and sink viewed in natural light (**B** and **D**) or after switching on the ultraviolet light (**C** and **E**). The red arrows in **C** and **E** show the fluorescent areas which have been touched by the healthcare worker.

Pathogenic biofilms most frequently form on the surfaces of manufactured medical devices designed to replace damaged or diseased parts of the body. They include heart valves, pacemakers, joints, eye lenses or the screws and plates inserted by surgeons to help broken bones to heal. These devices are usually made of metals or plastic and may contain electronic devices - some also require complicated surgical operations to position them in the right place in the body. In addition, many different types of catheters (thin flexible tubes) that are highly susceptible to biofilm formation are inserted by doctors and nurses into veins to take blood or to administer drugs or into the upper part of the lungs to help patients to breathe or into the bladder and urinary tract to help urine (wee) flow out. The most likely place to suffer with a biofilm-associated infection is, of all places, in a hospital.



Urinary tract catheters are inserted into the bladder via the urethra. **(A)** Catheter showing two ports, one for inflating the balloon after the catheter has been inserted into the bladder and one for attaching a urine drainage bag. **(B)** and **(C)** show insertion of the catheter into the bladder via the urethra and the position of the catheter balloon inside the bladder in a female and a male. Bacteria form pathogenic biofilms inside the catheter tube or on the outside between the catheter and the urethra.

Apart from these artificial implant surfaces, biofilm-associated infections are also highly problematic in cystic fibrosis, a genetically inherited disease, where pathogenic biofilms develop deep inside the lungs. Patients with diabetes frequently suffer with **foot ulcers**, wounds that fail to heal because they become chronically infected with pathogenic biofilms

3. ***When and how do bacteria build pathogenic biofilms?*** To form a pathogenic biofilm, bacteria must first gain access to a site in the body that they can't normally reach because it's protected by the skin, or deep within the body and only becomes accessible to bacteria after damage to the skin surface following surgery, or after the insertion of a **catheter** into the bloodstream or the urinary tract.

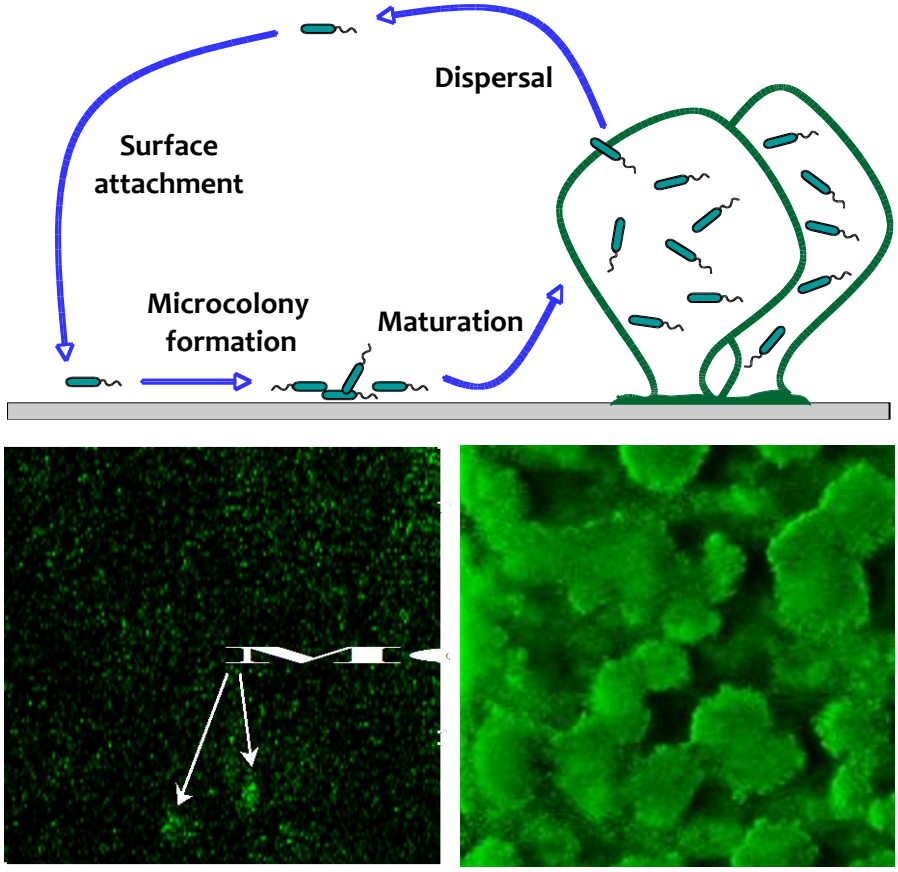
A good example of a pathogenic biofilm former is *P. aeruginosa* which mostly causes infections in people with weakened immune systems, **cystic fibrosis**, surgical wounds, **diabetes** or implanted medical devices. It is found in soil and water and in moist locations associated with human activities - especially in hospital sinks, drains and taps because it is very resistant to being killed by disinfectants and antibiotics. In fact, *P. aeruginosa* is on the World Health Organization's top priority list of antibiotic resistant bacteria.

To make a biofilm, bacteria like *P. aeruginosa* use a multi-step process. It starts with the bacterial cells exploring a surface before deciding whether or not to stick. Since *P. aeruginosa* is a motile bacterium, it can swim above and down to surfaces using its motor-driven flagellum, a hair-like structure that rotates and drives the bacterial cell body forwards or backwards (see **Video 1: BiofilmPaerug.mp4**).

After landing, *P. aeruginosa* moves on the surface using hair-like appendages called pili which are telescopic and can extend and retract allowing the bacterial cells to 'walk' or 'crawl'. Using their flagella and pili to 'feel' the surface, each bacterial cell then has to decide whether to stay or to leave by detaching and swimming away. If they like the surface, the cells that stay lose their flagella and start to secrete a sticky glue (see **Video 1: BiofilmPaerug.mp4**), become permanently attached and start to reproduce.

Each bacterial cell divides into two, the two divide into four, and so on, eventually forming a microcolony within a sticky matrix. As the microcolonies get bigger they merge together and develop into a 'slime city', a mature biofilm with its own internal architecture including water channels that can deliver food and take waste products to and from the cells

inside the biofilm. As the biofilm gets older and more overcrowded, it will deliberately start to break itself down with some of the bacterial cells re-growing their flagella so that they can swim away to search for a new surface site on which to build a new biofilm.

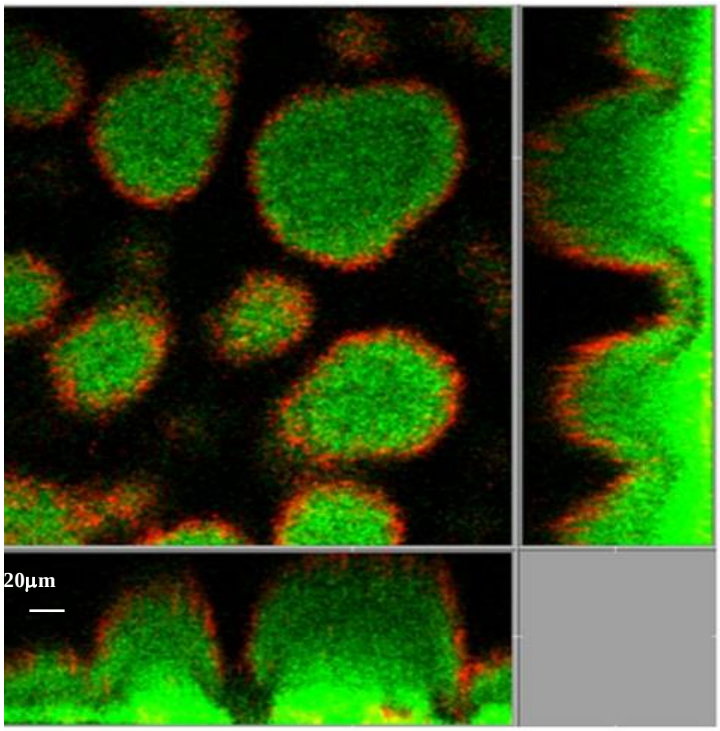


Top. Stages in biofilm development by *Pseudomonas aeruginosa* cells. Single bacterial cells swim down and land on a surface. Some stick firmly and start to divide and grow, forming an aggregate of bacterial cells called a microcolony which matures as it expands. As the biofilm ages, the bacterial cells start to leave to look for new surfaces to start biofilm formation over again. **Bottom**. Early stage (left) and mature (right) 'bird's eye' views of a biofilm. MC = microcolony.

4. ***Why can't our immune systems kill pathogenic biofilms?*** One of our first lines of defence are white blood cells called neutrophils, which patrol the bloodstream and internal organs and tissues looking for bacterial invaders which they capture and kill. However, by forming a biofilm, bacteria can protect themselves from neutrophil attacks. The slimy matrix covering the bacterial cells within a biofilm acts as camouflage, so that the neutrophils fail to sense the presence of the invading bacteria, as well as a shield, preventing them from capturing and engulfing the bacterial cells. This makes the neutrophils 'angry' so that they start releasing the toxic chemical cocktail they produce and, instead of killing the biofilm bacteria, cause collateral damage to the local tissues around the biofilm. This leads to pain, inflammation and tissue damage and, if present, the failure of a medical device which if left untreated can lead to recurrent and chronic infections, and sometimes organ failure and ultimately death.

5. ***Why are pathogenic biofilms resistant to antibiotics?*** Biofilms are up to 1,000 times more resistant to antibiotics than individual bacterial cells. This means that treating a biofilm infection with an antibiotic usually fails to kill all the bacteria and cure the infection so that the

relapses which occur have a major impact on the patient and their treatment. Consequently, the only way to eradicate a pathogenic biofilm is to surgically remove both the infected tissues and implanted medical device if present. For individuals with cystic fibrosis, this can sometimes mean that they need a lung transplant, for patients with non-healing diabetic foot ulcers, the amputation of the infected foot, for those with artificial hip joints, invasive surgery to remove and replace the biofilm-infected hip joint.



Biofilms are highly resistant to antibiotic treatment. Bird's eye and side views of a *P. aeruginosa* biofilm treated with an antibiotic and imaged under a microscope. The live bacterial cells inside the biofilm microcolonies stain green whereas those that are dead stain red. The antibiotic cannot kill all the biofilm bacteria because it is unable to penetrate so the bacterial cells deep inside the biofilm survive.

While individual bacterial cells are usually easily killed by antibiotics, the same antibiotics are unable to kill all the bacteria in a biofilm – they may kill those at the surface but not those deep inside. This is because the slimy matrix traps antibiotics and stops them penetrating through the biofilm to reach the cells deep within. Even when antibiotics get through, the bacterial cells in the biofilm have protective mechanisms that stop antibiotics reaching their targets inside the cells. These include enzymes that chemically inactivate the antibiotic, pumps that stop antibiotics concentrating inside the bacteria, as well as some of the biofilm population adopting a 'dormant-like' state where they stop growing. Since most antibiotics only kill actively growing bacteria, these dormant cells survive, and subsequently re-awaken and re-grow once the antibiotic threat has passed. For bacteria living in a mixed species biofilm, some species may protect the others from antibiotic treatment.

6. ***What are we doing to improve prevention and treatment of pathogenic biofilms?***

Pathogenic biofilms have evolved many ways of protecting themselves so that antibiotics and immune system's neutrophils are not effective at killing and clearing them. This means that we have to take special care to prevent biofilms forming and causing infections in the first place.

First and foremost, practising good hygiene is essential for infection control. Regular hand washing and cleaning of all surfaces with disinfectants, especially in hospitals, is very important. Medical and nursing staff need to wear personal protective clothing and disposable gloves in order to safely manage blood, body fluids and any sharp items, such as needles used for injecting patients. Good ventilation is also needed to help stop the spread of microbes in the air.

Since preventing pathogenic biofilms forming in the first place is of the highest priority, there is a need to discover new ways to do this. Many implanted medical devices are made of plastics such as PVC or silicone to which bacteria readily attach and form biofilms. One way to stop pathogenic biofilms forming would be to impregnate silicone for example with antibiotics which can slowly leak out and kill the attached bacteria before they have time to build a biofilm. Another strategy would be to find new materials or surface patterns to which bacteria cannot stick. One example adapted from nature is Sharklet, a micropatterned surface modelled on shark skin with millions of tiny, microscopic teeth that disrupt pathogenic biofilm formation. Catheters, breathing tubes and wound dressings with surface Sharklet micropatterns are now available for preventing biofilm-associated infections. Discovering new drugs that disperse biofilms so that the bacteria can be killed by antibiotics is another approach that could be very useful for treating patients with pathogenic biofilm infections.

7. ***What are the personal, economic and environmental costs of pathogenic biofilms?*** In the USA, hospital patients have been estimated to have caught ~1.7 million bacterial infections resulting in ~99,000 associated deaths every year. About 60%–70% of these hospital-associated infections are caused by pathogenic biofilms. They are therefore economically highly significant because every day a patient spends in hospital results in a major financial cost to both hospital/health service, to the individual including the loss of earnings for patients unable to work, and to the economy more broadly. Worldwide, the annual costs run into many billions of dollars.

Implanted medical devices also come with high environmental costs. They are manufactured mostly from plastics that are made from oil extracted from the earth. Many are not recyclable or biodegradable. While single use disposable devices reduce the risk of infection, they increase pollution as they are usually either incinerated or end up in land-fill sites. For example, in the USA, there are approximately 300,000 individuals with spinal injuries who need to change their urinary catheters up to 5 times per day. They have been estimated to produce 38 million kgs of waste per year which is equivalent to more than 26,000 cars or 80 Olympic-sized swimming pools. Implanted medical devices have to meet many different requirements including functionality, performance, safety, cost and environmental impact, all of which have to be considered and optimized. There is clearly a need for environmentally-friendly new materials which fulfil all these needs and in addition are able to prevent pathogenic biofilm infections.

Relevance for Sustainable Development Goals and Grand Challenges

The microbial dimension of pathogenic biofilms relates to three main SDGs:

- **Goal 1. End poverty.** Pathogenic biofilm infections result in loss of time from work and, in some cases, loss of income, which can create or exacerbate poverty in some families.
- **Goal 3. Ensure healthy lives and promote well-being for all at all ages** (*improve health, reduce preventable disease and premature deaths).* Good hygiene practices especially in hospitals and clinics reduces the risk of pathogenic biofilm infections. Healthy eating and exercise boosts our immune systems and reduces people becoming overweight,

helping to prevent diabetes and the problems associated with non-healing ulcers requiring foot amputations.

- **Goal 8.** Promote economic growth and full employment. Pathogenic biofilm infections result in loss of time from work which, in some cases, can lead to loss of employment of the affected individual and, because of the loss of productivity, to failure of the employing enterprise, if small and dependent upon the output of the infected person. Pathogenic biofilms thus have significant economic impacts on individuals, families, enterprises and local and national economies.
- **Goal 12. Ensure sustainable consumption and production patterns** (*achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero waste lifecycles, inform people about sustainable development practices)*. Most implanted medical devices are made of plastics that are single use and not recyclable or biodegradable. They must be supplied in sterile packaging, further increasing waste. Most cannot be re-used unless taken apart and reprocessed to make them clinically safe to use again. Medical devices that can be made from new environmentally friendly materials that reduce waste and pollution are needed. It has been suggested that if the health care sector was a country, it would be the fifth largest emitter of global emissions in the world. A change to more sustainable and reusable products could bring many environmental and financial benefits.

Potential Implications for Decisions

1. ***Individuals***
    - ***a.*** Personal hygiene
    - ***b.*** The need to complete courses of any antibiotics prescribed
    - ***c.*** The need to return unused antibiotics to a local pharmacy for disposal, not to dispose of them down the sink

2. ***Community policies***
    a. Public education campaigns about pathogenic biofilms and how to minimize them
    b. Public education campaigns about antibiotic resistance, how to minimize its spread, and the need to return unused medications to pharmacies for disposal

3. ***National policies***
    a. Antibiotic stewardship
    b. Health economics
    c. NHS plastic waste disposal
    d. Support of research and development targeting the development of new anti-biofilm strategies

Exercises

1. How does formation of biofilms by pathogenic bacteria make infections harder to treat or prevent? What methods could prevent biofilm forming on surfaces? How could biofilms be problematic in hospitals? Can you think of any other biofilms that you come across in daily life? How do biofilms contribute to antibiotic resistance and its spread?

2. Urinary catheter associated biofilm formation. Watch the video **(Video 1 BiofilmPaerug.mp4)** and write a story about what you see.

3. What are the different parts of a urinary catheter? Where could pathogenic biofilms form on a catheter? Look at the image of a hospital ward and identify the places where bacteria may be located. Watch **Video 2 (Hospital Ward Contamination Sites.mp4)** for a simulation of bacterial transfer during real-time nursing care. Where do the bacteria that form pathogenic biofilms come from? How could you prevent biofilms forming?

4. See **Superbugs sheets.**

5. If a catheter is 25 cm long, and placed end to end, how many will go around the circumference of the earth which is 40,000 km?

   (Answer: 160 million catheters. Laid end-to-end, there is enough catheter length used in hospitals to circumscribe the earth more than 5.5 times. (880 million catheters!) (Sun et al 2018)

The Evidence Base, Further Reading and Teaching Aids

Teaching aids

Video: Biofilm Paerug.mp4
Video: Hospital Ward Contamination Sites.mp4
Stick and slide lesson.ppt

Scientific review articles

Lebeaux, D., Ghigo, J. M., & Beloin, C. (2014). Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiology and molecular biology Reviews* 78(3), 510–543. https://doi.org/10.1128/MMBR.00013-14
Dadi, N., Radochová, B., Vargová, J., & Bujdáková, H. (2021). Impact of Healthcare-Associated Infections Connected to Medical Devices-An Update. *Microorganisms*, 9(11), 2332. https://doi.org/10.3390/microorganisms9112332
Ciofu, O., Moser, C., Jensen, P. Ø., & Høiby, N. (2022). Tolerance and resistance of microbial biofilms. *Nature reviews. Microbiology*, 10.1038/s41579-022-00682-4. https://doi.org/10.1038/s41579-022-00682-4
Sun, A. J., Comiter, C. V., & Elliott, C. S. (2018). The cost of a catheter: An environmental perspective on single use clean intermittent catheterization. *Neurourology and urodynamics*, 37(7), 2204–2208. https://doi.org/10.1002/nau.23562

Glossary

Biofilm: A community or group of microbes embedded in a protective matrix and usually attached to a surface.
Catheter: a flexible tube that can be inserted into the body to drain fluids such as urine from the bladder.

Cystic fibrosis: is a progressive, genetic disease that affects the lungs, pancreas and intestines and results in persistent lung infections most commonly associated with *Pseudomonas aeruginosa*.
Diabetes: a chronic disease characterized by high levels of blood sugar, which leads to serious damage to the heart, blood vessels, eyes, kidneys and nerves.
Diabetic foot ulcer: an open sore or wound that commonly forms on the bottom of the foot of a diabetic
Enzyme: a biological catalyst, usually a protein that is used to increase the rate of a chemical reaction and can be-re-used again and again.
Neutrophil: a type of white blood cell that fights infection by ingesting and killing bacteria
Oxygen radical: A type of unstable molecule that contains oxygen and that easily reacts with other molecules in a bacterial cell causing damage and cell death
Pathogen: a micro-organism that causes infection/disease