Simple cloth filter method to reduce cholera infections from drinking contaminated water

Straining water through a piece of clean cloth is an extremely simple, low-resource method and widely used for household water treatment. Cloth filters have been used in many cultures for centuries. Typically in South Asia, a sari or saree (a strip of unstitched cloth ranging from four to nine meters in length that is draped over the body of women (a traditional garment) is folded 7 to 8 times and used as a filter.

In laboratory experiments using electron microscopy, it was found that an inexpensive sari cloth, folded four to eight times provides a filter of about 20 µm mesh size, was small enough to remove all zooplankton, most phytoplankton, all Vibrio cholerae attached to the plankton and other particulates larger than 20 µm. The risk of cholera is therefore reduced by about 50% (HUQ et al., 1996). Water is poured through the folded sari cloth and collected in a pot underneath. The efficiency of straining depends on the weave of the cloth and the number of times it has been folded.

"Primary" versus "Opportunistic Pathogen"

- **Primary pathogen**: Implies that the organism has virulence mechanisms that allow it to cause disease in normal, healthy animals. Often requires only a low infectious dose (ex. *E. coli*, *Shigella*, *Salmonella*, certain *E. coli* pathotypes)

- **Opportunistic pathogen**: Implies that the organism can only cause disease in animals with impaired host defenses (antimicrobial treatment with loss of normal protective flora, catheters, surgery, burns, wounds, concurrent immunosuppressive disease or cancer, immunosuppressive drugs, genetic conditions...) Opportunists are often considered to be "normal host and environmental flora" type organisms, but primary pathogens are also opportunistic.

- **Accidental pathogens**: Don't really mean to cause disease, just minding their own business, but just happen to get in the "wrong" place.
  - Ex - *Clostridium tetani* in a wound.
Q. How do we decide if the isolation of one of these Enterobacteriaceae B TEAM species from a clinical specimen is significant?

- They are considered significant if isolated in large numbers and in pure culture from a normally sterile site or from a disease condition.
- Examples - from organs (septicemia) or bladder by cystocentesis (UTI), pleural or abdominal cavity, surgical site, abscess aspirate
- It is harder to decide if they are significant when isolated in mixed culture from a sample from a body site that has normal flora, or could be contaminated, such as a wound.

Remember, large numbers, pure culture...
The LFers B TEAM
*Klebsiella, Enterobacter, & Citrobacter*

- All are Lactose-fermenters (pink on MacConkey agar)
- All can be confused with *E. coli* on culture

Generically are called "fecal coliforms" along with *E. coli* (*E. coli* is considered the best indicator of fecal contamination)

- All are common in environment (water, sewage, soil, plants) and commensals of the GIT of animals

The LFers B TEAM

Q. What does "The LFers B TEAM" do?

- *Klebsiella, Enterobacter, & Citrobacter* are opportunistic & nosocomial pathogens
- All can cause infections in immunocompromised animals and humans
- All can be antimicrobial-resistant, nosocomial (hospital-acquired) pathogens causing infections in:
  - animals and humans with catheters, IVs
  - patients on prolonged antibiotic therapy, chemotherapy, surgical cases
The LFers B Team: Important *Klebsiella* species: *Klebsiella pneumoniae* subspecies *pneumoniae* and *K. oxytoca*

- The two important *Klebsiella* animal pathogens are:
  - *K. pneumoniae* sp. *pneumoniae* & *K. oxytoca*

  *Klebsiella pneumoniae* sp. *pneumoniae*:
  - Has large mucoid colony due to its capsule
  - Capsule is an adhesin (adheres to epithelial cells)
  - and is anti-phagocytic, blocks complement, which gives it serum resistance
  - Has plasmid-mediated resistance to beta-lactam drugs
  - *K. pneumoniae* strains can be NDM 1 SUPERBUGS!

*Klebsiella* and New Delhi 1 beta-lactamase enzyme
NDM 1 SUPERBUGS

- *Klebsiella* were the first bacteria identified (in 2009) to produce NDM-1 in a patient that traveled from India to England with an infection that did not respond to many antibiotics. The organism was resistant to beta-lactams and, after the organism’s genetic and antibiotic resistance mechanisms were studied, NDM-1 and its genetic source were discovered. The genetic source was a plasmid termed “*bla*NDM-1,” and since that discovery, other bacterial genera have been found to have *bla*NDM-1 integrated into other plasmids or into the bacterial chromosome, thus allowing the bacteria to produce NDM-1.

- NDM-1 is effective against both the old and newer antibiotics (carbapenems such as imipenem) that contain a beta-lactam ring.

- Because bacteria that contain NDM-1 are often resistant to almost every antibiotic, bacteria with NDM-1 have been termed a superbug; some investigators consider these bacteria to represent the most dangerous superbug of all that have developed so far.
The emergence and spread of *Klebsiella pneumoniae* and other Enterobacteriaceae producing novel plasmid-mediated $\beta$-lactamases active against third-generation cephalosporins contribute to the difficulty in treating nosocomial infections.

**Opportunistic nosocomial multiply resistant bacterial infections—their treatment and prevention**


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*Klebsiella pneumoniae* & *K. oxytoca* - opportunistic pathogens

- **Bovine (pigs, & goats too) mastitis:**
  - Disease scenario and clinical picture are similar to *E. coli* mastitis (coliiform mastitis)
  - Associated with bedding on contaminated wood shavings, contamination of udder with feces

- **Equine metritis** - *K. pneumoniae* and *K. oxytoca* can colonise stallion’s prepuce & infect mare during breeding

- **Neonatal septicaemia** (navel ill) in foals, calves, lambs and kids

- **Canine infections** - UTI, pyometra, neonatal septicaemia, otitis externa, pneumonia, enteritis

- **Nosocomial infections** in humans and animals

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Sand makes an ideal bedding choice because of its inorganic nature and inability to support environmental organisms.
**Klebsiella pneumoniae & K. oxytoca - opportunistic pathogens of pet birds and reptiles**

- Gram-negative bacteria are only a small part of normal flora of birds

- Contaminated seeds, fruits, & veggies allow colonisation of pet birds; can cause opportunistic respiratory tract infections, diarrhoea, & septicaemia

- Cause opportunistic infections in snakes, iguanas, reptiles (pneumonia, osteomyelitis, abscesses)

Any organism, no matter how obscure, can have its day.
More LFers B TEAM

**Enterobacter** species:
*E. aerogenes, E. cloacae*, & 10 other species

and

**Citrobacter** species:
*C. freundii* & 10 other *Citrobacter* species

• All can cause various opportunistic infections, similar to *Klebsiella* species

• Q. - What is their significance to veterinary clinicians and diagnosticians?

• These are opportunistic and nosocomial pathogens, encountered less frequently than *E. coli*

• Must do antimicrobial susceptibility testing of isolates from clinical samples, as they are frequently multi-antimicrobial drug-resistant

• *Enterobacter* species can be NDM 1 SUPERBUGS

• Q. How can these infections be prevented? (Hint - think about predisposing causes/ host/pathogen/environment relationships)

There is one special *Citrobacter* species that causes disease in mice. (but not Pinky and the Brain)
Q. What is an important disease of lab mice?

• The disease, transmissible murine colonic hyperplasia, caused by *Citrobacter rodentium*, (the EPEC/ETEC-like pathogen of mice and gerbils, NOT rats or hamsters)

• Infects by fecal-oral route causing epithelial cell hyperproliferation of colon

• A potential problem in rodent colonies; spread by fomites; can be triggered by sudden change in diet, poor husbandry, sanitation, stress

• Some rodent lines are very susceptible; diarrhea, stunted growth, rectal prolapse, high mortality

• Screening done in rodent health monitoring programmes for specific pathogen-free lab rodents using PCR for *C. rodentium*

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The NLFers B TEAM

*Proteus, Morganella, & Edwardsiella*

• Enteric & environmental bacteria

• All are nonlactose-fermenters

• Can be confused with *Salmonella* colonies on selective enteric media

• *Proteus* colonies must be distinguished from *Salmonella*

  (Do you know how we do this? – check in your Lab Manual)

• Two important species:

  *Proteus mirabilis & P. vulgaris*
The NLFers B TEAM - *Proteus* species
Are also on the Combined SWIMMING & ROWING TEAM

- *Proteus* species have flagella, are MOTILE
- *P. mirabilis* in particular can "swarm" on BA (nutrient rich medium).

- *Proteus* swarming is a problem in the diagnostic lab. Why?
  - It grows over other bacterial colonies.
  - It commonly contaminates post-mortem tissue samples + primary isolation plate cultures.
  - Swarming is inhibited on phenyl ethyl alcohol agar (PEA) or MacConkey.

- *P. mirabilis* has a strong odour ("musty", or "burnt chocolate" smell)

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*Proteus mirabilis* - Swarming

Vegetative (swimmer) cells differentiate into elongated, hyper-flagellated swarm cells that assemble into multicellular rafts & migrate away from the colony. Migration is facilitated by capsule or secreted biosurfactants that reduce surface tension, & is inhibited by increasing agar concentration. When migration ceases, swarm cells revert to the vegetative form.

- Q. - WHY IS THIS IMPORTANT?
  - In *vivo*, swarming permits rapid bacterial population migration; ascending colonisation of the urinary tract and biofilm formation on catheters; & entry into host cells.
Q. What does this bug - *Proteus* do?

- *Proteus mirabilis* and *P. vulgaris* are more opportunists
- Can cause:
  - urinary tract infections (UTI)
  - otitis externa, wound infections, prostatitis
  - nosocomial bacteremia, diarrhea in animals on prolonged antibiotic treatment
- Resistant to many antibiotics due to conjugative resistance (R-factor) plasmids

Q. What is special about *Proteus* Urinary Tract Infections (UTI)?

- *Proteus* has virulence factors which help it cause UTI:
  - 1. *Flagella* facilitate movement from peri-urethral area to bladder
  - 2. *Fimbriae* allow adherence to bladder epithelial cells
- Flagella and swarming behaviour allows biofilm formation and ascending colonisation of urinary tract → pyelonephritis, septicemia

If *Proteus* was the size of human, it could travel at the speed of 100 mph!
Q. What is special about *Proteus* Urinary Tract Infections (UTI)? (continued...)

3. Urease enzyme

- Q. Why is urease such an important virulence factor?
  - Urease hydrolyzes urea in the urine to form ammonia & CO$_2$.
  - Ammonia is toxic to cells & causes tissue damage.
  - Ammonia changes pH to basic (alkaline).
  - Alkaline pH causes *urinary calculi* (struvite or apatite crystals), bacteria become sequestered in the stones.

"Urolithiasis is the hallmark of Proteus UTI"

More of the NLFers B Team

*Morganella morganii*

- *Morganella* species (only one):
  - *Morganella morganii* was previously called *Proteus morganii*, but does not swarm.
  - Q. What is its significance to veterinary clinicians and diagnosticians?
  - A not too important *opportunistic* animal pathogen, but has intrinsic resistance to many antibiotics, so it can be difficult to treat.
### More of the NLFers B Team

**Edwardsiella species**

- *Edwardsiella species*:
  - Live in water and intestines of cold-blooded animals
  - Mainly cause disease in fish, eels, and humans (septicemia)
  - *Ex - E. ictaluri* causes “enteric septicemia” in catfish, also called “hole in the head” disease

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<tr>
<th>Image</th>
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<tr>
<td><img src="image1.png" alt="Fish with septicemia" /></td>
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<tr>
<td><img src="image2.png" alt="Eel farming" /></td>
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<td><img src="image3.png" alt="Catfish farming" /></td>
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