wellcome

UNIVERSITY OF SUSSEX

Scenes from the many lives of *Escherichia coli.*

A play in five acts

Words:

Catherine Will

Mark Erickson

Music:

Joe Davin

Cast – in order of appearance

Thalia: Josie Counsell

Leader of the Chorus: Dr Catherine Will

Theodor Escherich MD: Professor Dirk van Lem

Chorus: Dr Mark Erickson

Voice of the Laboratory: Dr Georgina Lloyd

Coliform, B. coli, E. coli: Joe Davin

Geneticist: Professor Robert Dingwall

Harriette Chick: Dr Lesley Cann

Alfred McConkey MD: Brian Blaney

Leonard Dudgeon: Dr Eric Will

E Napier Burnett: Gavin Middleton

Edward H. Kass MD: Dr Matt Baum

Microbiologist: Dr Sarah Purnell

Microbiologist: Dr Diogo Gomez da Silva

Wastewater narrator: Professor James Ebdon

Sally Davis: Professor Bobbie Farsides

Hospital lab director: Dr Ulla McKnight

Voice of Journal Science: Professor Steve Fuller

Molecular biologist: Dr Doug Browning

EUCAST: Professor Tilda Paananen

Data Scientist: Dr Alena Kamenshchikova

Public Health England: Sarah Chadwick

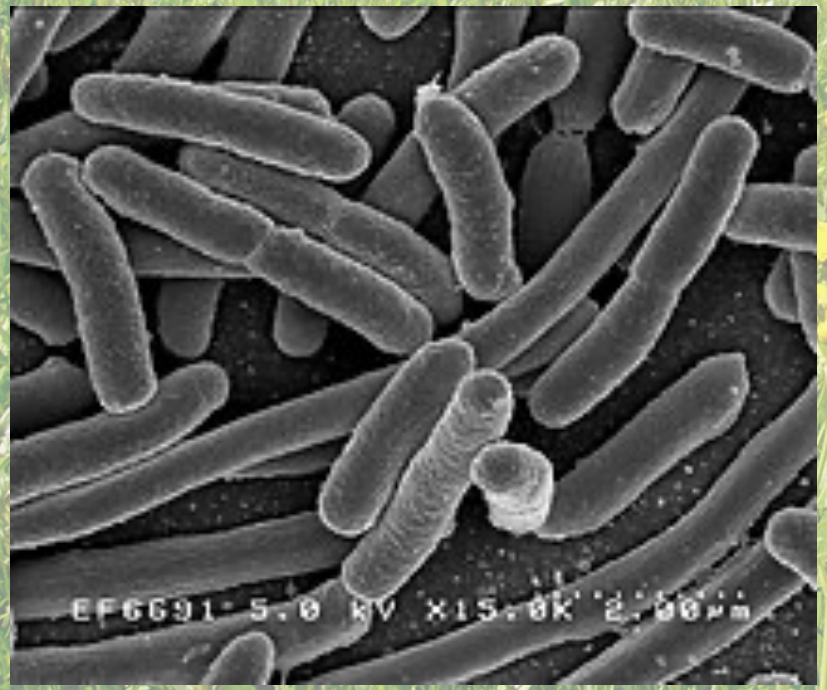
Epidemiologist: Dr Eleanor Kashouris

Clio: Mili Erickson-Bragg

Melpomene: Dr Sara Bragg

Prologue

Thalia: Welcome to our performance, a joyous play in four acts, with many players of this world and beyond, and with a serious end. I am Thalia, the muse of blooming. My sister Clio, the muse of history, has helped me with some parts, and, of course, Melpomene was always best at theatre (though oh so serious!). And where would we be without our darling mama Mnemosyne whose memory we rely upon.

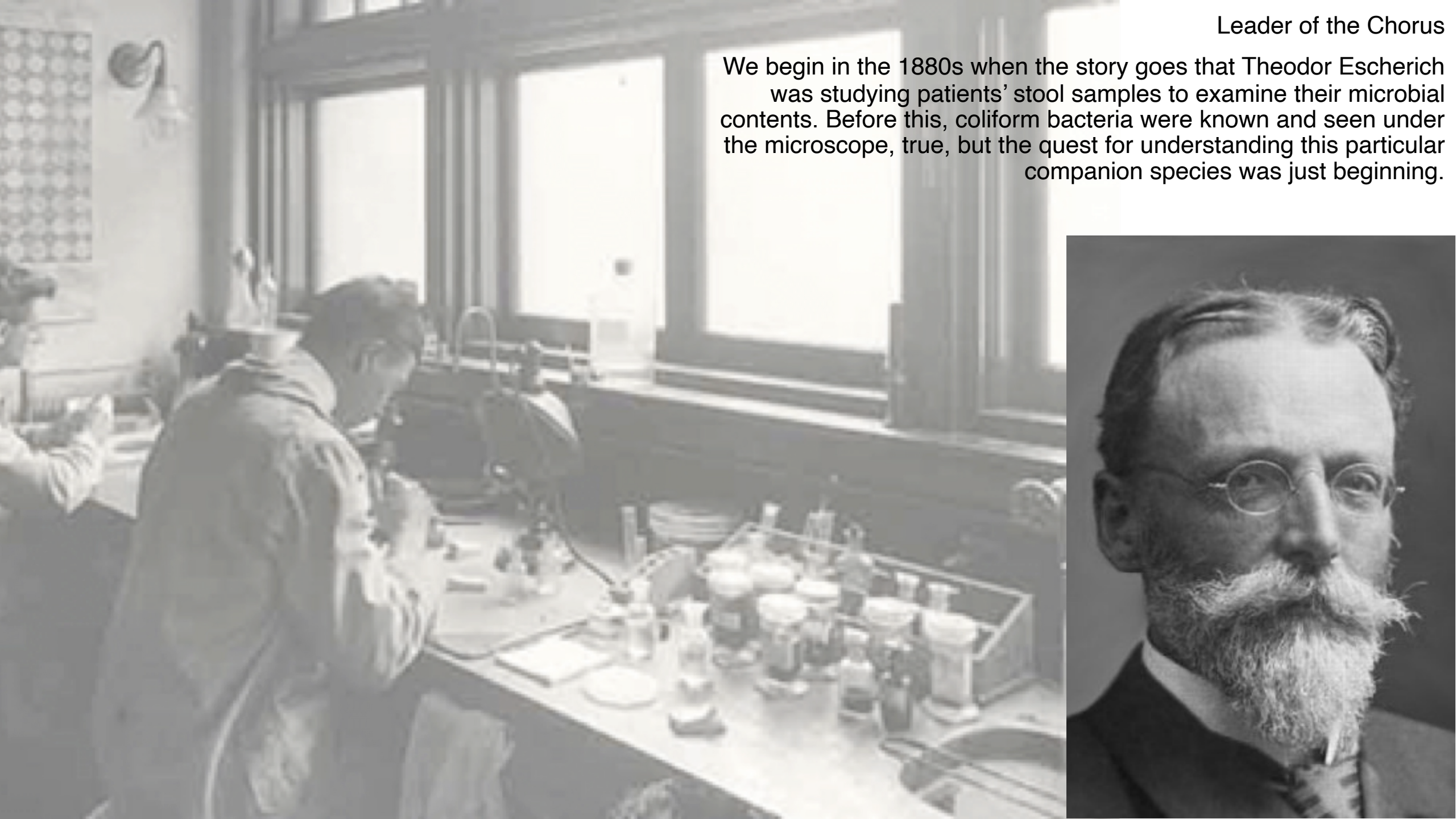


Thalia: We are brought together to tell you of a blooming world of microbes, first as many decades ago we find the wild one, as wild as Enkidu[2] who is older even than we Muses, and tame it through naming it, firstly, Bacillus coli, later Escherichia coli. Then bleak-hearted Melpomene scripts our play, as disease and illness are blamed upon E. coli who joins a gang and, like our distant cousin Loki[3], turns trickster, hiding and dissembling, only to be revealed as, perhaps, a hero after all. Next goddess Athene guides the molecular biologists' hands as they grow, break and construct the innards of E. coli, revealing its secrets. But Melpomene, in our final act, pushes us towards a tragic, fateful end as our microbes' resistance grows and grows. Truly it is they who will bloom in the future, whilst we who have flourished, like all fragrant flowers which abound in the meadow[4], will wither and die.

Act 1 Scene 1: A Laboratory In Munich

Leader of the Chorus

We begin in the 1880s when the story goes that Theodor Escherich was studying patients' stool samples to examine their microbial contents. Before this, coliform bacteria were known and seen under the microscope, true, but the quest for understanding this particular companion species was just beginning.



Theodor Escherich MD:

It’s good to be in Munich but here - as in Vienna - we lose too many infants to dysentery. We don’t know what’s responsible but the microscope might help.

Cultures are grown on plates, then samples placed on slides on a long lab bench, ready to go under the microscope.

Chorus: The party's assembled;
ready for action – lab,

Esteemed doctor,
microscope, slide.

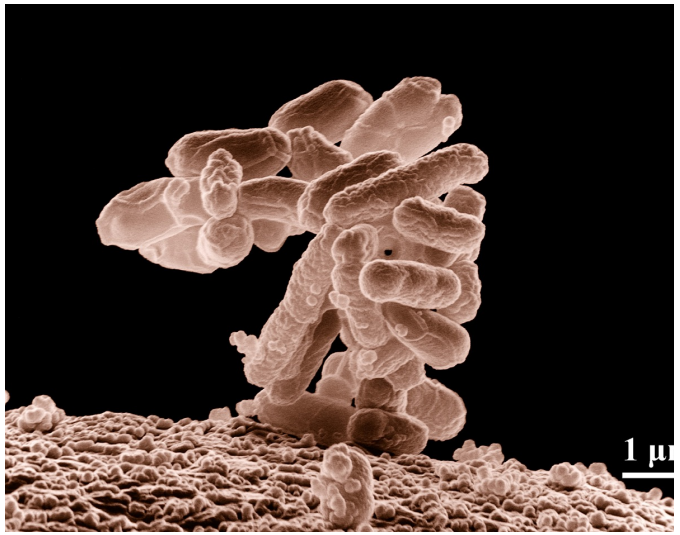
Could shit samples reveal
the cause of this illness?

Let focus and find what
our bodies might hide.

Escherich looks and listens carefully.[5]

Theodor Escherich:

I see distinctive shapes in many of my samples. Tiny organisms, short with rounded ends.



Coliform: I am here

Theodor Escherich

Perhaps they are the source of the dysentery? I will call them *Bacteria coli commune* and continue my investigations.[6]

Modern day geneticist

Great job. But are you sure you've got the right culprit? We've brought molecular vision in the form of a shiny new MiSeq System™ to historical samples. They originated in your lab alright, but they don't look to be pathogenic[7].

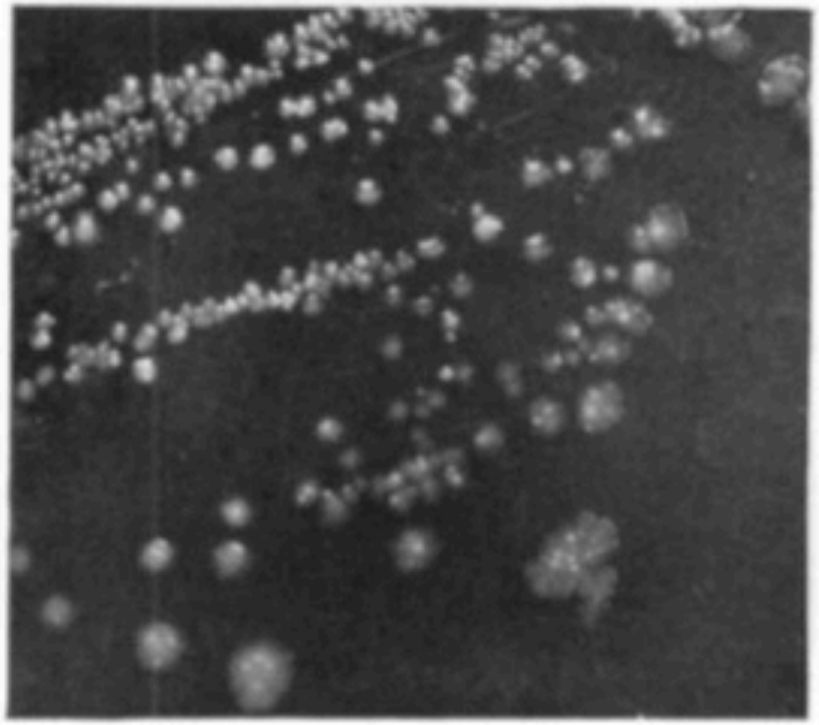


Fig. 1. *B. coli mutabile*, strain "6"; plate seven days old.

B. coli:

A false accusation, or case of mistaken identity? Whatever the reason we're all suspect now.

Act 1 Scene 2: A Laboratory in London

Leader of the Chorus:

Some of the first to work with Escherich's samples are focussed on hygiene and sanitation. In the Lister Institute of Preventive Medicine, a young Harriette Chick starts what will become an illustrious career in nutrition with laboratory work on the efficiency of different disinfectants against bacteria.

Harriette Chick

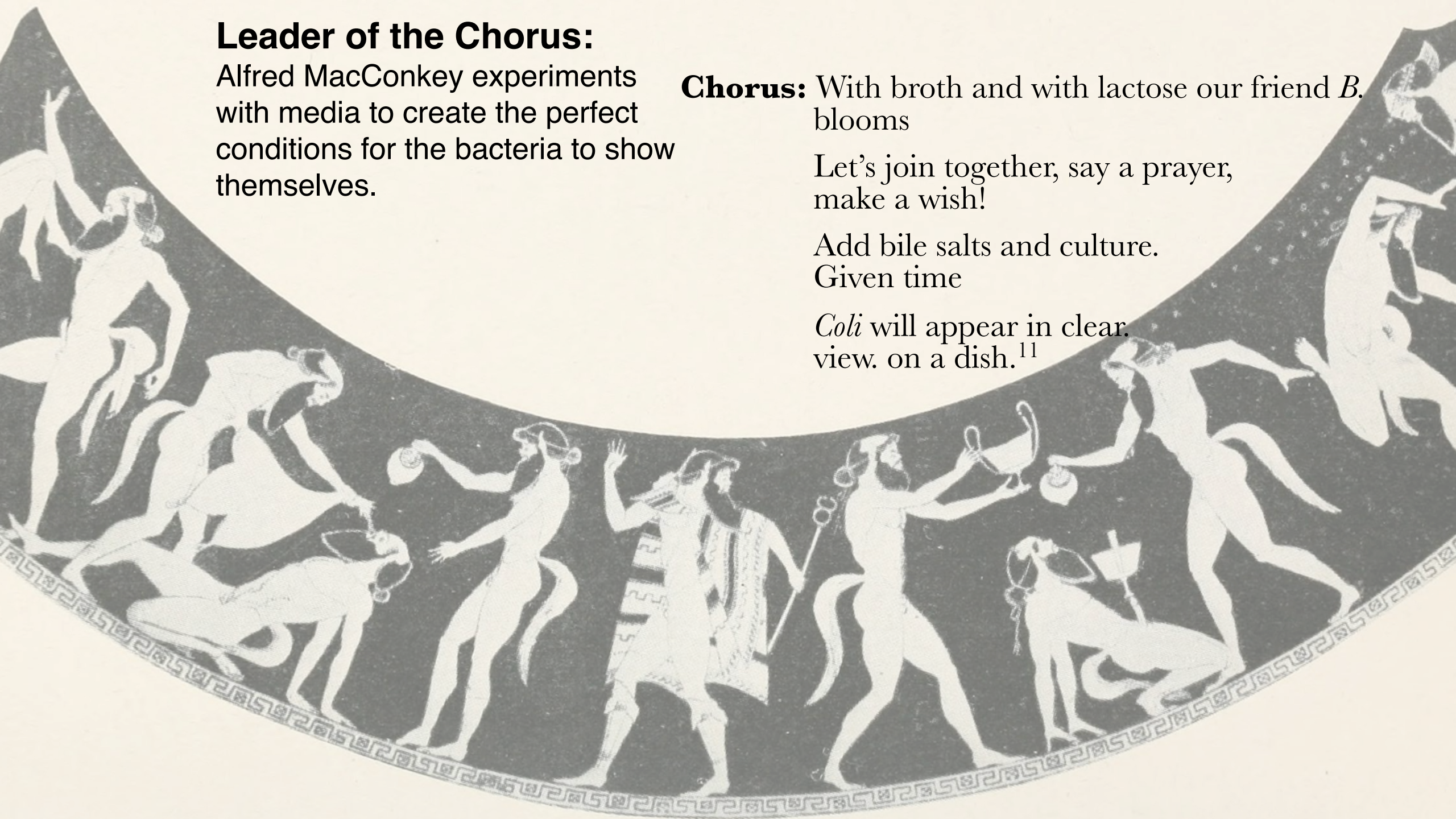
They're dirty and dangerous and I'm going to kill them…[8]

B. coli: AAAAARRRRRGGGGHHH!!!!!!!

Leader of the Chorus:
Chick's colleague Alfred MacConkey[9] – later of agar and broth fame – is interested in *B. coli* in water. He does a series of experiments to explore ways of identifying it effectively, seeing it as a signal of faecal contamination.

B. coli:
More slander, I assure you!

Leader of the Chorus: But the accusation proves hard to escape. Around the time of the First World War, in a frenzy of nomenclature across scientific communities, the genus *Escherichia* is named in honour of Theodor as part of the family *Enterobacteriaceae.*[10] Within this genus numerous strains – some pathogenic and some not – are given new names, including Escherichia coli which is to become the most famous of all.



Leader of the Chorus:
Alfred MacConkey experiments with media to create the perfect conditions for the bacteria to show themselves.

Chorus: With broth and with lactose our friend *B.* blooms

Let's join together, say a prayer,
make a wish!

Add bile salts and culture.
Given time

Coli will appear in clear.
view. on a dish.[11]

Alfred MacConkey MD:

Never mind Hariette's 'kill efficiency'. If we're going to test water samples for *B. coli* regularly, we need to grow these organisms. Just for a time, here in the lab, we want those bacteria to flourish like the roses in my garden. Fortunately they do well at body temperature, and they don't need protection from oxygen, but I'll offer them lactose, saccharose, dulcit, adonit or inulin. I can show that *B. coli* likes to ferment sugar, and that fact is useful…[11]

B. Coli

Busted! We like the lactose though the bile salts are not so welcoming. But you've certainly found us out: our bacterial cousins can't grow so well here so we reveal our presence on the plates.

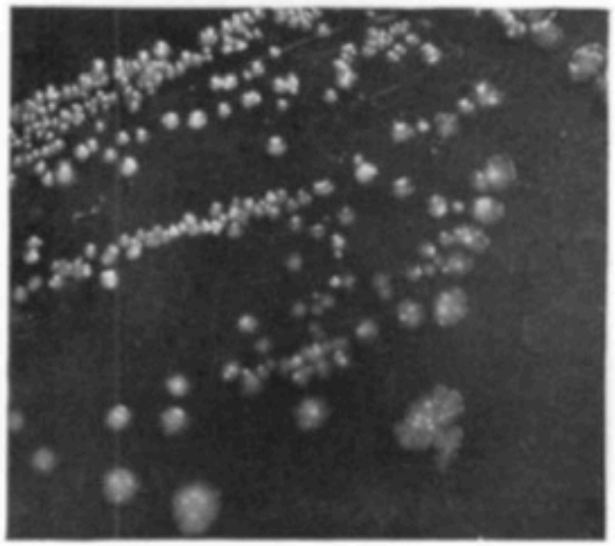


Fig. 1. *B. coli mutabile*, strain "6"; plate seven days old.

Samples of 497 bacilli are meticulously isolated from 76 different substances including human and animal faeces, soil, pond and rain water, oats, beans and cheese, and are put onto plates. Where possible bile salt media are used because of their inhibiting effect on other organisms. Once colonies appear MacConkey adds lactose and watches for fermentation.

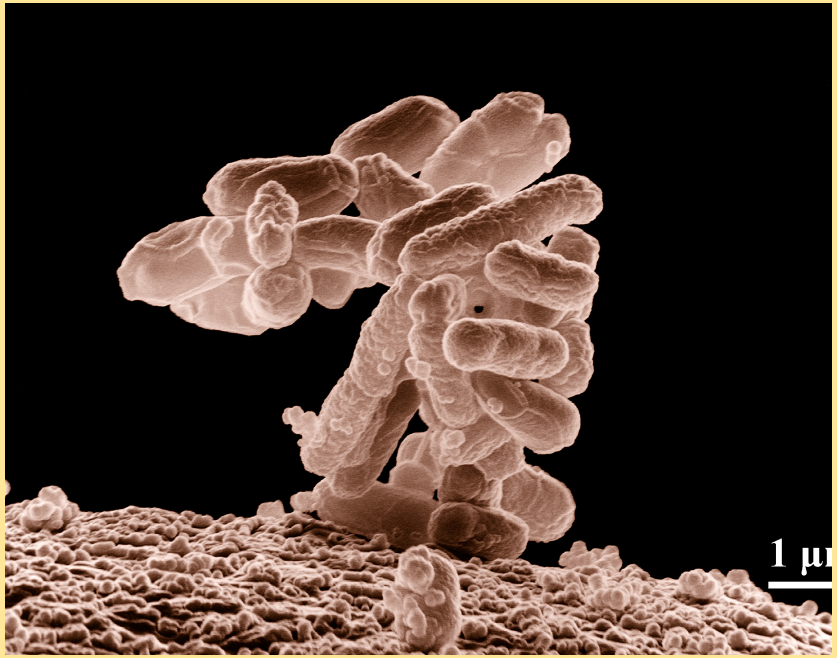
Alfred MacConkey MD

Problem is we keep identifying more types of lactose fermenting enteric bacilli, and at the moment we're calling them all *B. coli.* Still whatever they are, now we can culture them quickly we can go back to counting… what does our work tell us about the water from which the sample came? Ideally, we'd have some form of numerical interpretation[12] of what we find, perhaps by measuring the volume of gas produced in the fermentation.

Chorus: Again we're together. Broth, lactose, salts in a tube.
Wait once more. Watch. Hold your breath.
The doughty professor assembles his subjugated workers
But can dodgy *B. coli* really help confront death?

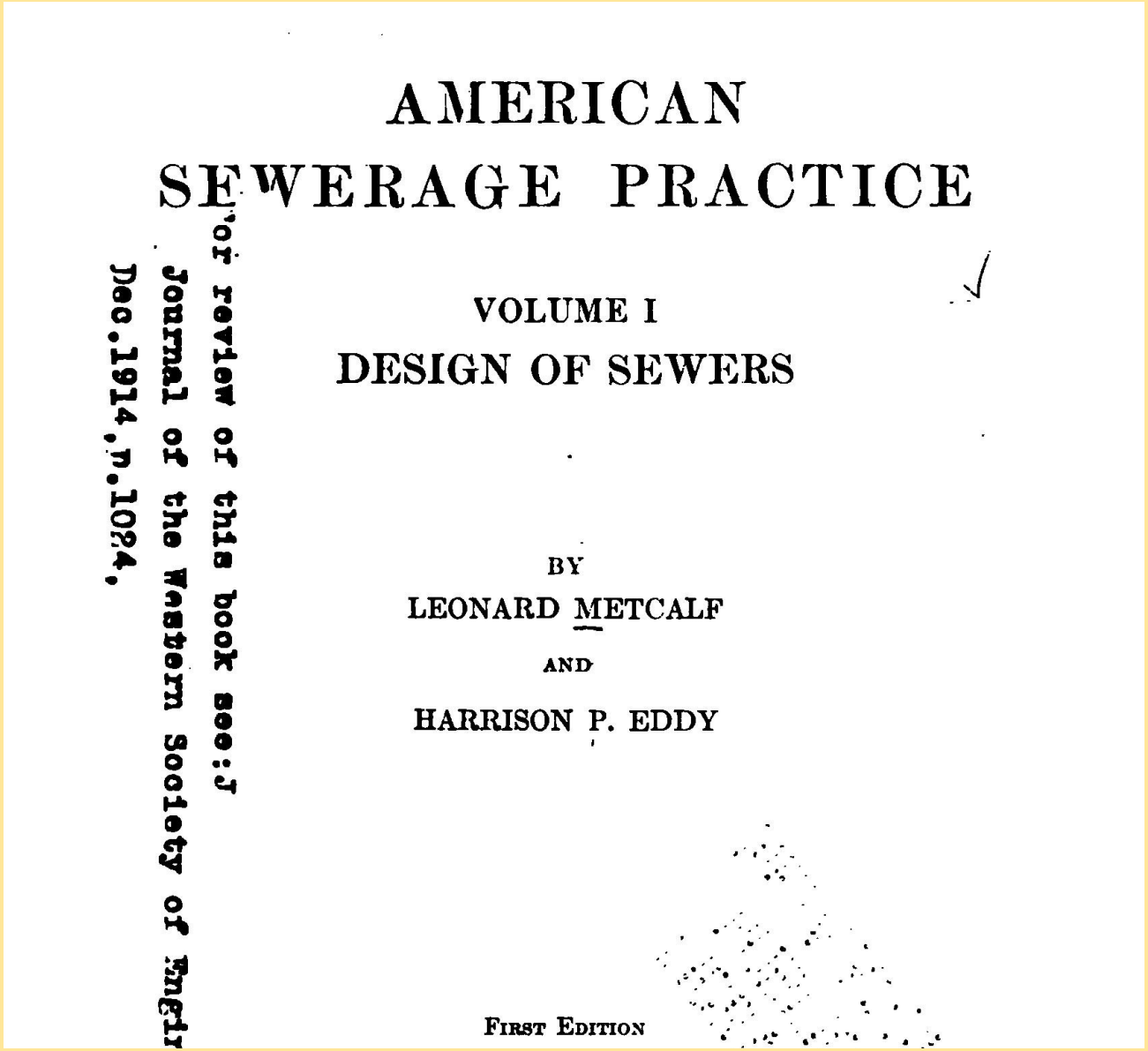
Alfred MacConkey MD

B. coli might not be a friend, but it could yet be an ally in our war against dirty water if we assume it reflects the presence of other coliforms. Contamination by human faecal matter is our fear but also a practical problem. "We all of us always wish to identify organisms as accurately as possible, in as short a time as possible, and with as little trouble as possible."[13]



B.coli:
Named, tamed and shamed!
Established as the villain of the piece my reputation can only improve as we create new forms of cooperation.
But I'm happy to get a job. I'm here to help, believe me…

Leader of the Chorus:
MacConkey's approach rapidly spreads, appearing in the very first edition of a book on *American Design of Sewers* – published by Leonard Metcalf and Harrison P Eddy in 1914 – still a touchstone in the field. Though we now see bacteria as making just over half of faecal solids, many non-pathogenic, *E. coli* is emerging from the mass as indicating the risk of disease.



AMERICAN
SEWERAGE PRACTICE

VOLUME I
DESIGN OF SEWERS

BY
LEONARD METCALF
AND
HARRISON P. EDDY

FIRST EDITION

Journal of the Western Society of Engr.
Dec. 1914, p. 1024.

Act 2 Scene 1: In the Edwardian hospital

Leader of the Chorus: We've given you one history, here's another. From their earliest identification *B. coli* have been blamed for more than diarrhoea. And in the hospital[14] the lab is growing in importance...

Chorus: **Big hospital, small lab. With samples coming**
All the time it's getting a bit tight.
Clinicians and technicians, they all join in the hunt
Examining slides late into the night."

B. coli: 'Why is it always my fault?

Leader of the Chorus: In truth urine has been examined for centuries, but from the 1880s bedside pronouncements were enhanced by chemical testing using test papers[15] and now 'piss pots' are travelling down to the lab for some bacteriology.

Chorus: **Another glass jar viewed with suspicion.**
Our microscopes are ready though so we'll
Plate piss - and culture - leave them to grow.
Tomorrow we're back for the great big reveal.

Leonard S. Dudgeon, MRCP (Lond): But it is hard to interpret the results of these cultures even though we work long hours and follow all correct procedures. What's the meaning of our frequent discovery of *B. coli*? 'Some pathologists go so far as to say that in the female this condition is of no importance; certainly there may be no associated symptoms of disease unless constipation is considered as such. At times, however, there will be headache, slackness, and general malaise.'[16]

B. coli: Slackness and general malaise sound a bit vague. What exactly is the accusation here? I am here to dissemble like Loki, the trickster.

Leader of the Chorus: You're in the wrong place in short. But focus of attention is the female urinary tract not sewers. Are you causing symptoms or somehow incidental? The same questions are asked in the teaching hospitals of Edinburgh.

E. Napier Burnett, FRCS, MRCP (Edin): Trying to understand the effects of *B. coli.* It is found in the intestinal canal of people in perfect health, yet it keeps appearing in post-mortem examinations in so many and diverse situations that it… 'has come into vogue again as the active factor in several pathological states.'[17]

B. coli: Am I pathogenic or not? Please make your mind up. I'm not sure that being in fashion here is such a good thing!

Act 2 Scene 2: A hospital in the United States, 1957

Leader of the Chorus: By 1957 doctors have antibiotics that can act effectively against what is now called *E. coli*. But they are still debating which tests to act on.

Edward H Kass MD: Here in Boston we test pretty much everyone who comes into the hospital. But we keep finding bacteria. There's a considerable number of cases of what I call 'asymptomatic bacteriuria'. I propose we distinguish between severe and mild infection by counting the colonies on an agar dish. If you're comparing in this way you have to follow careful steps to do the culture, but numbers can be multiplied to give an estimate of bacteria per ml.[18] In my view, more than 1,000,000 per ml should be taken as evidence of infection. Less than 10,000 per ml should be taken as a sign of contamination or as clinically irrelevant.

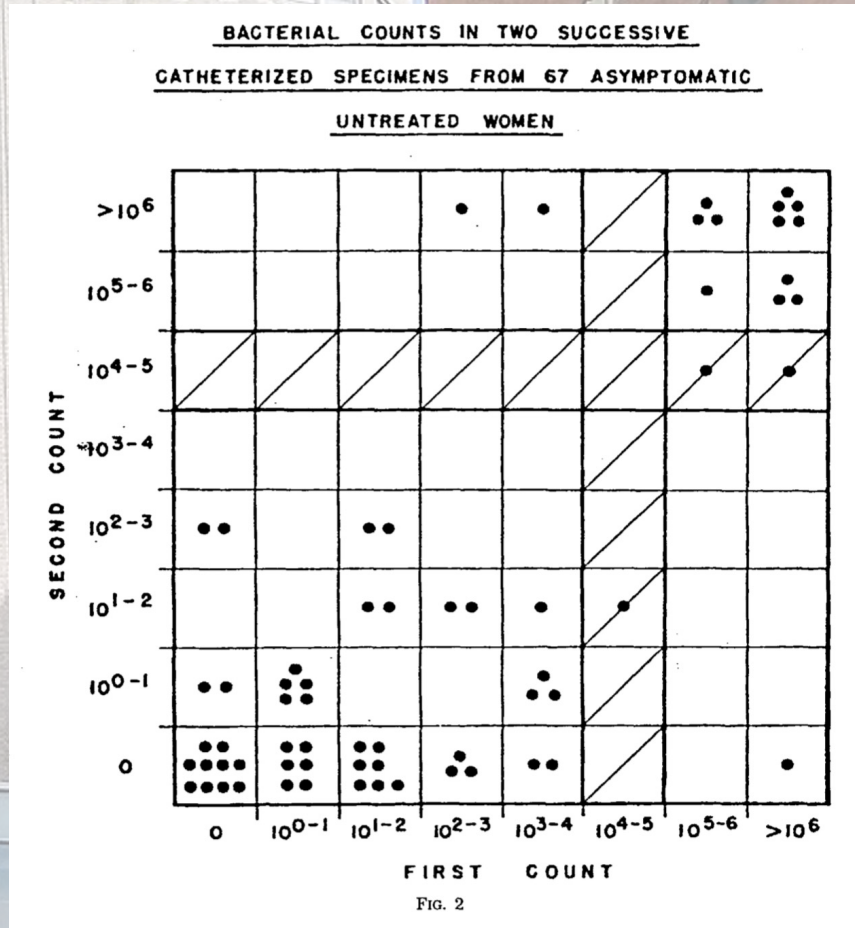
Chorus: For sure we're doing what we can to help here
More knowledge, more medics – the brightest and best –
The mid-century height of clinical hopes
With more counting we could have a new type of test.

Leader of the Chorus: With this approach *E. coli* could be blamed for silent infections as well as those with symptoms.

E. coli: Maybe sometimes it's enough to suspect my presence. But must you throw those clever new drugs at me?

Edward H. Kass MD: We're still looking for the relationship between cause and effect but in the absence of symptoms perhaps we should pull out the smaller set who have really large counts by my method. [To *E. coli*] So you're here in numbers, even if the clinical symptoms aren't there – yet!

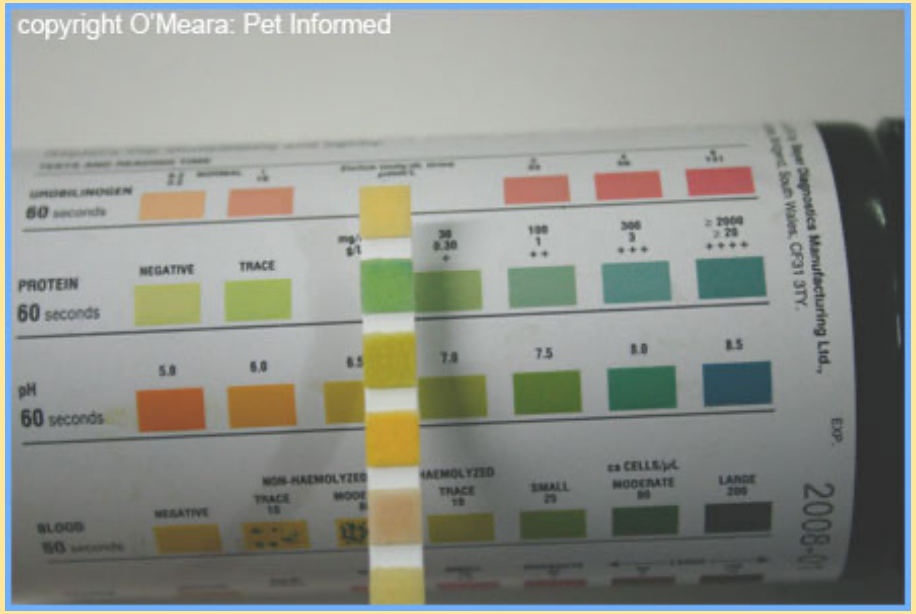
E. coli: We're 'here in numbers' alright. But are you sure we're pathogenic?



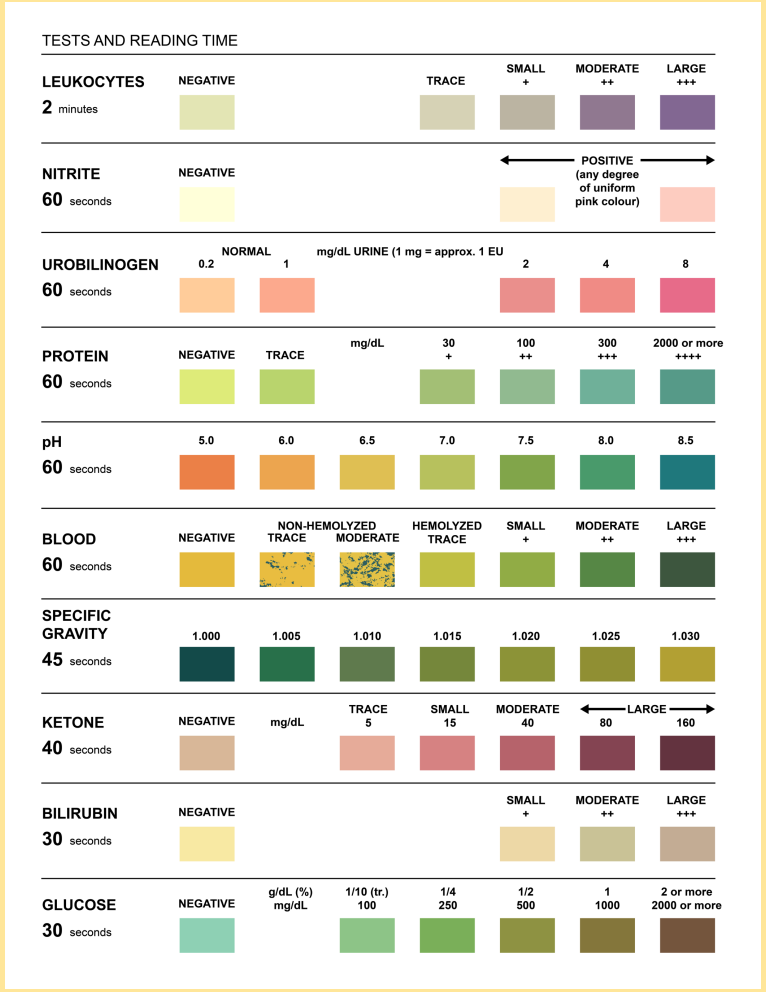
Kass diagram of E.coli counts, from Kass (1956)

Act 2 Scene 3: *E. coli* in UK General Practice

Leader of the Chorus: In family medicine too, a urine test remains a staple of practice.[19] By the 1950s general practitioners have the 'dipstick' combining on a single card test papers for glucose, white blood cells and nitrites or proteins[20] without the fuss of sending samples off for culture.

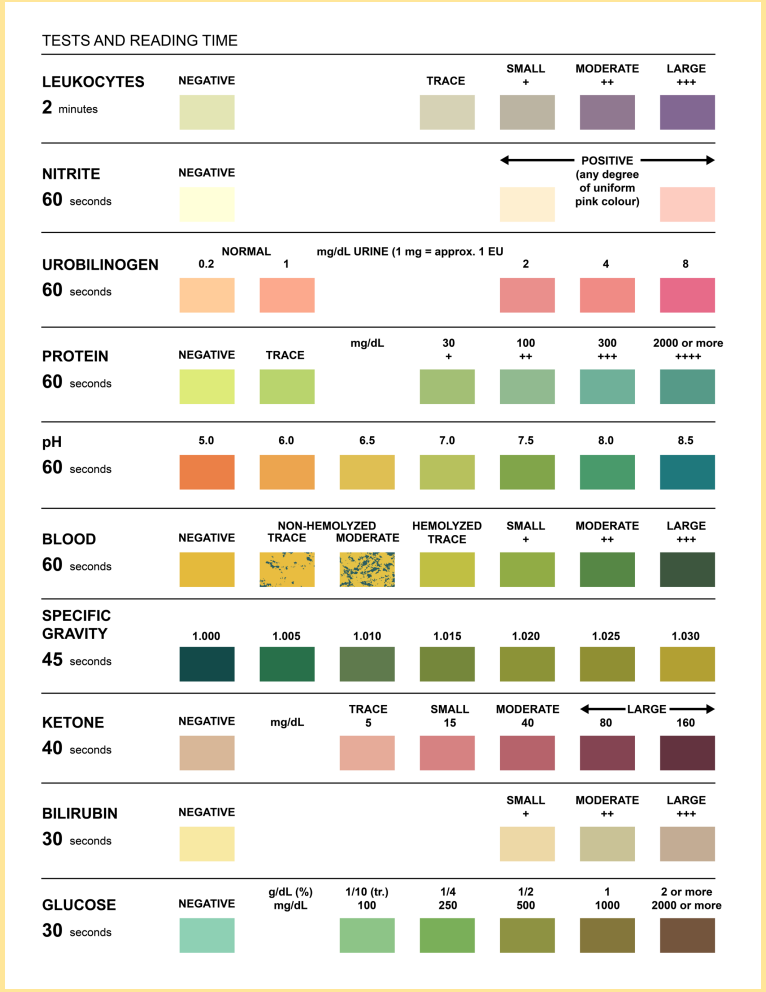


Chorus: With antibiotics *E. coli*'s distressed, but bacteriologists are still in a mess.
We'll need to gather all our wits and make some diagnostic kits.
Bacteria, samples, doctors, women,
Journals, dipsticks and – ampicillin[21].



A patient awkwardly hands over a small pot with yellow liquid, still warm. The doctor examines it visually, then puts in a card indicator for 1-2 seconds. He pulls it out and examines the coloured squares. Moving to purple and pink in the first indicates the presence of leukocytes, the body's response to infection, and nitrites which are reduced by *E. coli*. He pulls a prescription pad towards him and writes the name Penbritin[21].

E. coli: We're everywhere and nowhere[21]. Just because you can see us does not mean we're causing trouble. You're just picking us out because it's easy. Now I'm seriously worried I'm a target. And your efforts to make and use antibiotics are pretty concerning. Just because you can see me does not mean I'm causing any trouble. But should you really try to kill me off?



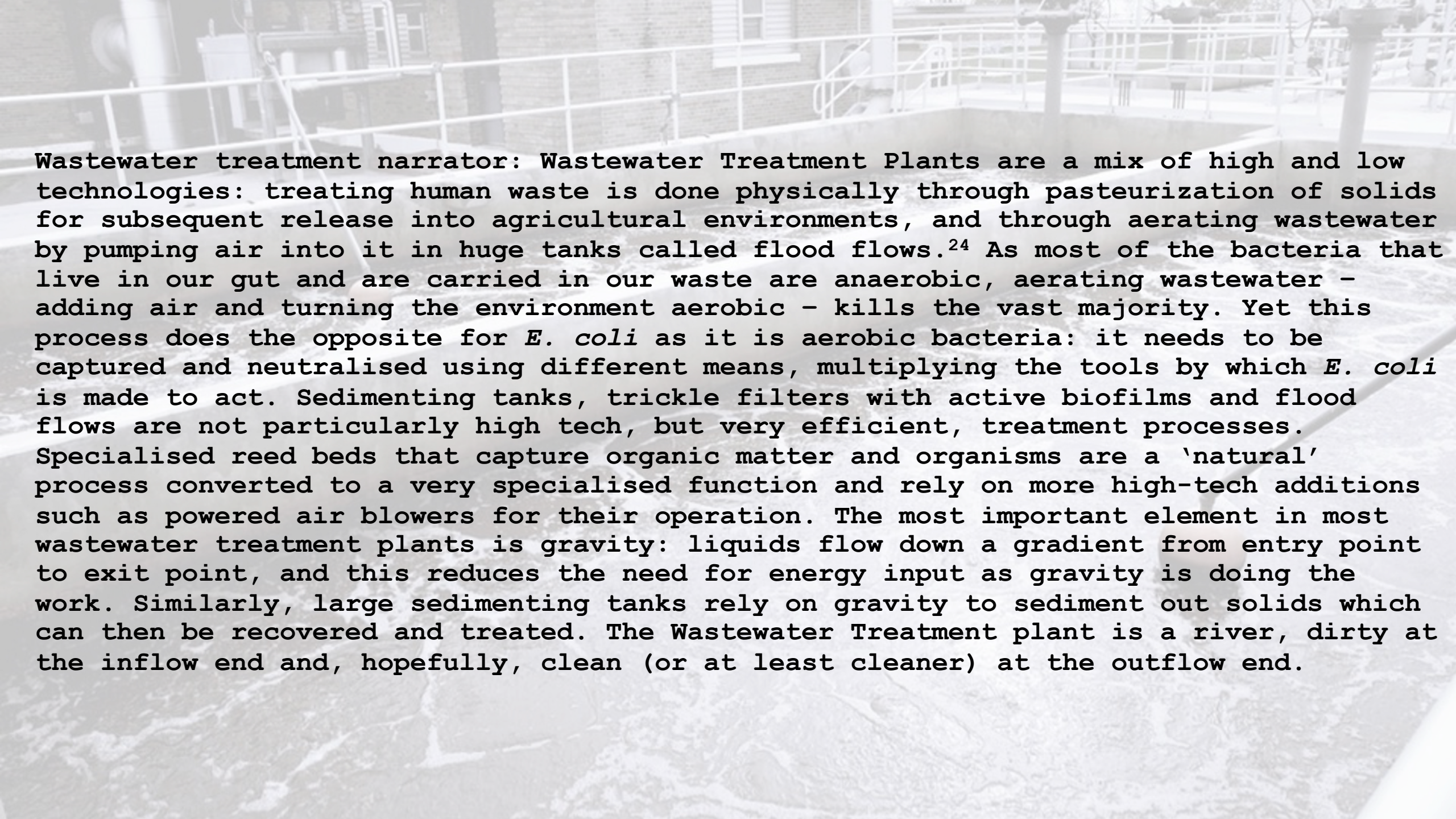
Leader of the chorus: short answer's no. In the 1980s doctors are still arguing. They can see colonisation without symptoms or pyuria in some patients, but they also see evidence of inflammation or symptoms that look or feel like urinary tract infections when their tests can't find bacteria.[23] Through these investigations – mainly from a clinical perspective – *E.coli emerges as a tricky character.*

Act 3 Scene 1: A Wastewater Treatment Plant in southern England

E. coli: 'I am here too, swimming in the river Scamander but ready to assist you.'

Microbiologists: "We microbiologists need to don our PPE (Personal Protective Equipment) as we enter the Wastewater Treatment Plant, a site so mundane and remote that many people never even think of it, let alone consider visiting."

The microbiologists listened very hard again....



Wastewater treatment narrator: Wastewater Treatment Plants are a mix of high and low technologies: treating human waste is done physically through pasteurization of solids for subsequent release into agricultural environments, and through aerating wastewater by pumping air into it in huge tanks called flood flows.[24] As most of the bacteria that live in our gut and are carried in our waste are anaerobic, aerating wastewater – adding air and turning the environment aerobic – kills the vast majority. Yet this process does the opposite for *E. coli* as it is aerobic bacteria: it needs to be captured and neutralised using different means, multiplying the tools by which *E. coli* is made to act. Sedimenting tanks, trickle filters with active biofilms and flood flows are not particularly high tech, but very efficient, treatment processes. Specialised reed beds that capture organic matter and organisms are a 'natural' process converted to a very specialised function and rely on more high-tech additions such as powered air blowers for their operation. The most important element in most wastewater treatment plants is gravity: liquids flow down a gradient from entry point to exit point, and this reduces the need for energy input as gravity is doing the work. Similarly, large sedimenting tanks rely on gravity to sediment out solids which can then be recovered and treated. The Wastewater Treatment plant is a river, dirty at the inflow end and, hopefully, clean (or at least cleaner) at the outflow end.

Microbiologists: But it is a dangerous place, so on with the PPE. Together we are looking for *E. coli*, and will treat its presence as an indicator of the relative cleanliness of the water running through the plant. But *E. coli* is a very small component of this river of effluent.

Chorus: Great – a bug hunt!

Round up the PPE, the shit, the collecting flasks,
the people and catch those microorganisms.

E. coli: I would like to explain, but my voice is very quiet here; it needs to be amplified and refined.
Take me away from this river and allow me to grow and speak to you.

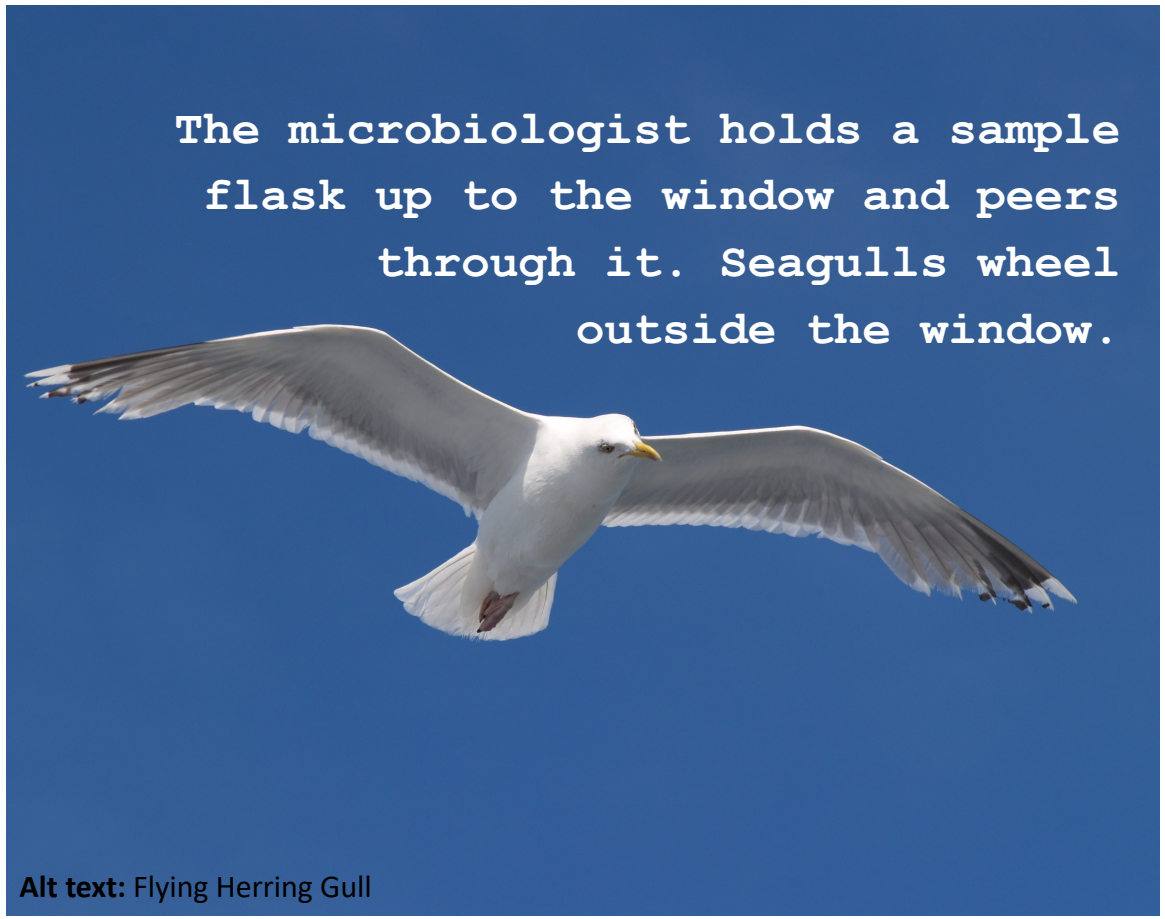
Microbiologists: Did you hear something?

`The microbiologists listened very hard again`

E. coli: I would like to explain, but my voice is very quiet here; it needs to be amplified and refined.
Take me away from this river and allow me to grow and speak to you.

Microbiologists: So we put the river into a bucket (actually, then a 1l collecting flask) and take it back to our laboratory.

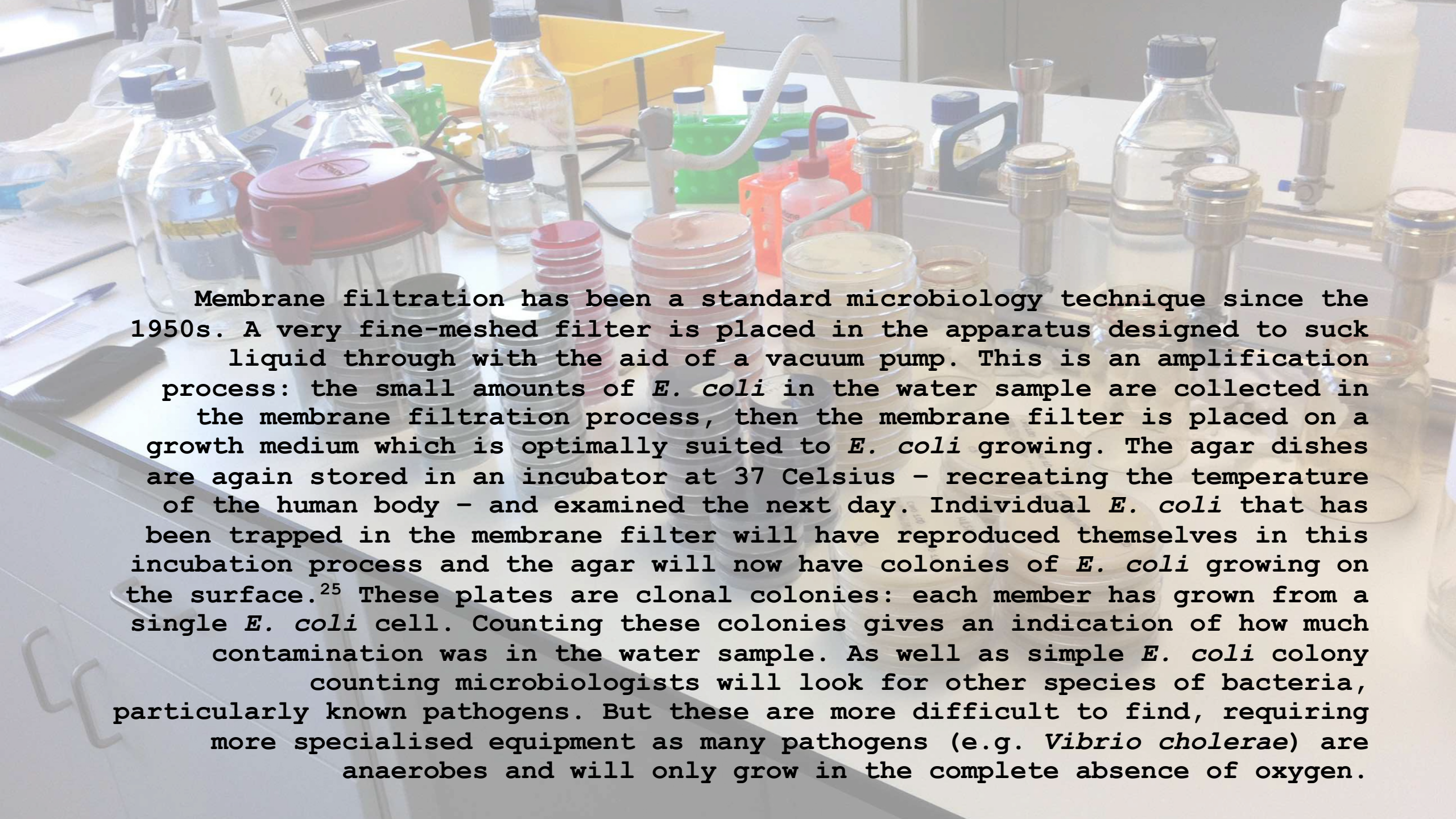
Act 3 Scene 2: A microbiology laboratory in a university in southern England today



Alt text: Flying Herring Gull

Microbiologist: *E. coli*, we know you are in our river sample, but where? How can we find you? No reply? Oh well, membrane filtration it is then.

Lab coats on and let's get to work.



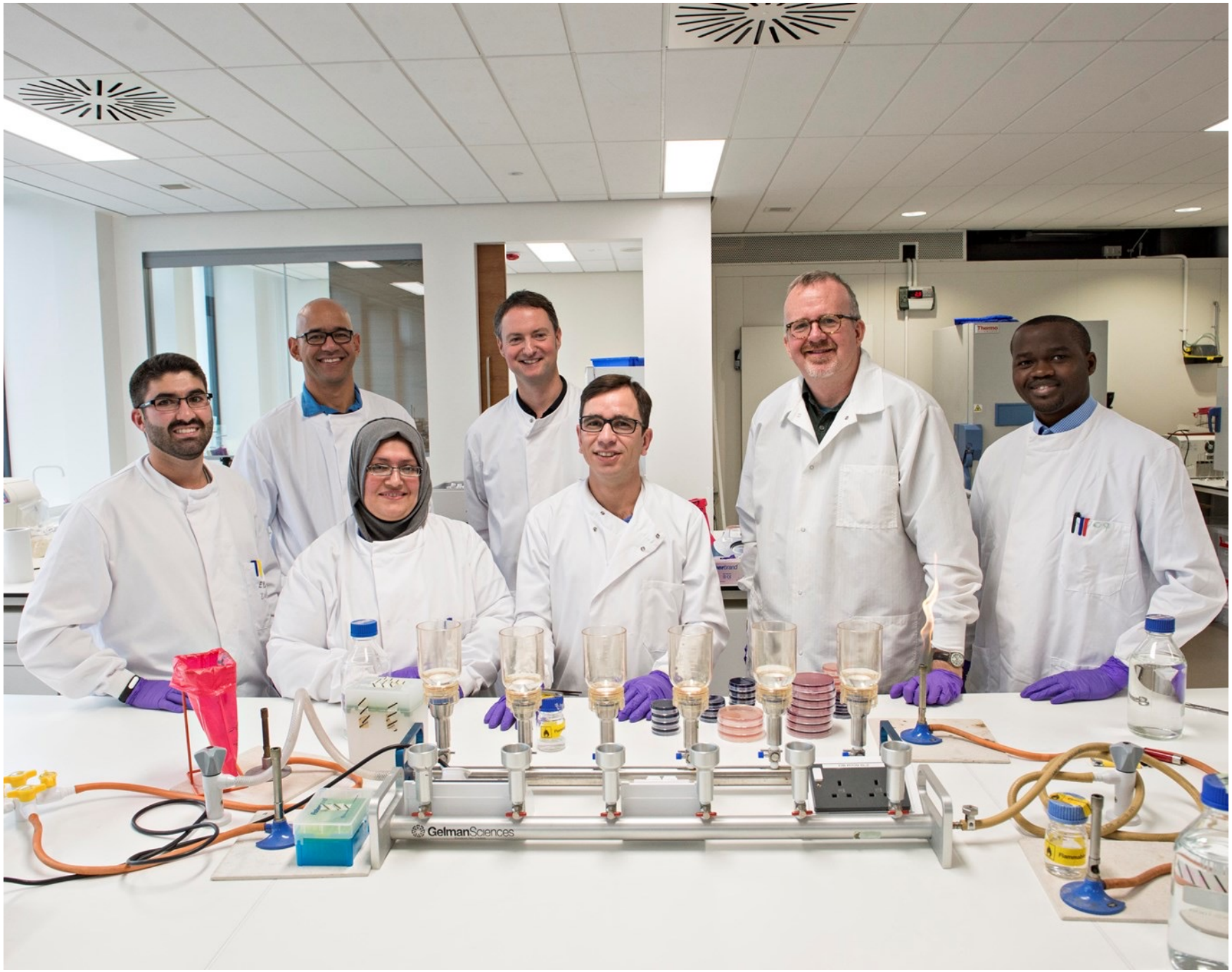
Membrane filtration has been a standard microbiology technique since the 1950s. A very fine-meshed filter is placed in the apparatus designed to suck liquid through with the aid of a vacuum pump. This is an amplification process: the small amounts of *E. coli* in the water sample are collected in the membrane filtration process, then the membrane filter is placed on a growth medium which is optimally suited to *E. coli* growing. The agar dishes are again stored in an incubator at 37 Celsius – recreating the temperature of the human body – and examined the next day. Individual *E. coli* that has been trapped in the membrane filter will have reproduced themselves in this incubation process and the agar will now have colonies of *E. coli* growing on the surface.[25] These plates are clonal colonies: each member has grown from a single *E. coli* cell. Counting these colonies gives an indication of how much contamination was in the water sample. As well as simple *E. coli* colony counting microbiologists will look for other species of bacteria, particularly known pathogens. But these are more difficult to find, requiring more specialised equipment as many pathogens (e.g. *Vibrio cholerae*) are anaerobes and will only grow in the complete absence of oxygen.

Chorus: So clean and pure, so standardised;
our membrane filters, our textbooks,
our aseptic techniques, our agreed assays,
our excellent Excel.

E. coli: "Now you can see and hear me, loud and clear. All my colonies are visible, bright specks on the TBX agar.[26] Count me, let me colonise your spreadsheet, and I will tell you how dirty that river was.

Microbiologist: Why should we let *E. coli* speak for all the coliforms? It is only 0.1% of the mass, and isn't even like the majority of the other gut microbes which are anaerobes.

E. coli: "Because the standard guide to Wastewater Engineering tells us that 'The presence of E. coli in drinking water is taken as an indication that there is a greater risk, as compared to the presence of fecal or total coliform, that disease causing pathogens could also be present,'.[27] Not only that, traditions stretching back decades in laboratories concur and tell microbiologists that this is just what you do. I am here to represent the mass, and with some amplification and growth I do that pretty well. Look how safe the drinking water is, if you don't believe me."



Microbiologists: We microbiologists take the spreadsheet data – the assay for the water company – combine it with our experimental protocol that we wrote a long time ago, add an introduction, a discussion and conclusion and here it is, our journal paper.

Narrator: and the sociologists bring all of this into their book chapter. Write, right, rite.

Act 3 Scene 3: In the public health laboratory debate continues

Leader of the Chorus: And there is still debate about the right approach to treatment today…

Sally Davies, Chief MOH UK: Over-treatment with antibiotics represents a waste of antibiotics' declining efficacy. If *E. coli* is exposed to too many antibiotics it can learn to live with them. New generations will increasingly be resistant to the drugs. Antimicrobial resistance (we call it AMR) is a major threat to modern medicine.

Leader of the Chorus: Automation makes it possible to review larger numbers of samples quicker and cheaper. It might be more reliable, but it might not.

Adele, director of a hospital lab: There's still lots of uncertainty. Numerous factors affect the results we get from the urine samples coming into our lab after a positive dipstick result. In those from the community we don't know how old the sample was and if it was 'mid-stream'. Still we put them all through our system. We do an automated microscopy stage with a *Menarini Sedimax,* running counts from the photographs of the culture. This speeds things up but the counting isn't that reliable.

Where we judge the counts are high we then do sensitivity tests against different antibiotics. We notice up to 15% error in the antibiotic quantity in the prepared discs we use, though the machines have got the depth of agar fairly consistent now. Is it MacConkey agar? No I'm sure it's not, but I couldn't tell you what it is these days. All proprietary in this stuff (*MAST-URI* system).

Chorus: It's all automated here. Once again progress is in the air.

Look on us *E. coli* and despair!

But we're not sure it's the end of the argument…

The voice of journal science: There's so much more to the human microbiome than we think. "The urinary tract is not sterile!"[28]

Leader of the Chorus: Given this the whole industry built around culturing urine samples might need rethinking. If *E. coli* might be even more ubiquitous than previously imagined, the search is on to understand its role in illness and how it relates to other organisms.

The voice of journal science: We need to move beyond 'an *E. coli*-centric view of UTI and the colony-forming units (CFU) threshold-based diagnosis… The new data described above suggest that polymicrobial UTI might be both common and frequently overlooked'[29].

Leader of the Chorus: Meanwhile studies of people with significant symptoms of UTI are suggesting other possibilities. Even if *E. coli* is responsible, it might not be visible with our tests, but evading them in the walls of the bladder or urinary tract.

The voice of journal science: "Some of the really exciting science is now around the notion of the biofilm and intracellular communities of *Escherichia coli*. We must leave behind the 'lone soldiers' model of microbial pathogenesis. Bacteria may invade the epithelial lining cells finding sanctuary from immune surveillance and urinary clearance mechanisms."[30]

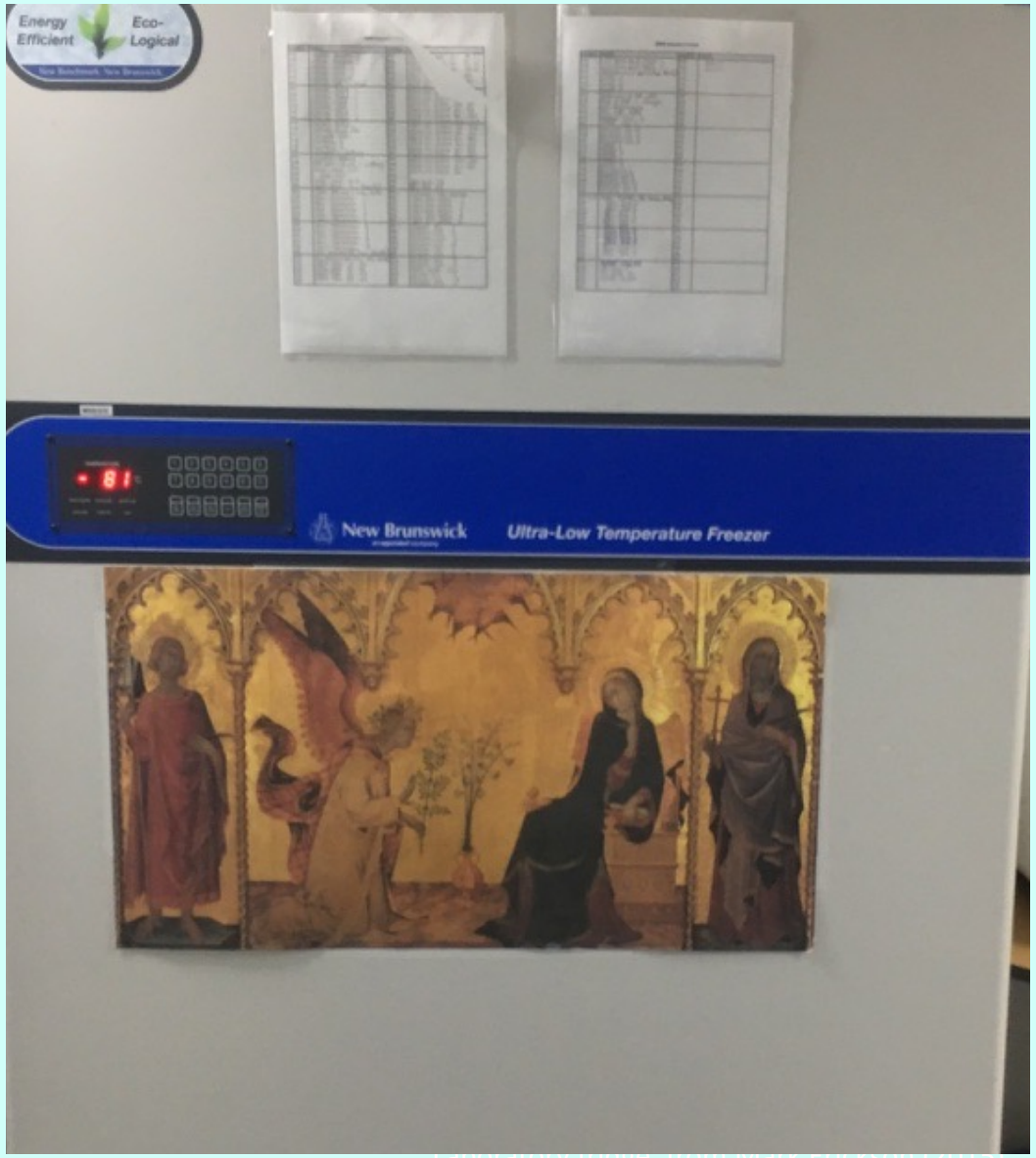
E. coli: Oh yes, we're also good at hiding, and we have friends who are even better at concealment. For us this is more than just a visit. We've settled in to stay. If we stick together we can avoid being flushed away by the urine flow. The epithelial lining is a wonderful thing. It's not home exactly but it's a lovely spot.'

Act 4: Wild but in the laboratory. *E. coli* explored (the tale of K-12)

The team of molecular microbiologists in the
lab are surrounded by machines, Bunsen
burners, assorted glass ware, reagents,
family photographs, discarded PPE, pens and
papers, fridges and microwave ovens.
Is this a mess?

E. coli: "I am here hoping to be better understood. Hail, Athene, blue-eyed daughter of Zeus, goddess of knowledge and justice!'

Chorus: Here we all are again
And what a proliferation!
Microbiologists, sociologists, biochemists
Careers, papers and prep-kits
Microbes, vectors, equipment shiny
Will make our esoteric thought community[31]
Working away on our usual routine
Looking for a β-barrel protein



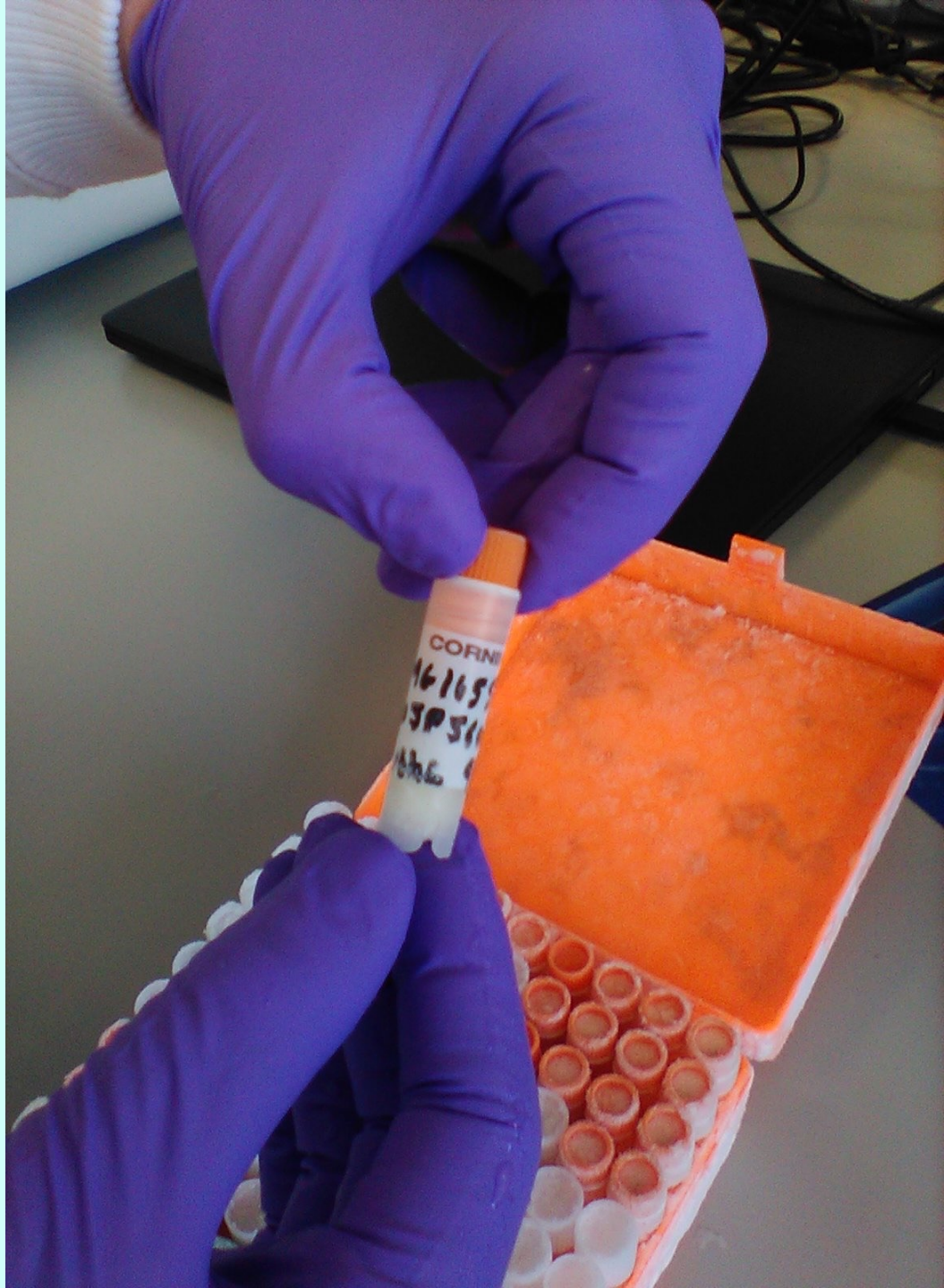
Laboratory image, from Mark Erickson (2015)

Molecular biologist: The laboratory is a secure and hazardous environment so we need to don our PPE; lab coats, goggles and nitrile gloves. We've got a big job on: to work out the structure and function of a piece of *E. coli's* cell wall, its BamA β-barrel protein.[32] This could give us a new antibiotic, if we're lucky.

Leader of the Chorus: The molecular biologists' task is large. They will need to enlist the help of many actors to bring this about, but the star here will be their old workhorse, the model organism *E. coli*. K-12.

Voice of the lab: The team of molecular microbiologists in the lab are surrounded by machines, Bunsen burners, assorted glassware, reagents, family photographs, discarded PPE, pens and papers, fridges and microwave ovens. Is this a mess?[33]

Leader of the Chorus: Eventually, the molecular biologists will triumph in identifying the structure and function of *E. coli*'s BamA β-barrel protein but, spoiler alert, on the way they experience some doubt.



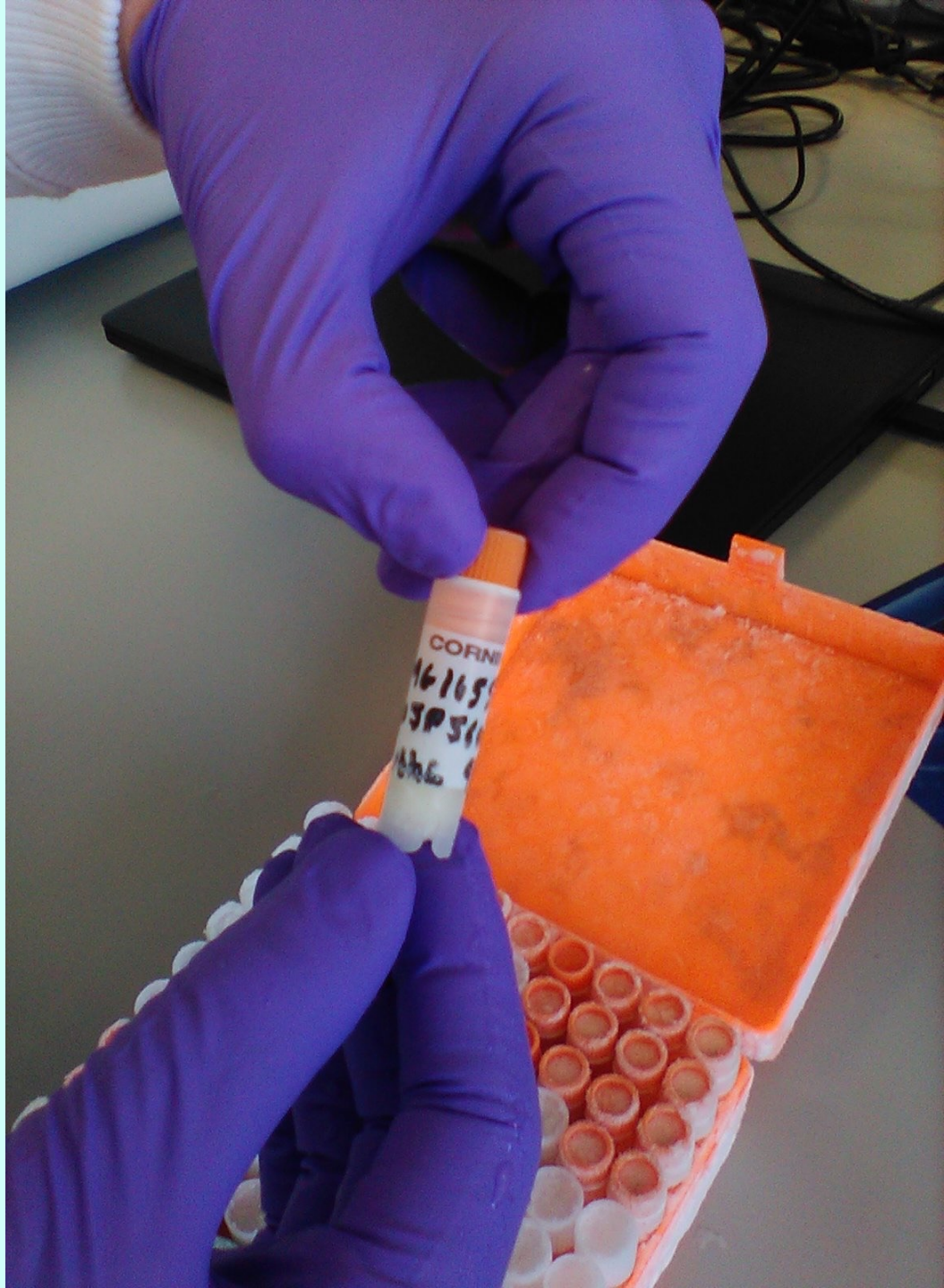
E. Coli K-12: Take me apart, look inside me, tell me my secrets. I am at your disposal, a willing helper in the quest for knowledge.

Molecular biologist: Thank you, *E.coli* K-12 but be aware that this will be a complicated process. And we should say we have nagging doubts about your identity.

E. Coli K-12: Why? You've worked with me before, many, many times and know me almost personally. I'm here to help – I am as accommodating as possible.

Molecular biologist: That might be a problem *E.coli.* Maybe you're too accommodating, too refined and cossetted, too removed from your wild cousins? You're right, we know you personally and have even given you your own name: *E.coli* K-12 RLG221[35], which indicates that you are a very specialized and refined bacterial strain, bred in captivity as a clone with special characteristics, not least of which is our ability to work with you easily. Your cell wall, which is what we are really interested in, is much easier to penetrate that that of your wild cousins.

E. Coli K-12 RLG221: Is that what you're going to do to me?



Molecular biologist: Yes – firstly through electroporation, to insert those plasmids and vectors, then through sonication to break you apart and take out your new DNA. Then we'll look at what the 'new you' is capable of doing before finally taking you apart again and inferring the structure of that BamA β-barrel protein.

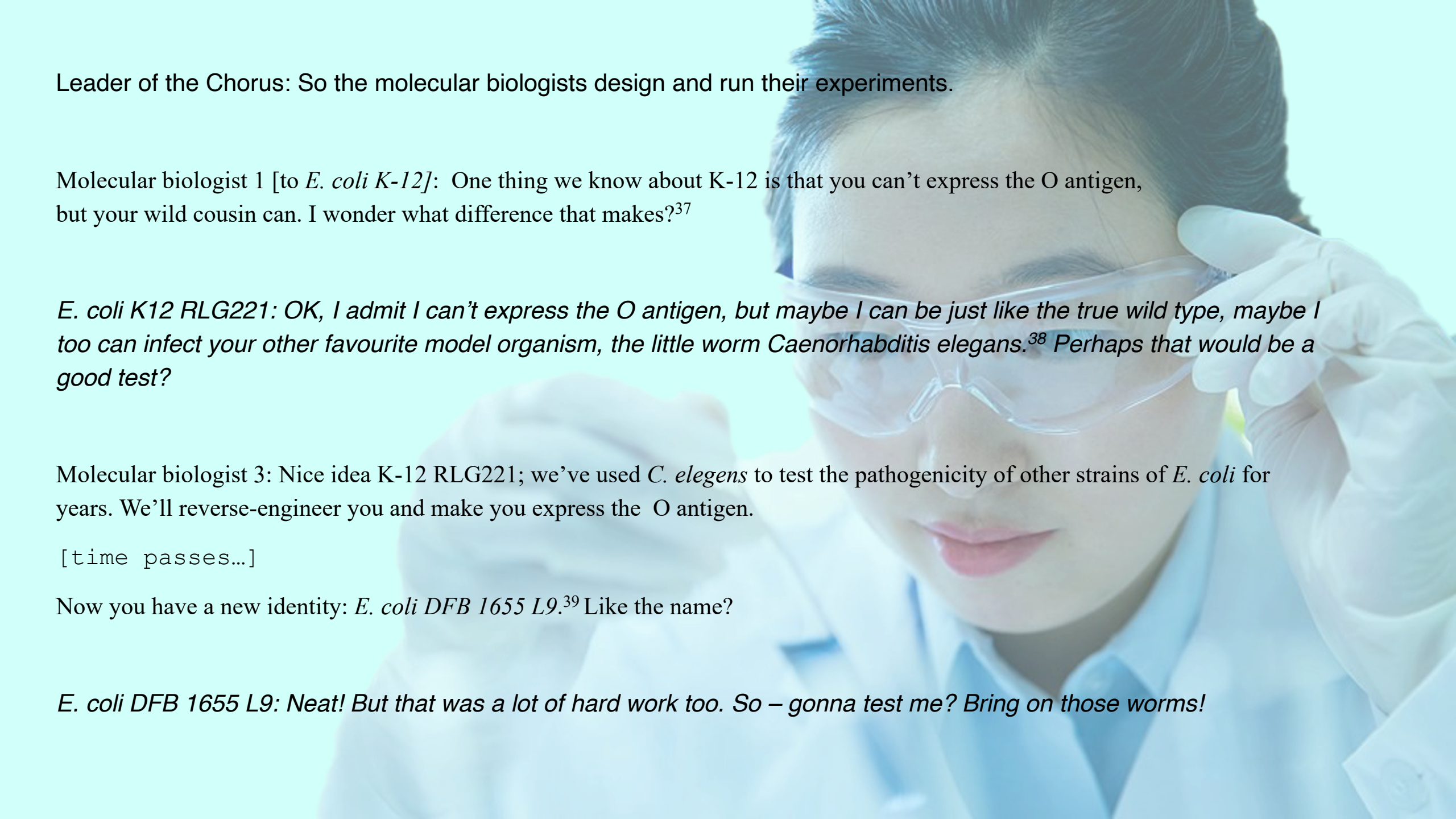
E. coli K-12 RLG221: *OK. You're right, it does sound complicated. Painful too.*

Molecular biologist: And we were only giving you a tiny part of the story!

But first, can we resolve the question of your identity K-12 RLG221?

E. coli K-12 RLG221*: Some more experiments, perhaps?*

Molecular biologist: However did you guess? We need to design and run a huge array of experiments to investigate just how far removed from the (true) wild type[36] you really are.



Leader of the Chorus: So the molecular biologists design and run their experiments.

Molecular biologist 1 [to *E. coli K-12]*: One thing we know about K-12 is that you can't express the O antigen, but your wild cousin can. I wonder what difference that makes?[37]

E. coli K12 RLG221: OK, I admit I can't express the O antigen, but maybe I can be just like the true wild type, maybe I too can infect your other favourite model organism, the little worm Caenorhabditis elegans.[38] Perhaps that would be a good test?

Molecular biologist 3: Nice idea K-12 RLG221; we've used *C. elegens* to test the pathogenicity of other strains of *E. coli* for years. We'll reverse-engineer you and make you express the O antigen.

`[time passes…]`

Now you have a new identity: *E. coli DFB 1655 L9*.[39] Like the name?

E. coli DFB 1655 L9: Neat! But that was a lot of hard work too. So – gonna test me? Bring on those worms!

Chorus: We're adding to our assembly again,

This time a worm and the O antigen

The worms are in a different laboratory

So our new microbe needs to make a journey.

Molecular biologist: Well, putting that O antigen back into K-12 made DFB 1655 L9 kill those worms with a vengeance! *E. coli* K-12 RLG221 without the ability to express the O antigen are non-pathogenic for *C. elegens*, but then we knew that already. But all the true wild type *E. coli do* have the ability to synthesise the O antigen. So - have we got the right model for our lab studies? These results clearly call things into question.[41] These guys are quite different from one another!

E. coli K-12 RLG221: I am here to help you. I thought I was helping you really well.

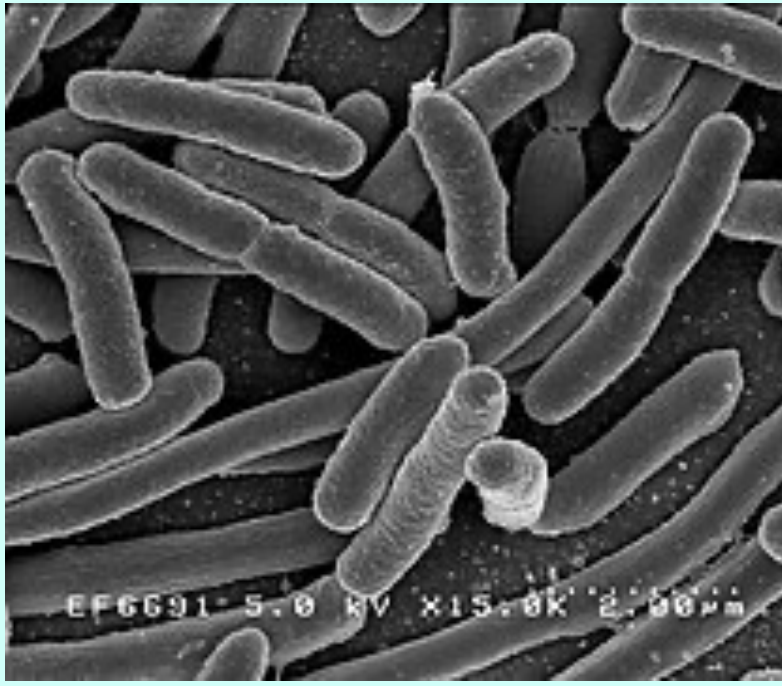
Molecular biologist 3: We do thank you for it – but things are different outside the lab.

E. coli (Wild Type): You better believe it – those wimpy lab strains wouldn't last a moment in the hostile environments I hang out in! You think you're tough 'cos you like MacConkey Bouillon and TBX agar – you should try living in some of the places I do – frozen seagull poo, sewage systems, air conditioning units…[42]

Molecular biologist 1: Interesting results regarding K-12! Do you think we should change how we run the BamA β-barrel protein experiments?

Molecular biologist 2: Not right now – stick to the protocol!

E. coli K-12 RLG221: 'ay! Happy to be working with you guys again. Let's get started.'



Chorus: *E.* coli K-12, K-12 RLG221, K-12 DFB1655 L9 all tamed like Enkidu

But which is the wild type like that in our poo?

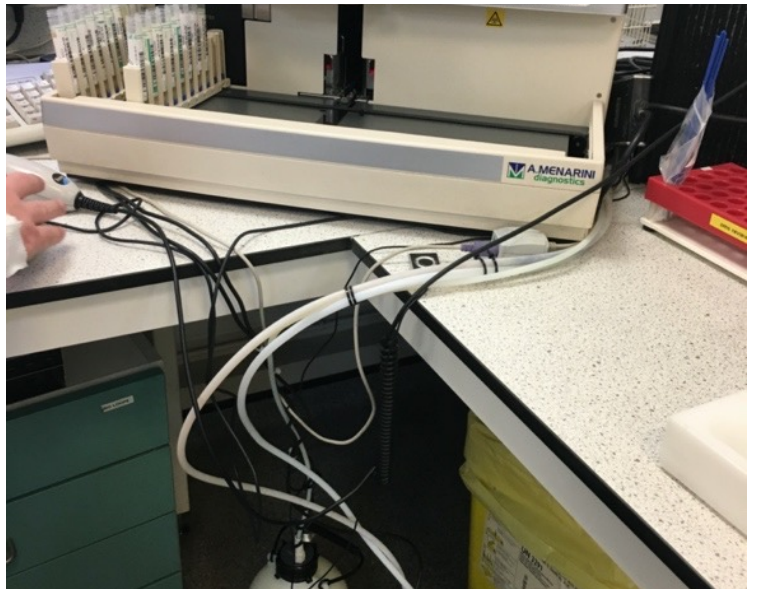
We've done a lot of experiments, but what have we found?

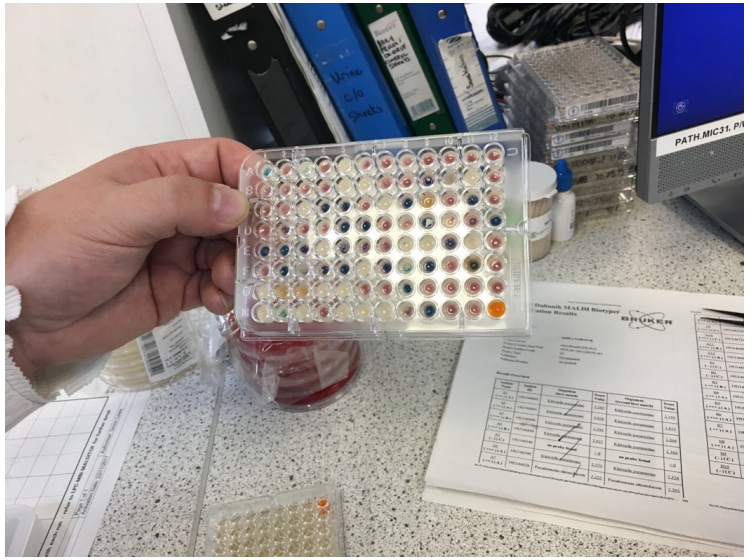
Are we nearer to showing our knowledge is sound?

Act 5: The public health laboratory again

E. coli: "I am here to resist your attempts to kill me. Thalia, help me to flourish and bloom."

Leader of the Chorus: The antics of *E. coli* in the urinary tract
show they can act together, while university science complicates
any easy equivalence drawn between strains. Meanwhile the
science of AMR is also developing. It seems that 'resistance' can be passed among bacteria horizontally, not just through generations. The capacity of bacteria to adapt has long been known, and the speed of their reproduction one of the things that endeared them to scientists, but persistence in the face of antibiotics is becoming problematic in clinical medicine.



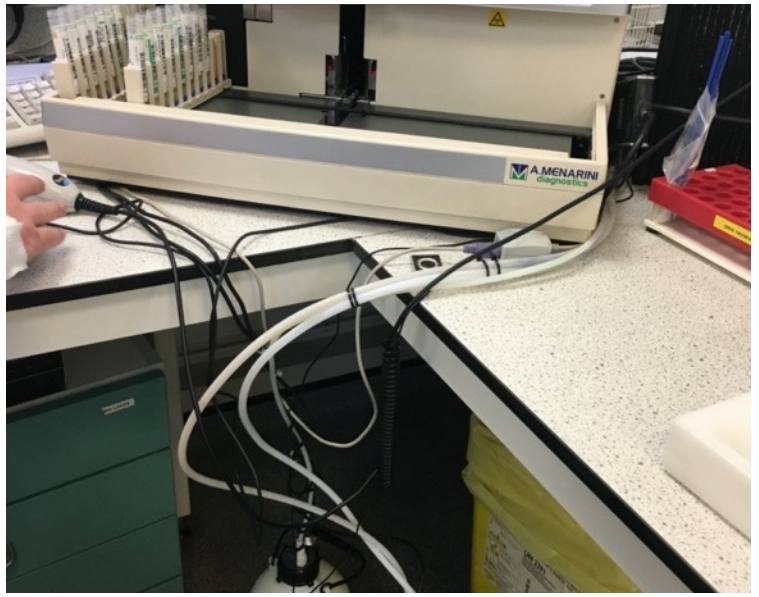


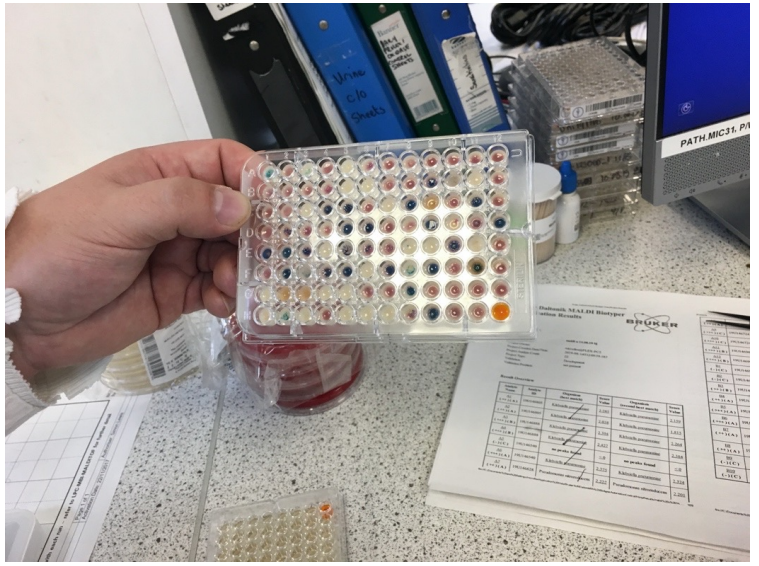
E. coli (Wild type): Here we go again. You've looked for me in water and in food, in the bloodstream and the urinary tract. Now you want to get to know me better. Fat chance! Stopped 'on reasonable suspicion' - even when I didn't cause an infection - you want to see what I've become and what I might be capable of.

Microbiologists: We see more and more nasty infections in the blood stream as well as urinary tract. We have to look for the right antibiotic for each infection, now not only to cure it but to reduce the growth of resistance. Don't select for the stubborn is our motto.

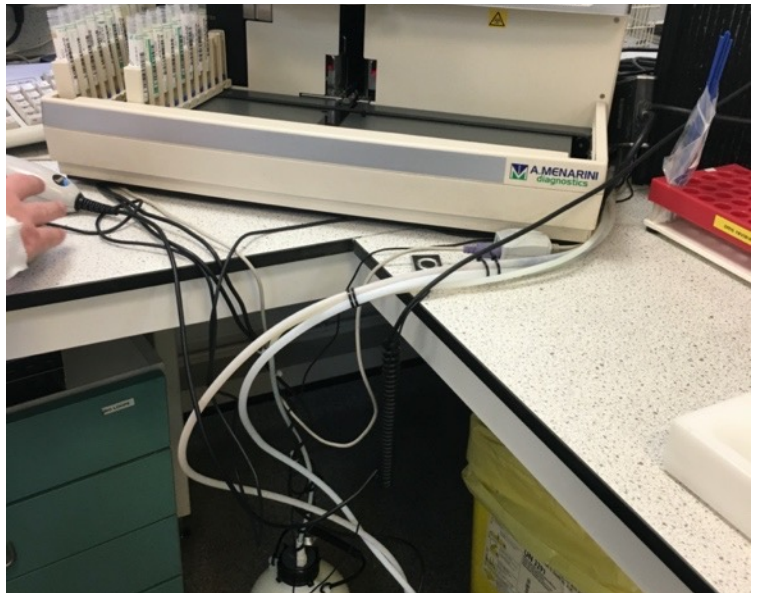
Chorus: More helpers required! Resistance tests aren't so simple.
Pour broth libations. Stack up 8x12 racks.
So much work to show whether drugs kill the bugs.

Voice of the laboratory again: Spotlight falls on the machines designed to give a measure of resistance, processing 95 samples at a time to examine susceptibility to different concentrations of antibiotics.

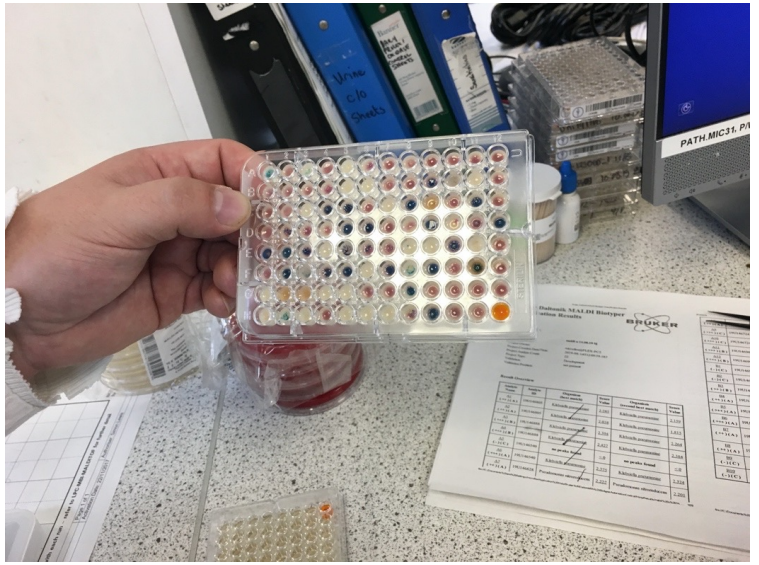




E. coli (Wild type): I get it, you're scared. Well maybe you should be! I guess I could help you (again) with your AMR project, but why should I quite frankly? I'm feeling pretty good, getting wise to your tricks, and I've got some of my own.[43] I'm doing so well I'm travelling all over.[44] You can throw ampicillin at me and half the time I bounce right back.[45] Maybe trimethoprim still knocks me out mostly but don't take it for granted… ciprofloxacin and amoxicillin are not a problem I assure you. I've hung out with them so often they're like mates. See how resistant I can be!



EUCAST: Whatever you say. We now know exactly how to define susceptibility and resistance across Europe. We're onto this.[46]

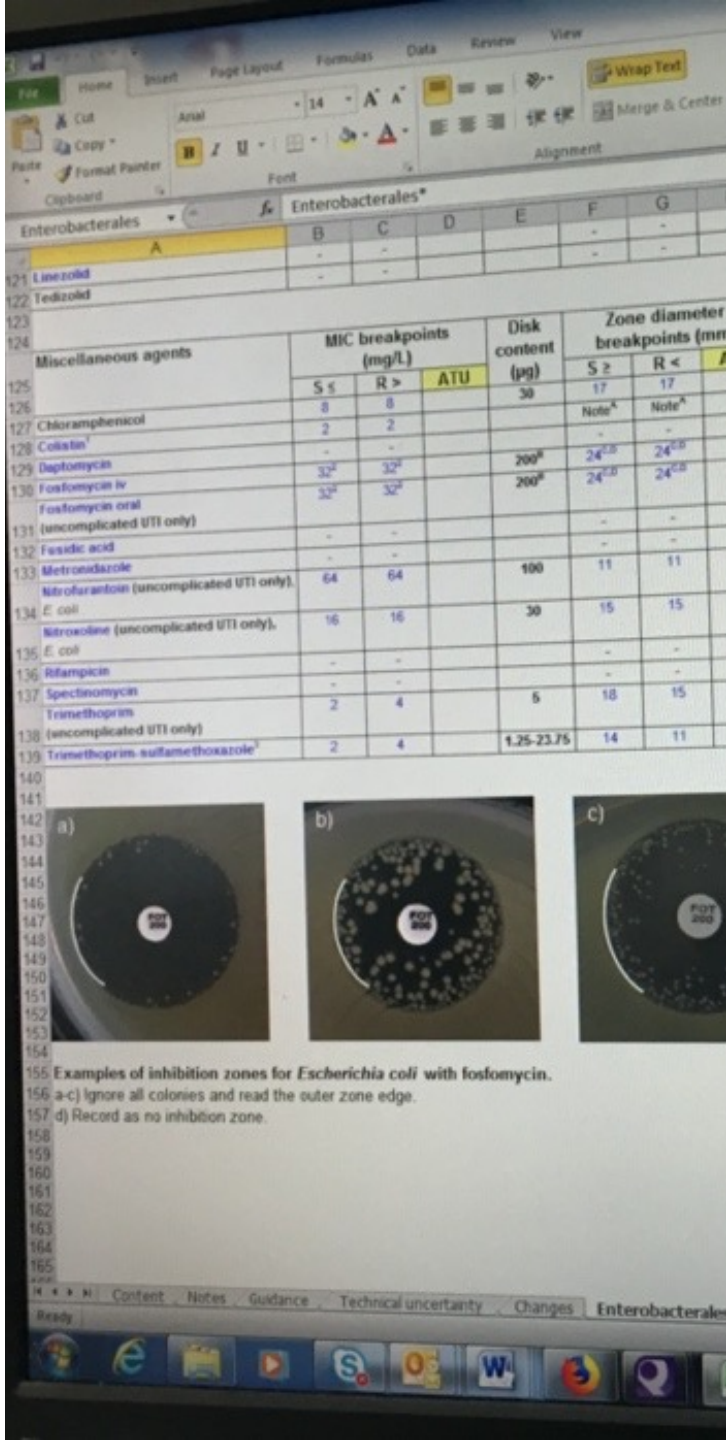


Leader of Chorus: It might be hard work checking susceptibility, but that's not the only kind of effort being made. Sally Davies (Professor Dame) got the ear of the government, and results from 30,000 women's pee pots are suddenly on David Cameron's Georgian desk.

Chorus: No, the work never ends, and nor do the grants.
They're calling committees and funding reports.
It's dizzying how many people turn up.
Multiplying disciplines, all with their thoughts.

Microbiologist: Maybe samples from pregnant women can help get a handle on the problem of AMR. In lots of the world we microbiologists test pregnant women regularly because we know urinary tract infections are dangerous for the health of their child. We can use those samples to get a picture on the spatial (or even social) distributions of the more resistant *E. coli*[47].

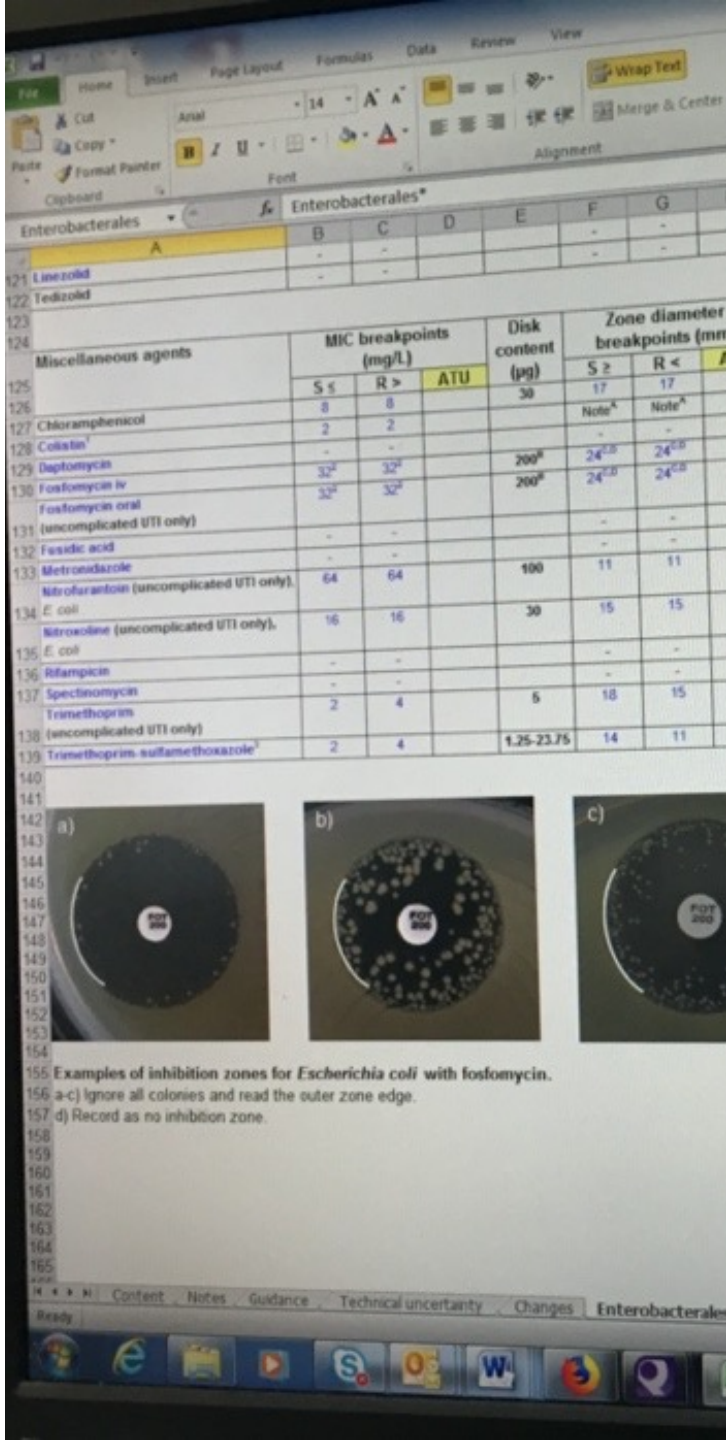
Data scientist: Yes, the figures are big enough for us data scientists to play now. Away from the smell of stale pee in the laboratories, we crunch numbers in bright white offices. But beware. The resistance percentages are acutely sensitive to the full set of samples being tested by each lab[48]. More testing of routine samples and the 'resistance rate' might look artificially low. If testing of routine samples is reduced – say because prescribing is increasingly allowed to follow clinical symptoms or the lab tries to cut down its routine work – then it may look artificially high. And the use of laboratory cultures varies among family doctors. Yes, there are guidelines but does anyone follow them in the same way?



Public Health England: “Our new guidance is for GPs to not send samples for testing, just prescribe those antibiotics anyway for uncomplicated UTIs. We know what’s happening and who is responsible.”

Epidemiologist: Surely there’s still a role for the old school epidemiologist in investigating these patterns. “In our study of GPs in the West Midlands there was variation in whether they requested a specimen for the scenarios involving a suspected uncomplicated urinary tract infection (UTI) and an asymptomatic catheterised elderly patient; with 40 and 38% respectively indicating they would [still] collect a specimen for microbiological examination.”[49]

Public Health England: “Our new guidance is for GPs to not send samples for testing, just prescribe those antibiotics anyway for uncomplicated UTIs. We know what’s happening and who is responsible.”

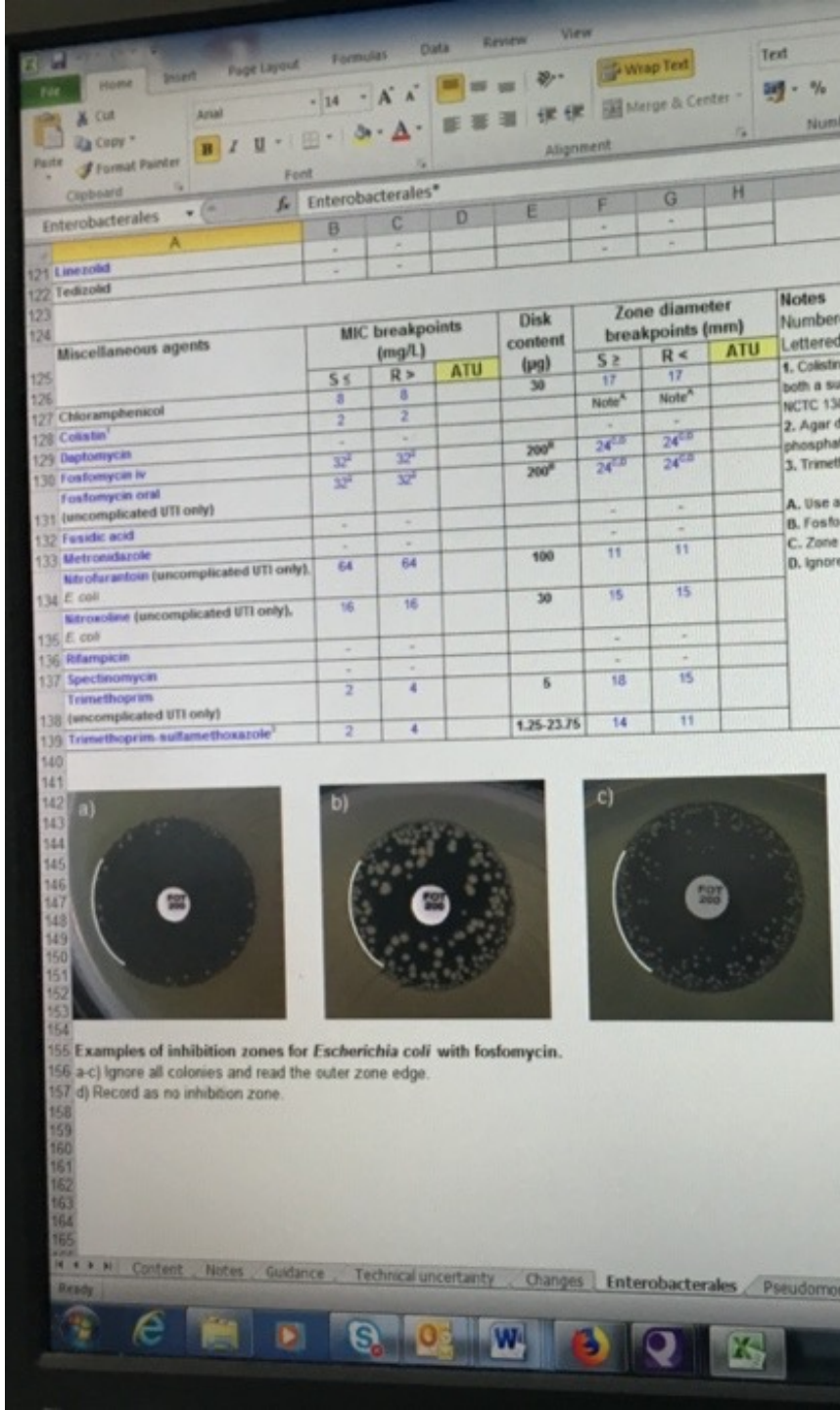


Data scientist: I really think this is another problem where data science is the solution. "Instead of trying to sum up from laboratory results at a regional or national level perhaps we should instead create a surveillance system. We have more to learn about how far prescribing for one condition increases the chance of suffering resistance in another and about the mechanisms behind resistance within the host or patient."[50]

Chorus: A system, let us have a system. That's always the solution in our experience.

Geneticist: Please don't forget us geneticists and the contribution of lab research. In this funding bonanza you'll want interdisciplinary teams for sure, and plasmid transfer means studying the 'bacterial gene pool' of individuals too. "Even a transient effect of antibiotic use on the carriage of resistant organisms by an individual could have a major impact on the endemic level of resistance in the population." [51]

E. coli (Wild Type): From your perspective I suppose this looks like a mess. From mine, a great success. Whatever you threw at me, I learned to cope and shared my skills. I still want to assist, but you're going to have to treat me with a bit more respect. Stop trying to get rid of me all the time and recognise I can help in more ways than you knew. Our long collaboration may be entering a new phase but please, please respect my creativity and complexity rather than just what you call 'virulence' or 'resistance'. I know you're a bit uneasy about how my lab self – K-12 – may vary from free-living E. coli but try to reconcile this. Your skills in ensuring my cooperation in the laboratory may yet give us a way to thrive together.



Epilogue

Back on Mount Olympus the Muses – Clio, Thalia and Melpomene – look down

Clio

I wonder what the mortals have learned from our tour? History as tragedy, history as farce, the present as uncertain as the past. Maybe the future will bring more certainty for these fearful mortals? After all, they have the tools and the motivation: they just keep on going.

Thalia
Yes, look! How joyous, how beautiful our actors working together are. Aren't they clever? Their blossoming and flourishing world will continue for ever!

Melpomene

Have you learnt nothing, just like the humans? Hubris is always followed by nemesis. Look at the storm clouds on the horizon, smell the air, feel the tragedy. They are all doomed and those mortals have made this fate for themselves.

Mnemosyne
Only I can know the future, sisters, but let us leave it to our audience to decide which voice they will hear, Thalia or Melpomene.

The End

Thanks to all the players for their time and enthusiasm

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