Scenes from the many lives of *Escherichia coli.*

A play in 5 acts[1]

Can bacteria be famous? If they can, then which are the most famous and why? In this paper we will put our candidate for the world's most famous bacterium onto the stage, literally, give it a voice – or rather a few voices – and show how *Escherichia coli* grew in importance and watched its identities proliferate at the same time as its discoverer – bacteriology.

So we present a play showing *E. coli* on an odyssey to discover itself that takes it from a children's clinic in Bavaria to high-tech contemporary university labs, via hospitals and medical schools, wastewater treatment plants and government offices tasked with slowing antimicrobial resistance. Names are important and we'll see how naming this bacterium changes our relationship to it[2], just as the disciplines, and tools, of those naming and making these microbes also shift[3].

It's not easy to put words in the mouth of a microbe. For a start we asked ourselves, 'Shouldn't that be mouths?' When encountered by humans *E. coli* is always plural. Yet that same plurality also introduces the issue of generation. The *E. coli* made visible in laboratories – clinical, environmental or scientific – tend to end their lives soon after in the autoclave. Confronted with these difficulties we use the singular to gesture to the ontological stakes[4], even as we multiply coliform identities[5]. Looking across the decades, our hero has a dizzying number of these identities, though as we show many of the practices involve familiar actors such as Petri dishes, Falcon® and Durham tubes, agar, Bunsen burners, slides and microscopes[6]. Nonetheless, from the perspective of *E. coli* we suggest it is unsettled, restless, fixed and stained, but misunderstood, accused and ignored.

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[1] Acknowledgements: Dr James Ebdon, Dr Doug Browning, Eric Will MD

[2] Here we are following Ian Hacking's 'dynamic nominalism' where kinds of things come into being at the same time as the kind itself is invented. Hacking, I. 2002. "Making Up People " in *Historical ontology*, Cambridge, MA.: Harvard University Press. pp. 99-114

[3] Hannah Landecker's 2016 work is particularly helpful here, reminding us that we can tell histories of biology through model organisms, practices, media, disciplines, practitioners as well as just through an examination of 'texts'. Landecker, H. 2016. "Antibiotic Resistance and the Biology of History." *Body & Society* 22(4):19-52.

[4] Here we (unwittingly) echo Andrew Balmer and Susan Molyneux-Hodgson's study (2013) which set out to compare 'bacterial ontologies' emerging from different practices, in the case of their research between wastewater treatment plants and synthetic biology (synbio) laboratories. They looked at differences between different proponents of 'engineering' hence the site of publication. Here we take another route by centring the bacteria in its encounters with a wider range of actors who share neither discipline nor practices. Balmer & Molyneux-Hodgson (2013) "Bacterial Cultures: ontologies of bacteria and engineering expertise at the nexus of synthetic biology & water services." *Engineering Studies* 5 (1): 59-73

[5] Our debt to Annemarie Mol's work is most clear in the notion of practices making multiple versions of something with the same name. The obvious reference is her work on atherosclerosis, Mol A (1999) *The Body* Mulitple. However in her more recent paper on 'schoon' she directs our attention to notions and practices for ensuring 'cleanliness' including how we treat waste water and gave us the lead to and the paper by Balmer and Molyneux-Hodgson (2013) (see also Erickson 2018 for details on how *E. coli* is a key indicator of water cleanliness). Mol, A. (2020). Not quite clean: Trailing schoon and its resonances. *The Sociological Review*, *68*(2), 385–400. Erickson, M. 2018. "Homer in the Laboratory: A Feyerabendian Experiment in Sociology of Science." *Social Epistemology* 32(2):128-141.

[6] With Bruno Latour we are reassembling the assemblages that we have encountered in our various researches. Latour, B. (2005) *Reassembling the Social: an Introduction to Actor-Network-Theory* Oxford: Clarendon.

Joining a tradition of experimental writing in STS[7] we take inspiration from classical Greek theatre, using a Chorus to represent the assemblages forming and decomposing, and its Leader to help us navigate. Like the writers of this time we also invoke higher powers, the Gods and Muses, to help us tell our tales.

[7] Here we are particularly drawing on the New Literary Form movement from the late 1980s (Ashmore, M. 1989. *The reflexive thesis: wrighting sociology of scientific knowledge*. Chicago ; London: University of Chicago Press.; Mulkay, M. 1991. *Sociology of science: a sociological pilgrimage*. Milton Keynes: Open University Press.; Woolgar, S.and M. Ashmore. 1988. "The next step: an introduction to the reflexive project." Pp. 1-11 in *Knowledge and reflexivity: new frontiers in the sociology of knowledge*, edited by S. Woolgar. London: Sage.), and later work that voices actants as well as humans (Latour, B. 1996. *Aramis or the Love of Technology*. Cambridge MA: Harvard University Press.), or presents STS analyses in counterposed textual forms (Mol, A. 2003. *The body multiple: ontology in medical practice*. Durham, N.C. ; London: Duke University Press.).

Prologue[8]

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 [klee-o], the muse of history, has helped me with some parts, and, of course, Melpomene [mel-po-min-ay] was always best at theatre (though oh so serious!). And where would we be without our darling mama Mnemosyne [ne-mo-zin-ay] whose memory we rely upon?

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[9] [en-kid-u] who is older even than we Muses, and tame it through naming it, firstly, *Bacillus [bac-kill-us] coli*, later *Escherichia [esh-e-rik-chi-a] coli*. Then bleak-hearted Melpomene [mel-po-min-ay] scripts our play, as disease and illness are blamed upon *E. coli* who joins a gang and, like our distant cousin Loki[10], 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 [mel-po-min-ay], in our final act, pushes us towards a tragic, fateful end as our microbes' resistance grows and grows. Truly it is they who

[8] Not all Greek dramatists use the prologue form and the conventions differ from later authors. Here we take as a model Euripides, whose prologues may be by a god or goddess, but where these usually give a background we reflect later traditions in using the prologue to summarise what will follow (as did Shakespeare or Chaucer). We invoke the muse of comedy and idyllic poetry Thalia (where translations including blooming (*Theogony* 77 (and Liddell & Scott's translation of θαλεια is "*blooming, luxuriant, goodly, bounteous*" and "Θαλεια, η, one of the Muses, the *blooming* one, Hes.") / or sprouting (C.f. Thallo (Graves 19760: 13.2 p. 55). Thalia as 'blooming' has good microbial connotations – bacterial blooms have been described in the literature (named analogously to algal blooms which are more common) e.g. Fuentes, S., et al. (2016). "From Rare to Dominant: a Fine-Tuned Soil Bacterial Bloom during Petroleum Hydrocarbon Bioremediation." *Applied and Environmental Microbiology* 82(3): 888).

[9] The 'wild man' companion to Gilgamesh in that eponymous epic poem composed sometime in the 2nd millennium BCE.

[10] Of Norse mythology

will bloom in the future, whilst we who have flourished, like all fragrant flowers which abound in the meadow[11], will wither and die.

[11] Theocritus *Idyll XXII: The Dioscuri* (from lines 27-52)

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.

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

Coliform: I am here.

Theodor Escherich MD: Perhaps they are the source of the dysentery? I will call them *Bacteria coli commune*[12] and continue my investigations.

Modern day geneticist: Great job. But are you sure you've got the right culprit? We geneticists 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[13].

[12] Escherich, T. 1885. Die darmbakterien des neugeborenen und sauglings. *Fortshr. Med.* 3:5-15-522, 547-554.
[13] Méric, G., Hitchings, M. D., Pascoe, B., & Sheppard, S. K. (2016). From Escherich to the *Escherichia coli* genome. *The Lancet Infectious Diseases*, *16*(6), 634-636. https://doi.org/10.1016/S1473-3099(16)30066-4. The authors tested strains reputed to be from samples reputed to be from Escherich's originals, that had made their way to the UK in this period.

B. coli: A false accusation, or case of mistaken identity? Whatever the reason we're all suspect now. [Ulla]

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 (later DBE): They're dirty and dangerous and I'm going to kill them[14].

B. coli: "AAAAAAAARGGGGHHH!!!!"

Leader of the Chorus: Her colleague Alfred MacConkey[15] – 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[16].* 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

[14] Chick, Harriette. (1908). "An Investigation of the Laws of Disinfection." *The Journal of Hygiene*. 8:1 92-158.
[15] See MacConkey's obituary in *Nature* 127, 980–9811931, https://www.nature.com/articles/127980a0, which also mentions his love of roses. MacConkey gave his name to a selective medium widely used to encourage the growth of *E.Coli* in laboratories across the 20th century.
[16] Buchanan, RE (1916). "Studies in the Nomenclature and Classification of Bacteria: The Problem of Bacterial Nomenclature". *Journal of Bacteriol*ogy 1: 591–6. doi:10.1128/jb.1.6.591-596.1916.

Coli will appear in clear view on a dish.[17]

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...

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.

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.

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[18] 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

[17] MacConkey was drawing on older ideas about differential media but exploring the best way to tailor the medium to *B.coli* from 1897 onwards, publishing a summary of work in MacConkey AT. (1908) Bile Salt Media and their advantages in some Bacteriological Examinations. The *Journal of Hygiene*. 8(3):322-334. DOI: 10.1017/s0022172400003375.

[18] This urge to count was by no means new, see Hamlin (1990) for a fuller account of the 19th century history of water analysis in Britain, in Hamlin, C. (1990) A Science of Impurity: Water Analysis in Nineteenth Century Britain. Berkeley: University of California Press. It was continued in publications like *HMSO (1934) The Bacteriological Examination of Water Supplies. Reports on Public Health and Medical Subjects, 1st edn, No. 71, London.*

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"[19].

E. 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…

Scene 3 Contemporary sewerage

Leader of the Chorus: MacConkey's approach rapidly spreads, appearing in the very first edition of the textbook *American Sewerage Practice* – published by Leonard Metcalf and Harrison P Eddy in 1914/5 – still a touchstone in the field. Though we now see bacteria as making just over half of faecal solids, many non-pathogenic[20], *E. coli* is emerging from the mass as indicating the risk of disease.

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

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."

E. coli: "Now you can see and hear me, loud and clear. All my colonies are visible, bright blue specks on the TBX[21] agar. Count me, let me colonise your spreadsheet, and I will tell you how dirty that river was."

Bacteriologists: Why should we let *E. coli* speak for all the coliforms? It is only 0.1% of the microbial flora[22], and isn't even an anaerobe like most of the others.

E. coli: Because the standard guide to Wastewater Engineering tells you 'The presence of E. coli in drinking water is an indication that there is a greater risk that disease causing pathogens could also be present.'[23] In this field you respect traditions, and these stretch back decades. I can represent the bigger group, and with some amplification I do that pretty well. Look how safe the drinking water is, if you don't believe me.

[19] MacConkey (1909) Further observations on the differentiation of lactose-fermenting bacilli, with special reference to those of intestinal origin. *Journal of Hygiene, 9*(1), 86-103. doi:10.1017/S002217240001615 p99

[20] Almeida, A., Mitchell, A.L., Boland, M., Forster, S.C., Gloor, G.B., Tarkowska, A., Lawley, T.D. and Finn, R.D. (2019) 'A new genomic blueprint of the human gut microbiota', *Nature*, 568, 7753, 499-504. (p. 499)

[21] Triptone Bile Agar with X-Glucuronide. The other standard agar is MUG: 4-methylumbelliferyl-β-D-glucuronide. *E. coli* can make an enzyme, β-glucuronidase, that can cleave the fluorogenic substrate from the growth medium. It is this that turns the colonies bright blue.

[22] Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E. and Relman, D.A. (2005) 'Diversity of the human intestinal microbial flora', *Science* 308, 5728, 1635-1638.

[23] Tchobanoglous, G. a., et al. (2014). *Wastewater engineering : treatment and resource recovery*. New York, McGraw-Hill Education. This is the current version of 'Metcalf and Eddy' – first published in 1914-15 as *American Sewerage Practice* (3 volumes) p160

Act 2 *E. coli* makes trouble in the hospital lab

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 the lab[24] 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[25] 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.' [26]

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.

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[24] Wall, R. (2013) *Bacteria in Britain, 1880-1939*, London: Pickering and Chatto.
[25] Voswinckel, P. (1994) A Marvel of Colours and Ingredients, *Kidney International,* 47,4 (with thanks to Eleanor Kashouris for these aspects of the history).
[26] Dudgeon, L.S (1908) Acute and Chronic Infections of the urinary tract due to the bacillus coli. *Lancet* 616-620.

E. Napier Burnett, FRCS, MRCP (Edin): We're all 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.'[27]

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!

Scene 2: In the hospital of 1957 United States

Leader of the Chorus: By 1957 doctors have antibiotics that can act effectively against *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 mil[28]. 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!

[27] Burnett, E.N. (1910) *B.Coli* Infection of Urinary Tract Complicating Pregnancy. *Journal of Obstetrics and Gynaecology of the British Empire*. 18:2:81-94.
[28] Kass, E. (1956) Asymptomatic infections of the urinary tract. *Journal of Urology* 167:106-120.

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

Scene 3: *E.coli* in UK general practice

Leader of the Chorus: In family medicine too, a urine test remains a staple of practice[29]. By the 1950s general practitioners have the 'dipstick' - combining on a single card test papers for glucose, white blood cells and nitrites or proteins[30] 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.

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[31].

E. coli: "We're everywhere and nowhere. Just because you can see us does not mean we're causing trouble. You're just picking us out because it's easy."[32] 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 no, in the 1980s doctors are still arguing. They can see colonisation without symptoms or pyuria [white blood cells] in some patients, but they also see evidence of inflammation or symptoms that look or feel like urinary

[29] In part because of interest in diabetes as a treatable condition. For more on the history of hormones in medicine see Oudshoorn, N. (1994) *Beyond the Natural Body: An Archaeology of Sex Hormones*. London: Routledge.

[30] In 1957, Ames launched *Albustix,* similarly with a colour indicator, which offered a semi-quantitative estimate of protein concentration.

[31] The brand name for Beecham's new antibiotic, ampicillin, on the market since 1961.

[32] In the 1970s Rosalind Maskell (1928-2016) among others asks whether other bacteria might be responsible for UTIs where a culture did not immediately show up *E. coli* or another that people were expecting. The problem was that laboratory conditions and tests were encouraging *E. coli* detection but this did not always match symptoms. Perhaps more 'fastidious' or fussy bacteria were to blame, but did not show themselves (Maskell R, Pead L, Sanderson RA. Fastidious bacteria and the urethral syndrome: a 2-year clinical and bacteriological study of 51 women. *Lancet.* 1983 Dec 3;2(8362):1277–1280). This was a particular concern for her when faced with children with symptoms but no positive culture from her work in general practice. See her books *Urinary tract infection* (London, Edward Arnold, 1982) and *Urinary tract infection in clinical and laboratory practice* (London, Edward Arnold, 1988)

tract infections when their tests can't find bacteria[33]. Through these investigations – mainly from a clinical perspective - *E. coli* emerges as a tricky character.

Act 3[34] Faecal *E. coli* at work

Scene 1: Wastewater Treatment Plant in rural 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."

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[35]. 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.

[33] Maskell R, Pead L, Sanderson RA. (1983) Fastidious bacteria and the urethral syndrome: a 2-year clinical and bacteriological study of 51 women. *Lancet.* 2(8362):1277–1280). Stamm, W. E. (1983) Measurement of pyuria and its relation to bacteriuria. *Am. J. Med*. 75 (Suppl.)53.

[34] We cut this whole act in producing the versions for EASST and *With Microbes.* This version made it to May 2020 and is, I think, the most recent one.

[35] The water in the flood flows has so much air in it that a human body, no matter how good a swimmer, would immediately sink to the bottom if it fell in. Hence my health and safety officer's advice prior to entering the site: "don't drown in shit".

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, a 1l collecting flask) and took it back to our laboratory."

Scene 2: A microbiology laboratory in a university in southern England

The microbiologist holds a sample flask up to the window and peers through it. Seagulls wheel outside the window.

Microbiologists: *"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."

Narrator: 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 plate will now have colonies of *E. coli* growing on the surface.[36] These are clonal colonies: each member has

[36] Sample and dilution designed for accuracy given the size of the agar plate: '*To be effective, the dilution of the original sample must be arranged so that on average between 30 and 300 colonies of*

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 blue specks on the TBX[37] agar. Count me, let me colonise your spreadsheet, and I will tell you how dirty that river was."

Microbiologists: "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 '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.' (Tchobanoglous[38] et al. 2014 p. 160). 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 of this all of this into their book chapter. Write, right, rite.

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*the target bacterium are grown [*on a single agar plate*]. Fewer than 30 colonies makes the interpretation statistically unsound whilst greater than 300 colonies often results in overlapping colonies and imprecision in the count. To ensure that an appropriate number of colonies will be generated several dilutions are normally cultured.*' EU Water Directive. 1980 and 1998.

[37] Triptone Bile Agar with X-Glucuronide. The other standard agar is MUG: 4-methylumbelliferyl-β-D-glucuronide. *E. coli* can make an enzyme, β-glucuronidase, that can cleave the fluorogenic substrate from the growth medium. It is this that turns the colonies bright blue.

[38] Tchobanoglous, G. a., et al. (2014). Wastewater engineering : treatment and resource recovery. New York, McGraw-Hill Education. This is the current version of 'Metcalf and Eddy' – first published in 1914-15 as *American Sewerage Practice* (3 volumes)

Scene 3 In a UK public health lab

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!"[39]

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.

[39] Evann E. Hilt Hilt EE, McKinley K, Pearce MM, et al. (2014) Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *Journal of Clinical Microbiology*. 52(3):871–876. doi:10.1128/JCM.02876-13

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'[40].

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."[41]

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) Molecular biology laboratories in universities in the West Midlands

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[42]
Working away on our usual routine
Looking for a β-barrel protein

[40] Travis K. Price, Tanaka Dune, Evann E. Hilt, Krystal J. Thomas White, Stephanie Kliethermes, CynthiaBrincat, Linda Brubaker, Alan J. Wolfe, Elizabeth R. Mueller, Paul C. Schreckenberger (2016) The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms. *Journal of Clinical Microbiology* 54 (5) 1216-1222; DOI: 10.1128/JCM.00044-16.
[41] See Blount ZD. (2015) The unexhausted potential of E. coli. *Elife*. 2015;4:e05826. doi:10.7554/eLife.05826
[42] Fleck, L. 1979. *Genesis and Development of a Scientific Fact*. Chicago: University of Chicago Press.

Molecular biologist 1: 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. 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 a large number of actors to bring this about, but the star here will be their old workhorse, the model organism *E. coli*. K-12[43].

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?[44]

Leader of the Chorus: Eventually, the molecular biologists will triumph in identifying the structure and function of *E. coli*'s BamA β-barrel protein[45] 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 complete knowledge.

Molecular biologist 1: Thank you *E.coli* K-12 but be aware that this will be a long, 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 2: 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[46] to be very precise, which indicates that you are a very specialised 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 than that of your wild cousins.

[43] Browning, D. F., et al. (2013b). "Mutational and Topological Analysis of the *Escherichia coli* BamA Protein." *PLoS ONE* 8(12): e84512.
[44] Erickson, M. (2015) *Science, culture and society: understanding science in the twenty-first century*. 2nd edition, Cambridge: Polity. Chapter 2.
[45] Browning, D. F., et al. (2013b). op cit.
[46] Browning et al. 2013b: Table S1 shows the relevant genotype of this bacterial strain.

E. coli K-12 RLG221: Is that what you are going to do to me?

Molecular biologist 1: 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 2: 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 1: However did you guess? We need to design and run a huge array of experiments to investigate just how far removed from the (true)[47] wild type you really are[48].

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 you K-12 is that you can't express the O antigen, but your wild cousin can. I wonder what difference that makes[49]?

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*[50]. 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 O antigen.

[time passes…]

Now you have a new identity: *E. coli DFB 1655 L9*[51]. Like the name?

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[47] We are using this nomenclature, rather than extended strain names, to make this an easier read. Our definitions are as follows: 'wild type' refers to an organism that has been unmodified, but may be a laboratory strain such as K-12; 'true wild type' refers to an organism that has not been modified and exists in the environment beyond the laboratory; 'strain' refers to an organism that exists in the laboratory but has been deliberately modified to take on certain phenotypical characteristics.

[48] Browning, D.F., et al. 2013a. "Laboratory adapted Escherichia coli K-12 becomes a pathogen of Caenorhabditis elegans upon restoration of O antigen biosynthesis." *Molecular Microbiology* 87(5):939-950.

[49] Ibid.

[50] Another model organism, a worm, microscopic, that eats bacteria; if the worm dies it shows pathogenicity of the microbe. See https://www.ncbi.nlm.nih.gov/books/NBK453431/.

[51] Under this name the strain is stored in extra cold freezers in the West Midlands lab, lying dormant until it's needed again. But it has also

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 1: Well, putting that O antigen back into K-12 made DFB 1655 L9 kill those little 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 certainly call things into question[52]. 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: And 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[53], sewage systems[54], air conditioning units[55]…

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*: 'Yay! 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

travelled the world, as the microbiologists send out samples in response to requests from places as far flung as Argentina, Canada and Singapore, just as Theodor Escherich shared his original *E. coli* with colleagues in Cambridge.

[52] Browning 2013a op cit..

[53] Rabbia, V., H. Bello-Toledo, S. Jiménez, M. Quezada, M. Domínguez, L. Vergara, C. Gómez-Fuentes, N. Calisto-Ulloa, D. González-Acuña, J. López, and G. González-Rocha. 2016. "Antibiotic resistance in Escherichia coli strains isolated from Antarctic bird feces, water from inside a wastewater treatment plant, and seawater samples collected in the Antarctic Treaty area." *Polar Science* 10(2):123-131.

[54] Sozzi, E., K. Fabre, J.-F. Fesselet, J.E. Ebdon, and H. Taylor. 2015. "Minimizing the Risk of Disease Transmission in Emergency Settings: Novel In Situ Physico-Chemical Disinfection of Pathogen-Laden Hospital seas." *PLoS Negl Trop Dis* 9(6).

[55] Gołofit-Szymczak, M., A. Stobnicka-Kupiec, and R.L. Górny. 2019. "Impact of air-conditioning system disinfection on microbial contamination of passenger cars." *Air Quality, Atmosphere & Health* 12(9):1127-1135.

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 their speed of 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.

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[56]. I'm doing so

[56] Target alteration, reduced drug concentration, inactivation of the drug.

well I'm travelling all over[57]. You can throw ampicillin at me and half the time I bounce right back[58]. 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 right across Europe. We're onto this[59].

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 problem of AMR. In lots of the world we microbiologists test pregnant women regularly because we know urinary tract infections are dangerous in 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*[60].

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[61]. 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 everyone follow them in the same way?

[57] Adapted *E. coli* 131 (ST131) improved fitness and growth rate and spread worldwide as extraintestinal pathogenic organism.
[58] Nomamiukor B., Horner C., Kirby A., Hughes G.J. (2015) Living conditions are associated with increased antibiotic resistance in community isolates of Escherichia coli. *Journal of Antimicrobial Chemotherapy* 70:3154-8.
[59]See http://www.eucast.org/documents/rd/ and explanations there of new definitions of susceptibility and resistance published in January 2019 after review by the European Committee on Antimicrobial Susceptibility Testing. Website accessed 9th December 2019.
[60] Nomamiukor et al (2015) op cit.
[61] Pouwels KB, Muller-Pebody B, Smieszek T, Hopkins S, Robotham JV (2019) Selection and co-selection of antibiotic resistances among *Escherichia coli* by antibiotic use in primary care: An ecological analysis. *PLOS ONE* 14(6): e0218134. https://doi.org/10.1371/journal.pone.0218134

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.

Epidemiologists: 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."[62]

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."[63]

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."[64]

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 –

[62] Dean Ironmonger, Obaghe Edeghere, Neville Q Verlander, Savita Gossain, Susan Hopkins, Bridget Hilton, Peter M Hawkey, (2018) Effect of general practice characteristics and antibiotic prescribing on *Escherichia coli* antibiotic non-susceptibility in the West Midlands region of England: a 4 year ecological study, *Journal of Antimicrobial Chemotherapy*, 73: 3: 787–794, https://doi.org/10.1093/jac/dkx465

[63] Costelloe Céire, Metcalfe Chris, Lovering Andrew, Mant David, Hay Alastair D. (2010) Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis *BMJ* 340 :c2096.

[64] Knight GM, Costelloe C, Murray KA, Robotham JV, Atun R, Holmes AH. (2018) Addressing the Unknowns of Antimicrobial Resistance: Quantifying and Mapping the Drivers of Burden. *Clin Infect Dis*. 66(4):612–616. doi:10.1093/cid/cix765

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.

Clio: 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.

Curtain(s)