HOSPITAL WASTEWATER SYSTEMS: ORIGINS OF NOVEL NOSOCOMIAL BACTERIA

Professor Colum Dunne,
School of Medicine, University of Limerick
Email: colum.dunne@ul.ie

Hosted by Prof. Jean-Yves Maillard
Cardiff University

4i Centre for Interventions in Infection Inflammation & Immunity

Objectives

Hospital-acquired infection (HAI) is a challenge globally, often associated with incidence of infectious agents exhibiting antimicrobial resistance (AMR).

Internationally, there are numerous publications describing outbreaks of nosocomial infections, typically providing characterisation of recognised microorganisms, their antimicrobial susceptibilities and details of infection prevention and control (IPC) interventions.

Environmental patient-facing wastewater (WW) apparatus such as handwashing sinks, showers and toilets are identified frequently as the sources of these infectious agents and associated AMR genes (ARGs).

We have undertaken:

a large-scale metagenomic analysis of the WW system in a large teaching hospital in the Republic of Ireland that has experienced ongoing multidrug-resistant HAI outbreaks.

Diverse taxonomic and resistome profiles were observed across all pipe sections studied, with members of phyla Proteobacteria and Actinobacteria dominating.

The highest numbers of ARGs observed were those encoding resistance to tetracyclines, fluoroquinolones, diaminopyrimidines, β-lactams, and macrolides, all of which are significant clinically and are commonly used antibiotic classes.

These unique large-scale analyses reinforce the need for regular decontamination of patient-facing hospital WW pipes and effective infection control policies to prevent the transmission of nosocomial infection and emergence of AMR within potential WW reservoirs.

Introductions

Me:

Microbiologist.

Dean - School of Medicine

University of Limerick

Email: colum.dunne@ul.ie

Colleagues:

Clinical and Medical Microbiologists and Biochemists.

UHLG and international.

Anatomy, engineering, gastroenterology, hospital management, immunology, infection prevention and control, nursing, paediatrics, physiology, respiratory medicine, surgery, surveillance, technology transfer.

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Challenges

Journal of Hospital Infection xxx (2016) 1–7

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

An Irish outbreak of New Delhi metallo-β-lactamase (NDM)-1 carbapenemase-producing Enterobacteriaceae: increasing but unrecognized prevalence

C. O'Connor [a,b], M. Cormican [c,d], T.W. Boo [c], E. McGrath [c,e], B. Slevin [d], A. O'Gorman [d,e], M. Commane [e], S. Mahony [b], E. O'Donovan [e], J. Powell [a], R. Monahan [a], C. Finnegan [a], M.G. Kiernan [b], J.C. Coffey [b], L. Power [a], N.H. O'Connell [a,b], C.P. Dunne [b,*]

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K. pneumoniae Carbapenemase

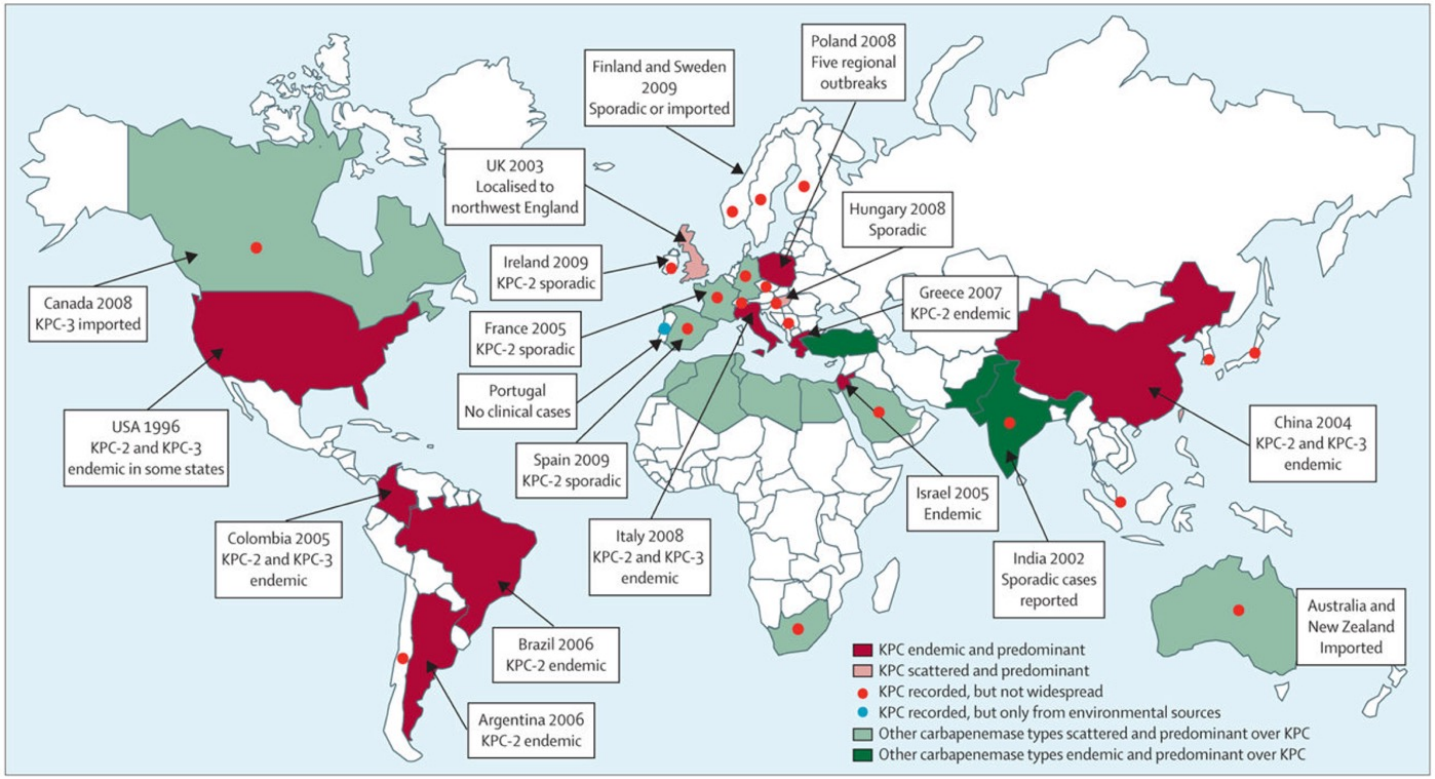
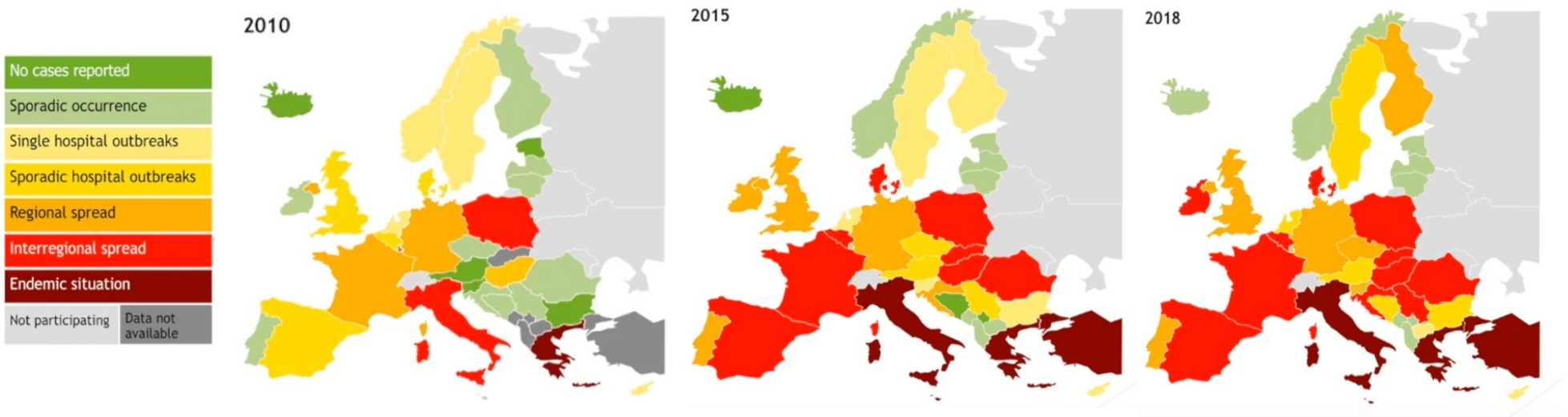


Figure. Epidemiological features of producers of *Klebsiella pneumoniae* carbapenemases by country of origin
Other carbapenemase types include VIM, OXA-48, or NDM. KPC=*Klebsiella pneumoniae* carbapenemase.

Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. *Lancet Infect Dis*. 2013;13(9):785-796.

Progression of Outbreaks 2010 - 2018



ECDC. https://www.ecdc.europa.eu/en/antimicrobial-resistance/threats-and-outbreaks

Irish (Limerick) incidence

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Letter to the Editor

Limerick: forever associated with five lines of rhyme or infamous for irrepressible carbapenemase-producing Enterobacteriaceae for all time?

Our current analysis has identified two fatal KPC bacteraemias, three intra-abdominal theatre-derived samples positive for CPE, with *K. pneumoniae* ($N = 80$), *Klebsiella oxytoca* ($N = 30$), and *Citrobacter freundii* ($N = 17$) dominant. Indeed, our 2011 outbreak documented transmission of these strains between Irish hospitals.[6] Subsequent to this outbreak, CPE screening has been performed, in accordance with national guidelines, via rectal swab or stool specimen, for all patients

Journal of Hospital Infection xxx (2014) 1

Available online at www.sciencedirect.com

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journal homepage: www.elsevierhealth.com/journals/jhin

Letter to the Editor

Against the onslaught of endemic carbapenemase-producing *Klebsiella pneumoniae*, the war is being lost on the Irish Front

Madam,

In the context of the excellent report of successful control screening policy whereby all HDU and ICU transfers are isolated until determined to be negative for KPC carriage.

Despite these measures, control has been ineffective and we have experienced simultaneous incidences of seven cases in June 2012, five cases in January 2014, and four cases in April 2014, with significant morbidity and mortality. In light of our inability prevent KPCs, we are debating the value of completing a study of local community carriage and revision of empirical first-line treatments, or even prophylaxis.

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Incidence, management and outcomes of the first *cfr*-mediated linezolid-resistant *Staphylococcus epidermidis* outbreak in a tertiary referral centre in the Republic of Ireland

C. O'Connor[a,b,c], J. Powell[a], C. Finnegan[a], A. O'Gorman[b], S. Barrett[d], K.L. Hopkins[e], B. Pichon[e], R. Hill[e], L. Power[a,b], N. Woodford[e], J.C. Coffey[c], A. Kearns[e], N.H. O'Connell[a,b,c], C.P. Dunne[c,*]

JMM Case Reports (2014) DOI 10.1099/jmmcr.0.000075

Case Report

Clustered multidrug-resistant *Bordetella petrii* in adult cystic fibrosis patients in Ireland: case report and review of antimicrobial therapies

Aislie Carleton,[1] Brian Casserly,[1,2] Lorraine Power,[2] Barry Linnane,[1,2] Grainne O'Flaherty,[2] James Powell,[2] Peig Hartnett,[2] Jonathan Collins,[3] Philip Murphy,[4] Dervla Kenna,[5] Nuala H. O'Connell[1,2] and Colum Dunne[1]

[1]Graduate Entry Medical School and Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick, Ireland

[2]University Hospital Limerick, Dooradoyle, Limerick, Ireland

Correspondence
Colum Dunne
colum.dunne@ul.ie

JMM Case Reports (2015) DOI 10.1099/jmmcr.0.000089

A case of fatal daptomycin-resistant, vancomycin-resistant enterococcal infective endocarditis in end-stage kidney disease

Ciara O'Connor,[1,2] Liam F. Casserly,[3] Junaid Qazi,[3] Lorraine Power,[1] Cathriona Finnegan,[1] Nuala H. O'Connell[1,2] and Colum P. Dunne[2]

Infection Inflammation & Immunity

Patients, Carers, Families

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Letter to the Editor

Becoming patient-centred: sobering insight into CPE-positive patients' experiences of clinical care

by communication to them of their CPE and the explanatory leaflets provided. Further, they used emotive terms such as 'leper', 'pariah' and 'plague' to describe their treatment by staff, clearly demonstrating the need for consistent, effective education of healthcare professionals regarding multi-drug-resistant organisms and holistic needs of affected patients.

As healthcare professionals, we often do not look beyond specimens and infection control aspects of managing patients with CPE. However, the impact of a CPE diagnosis on patients

Journal of Hospital Infection

Volume 101, Issue 2, February 2019, Pages 194-195

Letter to the Editor

Opportunities lost may be the greatest cost of CPE outbreaks

C.P. Dunne [a], B.L. Slevin [b], P. Treacy [b], N.H. O'Connell [a, b]

[a] Graduate Entry Medical School and Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick, Ireland

[b] University Hospital Limerick, Dooradoyle, Limerick, Ireland

Received 11 October 2018, Accepted 15 October 2018, Available online 22 October 2018.

PLOS ONE

RESEARCH ARTICLE

A retrospective observational study of the impact of 16s and 18s ribosomal RNA PCR on antimicrobial treatment over seven years: A tertiary hospital experience

TeeKeat Teoh[1,2], Rachel McNamara[3], James Powell[1], Nuala H. O'Connell[1,2], Colum P. Dunne[2]*

1 Department of Clinical Microbiology, University Limerick Hospital Group, Limerick, Ireland, **2** Centre for Interventions in Infection, Inflammation & Immunity (4i) and School of Medicine, University of Limerick, Limerick, Ireland, **3** Department of Medicine and Infectious Diseases, University Hospital Limerick, Limerick, Ireland

Conclusion

There was limited impact of 16s PCR results on antimicrobial treatments. Relevance to practice was affected by relatively long turn-around-time for results. Utility may be increased in specialised surgical centres, or by reducing turn-around-time. Enrichment culture should be considered on samples where 16s PCR is requested. There remains limited evidence for use of 18s PCR in clinical management, and further studies in this area are likely warranted.

Teoh T, McNamara R, Powell J, O'Connell NH, Dunne CP. A retrospective observational study of the impact of 16s and 18s ribosomal RNA PCR on antimicrobial treatment over seven years: A tertiary hospital experience. PLoS One. 2021 Oct 12;16(10):e0258552.

PLOS ONE

RESEARCH ARTICLE

Outcomes of implementation of the FilmArray meningoencephalitis panel in a tertiary hospital between 2017 and 2020

TeeKeat Teoh[1,2,3], James Powell[1], Jillian O'Keeffe[1], Eoghan Donlon[4], Lisa Dillon[1], Marie Lenihan[1], Amanda Mostyn[1], Lorraine Power[1], Peter Boers[4], Patrick J. Stapleton[1,3], Nuala H. O'Connell[1,2,3], Colum P. Dunne[2,3]*

1 Department of Clinical Microbiology, University Limerick Hospital Group, Limerick, Ireland, **2** Centre for Interventions in Infection, Inflammation & Immunity (4i), Limerick, Ireland, **3** School of Medicine, University of Limerick, Limerick, Ireland, **4** Department of Neurology, University Limerick Hospital Group, Limerick, Ireland

* colum.dunne@ul.ie

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Journal of Hospital Infection 110 (2021) 45–51

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Outcomes of point-of-care testing for influenza in the emergency department of a tertiary referral hospital in Ireland

T.K. Teoh [a,b,d], J. Powell [a], J. Kelly [c], C. McDonnell [b], R. Whelan [c], N.H. O'Connell [a,b,d], C.P. Dunne [d,*]

[a] *Department of Clinical Microbiology, University Limerick Hospital Group, Limerick, Ireland*
[b] *Department of Serology and Immunology, University Limerick Hospital Group, Limerick, Ireland*
[c] *Department of Emergency Medicine, University Hospital Limerick, Limerick, Ireland*
[d] *Centre for Interventions in Infection, Inflammation & Immunity (4i) and School of Medicine, University of Limerick, Limerick, Ireland*

Journal of Hospital Infection 133 (2023) 103–104

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Letter to the Editor

Off-label novel application of diagnostic multiplex molecular platforms for environmental detection of carbapenemase-producing Enterobacterales

samples; and (c) ward layout and architecture (i.e. Nightingale wards accommodating 12–14 inpatients). Samples were analysed in parallel using two methods for comparison: EBCPE PCR assay and culture [i.e. 24-h enrichment prior to inoculation on to a selective chromogenic CPE agar medium (CARBA mSuper ChromID)]. Positive results were obtained from 36 of the EBCPE PCR assays and 29 of the cultures. Specifically, KPC and VIM were most prevalent according to the EBCPE assay, correlating with culture outcomes where KPC was also most common. While potentially of benefit in IPC, there are some challenges

Journal of Hospital Infection 140 (2023) 139–155

Available online at www.sciencedirect.com

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Systematic Review

Whole-genome sequencing to investigate transmission of SARS-CoV-2 in the acute healthcare setting: a systematic review

D. Hare [a, b, *], K.M. Dembicka [b], C. Brennan [a], C. Campbell [a], U. Sutton-Fitzpatrick [a], P.J. Stapleton [c], C.F. De Gascun [a], C.P. Dunne [b, d]

Journal of Hospital Infection 126 (2022) 1–9

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Repeated transmission of SARS-CoV-2 in an overcrowded Irish emergency department elucidated by whole-genome sequencing

D. Hare [a,b,c,*], C. Meaney [a], J. Powell [a,d], B. Slevin [e], B. O' Brien [e], L. Power [a], N.H. O' Connell [a,b,d], C.F. De Gascun [c], C.P. Dunne [b,d], P.J. Stapleton [a,b]

[a] Department of Clinical Microbiology, University Hospital Limerick, St Nessan's Road, Dooradoyle, Limerick, Ireland
[b] School of Medicine, University of Limerick, Limerick, Ireland
[c] UCD National Virus Reference Laboratory, University College Dublin, Dublin, Ireland
[d] Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick, Ireland
[e] Department of Infection, Prevention and Control, University Hospital Limerick, Limerick, Ireland

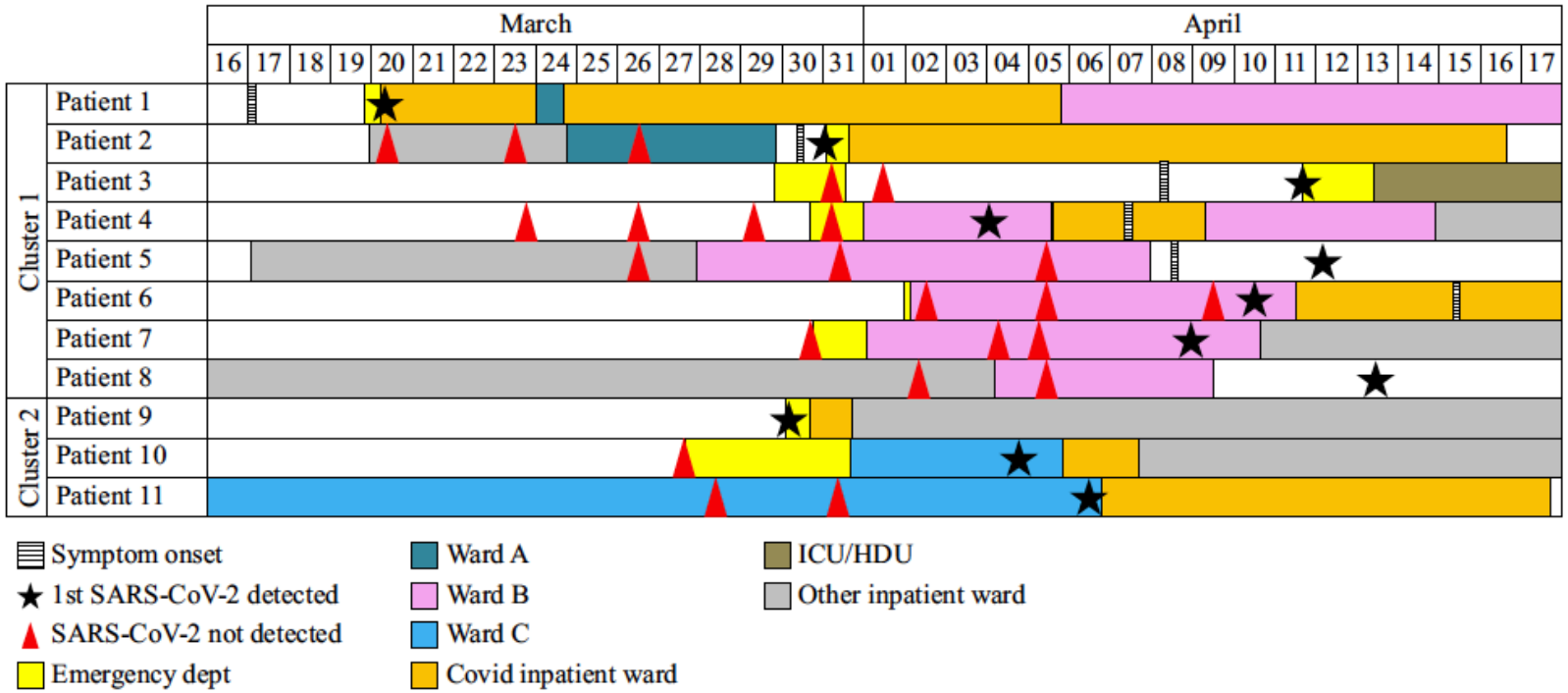
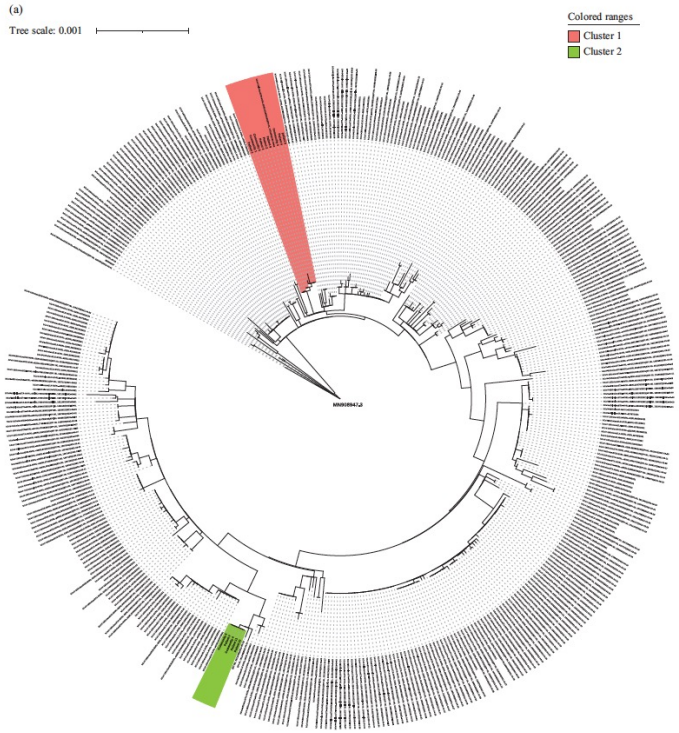


Figure 1. Gannt chart illustrating temporal and geographic links between cases. Within cluster 1, the putative chain of transmission from Patient 1 to Patient 8 is supported, beginning in Ward A, through the emergency department on 30th–31st March, and subsequently on to Ward B. Within Cluster 2, Patient 9 is the proposed source of introduction, overlapping with Patient 10 in the emergency department, who in turn shared time with Patient 11 on Ward C. HDU, high-dependency unit; ICU, intensive care unit.



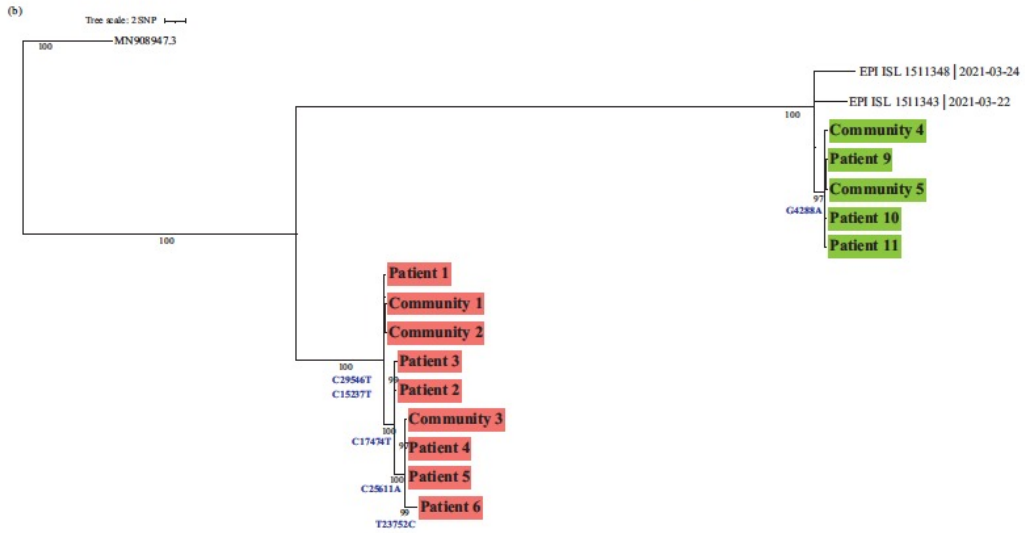


Figure 3. (a) Maximum-likelihood viral phylogenetic tree rooted in Wuhan MN908947.3, including all B.1.1.7 viral genomes submitted to Global Initiative on Sharing All Influenza Data (GISAID) from the Mid-West region of Ireland (including counties Limerick, Clare and Tipperary, $N = 422$) between 1st March and 30th April 2021. Cluster 1 is highlighted in red and Cluster 2 in green. (b) Singler nucleotide polymorphism (SNP) annotated viral phylogenetic tree rooted on MN908947.3 and pruned to highlight outbreak sequences: both clusters were preserved with 100% ultrafast bootstrap support (nodes with support values ≥97% are annotated). SNPs unique to each clade are highlighted in blue: Cluster 1 was defined by the presence of two nucleotide mutations C15732T and C29546T, while sequences in Cluster 2 all shared a unique G4288A mutation.

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Journal of Hospital Infection 141 (2023) 152–166

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Large-scale characterization of hospital wastewater system microbiomes and clinical isolates from infected patients: profiling of multi-drug-resistant microbial species

S.A. Kelly [a], N.H. O'Connell [b,c], T.P. Thompson [a], L. Dillon [d], J. Wu [a], C. Creevey [d], P. Kiely [c], B. Slevin [e], J. Powell [b,c], B.F. Gilmore [a], C.P. Dunne [c,*]

[a] School of Pharmacy, Queen's University Belfast, Belfast, UK
[b] Department of Clinical Microbiology, University Hospital Limerick, Limerick, Ireland
[c] School of Medicine and Centre for Interventions in Infection, Inflammation and Immunity (4i), University of Limerick, Limerick, Ireland
[d] School of Biological Sciences, Queen's University Belfast, Belfast, UK
[e] Department of Infection Prevention and Control, University Hospital Limerick, Limerick, Ireland

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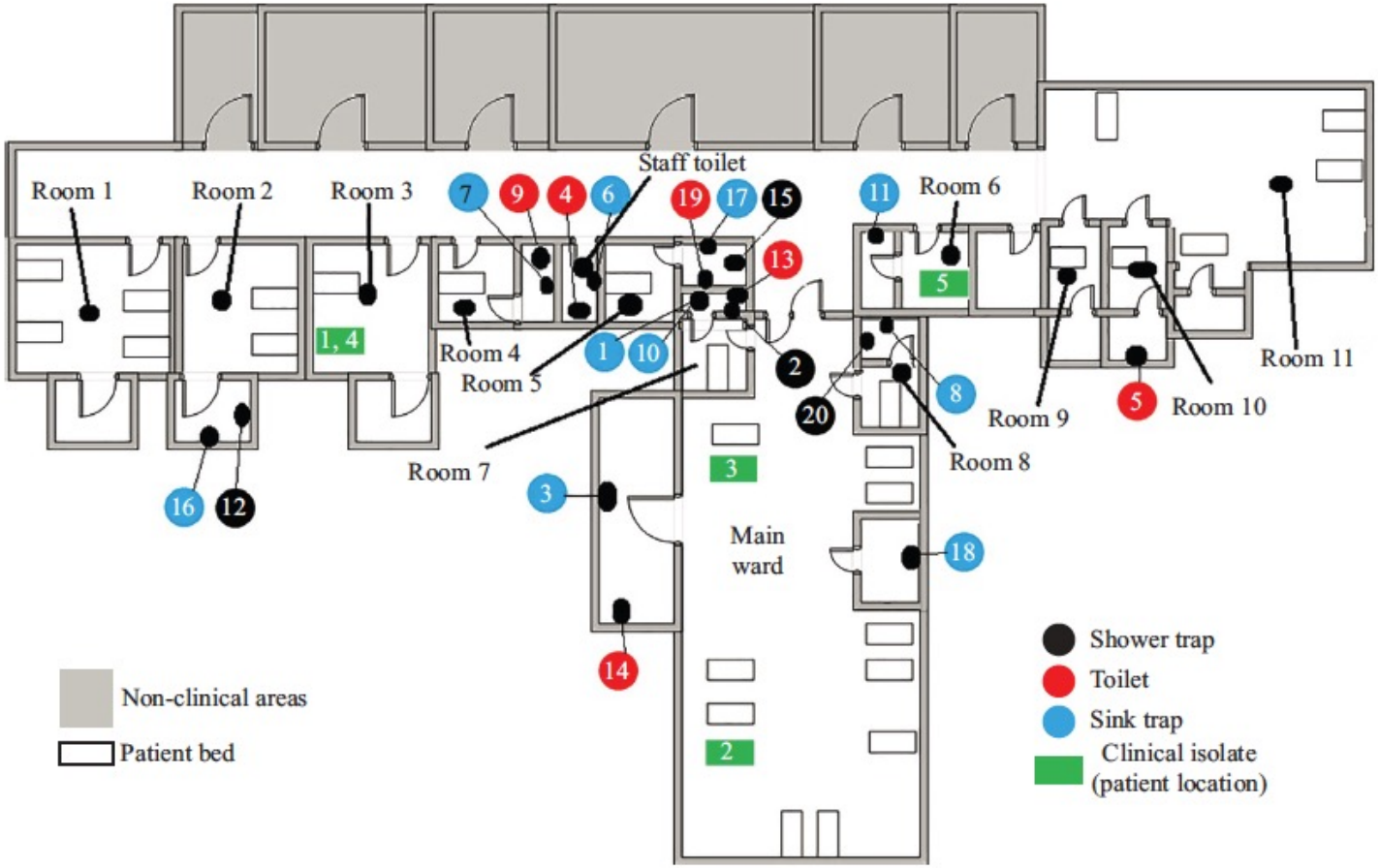

Figure 1. Layout of the ward showing the locations of the wastewater pipe sections used for metagenomic analysis, and the locations of the patients that acquired the clinical isolates investigated (not to scale). The numbers represent the sample numbers for the 20 pipe samples tested.

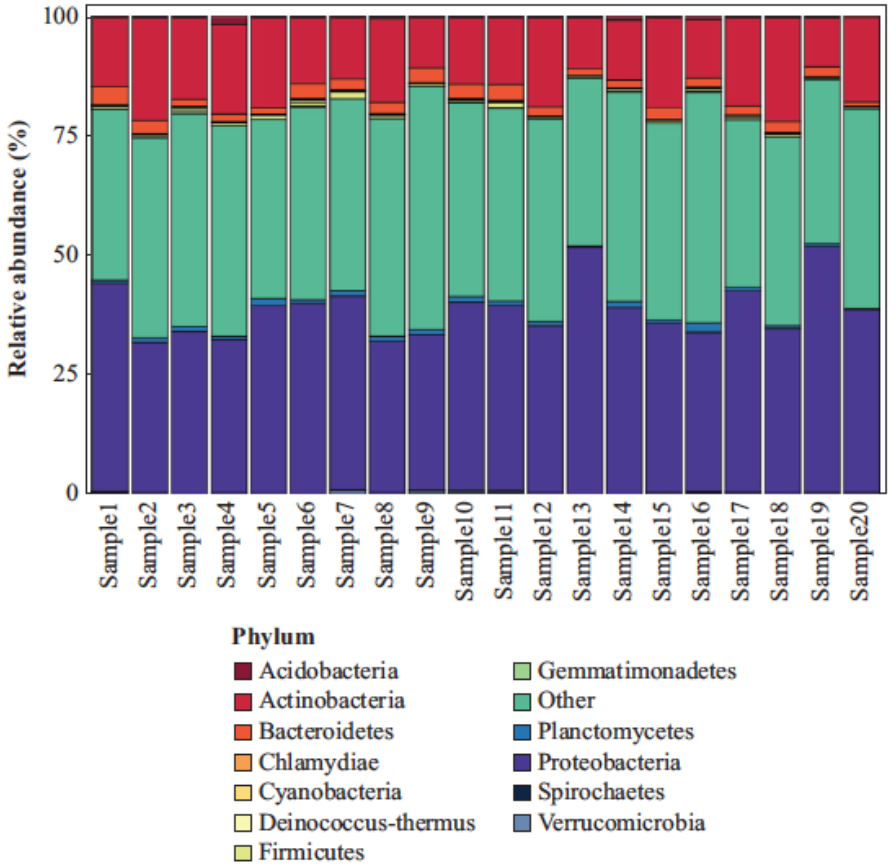

Figure 2. Metagenomic analysis of the clinical wastewater environment showing microbial taxonomic breakdown at phylum level across all 20 pipe sections examined. Taxonomic analysis was performed using Kraken2 v2.0.8 following assembly with SPAdes v3.14.0.

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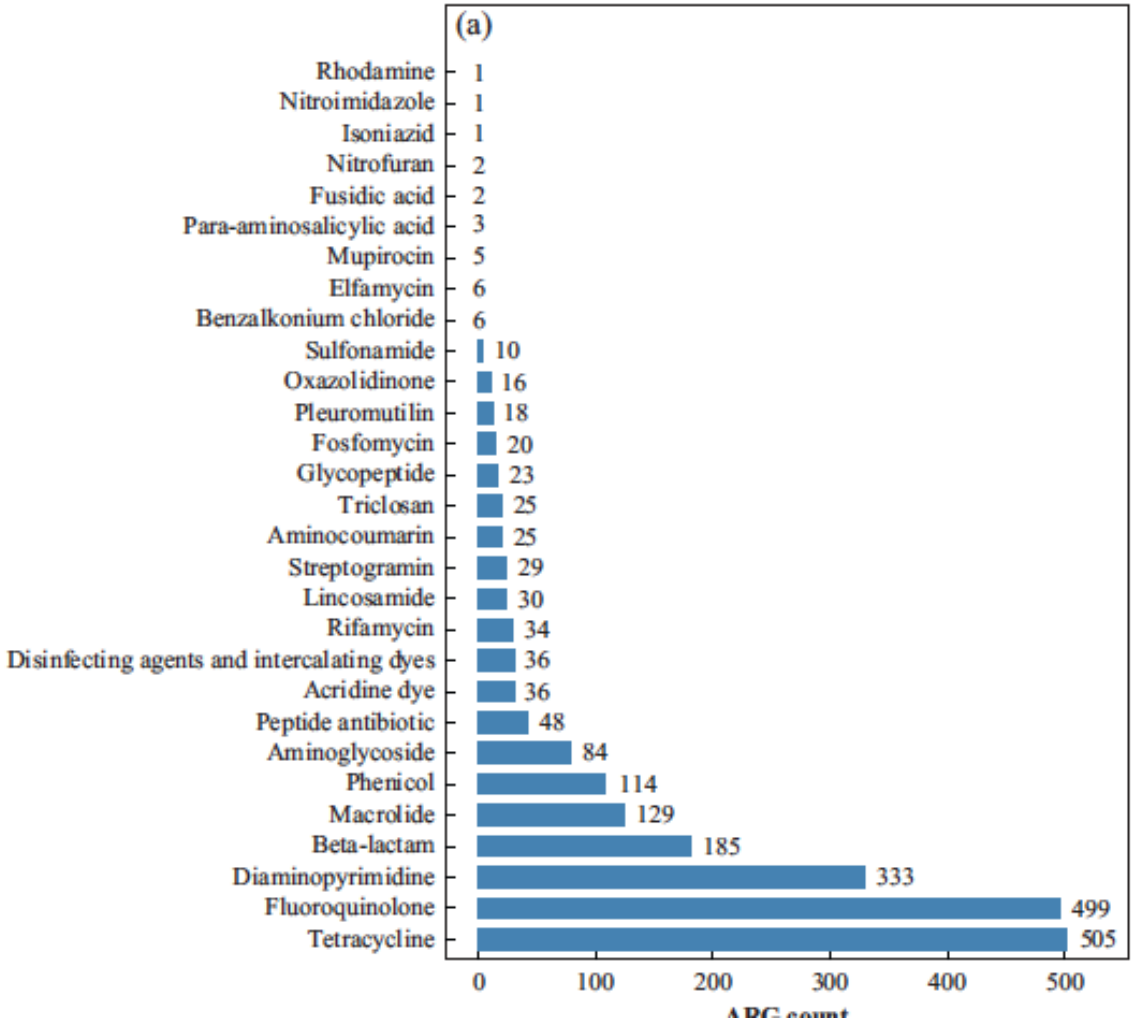

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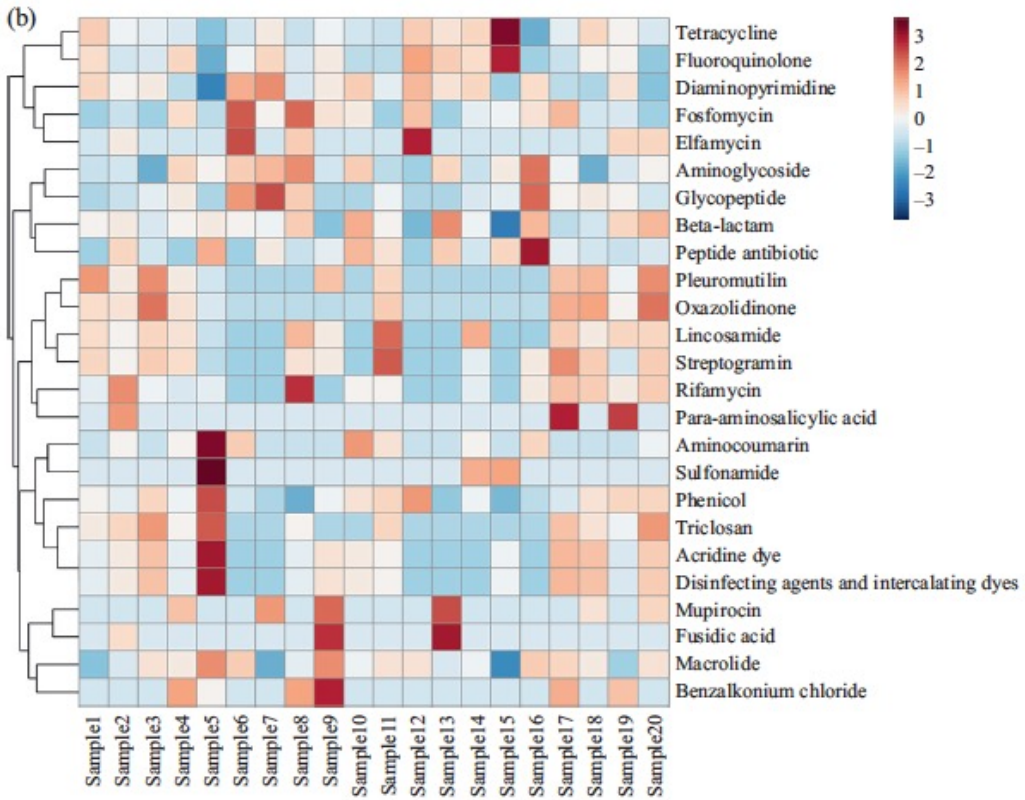

Figure 3. (a) Total combined antimicrobial resistance gene (ARG) count for each antibiotic class across all metagenomically-assembled genomes generated from wastewater pipe sections. (b) Resistome heatmap across all metagenomically-assembled genomes generated from wastewater pipe sections showing the relative density of ARG type by antibiotic class. (c) Principal co-ordinate analysis (PCoA) of resistome profiles of wastewater pipe sections generated by the Resistance Gene Identifier, grouped as showers, sinks and toilets. Heatmap and PCoA figures were generated using R v4.1.0, ggplot2 v3.3.6 and pheatmap v1.2.12.

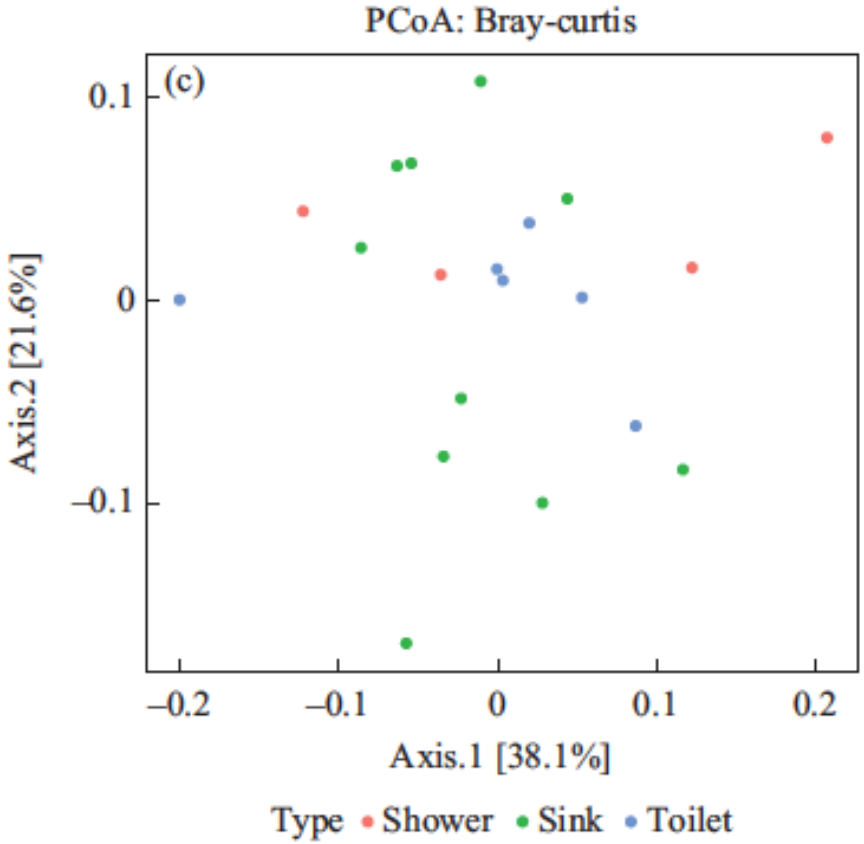

Figure 3. *(continued)*

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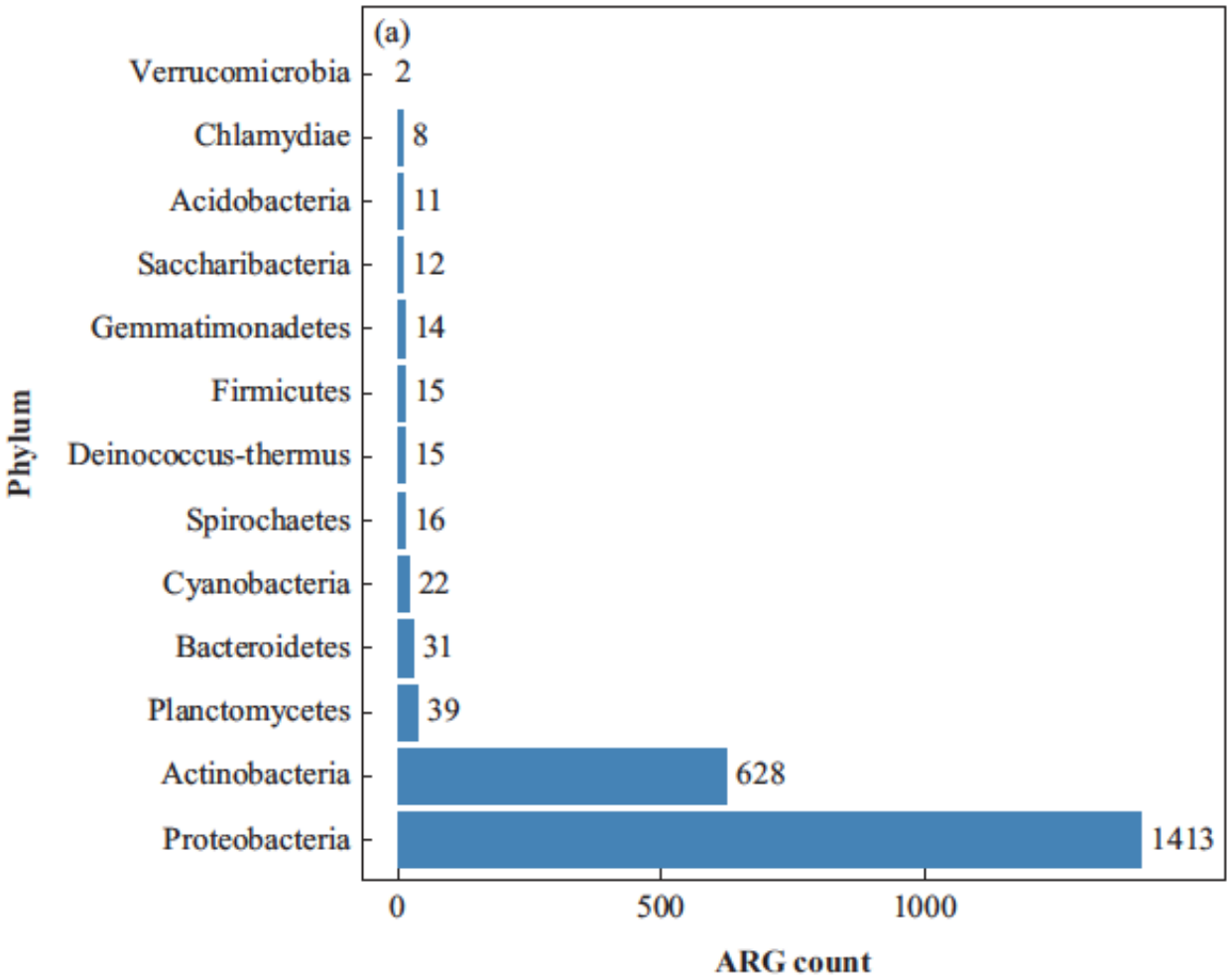

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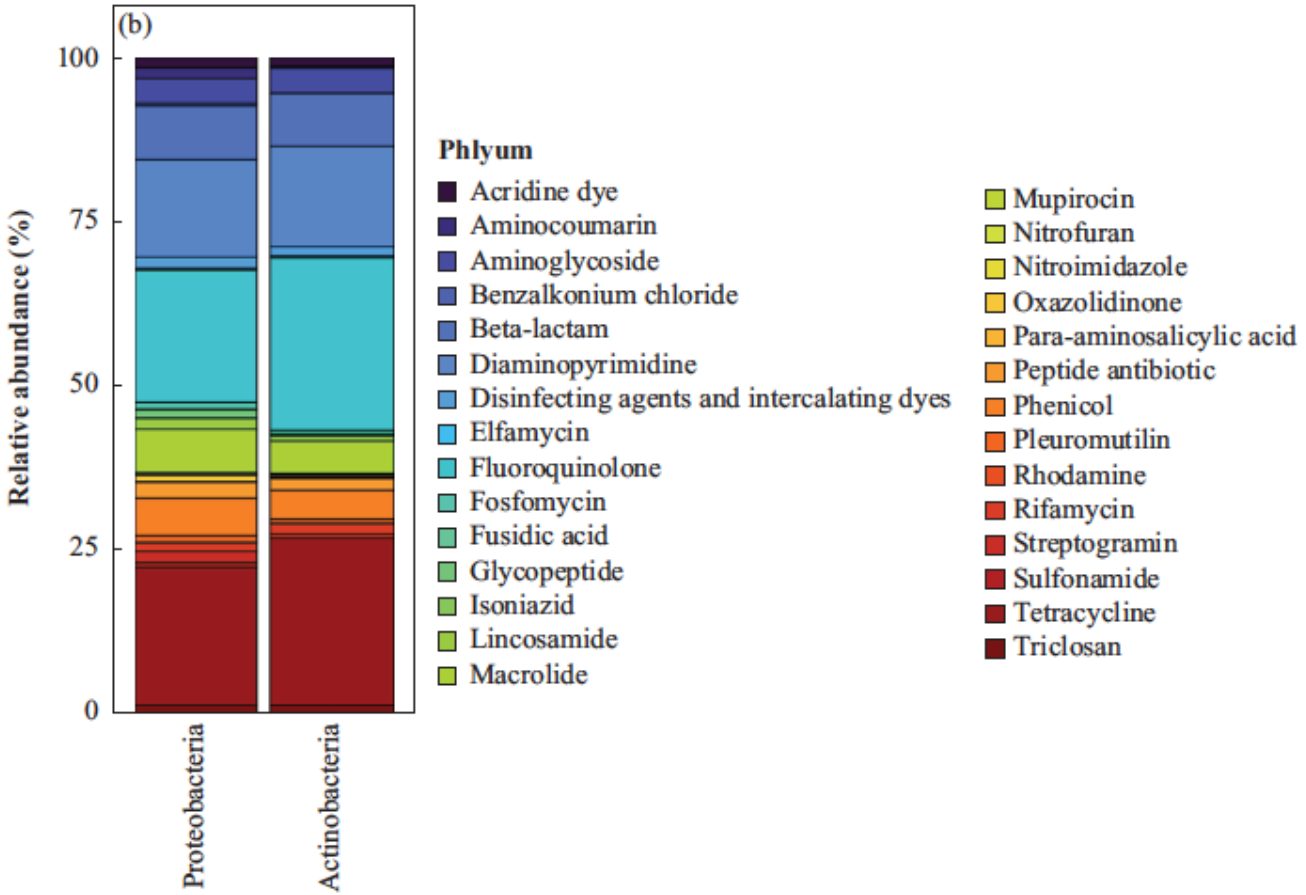

Figure 4. (a) Total combined antimicrobial resistance gene (ARG) count for each phylum across all metagenomically-assembled genomes generated from wastewater pipe sections. (b) Stacked abundance charts showing relative ARG class abundance for the two most dominant phyla, Proteobacteria and Actinobacteria.

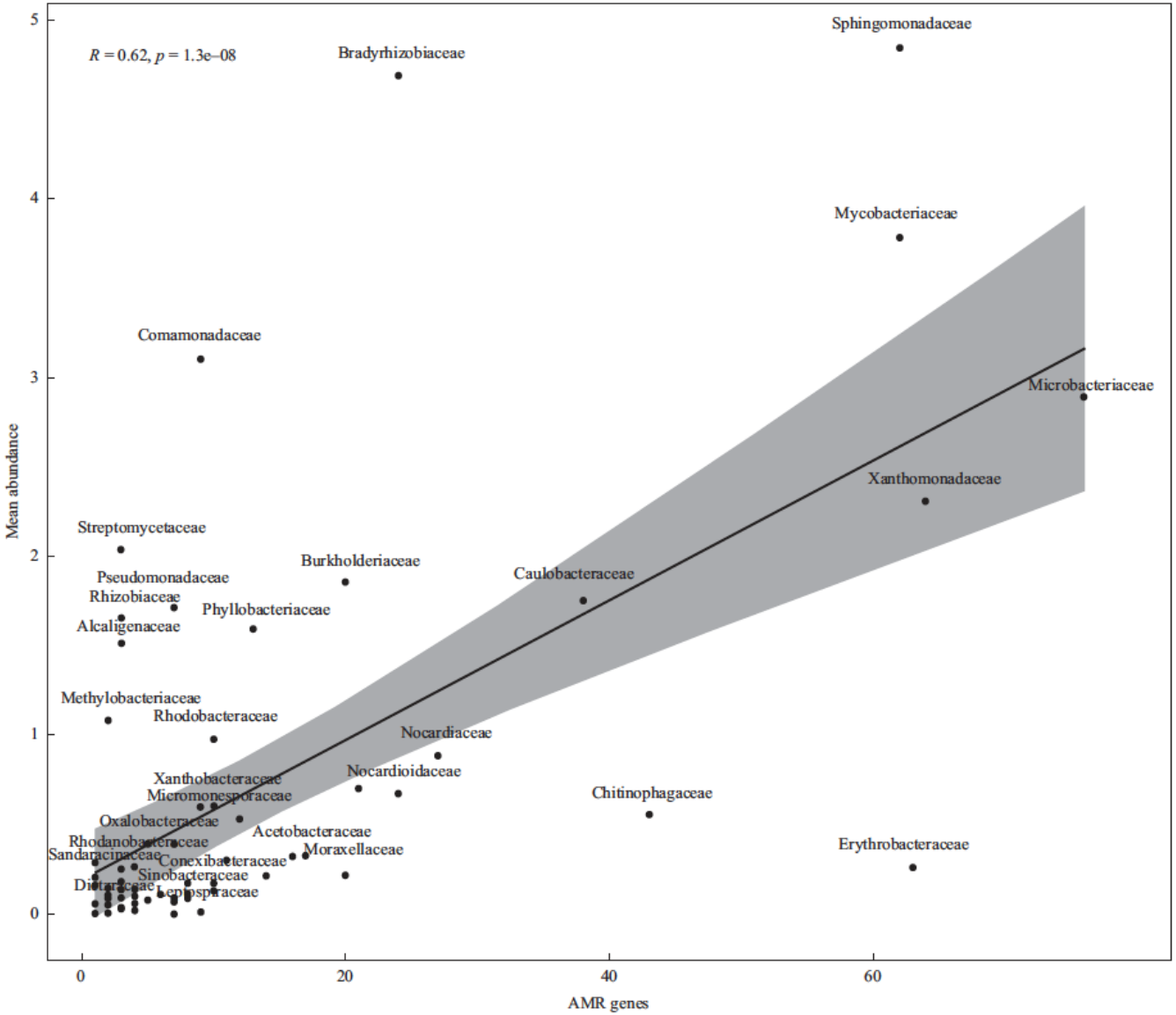

Figure 5. Correlation analysis comparing mean percentage abundance and antimicrobial resistance (AMR) gene count at family level, combined across all environmental wastewater pipe metagenomes (Pearson coefficient=0.62, r^2=0.3844). This correlation analysis figure was generated using R v4.1.0.

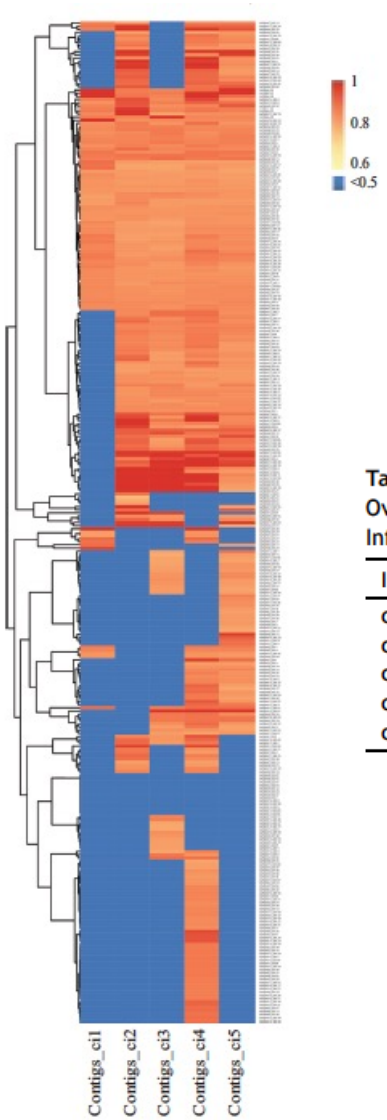

Figure 6. Average nucleotide identity analysis using pyani, showing the similarity of clinical isolate whole-genome sequences to metagenomically-assembled genomes generated from environmental DNA extracted from wastewater pipe sections. This pyani heatmap figure was generated using R v4.1.0.

Table I

Overview of clinical isolates used in this study, showing isolate designation, closest neighbour using the National Center for Biotechnology Information database, and number of antimicrobial resistance genes (ARGs) identified in each isolate using the Resistance Gene Identifier

Isolate designation	Closest neighbour (% identity)	Ward location	No. of ARGs identified
ci1	*Enterococcus faecium* (100)	Room 3, Bed 2	24
ci2	*Escherichia coli* (100)	Main ward, Bed 5	77
ci3	*Enterobacter kobei* (99.9)	Main ward, Bed 2	26
ci4	*Citrobacter youngae* (99.8)	Room 3, Bed 2	60
ci5	*Enterobacter hormaechei* (100)	Room 6, Bed 1	54

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Table 1. Average nucleotide identity (ANI) analysis comparing the genome of the UCI a series of genomes from the family Enterobacteriaceae.

	ANI with ci9
UCI	1.0000
Pseudocitrobacter corydidari strain G163CM	0.9562
Escherichia coli	0.8555
Shigella dysenteriae	0.8539
Shigella flexneri	0.8533
Escherichia fergusonii	0.8525
Kluyvera ascorbata	0.8514
Enterobacter hormaechei	0.8507
Citrobacter werkmanii	0.8495
Citrobacter freundii bd	0.8492
Citrobacter freundii b9-c2	0.8491
Citrobacter braakii	0.8488
Enterobacter cloacae	0.8487
Salmonella enterica	0.8485
Citrobacter youngae	0.8483
Klebsiella variicola	0.8465
Klebsiella pneumoniae	0.8459
Klebsiella aerogenes	0.8453
Klebsiella planticola	0.8429

Table 4. Average nucleotide identity (ANI) analysis comparing the genome of the unknown clinical isolate (UCI) with a series of genomes from the genus *Pseudocitrobacter*.

	ANI with UCI
UCI	1.0000
Pseudocitrobacter corydidari strain G163CM	0.9562
Pseudocitrobacter sp. 73	0.9493
Pseudocitrobacter sp. 260	0.9334
Pseudocitrobacter faecalis strain 25CIT	0.9300
Pseudocitrobacter faecalis strain DSM27453	0.9296
Pseudocitrobacter faecalis strain CCM8478	0.9291

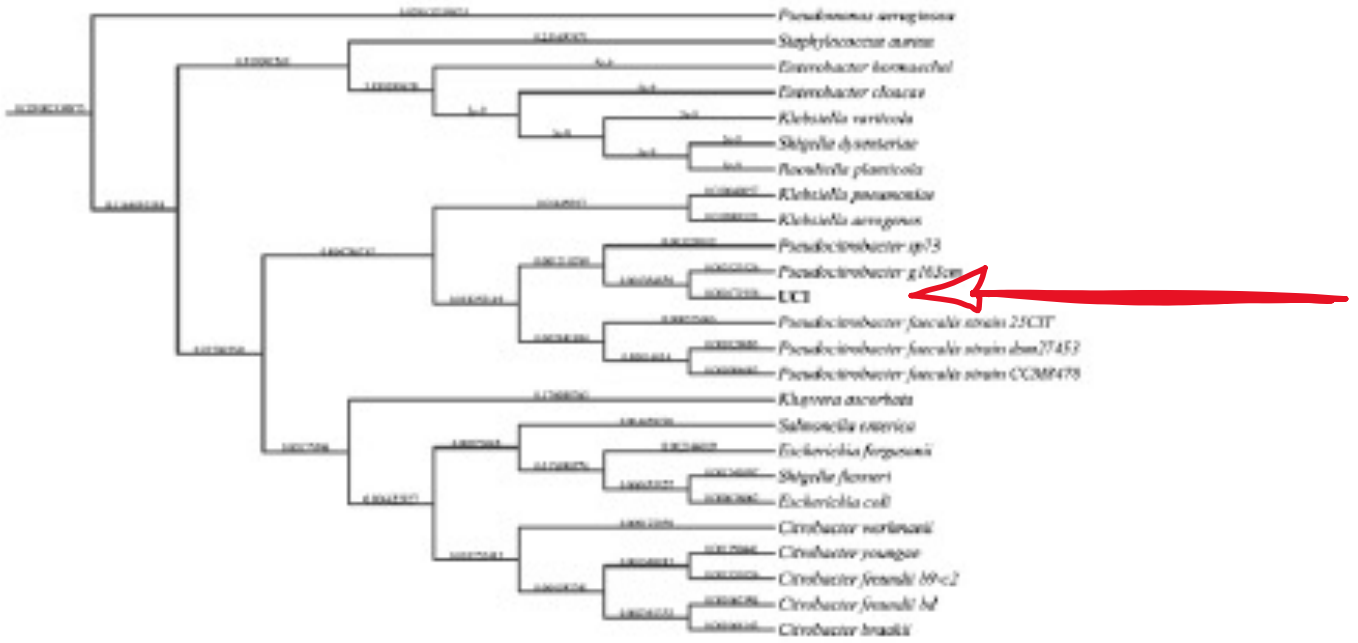

Figure 2. Phylogenetic analysis showing the position of UCI within the family Enterobacteriaceae. Sequences for each entry were based on 40 universally single copy genes, which were aligned using Clustal-Omega and a consensus tree was produced using iTOL. Bootstrap values (%) are shown in parentheses.

Table 3. Major fatty acid compositions (percentages) of the unknown clinical isolate (UCI) in comparison to other *Pseudocitrobacter* strains. Data for *P. corydidari* is taken from Guzman *et al.*[36]. Data for *P. faecalis* and *P. vendiensis* are taken from Kämpfer *et al.*[39,40]

Fatty acids	UCI	*P. corydidari*	*P. faecalis*	*P. vendiensis*
$C_{12:0}$	3.7	2.6	3.7	3.6
$C_{14:0}$	9.3	6.5	7.2	6.6
$C_{15:0}$	-	1.0	1.1	1.4
iso-$C_{15:0}$	-	Trace	-	-
anteiso-$C_{15:0}$	-	-	-	-
$C_{16:0}$	36.3	31.5	28.4	30.9
$C_{17:0}$ cyclo	20.8	15.3	13.2	18.6
$C_{18:1}$ ω7c	3.7	22.6	22.9	20.8
$C_{19:0}$ cyclo ω8c	7.8	2.5	5.5	2.6
Summed feature 2	7.5	9.4	8.1	8.0
Summed feature 3	3.9	8.6	9.1	6.6

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Thank you for your interest.

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December 14, 2023	*(FREE Teleclass)* **THE FUTURE OF OUTBREAKS** Speaker: **Prameet M. Sheth**, Queen's University, Canada
January 11, 2024	*(FREE Teleclass)* **DISCOURSE: HOW OUR LANGUAGE INFLUENCES OUR ATTEMPTS TO PREVENT AND CONTROL HEALTHCARE-ASSOCIATED INFECTION** Speaker: **Prof. Mark Cole**, The University of Manchester
January 18, 2024	**THROW IT AWAY: HOW INFECTION CONTROL PRACTICE DESTROYS PLANETARY HEALTH AND FUELS LABOUR ABUSE** Speaker: **Prof. Mahmood Bhutta**, University of Sussex
January 25, 2024	*(FREE Teleclass)* **ENCOUNTERING BED BUGS WHILE ON VACATION, OR JUST TRAVELLING** Speaker: **Dr. Marcia L. Anderson**, EPA Center for Integrated Pest Management
February 1, 2024	**DEVELOPING A BETTER UNDERSTANDING OF WHY HYGIENE IS KEY TO DEVELOPING EFFECTIVE HYGIENE BEHAVIOUR IN HOMES AND EVERYDAY LIVES** Speaker: **Dr. Sally Bloomfield**, International Scientific Forum on Home Hygiene
February 8, 2024	**TARGETED HYGIENE: A RISK-BASED APPROACH TO APPLYING POLICIES AND HYGIENE INTERVENTIONS IN PUBLIC SETTINGS AND LARGE EVENTS** Speaker: **Dr. Lisa Ackerley**, International Scientific Forum on Home Hygiene
	(Australasian Teleclass) **HUMAN AMR SURVEILLANCE – WHERE ARE WE NOW AND WHERE SHOULD WE**

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