Impact of antimicrobials on the resistome- an IPC dilemma

Prof Shaheen Mehtar

ICAN and SUN (retd)
Cape Town, South Africa

Outline of the talk

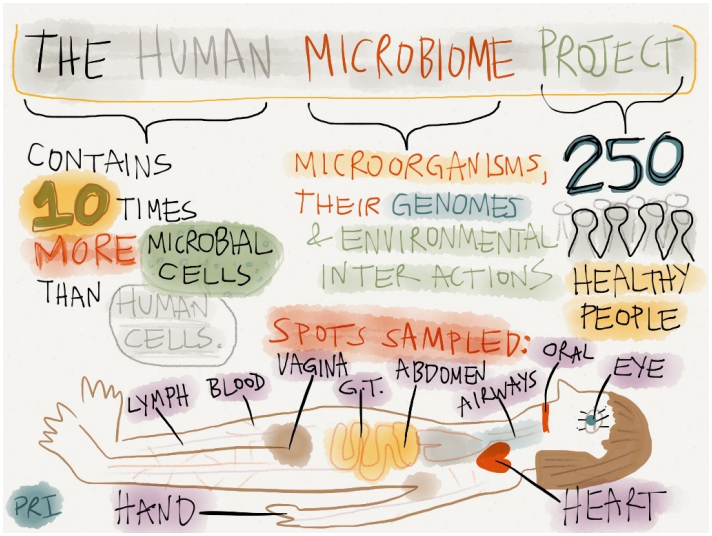
- Origins of the resistome
- Mobility of ARG
- Resistome in the clinical setting- an IPC concern?
- Impact of antimicrobials in the hospital setting
- Preserving the sensitive microbiome in a clinical setting

Consider this….

- Microbes were here many years before humans arrived on this earth and will be here many years after we have gone.
- Microbes do not become extinct- only resistant.
- How have microbes survived for so long?
- What is the role of IPC in reducing AMR?

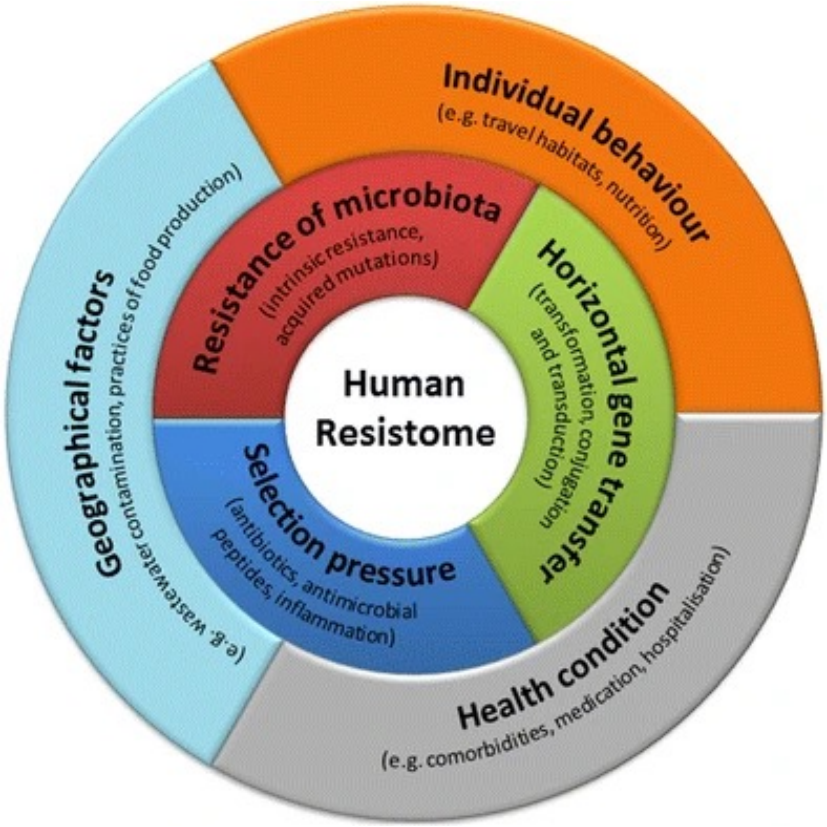
The microbiome- friend or foe?

- The microbiome is used to describe **all** microorganisms and their genomes, including bacteria, archaea, viruses, and fungi.
- Commensal microbiomes, such as the gut, skin, and vagina microbiome, contain beneficial microbes and pathogens, which contribute to host homeostasis in different locations.
- The environment and buildings have their own microbiome which often interchange when in contact with humans or animals.
- Modulating the microbiome is regarded to be an effective way to regulate host homeostasis and defeat diseases- or create disruption!



Zhang Y, Zhou L, Xia J, Dong C and Luo X (2022) Human Microbiome and Its Medical Applications.Front. Mol. Biosci. 8:703585. doi: 10.3389/fmolb.2021.703585

The resistome



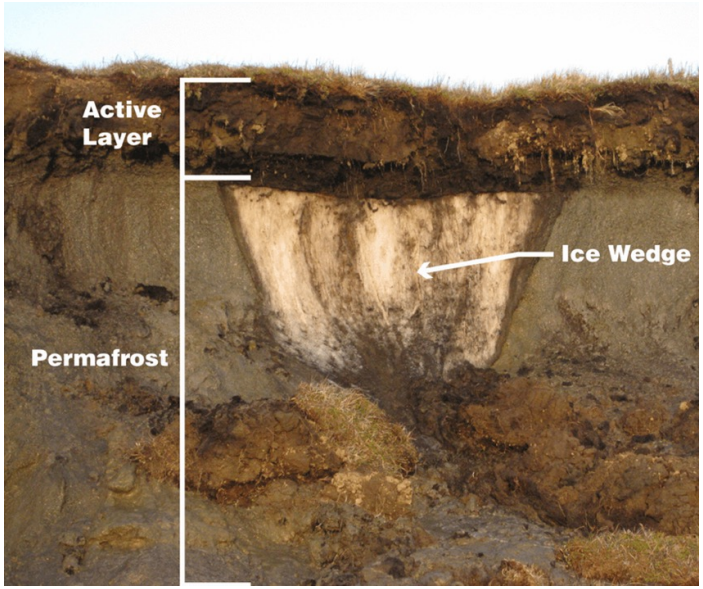
The microbiome carries a plethora of ARG which make up the resistome

The "resistome" is the collection of all antimicrobial genes that directly or indirectly contribute to antimicrobial resistance, both in the environment and the clinical setting.

The resistome has major significance and relevance to IPC and AMR

The environment resistome- these genes are ancient!

- Microbiome from Canadian high Arctic permafrost, harboured diverse resistance mechanisms at least 5,000 years ago.
- 8 genes isolated conferring clinical levels resistance against ***aminoglycoside, β-lactam*** and ***tetracycline*** antibiotics (naturally produced)
- 4/8 also conferred resistance against ***amikacin*** (cross resistance), a modern semi-synthetic antibiotic,



- Overlaying active layer, 10 different genes resistance to all six antibiotics -naturally produced and semi-synthetic variants.
- Resistance genes from permafrost bacteria conferred ***lower levels of resistance*** against clinically relevant antibiotics than resistance genes sampled from the active layer.

PLoS ONE 10(3): e0069533. doi:10.1371/journal.pone.0069533

But, how far back do these go?

- Metagenomic analyses of ancient DNA from 30,000-year-old Beringian permafrost sediments identified a highly diverse collection of genes encoding resistance to ***β-lactam, tetracycline*** and ***glycopeptide*** antibiotics.
- Analysis of resistance element *VanA* confirmed similarity to modern variants.
- The resistome is as ancient as bacterial metabolism itself, most likely >3 billion years- in response to natural product antimicrobials.
- Today, anthropomorphic factors clearly contribute to the mobilization, fixation, and dissemination of resistance genes!

D'Costa et al. Nature; 2011 Aug 31;477(7365):457-61. doi: 10.1038/nature10388

The environmental and clinical resistome interaction

- The environmental "resistome" is a substantial source of naturally occurring resistance genes.
- Strong evidence for exchange between environmental and clinical resistome:
  - clinical aminoglycoside, tetracycline and vancomycin resistance enzymes,
  - the extended-spectrum β-lactamase *CTX-M* (traced to environmental *Kluyvera* spp[1])
  - quinolone resistance gene *qnr (*broad host range conjugative plasmid from *K pneumoniae* Cip^R have direct links to the environmental resistome of water borne species[2,3]
- Response to **selective pressure** from human activities in the environment resulting in gene transfer to clinical pathogens
- In the clinical setting the resistome is further affected by **frequency and diversity of AMR pathogens** resulting in further gene transfer.

Frontiers in Micro, 2013;

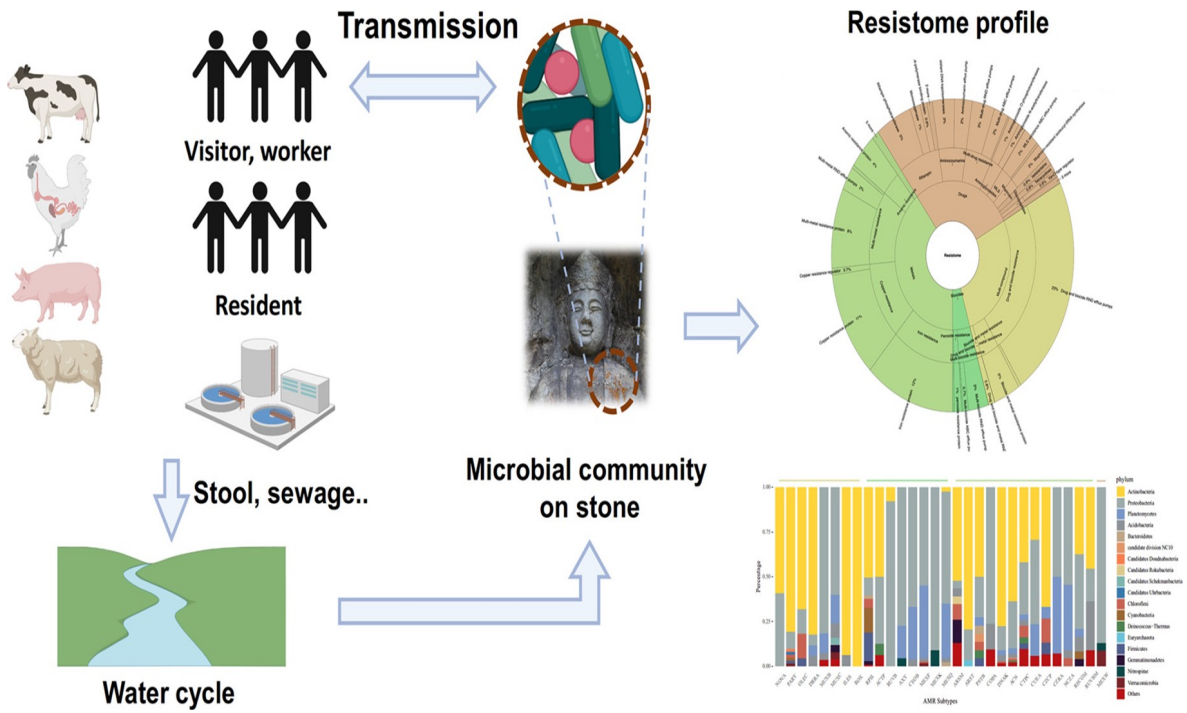
[1]*Antimicrob. Agents Chemother.* 46, 3045–3049. doi: 10.1128/AAC.46.9.3045-3049.2002
[2]*J. Antimicrob. Chemother.* 56, 1118–1121. doi: 10.1093/jac/dki371
[3]*Curr. Opin. Biotechnol.* 19, 260–265. doi: 10.1016/j.copbio.2008.05.006

What is a “mobilome”?

- **The mobilome is key to the spread of genes encoding resistance to antimicrobial drugs and heavy metals and for pathogenic traits among bacteria**.
- Functions are often **co-located** on the same mobile elements, selection for 1 phenotype inadvertently selects for its unintended (and often unrecognized) companion.
- **Cross resistance** between antibiotics, biocides (disinfectants) and heavy metals occurs by the movement and spread of ARG

ARG mobilization in the environment

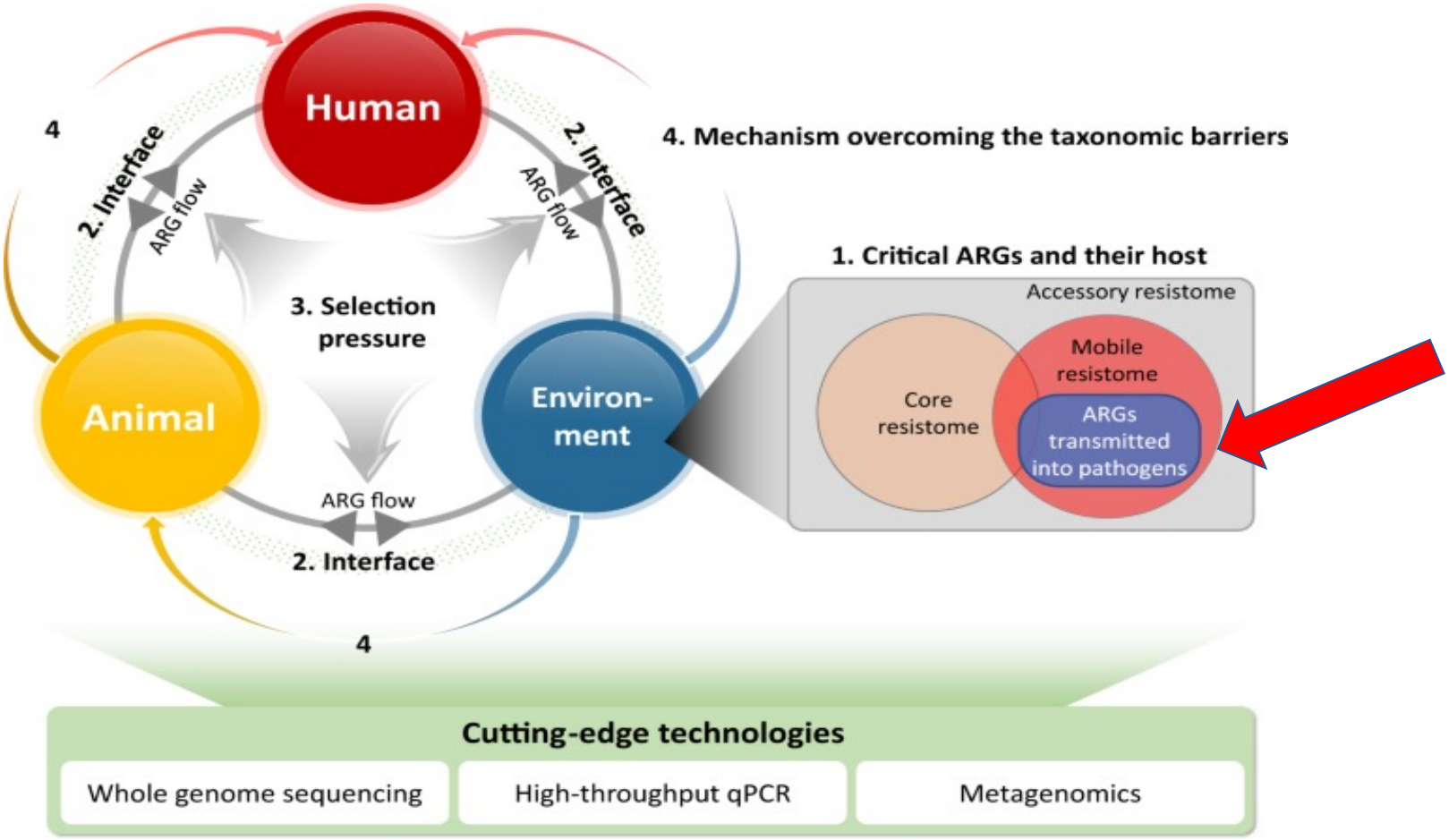
- Discharge of chemicals into the environment – accelerated lateral movement of ARG by increasing selective pressure to
  - maintain mobile gene elements
  - Increase rate of gene transfer
- Mixing opportunities between environmental bacteria and human pathogens in the presence of such selective agents through,
  - sewage and waste water treatment plants,
  - chemical production factories,
  - spreading manure on farmland.



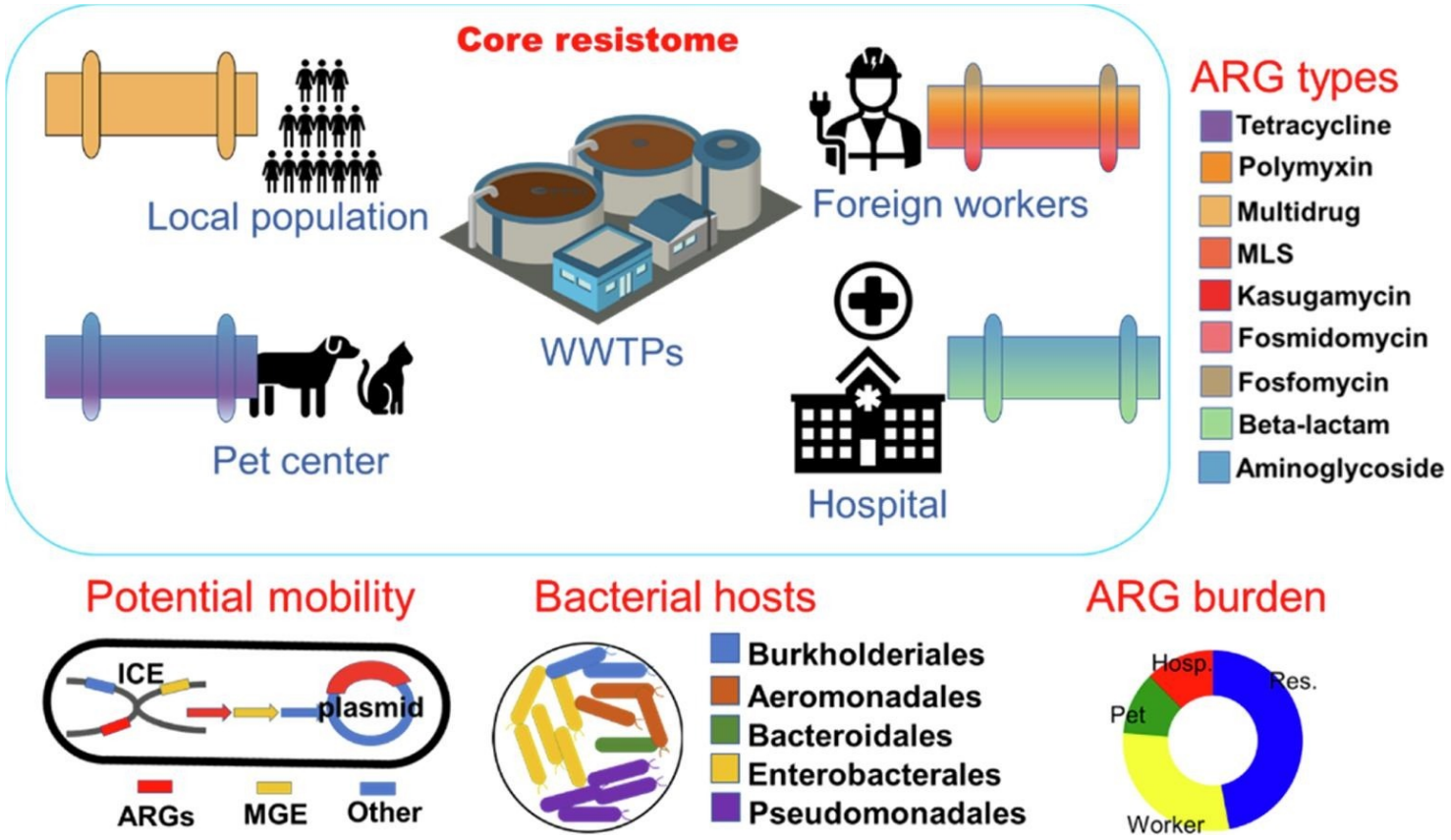
Resistome & mobilome from limestone

Fig. 2. Diverse resistome revealed by contigs assembled from deep shotgun sequencing. Krona graph showing the average relative abundance (TPM) of AMR genes, which were grouped according to the mechanism (outer ring) and type (middle ring). Each type was further grouped into Drugs, Multi-compound, Biocides, and Metals resistance (inner ring) according to MEGARes Ontology.

Movement of ARG in One Health



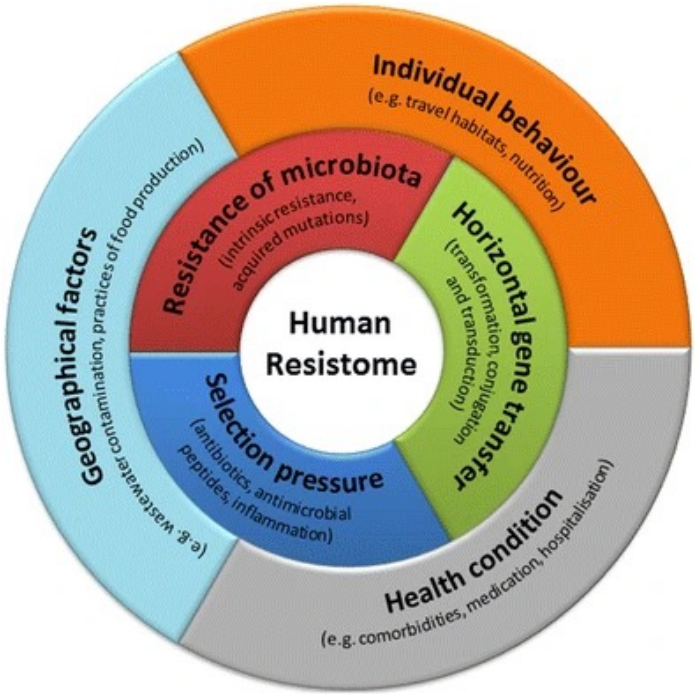
Urban sewage metagenome surveillance



Li W et al. Environment International 163 (2022), 107185

Resistome modified by antimicrobials

- Antibiotics are essential in treating infections, they may destroy the protective resident microbiota (environment).
- S Majumder : *Alteration of the composition and function of the microbiota as a result of antibiotic use, diet evolution and elimination of constitutive partners such as helminths worms has transformed our resident microbes into potential pathogens*. DOI: 10.20959/wjpps20198-14403
- Waste water effluent analysis from the community and hospitals showed no difference in the antibiotic resistance genes
- The effect of animal farming?



Willmann, M., Peter, S. Translational metagenomics and the human resistome: confronting the menace of the new millennium. J Mol Med 95, 41–51 (2017). https://doi.org/10.1007/s00109-016-1478-0

Current Opinion in Microbiology
Volume 21, October 2014, Pages 45-50
https://doi.org/10.1016/j.mib.2014.09.002

Antibiotic, biocide & metal linked res in pig stock farming

- Pig gut microbiome from 3 countries
  - China= on antibiotics (78/278)
  - Denmark = only at weaning (100/278)
  - France = no antibiotics (100/278)
- Metals as growth promoters and biocides widely used in stock farming in all three farms
- **Findings:** Metals and biocides displayed strong selective pressures on ARGs exerted by intensive farming, regardless of the current use of antibiotics
- Mechanisms of resistance….
  - resistance genes are physically located on the same genetic element (**co-resistance**);
  - the same gene confers resistance to both antibiotics and biocide/metal (**cross resistance**);
  - biocide resistance genes (BRGs)/multiple resistance genes (MRGs) share the same regulatory mechanism with ARGs (**co-regulation**).
- *Enterobacteriaceae* displayed increased co-occurrence phenomena

Li X et al: Environment International: 158 (2022) 106899

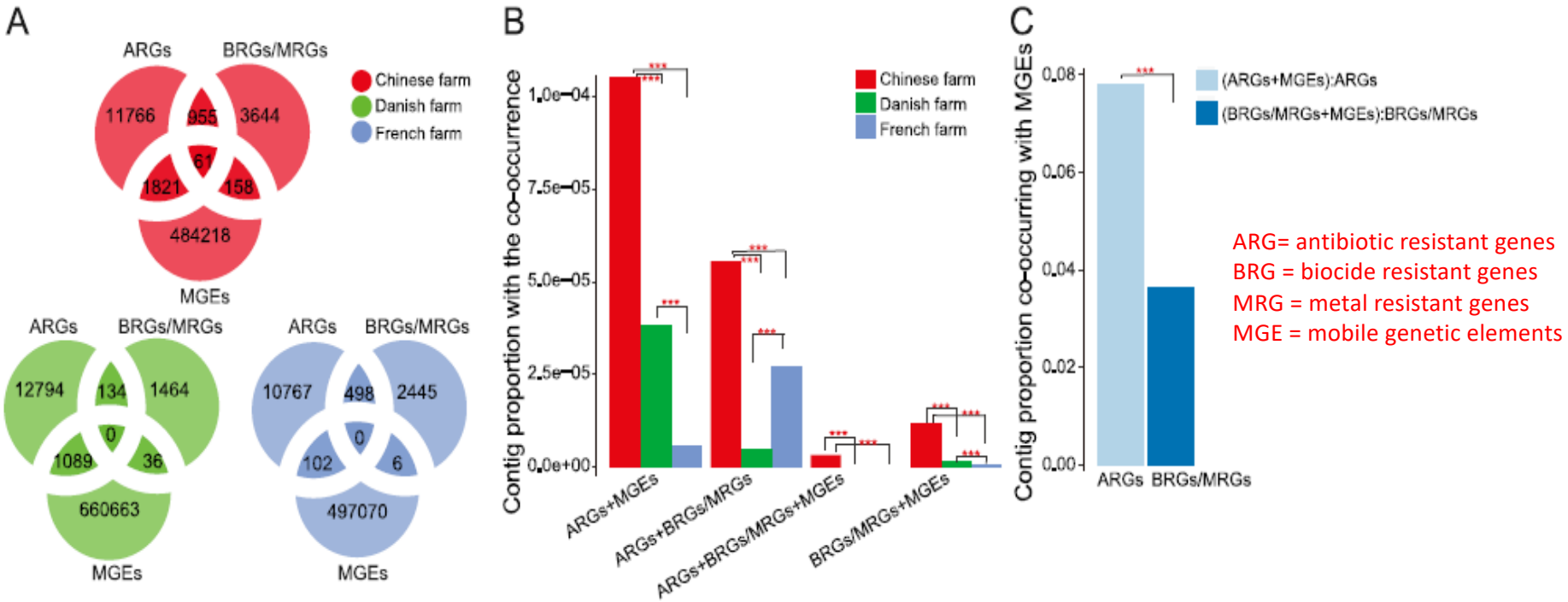
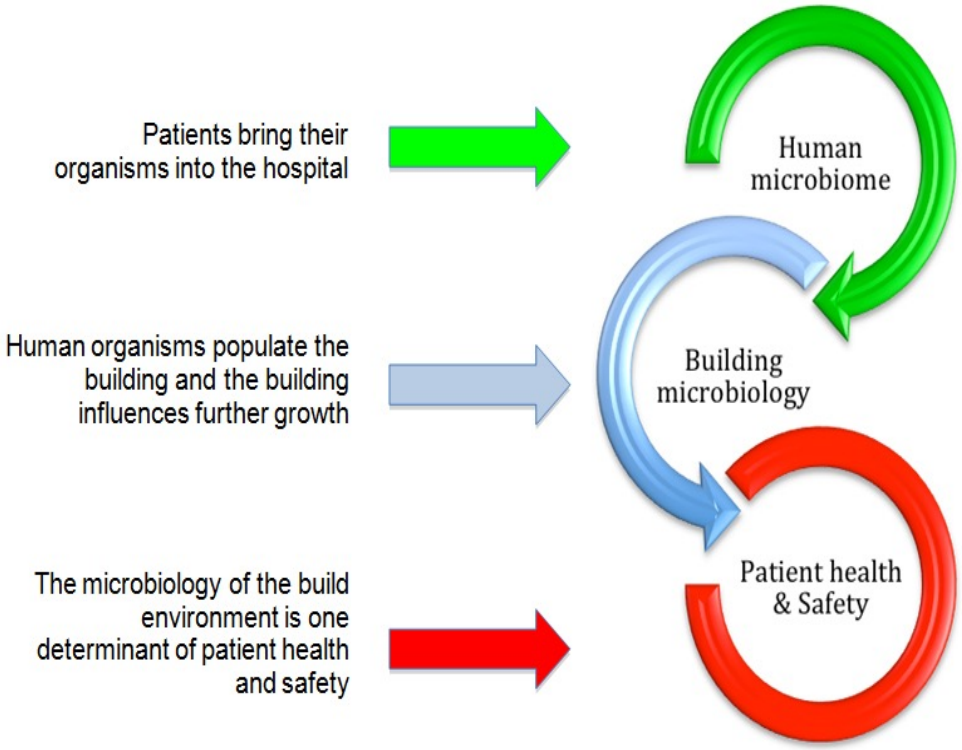


Fig. 2. Overview of co-occurrences of ARGs, BRGs, MRGs and MGEs in assembled contigs. A). Venn diagram showing the number of contigs carrying ARGs, BRGs/MRGs, MGEs and their combinations in pigs. **B).** The proportion of contigs with co-occurrences of ARGs and BRGs/MRGs, ARGs and MGEs, BRGs/MRGs and MGEs, BRGs/MRGs, ARGs and MGEs in pigs. Asterisks stand for significant statistical difference between groups (Fisher's exact test; $P < 0.05$*, $P < 0.01$**, $P < 0.001$***). **C).** The proportion of contigs carrying ARGs and BRGs/MRGs with MGEs. Asterisks stand for significant statistical difference between groups (Fisher's exact test; $P < 0.05$*, $P < 0.01$**, $P < 0.001$***).

The resistome of the built environment- an IPC concern?

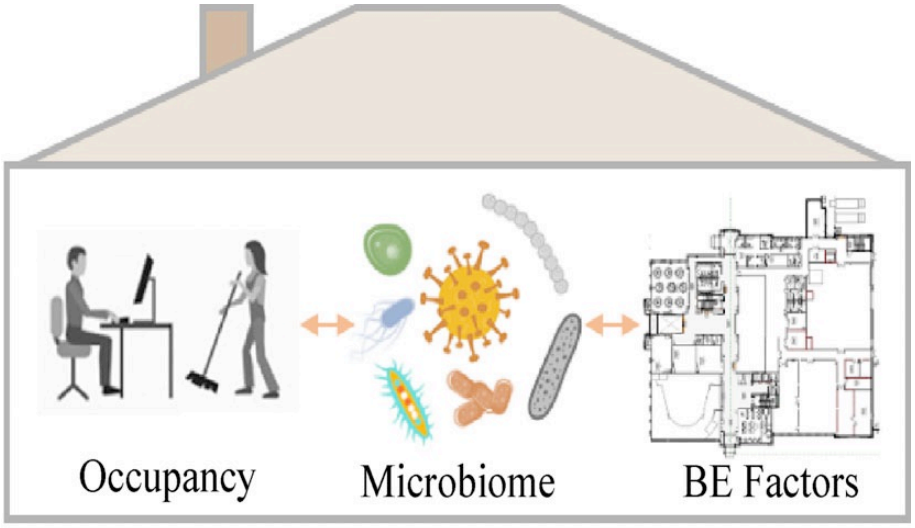
A constant movement of the microbiome



- Humans are the main source point of introduction of microorganisms especially in environments like the hospital.
- Carry microorganisms on our skin, hands, clothes, on the soles of our shoes, on our cell phones and other belongings.
- Emit 10^6 biological particles per hour into the environment.
- Patients come in with their (microbes and) resistome.
- These merge with the hospital microbiome.
- Antimicrobial agents (antibiotics and disinfectants) in the environment perpetuate this resistance.

NO SUCH THING AS A STERILE ENVIRONMENT

The microbiome and the built environment



Spread occurs from:

- The environment
- The pipes and sewage system
- The patients admitted
- The transfer to patients from the environment

Li S et al Front. Environ. Sci. Eng. 2021, 15(4): 65; Adams et all

- Built environments, occupants, and microbiomes **constitute a system of ecosystems with extensive interactions that impact one another.**
- The interactions between attributes of built environments and occupant behaviours shapes the structure and dynamics of indoor microbial communities.
- Can use this information for the development of transformative intervention strategies toward healthy built environments

IPC in the clinical setting

The goal of IPC is to

- reduce transmission of pathogens and AMR microbes between persons, from persons to and from the environment
- Achieved by
  - A clean environment
  - Good IPC practices such as hand hygiene
  - Antimicrobial stewardship
  - Outbreak and/ or transmission investigation

What is a sterile environment?

- There is no such thing as a sterile environment- will always have a microbiome present.

- A “Sterile Room” is a microbiologically controlled Clean Room built in such a way to prevent bacteria, viruses or parasites from being present. In order to create a sterile germ-free environment, operators install chemical sterilization systems and perform microbial tests as well as bacterial load analyses.
- While a sterile environment is completely free of microorganisms, aseptic describes something that has been made contamination-free.
- **Whenever a human operator enters a cleanroom, some level of microbial contamination can be expected.**

- What should IPC be trying to achieve? Sterile or clean?

What does a clean environment require?

- Cleaning an environment only requires to be cleaned using water and detergent. Use friction to remove dirt and keep it dry
- This does not disturb the environmental microbiome to a large extent because there is no antimicrobial agent present- selection pressure
- When does cleaning needs to be increased to disinfection? Specific indications
  - Infectious patient discharged- terminal cleaning
  - Spillage of contaminated blood or body fluids
  - Admitting an immunocompromised patient in an isolation facility
  - If there is an outbreak of an infectious disease
- Enhanced hand hygiene- before and after each patient contact (5MHH)- reduce environmental contamination from contact

Factors at play in the clinical setting affecting the resistome

Selection pressure

Factors

- Human activities: altered selection pressures through pollution by antimicrobials- antibiotics, heavy metals and disinfectants
- Stable molecule antibiotics- fluoroquinolones, sulphonamides, and tetracyclines, persist in water, sediment, or soil long after excretion from humans or animals after therapy.
- Increase selective advantages to resistant bacteria

Concentrations of antimicrobial drugs required to promote resistant strains,

- Well below the MICs (i.e., nanograms per litre)
- Residual effect of biocides
- Cross linkage from environment microbiome/ resistome

This translates to even more complex microbial communities!

http://dx.doi.org/10.3201/eid1907.120871

Areas of selective pressure (contd)

- Disinfection – animal husbandry
  - equipment disinfection(e.g., dairy)
  - housing (e.g., sheds),
  - preservatives for food
- Antibacterial agents- household products
  - QAC, (benzalkonium chlorides) -basis of many commercial and household disinfectants select for resistance encoded by the *qac* genes; recovered on class 1 integrons together with antimicrobial resistance gene cassettes.
- Antimicrobial drugs, metals, and biocides occur together in mixtures in the environment, frequently in hot spots of horizontal gene transfer (HGT). (sewage)
- A cocktail of selective pressures and biologic responses responsible for enriching resistance in the environment and promoting its transfer to pathogens.

Microbiome response to antimicrobials

- Communication between bacteria via quorum sensing (chemical sensors)
  - Detect and probe same species and friends
  - Avoid predators and toxic substances
  - Inhibit the growth of competitors
  - Find favourable conditions to survive
- Develop bioactive molecules to interact with other microbes and form defence systems
- Movement of genes-
  - vertically though population by cell division
  - Horizontally across species and genera
  - Supported by the mobilome which are genetic elements that enable and contribute to horizontal gene transfer
- Antimicrobial drugs (toxins) favour genetic exchange and increase gene diversity

Emerg Infect Disease (2013)
http://dx.doi.org/10.3201/eid1907.120871

Microbiome & resistome acquisition- hospital

- Pre-opening microbiome and the first 30 weeks after patient occupancy sampling. Used disinfectant 2 hrs prior to sampling
- Resistance reservoir - antibiotic resistance genes (ARGs) on floors, doorhandles and sinks,
- Findings: No increase of pathogenic bacteria in the hospital environment; a significant increase of ARG on the hospital floor.

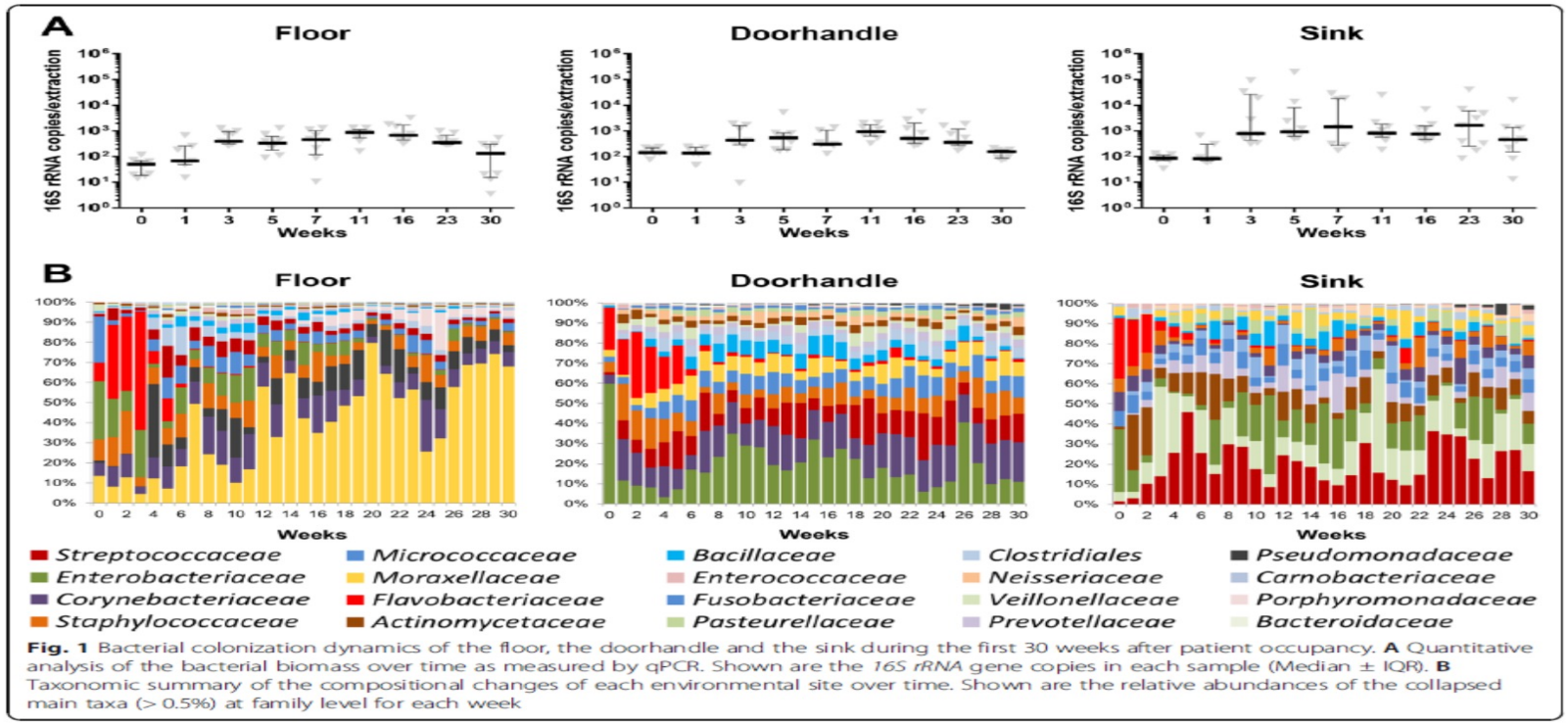


Fig. 1 Bacterial colonization dynamics of the floor, the doorhandle and the sink during the first 30 weeks after patient occupancy. **A** Quantitative analysis of the bacterial biomass over time as measured by qPCR. Shown are the *16S rRNA* gene copies in each sample (Median ± IQR). **B** Taxonomic summary of the compositional changes of each environmental site over time. Shown are the relative abundances of the collapsed main taxa (> 0.5%) at family level for each week

Klassert et al Microbiome 2021

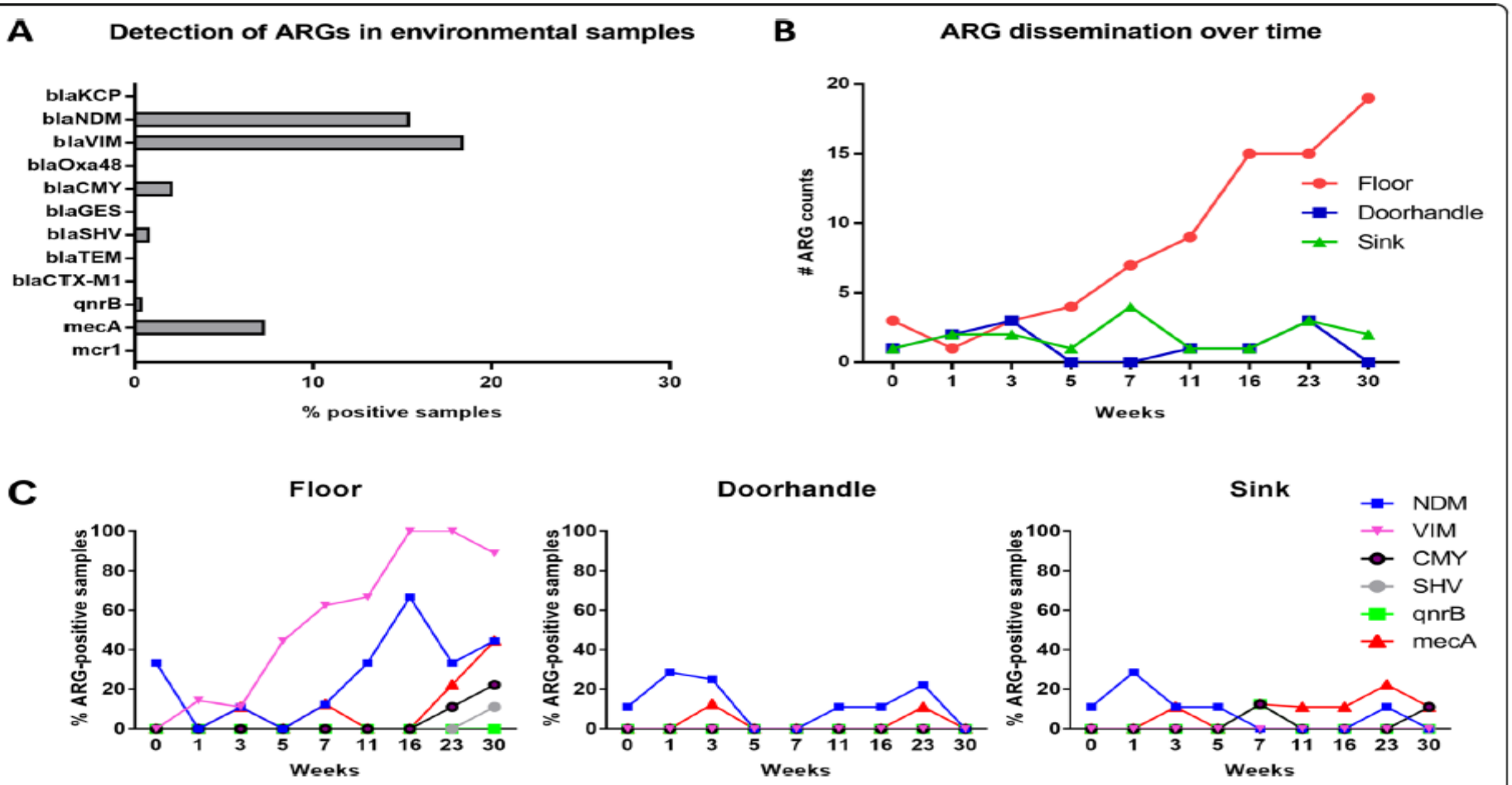
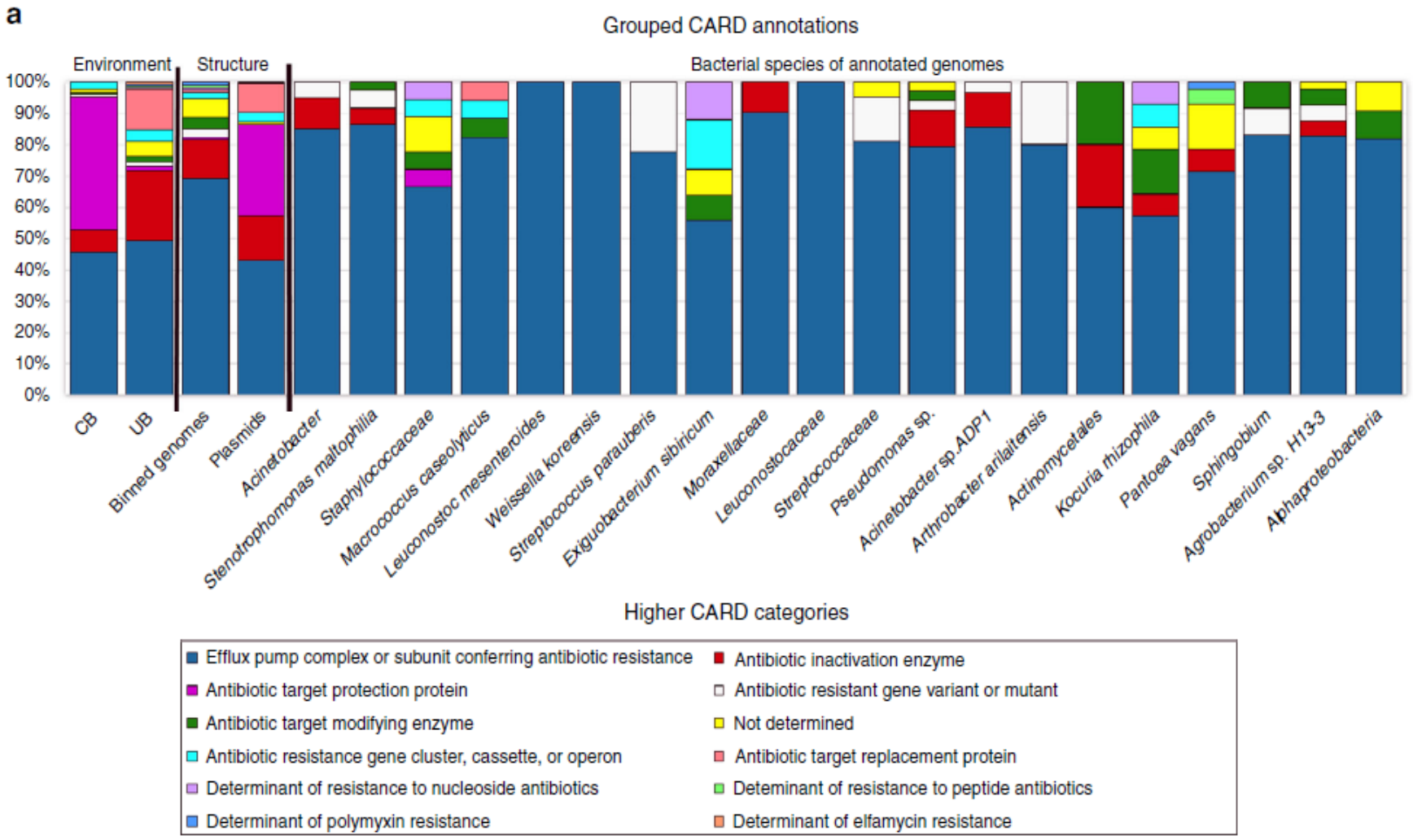


Fig. 6 ARG detection in the hospital environment after patient occupancy. **A** Bar chart depicting the ARG expression across all environmental samples analyzed. Bars represent percentage of samples with positive ARG detection. **B** ARG detection over time. Shown are the total ARG counts for each of the environmental sites over different weeks after hospital opening. **C** Chart depicting the specific ARGs detected at each site over time. Shown is the percentage of positive samples

Impact of antimicrobials on the resistome

	Confined built (CB) spaces	Unrestricted Buildings (UB)
Type of environment	Confined, clean space exposed to disinfectants, toxic chemicals to maintain clean/ sterile environment- ICU	Open space buildings with an outdoor, rural setting. Much less exposure to chemicals
Microbial diversity	Reduced	extensive
Degrade chemicals and compounds	increased functional capabilities to degrade xenobiotics and chemicals	Less capability to degrade chemicals
Virulence, defence mechanisms	Virulence factors more abundant for bacteria that need to survive in clean, nutrient-poor, and microbially controlled CB environments.	Not as virulent, with less resistance
Antibiotic resistance	Encode for a bigger diversity of genes involved in multidrug efflux	harboured more specific resistance features
Resistome	diverse, potentially mobile, and in increased contact to potential pathogens, but often less active	stable
Selection pressure	Antibiotics and biocides select broad spectrum and increase resistances against fluoroquinolones, triclosan, or elfamycins	Very little selection pressure

NATURE COMMUNICATIONS | (2019) 10:968 | https://doi.org/10.1038/s41467-019-08864-0


a
Grouped CARD annotations
Environment
Structure
Bacterial species of annotated genomes
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
CB
UB
Binned genomes
Plasmids
Acinetobacter
Stenotrophomonas maltophilia
Staphylococcaceae
Macrococcus caseolyticus
Leuconostoc mesenteroides
Weissella koreensis
Streptococcus parauberis
Exiguobacterium sibiricum
Moraxellaceae
Leuconostocaceae
Streptococcaceae
Pseudomonas sp.
Acinetobacter sp.ADP1
Arthrobacter arilaitensis
Actinomycetales
Kocuria rhizophila
Pantoea vagans
Sphingobium
Agrobacterium sp. H13-3
Alphaproteobacteria
Higher CARD categories
Efflux pump complex or subunit conferring antibiotic resistance
Antibiotic inactivation enzyme
Antibiotic target protection protein
Antibiotic resistant gene variant or mutant
Antibiotic target modifying enzyme
Not determined
Antibiotic resistance gene cluster, cassette, or operon
Antibiotic target replacement protein
Determinant of resistance to nucleoside antibiotics
Determinant of resistance to peptide antibiotics
Determinant of polymyxin resistance
Determinant of elfamycin resistance

Global use of disinfectants during Covid-19

- Immeasurable effect of the large scale use of disinfectants and sanitisers in the Covid-19 pandemic on the microbiomes of various ecological niches in humans, animals, and environments.
- Dysbiosis in host-commensal interactions is a likely outcome of such practices, thereby affecting the host's immune functioning, metabolism, physiological parameters, and susceptibility to infectious and non-infectious diseases.
- But the problems caused by excessive use of disinfectants and sanitisers globally extend beyond dysbiosis—for example,
  - emergence of alcohol resistance in *Enterococcus faecium*, a nosocomial pathogen, and its vancomycin resistant strains (as superbugs) has recently been reported.

The extensive use of disinfectants, sanitisers and antibiotics – immeasurable collateral damage by wiping out commensal and replaced by resistant pathogens.

BMJ 2020;370:m2795
http://dx.doi.org/10.1136/bmj.m2795

Preserving the sensitive clinical microbiome

Cleaning alternatives to chemicals- *Bacillus spp*

- The microbial cleaning products used in the field trials comprised spores of *Bacillus subtilis, Bacillus pumilus and Bacillus megaterium*, with a fixed quantity of 56 10^7 CFU per ml of product concentrate- contained few R genes (*OXA* gp. *mrsA*)
- comparison was made between cleaning with microbial cleaning products and the conventional hygiene protocols (using chemical cleaning products and disinfectants).
- Collected samples 7 hours after cleaning
- Followed up HAI in patients
- Such method was proved effective in counteracting surface recontamination by diverse pathogens, stably decreasing their presence of about 80– 90% compared to the microbial load detected on surfaces treated with conventional cleanser/ disinfectants
- No HAI related to these bacteria where observed
- No increase in AMR was found

Cleaning with *Bacillus* incorporated product.

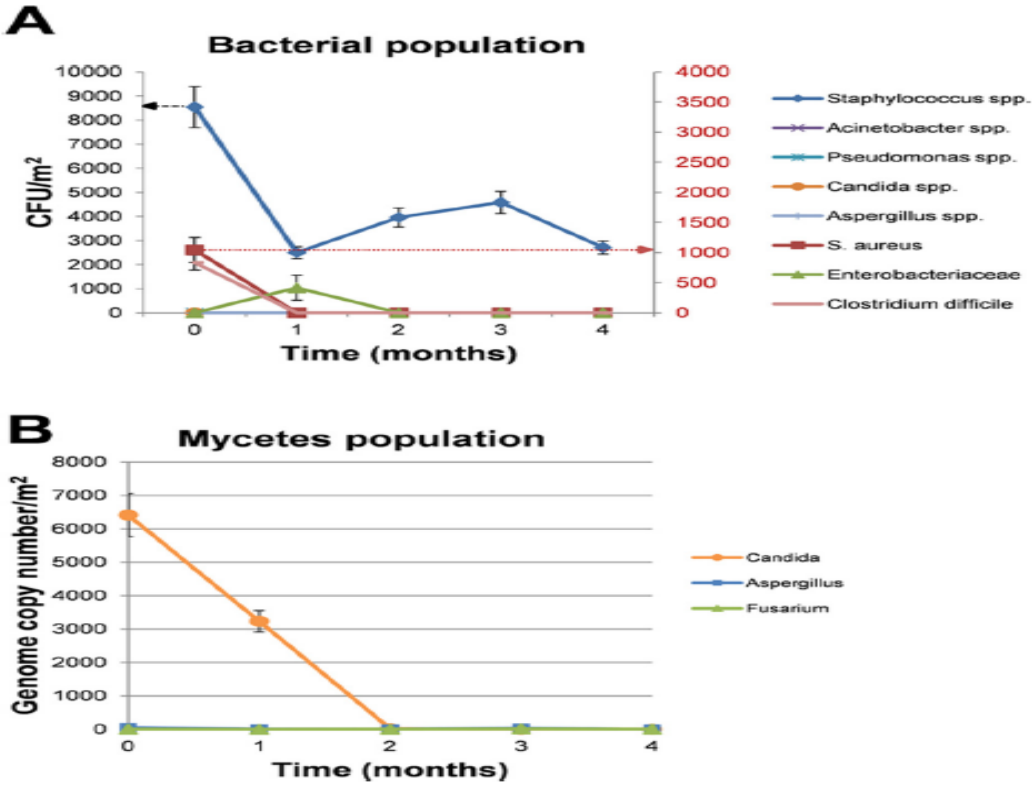


Fig 3. Effect of microbial cleaning on hospital surface contaminants. (A) Sampling was performed in triplicate by 24 cm^2 RODAC™ plates containing general or selective mediums for bacteria and mycetes. Results are expressed as median value of CFU counts/m^2 ± SE. *Staphylococcus* spp counts refer to the left Y axis (indicated by the black arrow), whereas all the other microbial counts refer to the right Y axis (indicated by the red arrow). **(B)** Mycetes were detected and quantified also by specific qPCRs. Sampling was performed by swab collection of a 100 cm^2 surface. Results are expressed as mean genome copy number/ m^2 ± SD.

PLOS ONE | DOI:10.1371/journal.pone.0148857 February 17, 2016

Preserving a sensitive microbiome- a win win situation for IPC

- Most surfaces will have human skin bacteria which are sensitive.
- Most frequently touched surfaces will have hand and elbow microbes
- The floor has more pathogenic microbes
- Interventions
  - Routine cleaning with water and detergent only
  - Disinfection at specific indications – terminal cleaning etc
  - Do not use disinfectants indiscriminately
  - Consider replacement with a relatively sensitive microbiome to prevent resistance selection

My thoughts

- The preservation mechanisms in the microbiome predate anthropomorphic activities.
- The longer we preserve the sensitive clinical microbiome the less ARG and AMR will spread
- Most microbes are normal human skin flora which colonises the hospital- these are of little or no threat to patients and staff
- Reduce the count by good cleaning without the need for disinfection.

Do we really need to remove a sensitive resistome using antimicrobial agents and chemicals to be replaced by a resistant one?

In order to achieve what????

www.webbertraining.com/schedulep1.php

May 18, 2023	**NON-CATHETER-ASSOCIATED URINARY TRACT INFECTIONS** Speaker: **Cindy Hou**, Jefferson Health, New Jersey
May 29, 2023	*(FREE Teleclass ... Broadcast live from the IPAC Canada conference)* **THE PSYCHOLOGY OF PANDEMICS** Speaker: **Dr. Steven Taylor**, University of British Columbia
May 29, 2023	*(FREE Teleclass ... Broadcast live from the IPAC Canada conference)* **SCIENTIFIC COMMUNICATIONS TO THE PUBLIC DURING A PANDEMIC AND BEYOND** Speaker: **Dr. Bonnie Henry**, Province of British Columbia, Victoria BC
June 8, 2023	**CHANGING HAND HYGIENE BEHAVIOUR: NUDGING CONCEPTS** Speaker: **Dr. Marco Bo Hansen**, Copenhagen University Hospital, Denmark
June 15, 2023	**ARE EMERGENCY DEPARTMENT WAIT TIMES AND PATIENT-REPORTED CLEANLINESS PREDICTORS OF HEALTHCARE-ASSOCIATED *CLOSTRIDIOIDES DIFFICILE* INFECTION, OR PREDICTORS OF POOR PROCESSES?** Speaker: **Prof. Danielle J. Durant**, Texas A&M University, College of Nursing and Health

Thanks to Teleclass Education

PATRON SPONSORS

diversey.com

virox.com

gamahealthcare.com