DE MONTFORT UNIVERSITY LEICESTER

INFECTIOUS DISEASE RESEARCH GROUP

The Dirty Truth Microbiome Build-Up on Bed Linen and Domestic Washing Machines

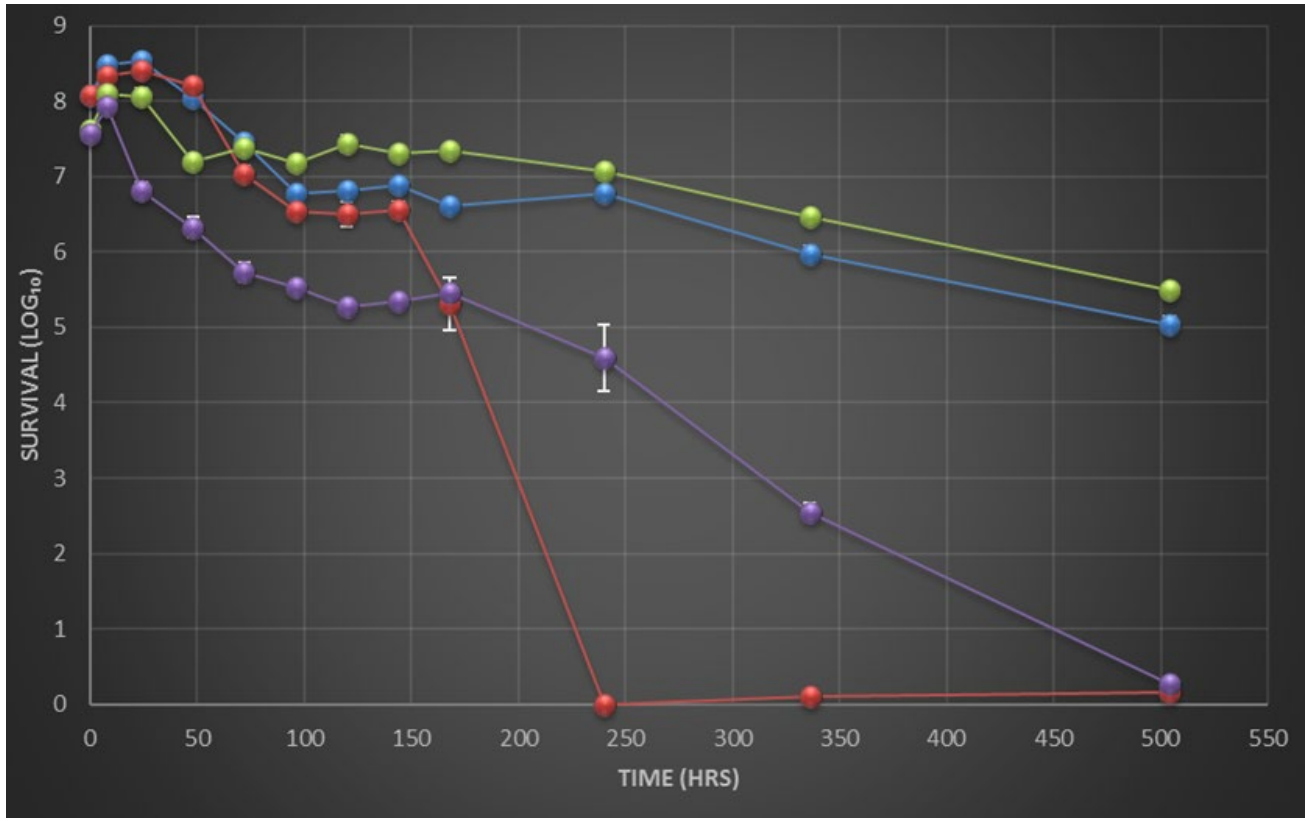
Prof Katie Laird – klaird@dmu.ac.uk

Overview

- Survival of bacteria on textiles
- Disinfection efficacy of domestic washing machines
- Domestic launderings contribution to antibiotic resistance
- Bedsheet bacterial and fungal colonisation

In the UK and the USA, domestic laundering machines are commonly used to clean healthcare worker uniforms, raising concerns about their effectiveness in microbial decontamination and role in AMR development

Survival of Bacteria on Textiles



Survival of *Escherichia coli* and *Staphylococcus aureus* on polyester and cotton fibres

Green -

S. aureus cotton

Blue -

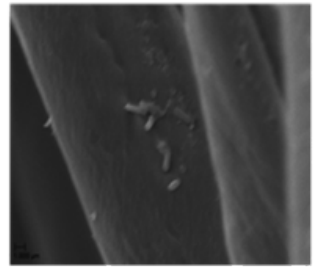
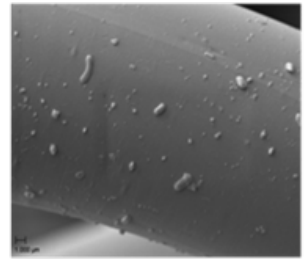
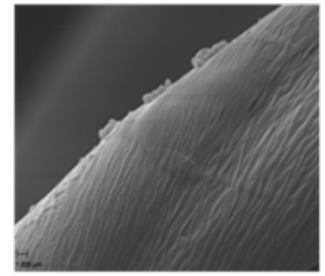
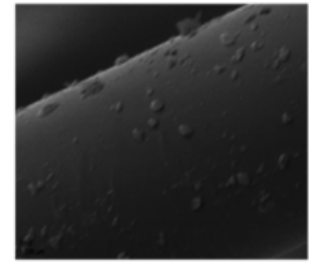
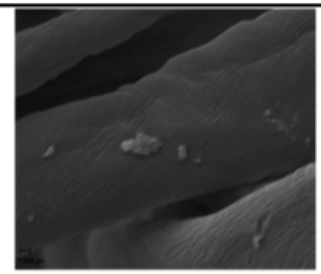
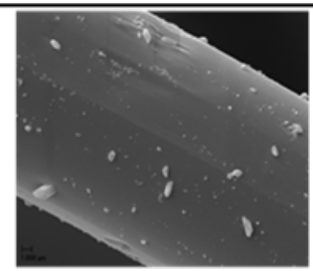
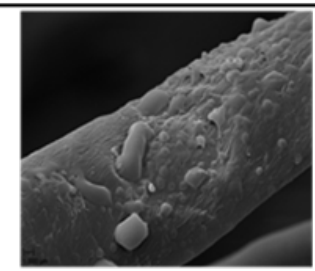
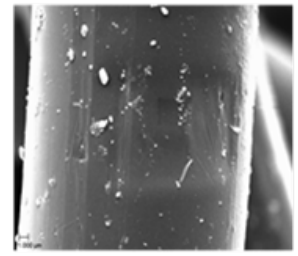
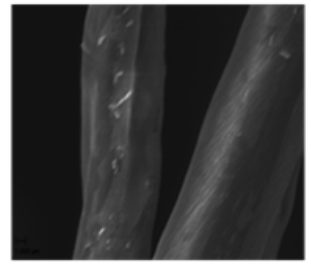
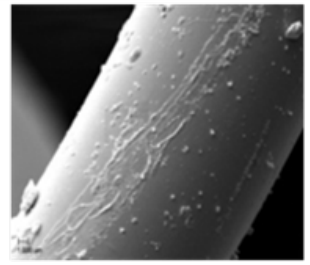
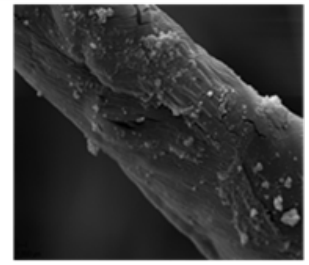
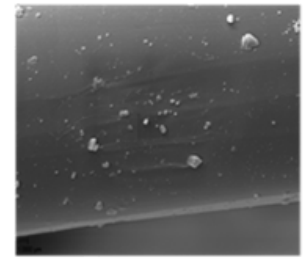
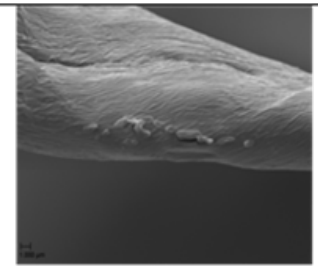
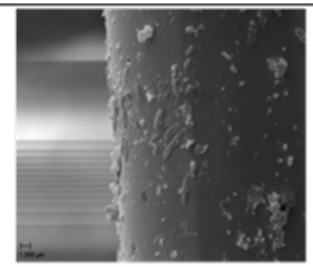
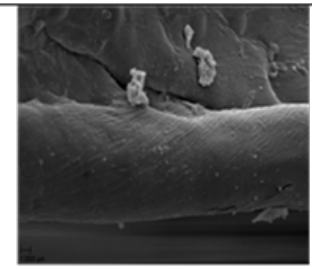
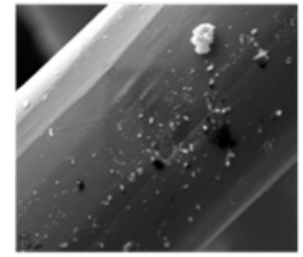
E. coli cotton

Red -

E. coli polyester

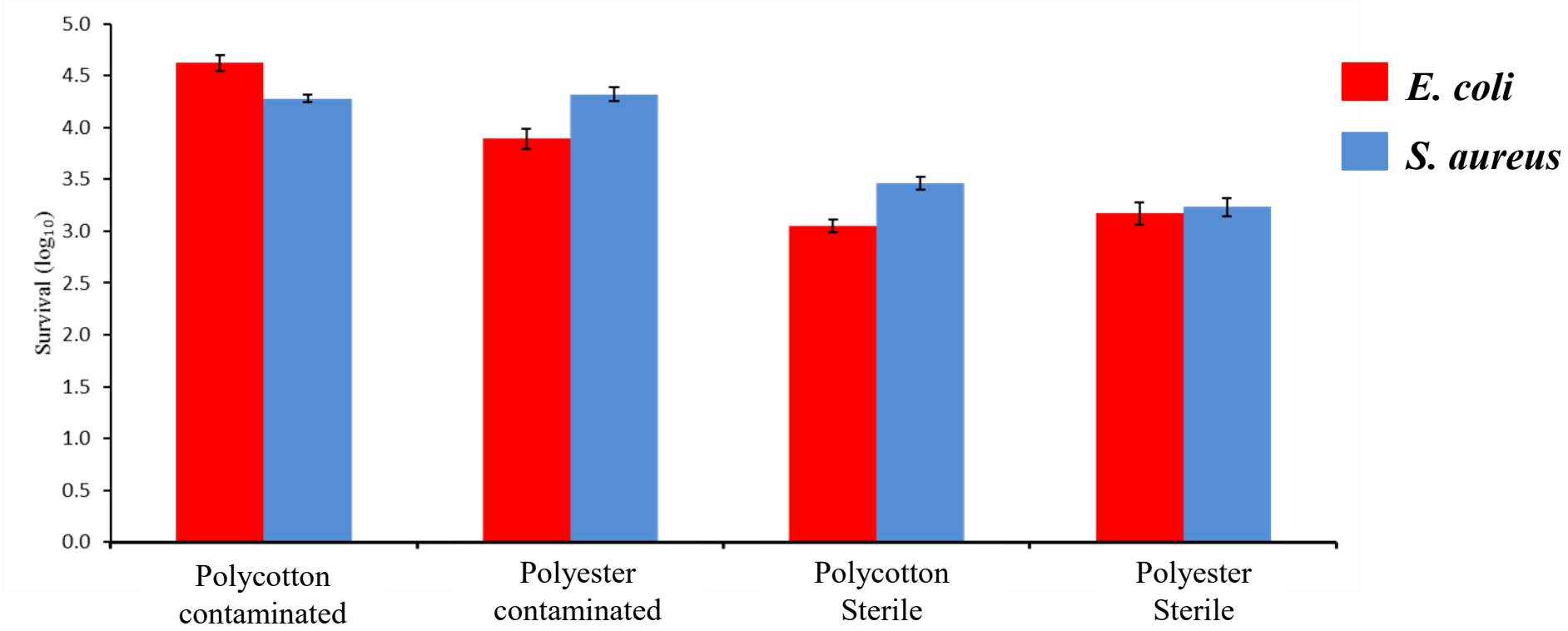
Purple –

S. aureus polyester

	Cotton (*E. coli*)	Polyester (*E. coli*)	Cotton (*S. aureus*)	Polyester (S. *aureus*)
8 hrs				
24 hrs				
168 hrs				
504 hrs				

Transmission of Bacteria During Laundering

Reductions 99.9% - 99.99%



Survival of *E. coli* (red) and *S. aureus* (blue) after laundering at 40ºC on contaminated and sterile samples of fabric A (65% polyester/35% cotton) and fabric B (100% polyester)

It is well documented in the literature that a range of microorganisms survive on textiles, including *E. coli*, *S. aureus*, and *Enterococcus* sp., *Candida* sp., SARS-CoV-2, HSV-1, etc.

Table 1 **In vitro survival of microorganisms on textiles.**

Microorganism	Surface	Survival	Reference
E. coli, *S. aureus*	Cotton and polyester	5 $\log_{10}$ survived on cotton for 21 days; 0.16–0.28 $\log_{10}$ survived on polyester for 21 days	*Riley et al. (2017)*
E. faecium, *S. aureus*, *P. aeruginosa*	Cotton	4–5 $\log_{10}$ *E. faecium* and *S. aureus* survived for 21 days. *P. aeruginosa* survived for 20 days.	*Fijan, Pahor & Turk (2017)*
Faecal coliforms	Cotton, blended textile and silk	Faecal coliforms survived for 120 days on cotton and blended textile at 25 °C (>1.1 $\times 10^4$ CFU/ml). 1.1 $\times 10^2$ CFU/ml survive on silk over 120 days.	*Colclasure et al. (2015)*
Candida spp., *Aspergillus* spp., *Fusarium* sp., *Mucor* sp., *Paecilomyces* sp.	Cotton, terry, blended textile, polyester and spandex	*Candida* spp. and *Aspergillus* spp. survived for 1 to >30. days *Fusarium* sp. for 4 to >30 days, *Mucor* sp. for 6 to >30 days and *Paecilomyces* sp. for <1 to 11 days.	*Neely & Orloff (2001)*
SARS-CoV	Cotton and disposable gowns	SARS-CoV survived on a cotton gown for 5 min at an inoculum of 10^4 $TCID_{50}$/ml and 24 h at an inoculum of 10^6 $TCID_{50}$/ml. Survival on a disposable gown was 1 h at 10^4 $TCID_{50}$/ml and 2 days at 10^6 $TCID_{50}$/ml.	*Lai, Cheng & Lim (2005)*
SARS-CoV-2	Cloth and surgical masks	SARS-CoV-2 persisted on cloth for 2 days, compared to 4 days on glass and bank notes to 7 days on surgical masks, stainless steel and plastic.	*Chin et al. (2020)*
HSV-1	Cotton	Herpes simplex virus 1 (HSV-1) in the presence of artificial soiling (bovine serum albumin and sheep erythrocytes) gradually reduces on cotton surfaces over time with a 1 $\log_{10}$ reduction after 30 min and complete inactivation within 48 h.	*Gerhardts et al. (2016)*
Poliovirus, adenovirus, hepatitis A virus and murine norovirus	Cotton, wool, gauze and diaper material	Poliovirus survives at room temperature for 84–140 days on wool and 42–84 days on cotton, adenovirus and hepatitis A remaining infectious for 60 days in cotton and murine norovirus surviving for 40 days on gauze and diaper material.	*Yeargin et al. (2016)*
HCoV OC43 and 229E	Cotton gauze sponge	HCoV 229E remained infectious for 12 h and OC43 for 3 h (initial titre 5 $\times 10^5$ $TCID_{50}$/ml).	*Sizun, Yu & Talbot (2000)*

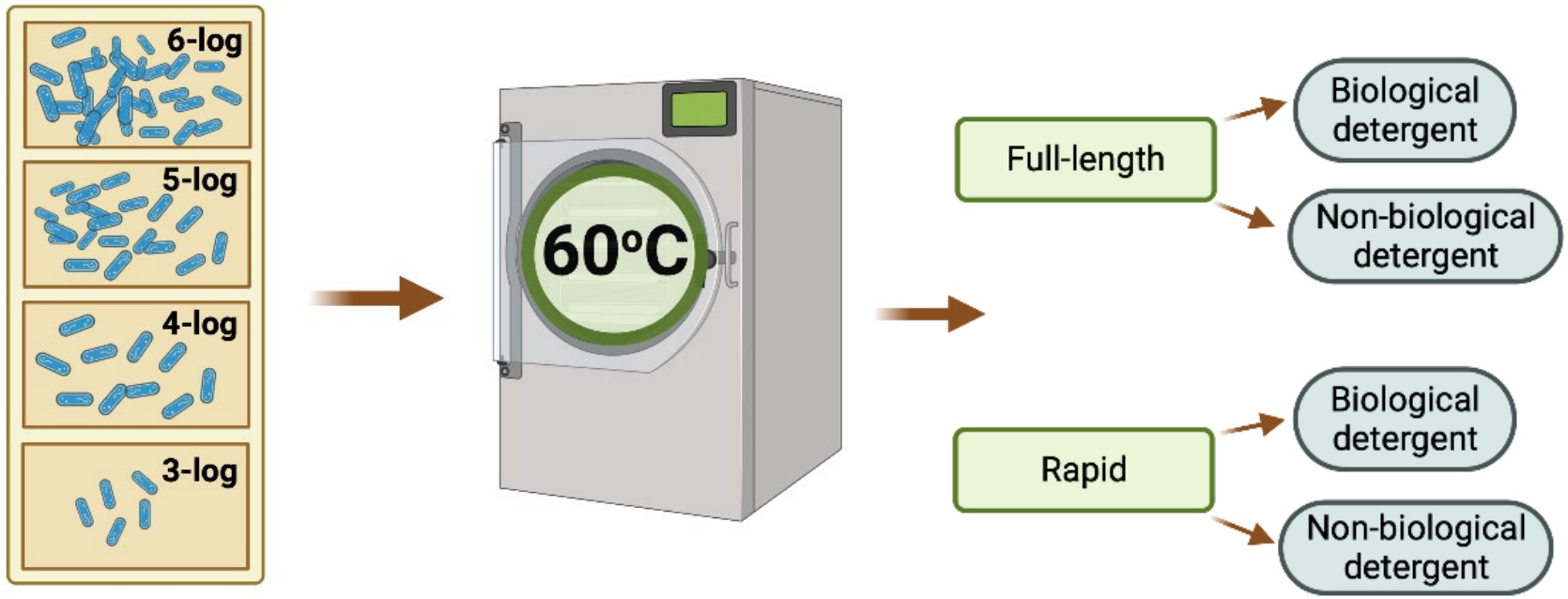
Microorganisms have also been shown to transfer between textiles and skin, stainless steel, vinyl flooring, surgical gowns and more.

Table 2 **In vitro studies on the transmission of microorganisms to/from textiles from other surfaces.**

Microorganism	Transfer material	Findings	Reference
Acinetobacter baumannii, MRSA, VRE	100% cotton white coats to porcine skin	Test species transferred onto porcine skin 1, 5 and 30 min after textile inoculation with 0.5 MacFarland standard or a 1:100 dilution of this suspension. The rate of transfer was not quantified.	*Butler et al. (2010)*
Bacillus thuringenesis, *E. coli*, *S. aureus*	Cotton or polyester to fingertips	Transfer efficiencies of cotton and polycotton were <6.8–0.37% for *E. coli*, <1.0–0.37% for *S. aureus* and <0.6% for *B. thurengenesis*. Transfer was higher for non-porous surfaces at 40.7–3.8%, 20.3–2.7% and 57–0.04%, respectively.	*Lopez et al. (2013)*
C. difficile spores	Stainless steel or vinyl flooring to polypropylene laminate surgical gowns	10^1-10^3 CFU *C. difficile* spores transferred onto surgical gowns after 10 s to 1 min contact with stainless steel or vinyl surfaces spiked with 10^5 CFU spores.	*Dyer et al. (2019)*
MRSA	Cotton bedsheets and towels to porcine skin	MRSA was transmissible for up to 14 days; 10^3–10^4 CFU transferred on to porcine skin 1 day after the textile was inoculated (10^6 CFU inoculum) and 10^2–10^3 CFU transferred 7 days post-inoculation.	*Desai et al. (2011)*
Acinetobacter calcoaceticus, *E. coli* and *S. aureus*	Textile to textile: cotton, polycotton, polyester, silk, wool, polypropylene and viscose	Friction increased the transfer of *S. aureus* by two to five-fold and *E. coli* and *Acinetobacter calcoaceticus* by 5.7–61% compared to direct contact without friction. Transfer of *S. aureus*, *A. calcoaceticus* and *E. coli* was also significantly greater for wet fabrics compared to dry fabrics. *A. calcoaceticus* and *E. coli* transferred more efficiently from smoother textiles (viscose and polyester) compared to rougher textiles (polypropylene).	*Varshney et al. (2020)*
S. aureus	Textile (cotton/polycotton) to textile or fingers.	Transfer of *S. aureus* to fingers was generally low (<3% transfer), however polycotton had a greater rate of transfer than cotton. Friction increased transfer by up to 5-fold. Transfer was significantly greater from textile to other textile or fingers when the textile was moist and when friction was applied.	*Sattar et al. (2001)*

Domestic Laundering of Textiles

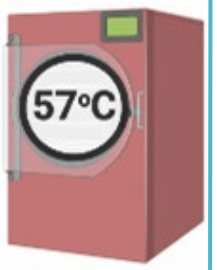
This study aimed to investigate the bacterial decontamination efficacy of a range of domestic washing machines & cycle parameters using a commercially available bioindicator test strip containing *Enterococcus faecium*.



Materials: Domestic Washing machines

						
Make	Indesit	Beko	AEG	Hoover	Indesit	Indesit
Model	IWSD61251 Eco	WDEY854 P44QW	7000 series kombi	HBWD851 4DC-80	IW007143	IWE91281
Machine Age (years)	8	1.4	3	4	2	9

Results: Full-length cycle

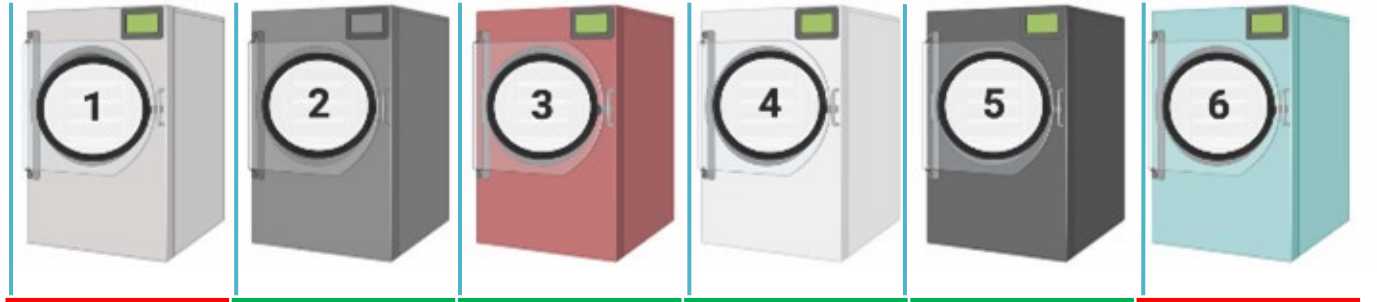
	1	2	3	4	5	6
Cycle Length:	1h 52 min	2h 5 min	2h 30 min	1h 55 min	2h 40 min	3h 5 min
Peak Temperature:						
Holding time (± 1°C):	**5 min**	**39.25 min**	**9.5 min**	**8 min**	**10.5 min**	**28.5 min**
Machine Age (years)	8	1.4	3	4	2	9

Results: Rapid Cycle / Eco Cycle

	1	2	3	4	5	6
Cycle length:	1h 25 min	44min	1h 4 min	59min	2h 30 min	1h 44min
Peak Temperature:						
Holding time (± 1°C):	**38 min**	**10.5 min**	**2.5 min**	**7 min**	**12.75 min**	**20.5 min**
Machine Age (years)	8	1.4	3	4	2	9

Results: Decontamination efficacy

Log_{10} reduction of *E. faecium* bioindicators following domestic laundering using a 60°C full-length or rapid wash cycle (n=4).



Full length 60°C	**Biological**	0	>5	6	6	6	0
	Non-Biological	0	5	6	6	6	0
Rapid 60°C	**Biological**	0	5	0	>4	6	0
	Non-Biological	0	6	0	6	6	0

Domestic washing machine microbiome and resistome: Sampling and metagenomic sequencing

Biofilm sampling (8 DLMs)

Drum rubber

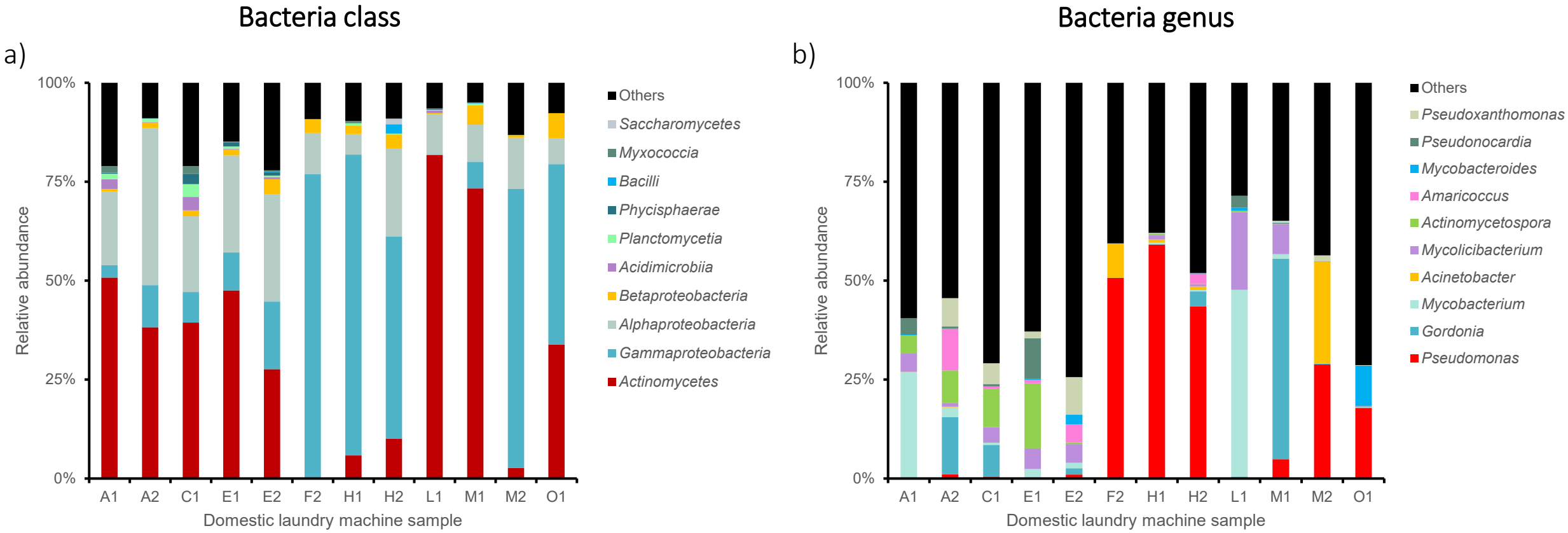
Drawer pipe

DNA extraction

Shotgun metagenome sequencing using the PE150 strategy

Illumina NovaSeq 6000 S4 platform

Bacteria Found In Domestic Machines

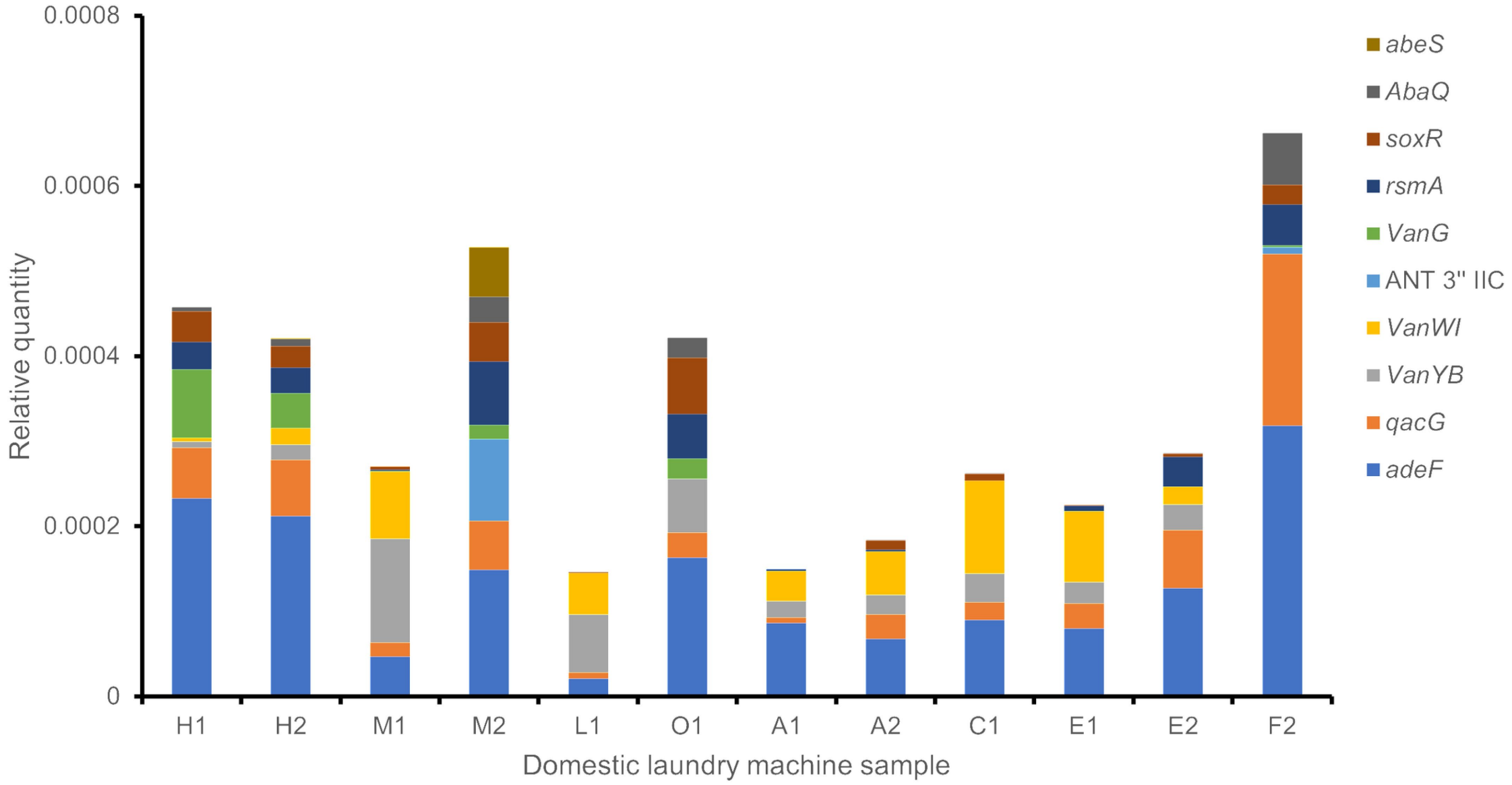


Bacteria classes and genera distribution in domestic laundry machine samples. The 10 most abundant classes and genera in the samples are displayed.

Antibiotic Resistance Found in Domestic Laundry Machines

Antibiotic resistance genes detected in domestic laundry machines. The resistance genes are ranked from the highest frequency (top) to the lowest frequency (bottom) among all the samples. Only the 10 most frequent ones are listed. Gene data were extracted from the CARD database.

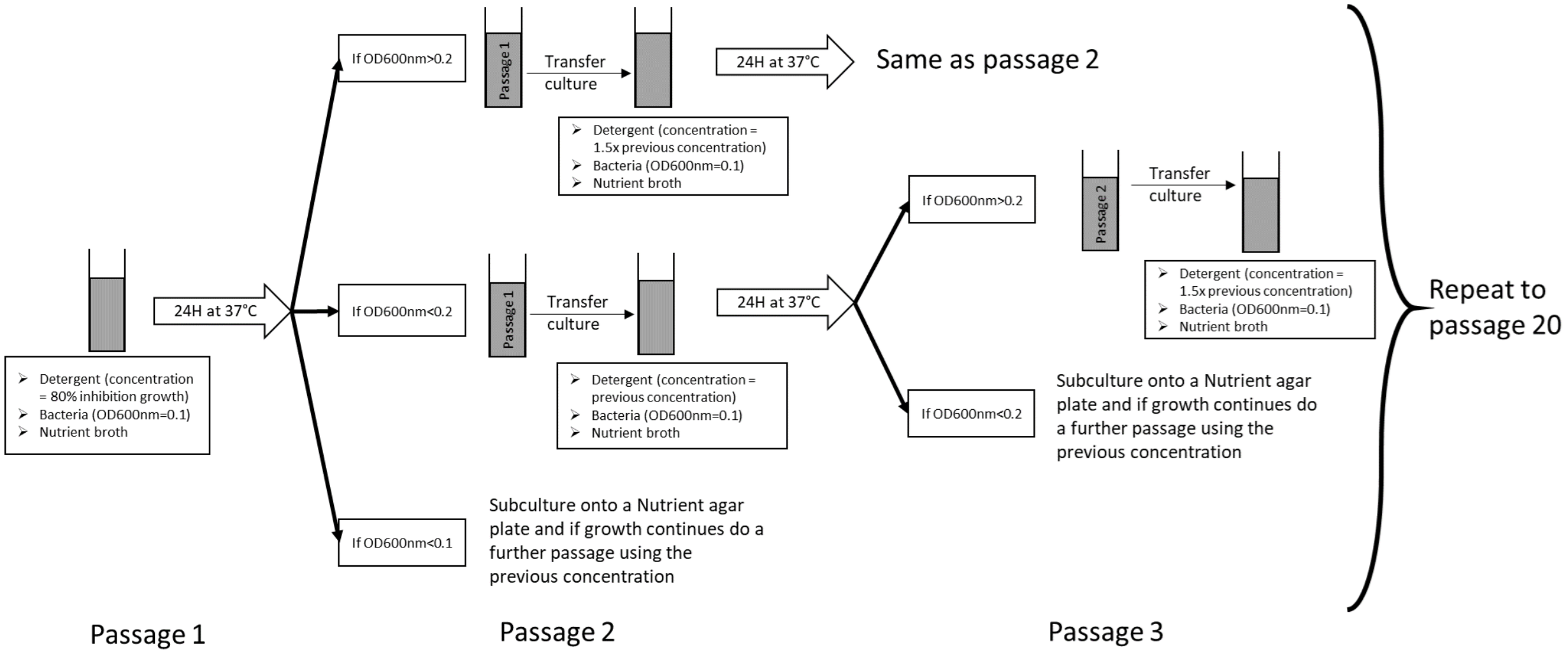
Gene	Resistance mechanism	Drug class
adeF	Antibiotic efflux	tetracycline antibiotic, fluoroquinolone antibiotic
qacG	Antibiotic efflux	disinfecting agents and antiseptics
VanYB	Antibiotic target alteration	glycopeptide antibiotic
VanWI	Antibiotic target alteration	glycopeptide antibiotic
ANT 3'' IIC	Antibiotic inactivation	aminoglycoside antibiotic
VanG	Antibiotic target alteration	glycopeptide antibiotic
rsmA	Antibiotic efflux	diaminopyrimidine antibiotic, phenicol antibiotic, fluoroquinolone antibiotic
soxR	Antibiotic efflux + Antibiotic target alteration	tetracycline antibiotic, cephalosporin, penam, fluoroquinolone antibiotic, phenicol antibiotic, disinfecting agents and antiseptics, rifamycin antibiotic, glycylcycline
AbaQ	Antibiotic efflux	fluoroquinolone antibiotic
abeS	Antibiotic efflux	macrolide antibiotic, aminocoumarin antibiotic



Abundance of resistance genes in each DLM samples.
The 10 most abundant resistance genes in the samples are displayed

Detergent & Disinfection Resistance and cross resistance to antibiotics

Inducing Detergent and Antibiotic Resistance



Sublethal laundry detergent concentration and Evolution of bacteria tolerance to laundry detergent after repeated exposure

Bacteria	Non-Biological Detergent	Starting concentration (80% inhibition of growth)	Maximum concentration reached	% increase in concentration
S. aureus	Powder	180 µg/ml	270 µg/ml	50%
	Liquid	0.000675 µl/ml	0.60 µl/ml	89,000%
K. pneumoniae	Powder	1800 µg/ml	4100 µg/ml	128%
	Liquid	0.000675 µl/ml	10.36 µl/ml	>1.5m%
P. aeruginosa	Powder	1800 µg/ml*	2700 µg/ml	50%
	Liquid	0.00675 µl/ml	2.05 µl/ml	30 000%

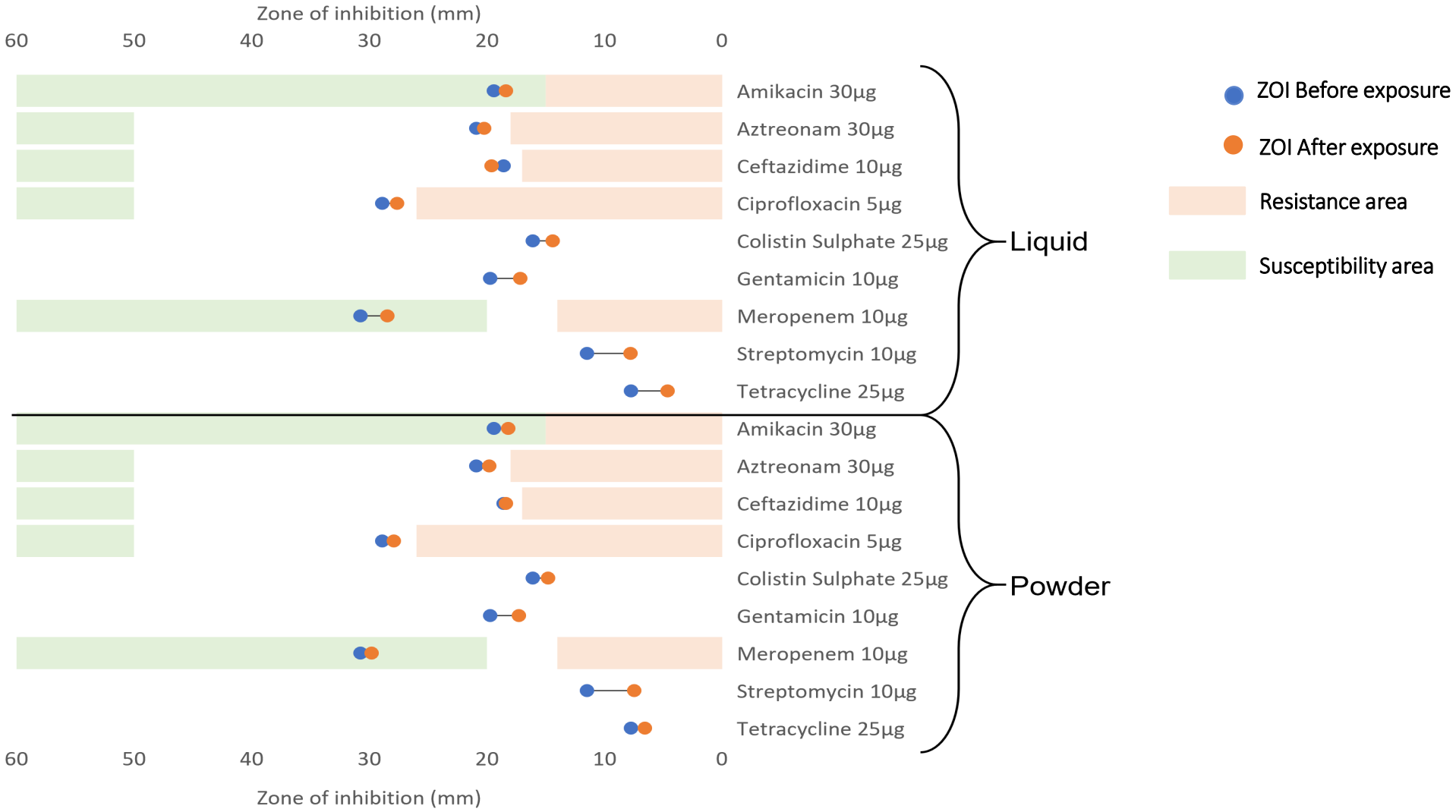


Figure 2 . *P. aeruginosa* antibiotic resistance profile before and after repeated exposure to laundry detergents (liquid or powdered). Blue markers represent the mean ZoI before exposure to the detergents. Orange markers represent the mean ZoI after exposure to the detergents. The green areas indicate each antibiotic susceptibility area based on the EUCAST (2024) breakpoints, the white areas represent the intermediary susceptibility, and the light red areas represent the antibiotic resistance area. When the antibiotic is not clinically relevant no areas is highlighted.

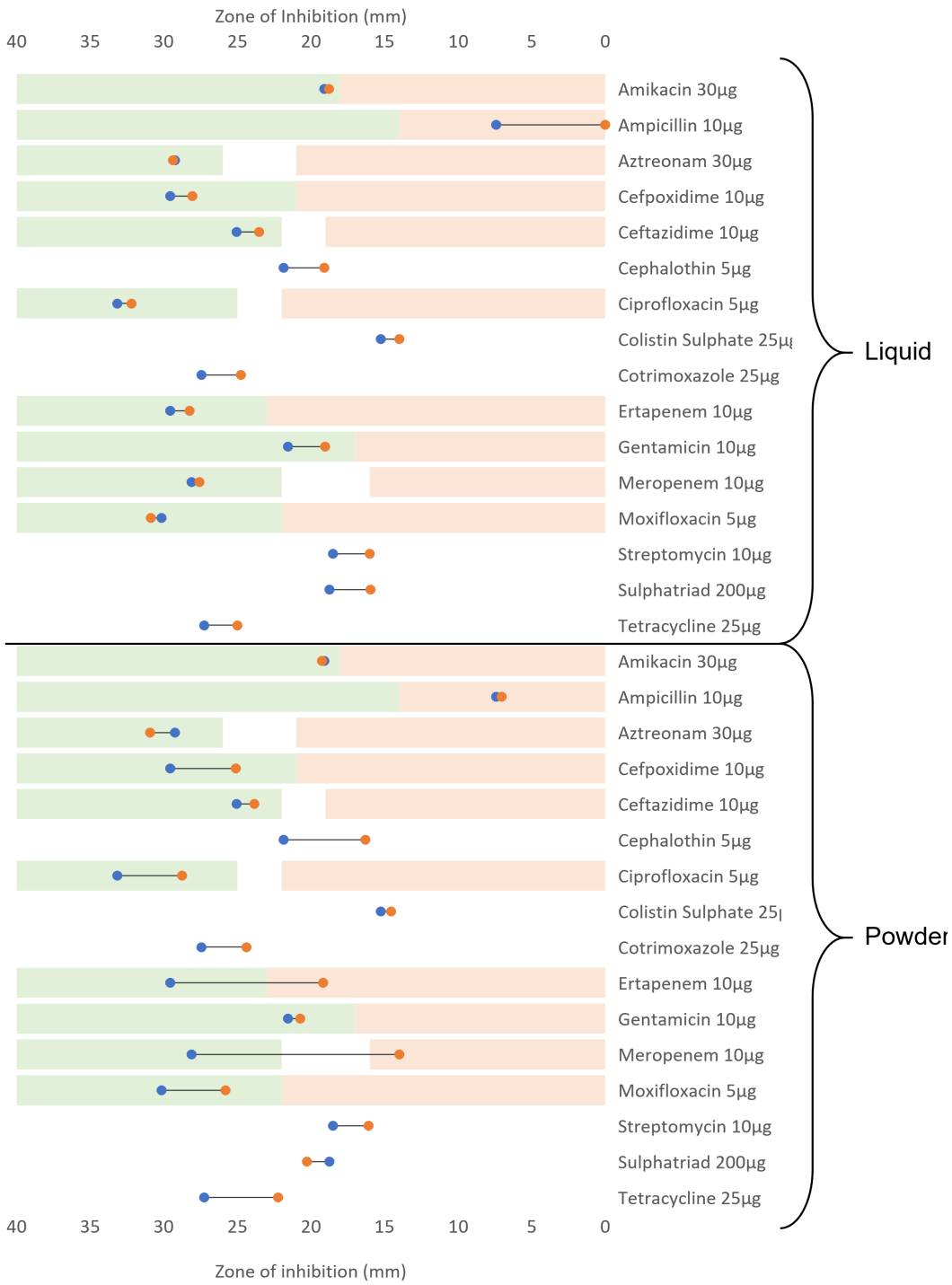


Figure 3. *K. pneumoniae* antibiotic resistance profile before and after repeated exposure to laundry detergents (liquid or powdered). Blue markers represent the mean ZoI before exposure to the detergents. Orange markers represent the mean ZoI after exposure to the detergents. The green areas indicate each antibiotic susceptibility area based on the EUCAST (2024) breakpoints, the white areas represent the intermediary susceptibility, and the light red areas represent the antibiotic resistance area. When the antibiotic is not clinically relevant no areas is highlighted.

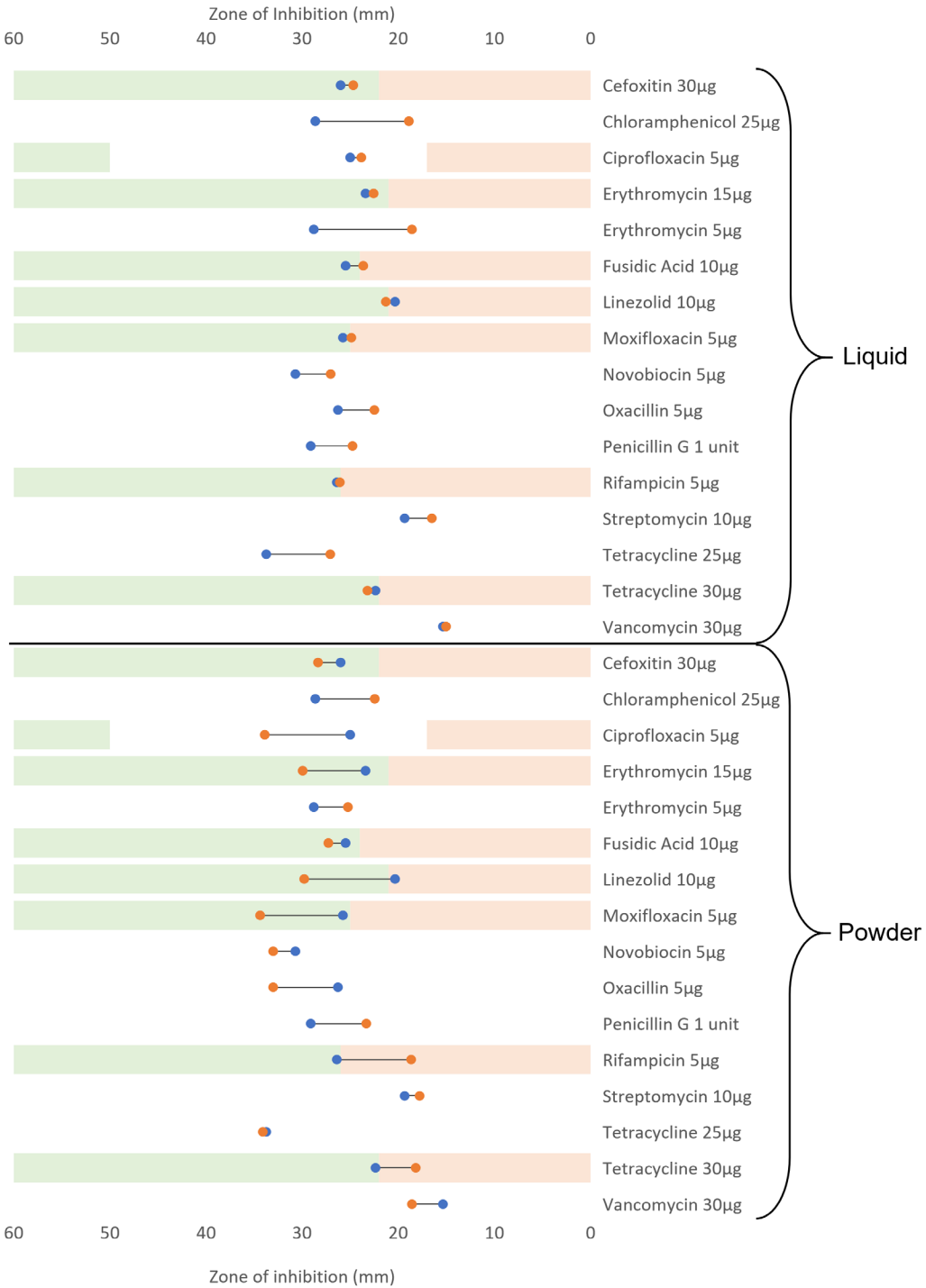


Figure 4. *S. aureus* antibiotic resistance profile before and after repeated exposure to laundry detergents (liquid or powdered). Blue markers represent the mean ZoI before exposure to the detergents. Orange markers represent the mean ZoI after exposure to the detergents. The green areas indicate each antibiotic susceptibility area based on the EUCAST (2024) breakpoints, the white areas represent the intermediary susceptibility, and the light red areas represent the antibiotic resistance area. When the antibiotic is not clinically relevant no areas is highlighted.

Isolates that produced clinically relevant resistances

Organism	Detergent	Antibiotic	Zone of inhibition (mm)			
			Pre-Exposure		Post-Exposure	
			Mean	SD	Mean	SD
Staphylococcus aureus	Liquid	Moxifloxacin 5µg	25.77	0.19	24.87	0.44
		Fusidic Acid 10µg	25.48	0.28	23.65	0.64
		Penicillin G 1 unit	**29.08**	**0.58**	**24.76**	**1.31**
Staphylococcus aureus	Powder	Tetracycline 30µg	22.37	0.12	18.16	0.31
		Rifampicin 5µg	26.4	0.05	18.67	0.5
		Penicillin G 1 unit	**29.08**	**0.58**	**23.33**	**1.53**
		Oxacillin 5µg**	**26.27**	**0.34**	**19.55**	**0.45**
Klebsiella pneumoniae	Powder	Ertapenem 10µg	29.56	0.24	19.18	0.39
		Meropenem 10µg	28.10	0.12	13.98	0.38

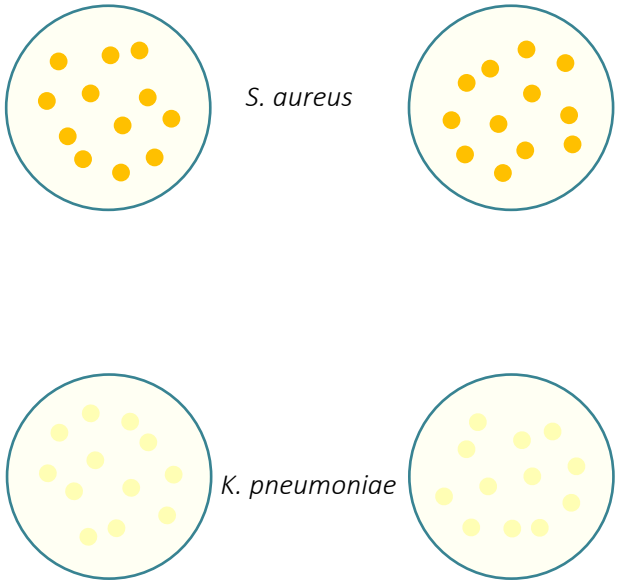
From initial screening

** Breakpoint is for 1µg, but still below this despite higher strength antibiotic exposure

Whole Genome Sequencing of antibiotic cross-resistant strains

Passage 0

Passage 15



DNA extraction

Shotgun metagenome sequencing using the PE150 strategy

Illumina NovaSeq 6000 S4 platform

Whole Genome Sequencing of antibiotic cross-resistant strains

S. aureus

SNP in the *MgrA* gene of *S. aureus* after liquid detergent exposure.

MgrA is a major global regulator of *S. aureus.*

This gene has links to increased virulence and antibiotic resistance, as it regulates efflux pumps.

K. pneumoniae

SNP identified within the *AcrB* gene after powder detergent exposure.

A stop codon in the sequence was substituted for a tryptophan, which is likely to influence the gene's ability to produce a functioning protein.

AcrB encodes a subunit of the AcrAB efflux pump, which has links to increased antibiotic resistance.

Observations during industrial chemistry passages (using *S. aureus*)

- SDS reached 7 passages
- Peracetic acid reached just 3 passages before dying out
- Hydrogen peroxide has not been tested as laundries have suggested this is not used
- Chlorine reached 2 passages before the *S. aureus* culture died

Antibiotic susceptibility testing pre and post exposure of *S. aureus* to SDS. Red indicates clinical resistance

Antibiotic	Zone of inhibition (mm)				
	Pre-exposure		Post-exposure		Change in ZoI
	Mean	SD	Mean	SD	
Chloramphenicol 25µg	28.62	0.29	23.23	0.26	-5.39
Erythromycin 5µg	28.78	1.21	25.17	0.17	-3.62
Fusidic acid 10µg	33.28	0.55	29.30	0.62	-3.98
Oxacillin 5µg	26.27	0.34	25.52	0.52	-0.75
Novobiocin 5µg	30.71	0.83	27.49	0.56	-3.23
Penicillin G 1 unit	29.08	0.58	25.05	0.53	-4.03
Streptomycin 10µg	19.33	0.80	17.82	0.29	-1.52
Tetracycline 25µg	33.71	0.70	25.42	0.09	-8.29

Paper

PLOS ONE

RESEARCH ARTICLE

Domestic laundering of healthcare textiles: Disinfection efficacy and risks of antibiotic resistance transmission

Caroline Cayrou, Katie Silver, Lucy Owen[¤], Jake Dunlop, Katie Laird*

Infectious Disease Research Group, Leicester School of Pharmacy, De Montfort University, Leicester, United Kingdom

¤ Current address: ECHA Microbiology Ltd., Units 22 & 23 Willowbrook Technology Park, Cardiff, United Kingdom
* klaird@dmu.ac.uk

Recommendations

Healthcare workers		Healthcare workers employer	
Recommendations	**Reasons**	**Recommendations**	**Reasons**
Do not use short cycles	Short cycles are more likely to underperform	Establish a list of domestic detergents/ supply detergents, that are proven to provide chemical disinfection at room temperature with contact time typical to those found in short cycle wash programmes.	To ensure that disinfection can be achieved in DLMs that do not reach the requested programme temperature in the minimum contact time typically found in DLMs
If relying on thermal killing select cycle temperature >70°C	By targeting 70°C it is more likely that the temperature will at least reach >60°C for 10min	Offer a servicing and performance (temperature monitoring) check of healthcare workers DLM.	Ensuring that healthcare uniforms are being disinfected effectively.
Disinfect and clean DLM regularly with washing machine cleanser and/or by performing a very high temperature empty wash (>90 °C)	Cleaning the DLM regularly aids with reducing biofilm formation	Offer On Premise Laundering (OPL) with a regularly controlled washing machine (performance and biofilm formation) and efficient detergent	By offering OPL the employer will have control over the decontamination performance of healthcare uniforms in term of thermal and chemical disinfection
Do not mix the laundering of uniforms with any other garment.	Avoid the possibility of cross contamination	Outsource the laundering of healthcare uniforms to industrial launders accredited to BS EN 14065:2016. Textiles - Laundry processed textiles - Biocontamination control system [46]	Industrial laundries are obligated to check the performance of their laundering process and ensure disinfection is achieved
Renew the DLM regularly preferably every four years	DLM performance reduces over time and regular renewing of the DLM will ensure that the laundry is performed under optimal conditions	Source uniforms that can be laundered at temperatures at or above 60°C	A combination of thermal and chemical disinfection provides more effective cleaning for healthcare uniforms, but it can only be applied if the uniform's textile can withstand the process without damage
		If laundry is performed on-site without performance controls in place, the DLM should be renewed regularly, ideally every four years.	DLM performance reduces over time and regular renewing of the DLM will ensure that the laundry is performed in optimal conditions

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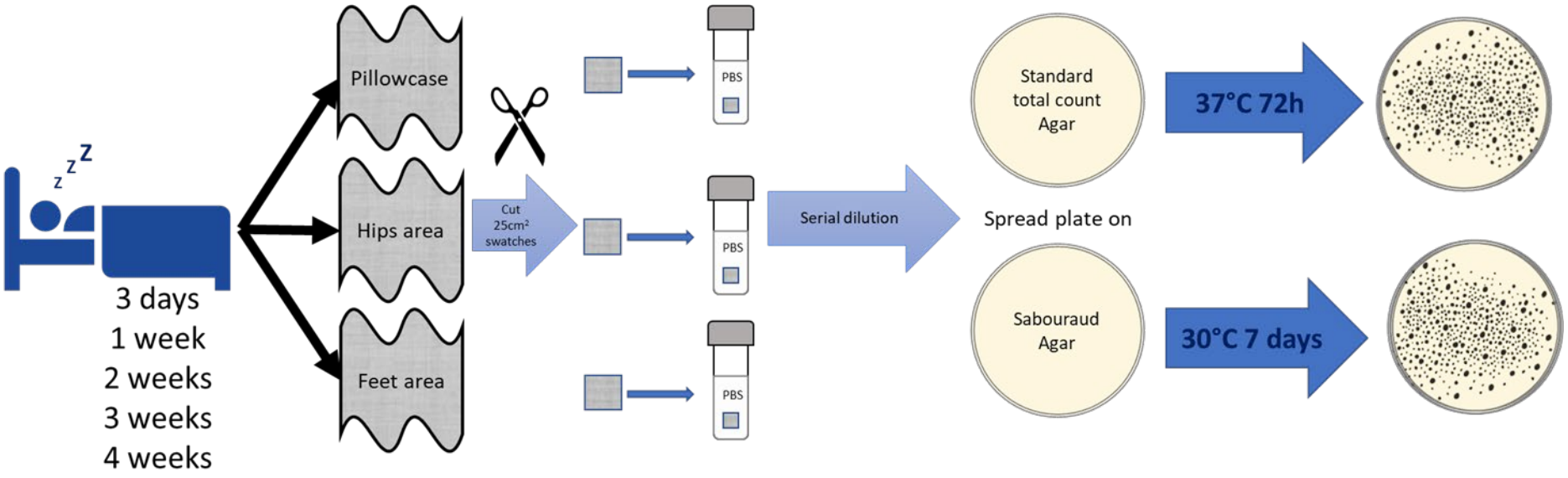
Evolution of Bacterial Colonisation of Bed Linen Over Time

Nine volunteers used clean polycotton (50/50) fitted sheets and pillowcases for a period of 3 days, 1 week, 2 weeks, 3 weeks and 4 weeks.

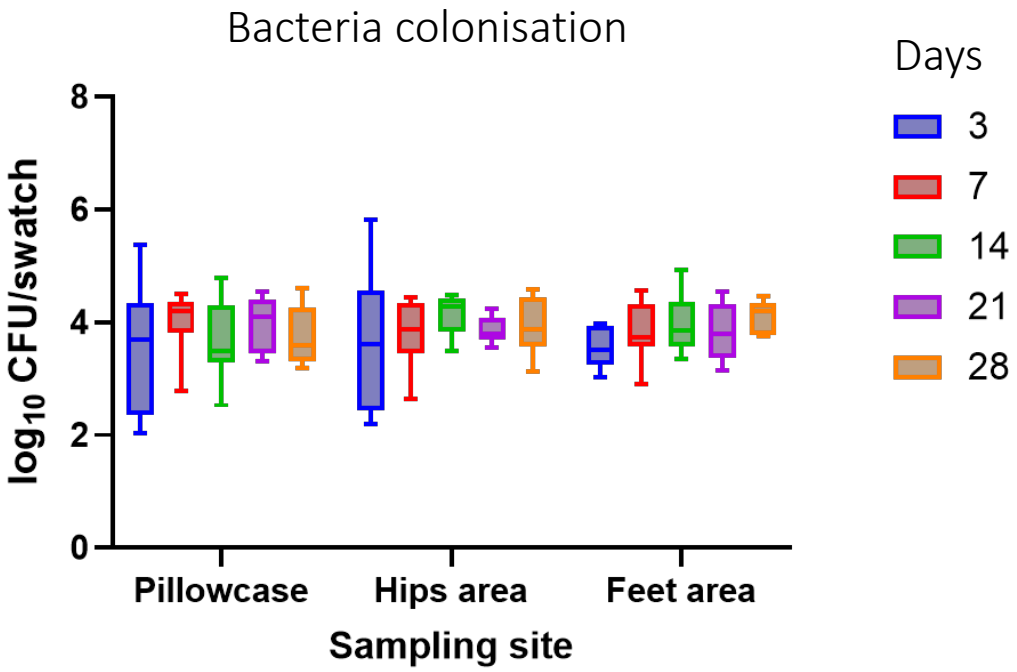


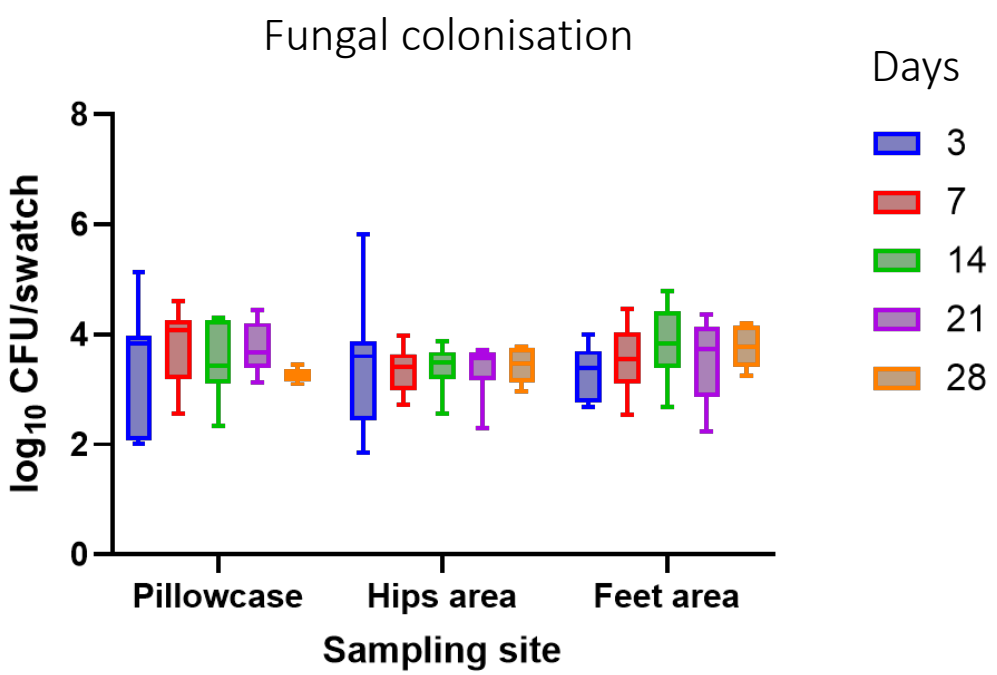
After incubation the higher concentration plate was washed with 20% glycerol stock (in NB) and store at-80°C

DNA was extracted from 81 bacteria samples and 81 Fungi samples from 3 days, 1 week and 4 weeks time points.

The DNA was used for 16S and ITS full amplicon metagenomic analysis

Results: Viable counts

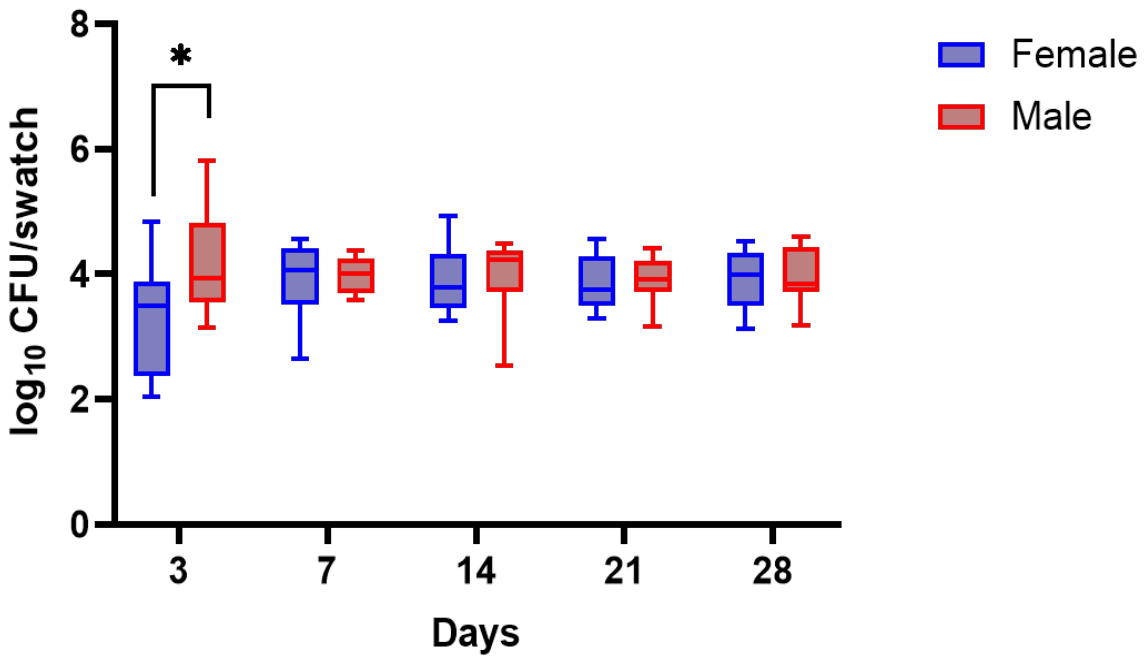




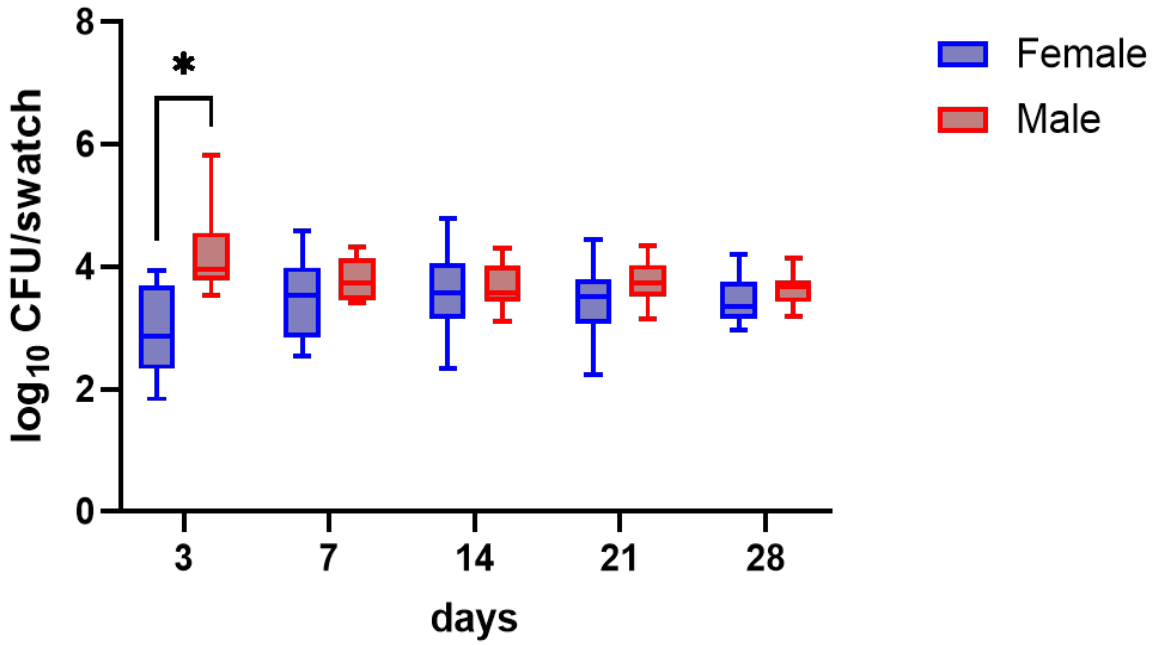
- The bacterial colonisation was in average 3.8 $\log_{10}$ CFU/swatch (2 to 5.8 $\log_{10}$ CFU/ swatch)
- Fungal colonisation was in average 3.5 $\log_{10}$ CFU/ swatch (1.8 to 5.8 $\log_{10}$ CFU/ swatch)
- No significant change of the microbial colonisation level over time. However a trend of lower level of colonisation at 3 days is observed for the feet area.

Results: viable counts and gender

Bacteria colonisation

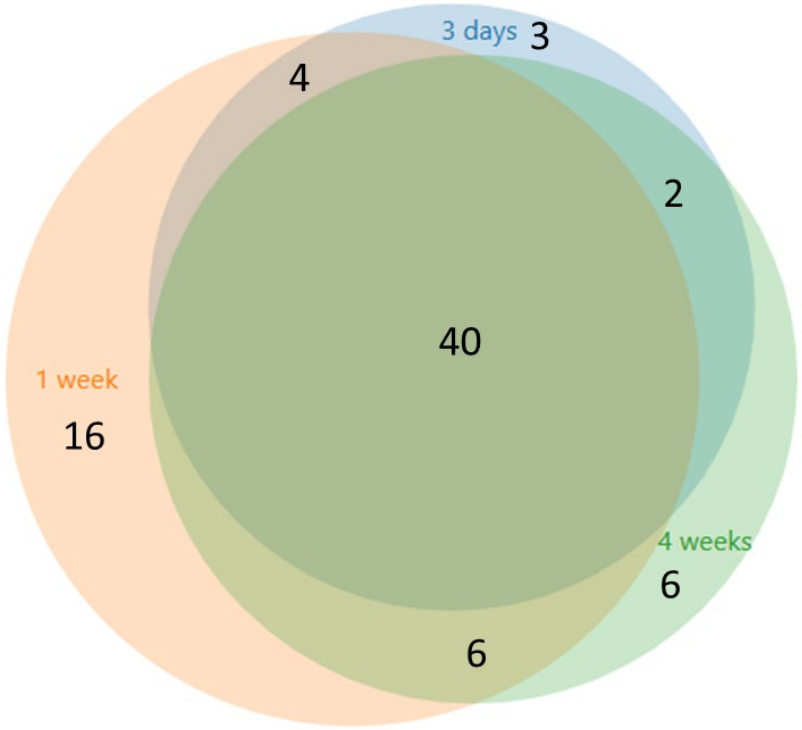


fungal colonisation



- At 3 days female volunteers exhibited significantly lower level of bed linen colonisation than male volunteers.

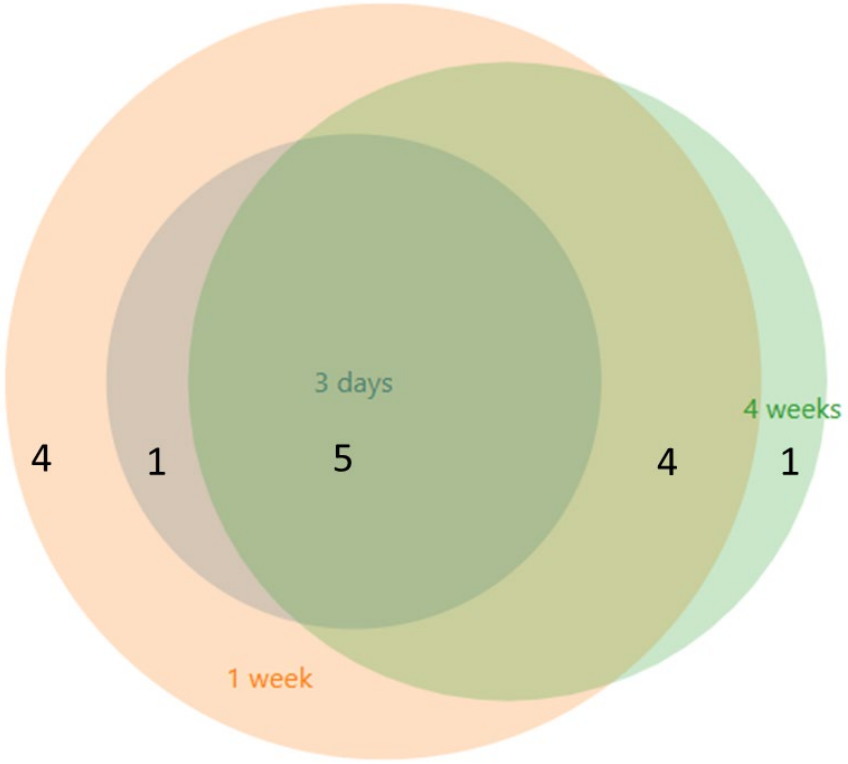
Results: Bacteria identification



Bacterial species present in ≥80% of samples at each time point

- In total (among the 81 samples) 1251 bacterial species were identified.
- 51 species are detected in at least 80 % (N≥65 samples) of the samples.
- Among those 51 species, 7 species are the one exhibiting the highest DNA concentration (Higher presence in one sample) and frequency (present in the highest number of samples).
- Those species belonged to *Staphylococcus* (4 species), *Streptococcus*, *Moraxella* and *Kocuria* genera.
- When considering the species identified at each time point 25 species seems to be associated with particular time point (Venn diagram)

Results: fungal identification



Fungal species present in ≥50% of samples at each time point

- In total (among the 81 samples) 293 bacterial species were identified.
- 3 species are detected in at least 80 % (N≥65 samples) of the samples.
- Among those 3 species, one specie is exhibiting the highest DNA concentration (Higher presence in one sample) and frequency (present in the highest number of samples).
- The species detected the most is *Naganishia diffluens*.
- When considering the species identified at each time point 5 species seems to be associated with particular time point (Venn diagram).
- All species present at 3 days were present at later time points.

Conclusions

- Microorganisms are able to survive on textiles and through domestic wash cycles
- Domestic washing machines often do not perform to their set thermal disinfection
- Domestic washing machines harbour antibiotic resistant bacteria
- Resistance to non-biological washing detergents leads to cross resistance to antibiotics
- It is recommended that bedsheets are changed within the healthcare setting on every fourth day if the sheets remain unsoiled and the patient is not susceptible to infection.

Questions?