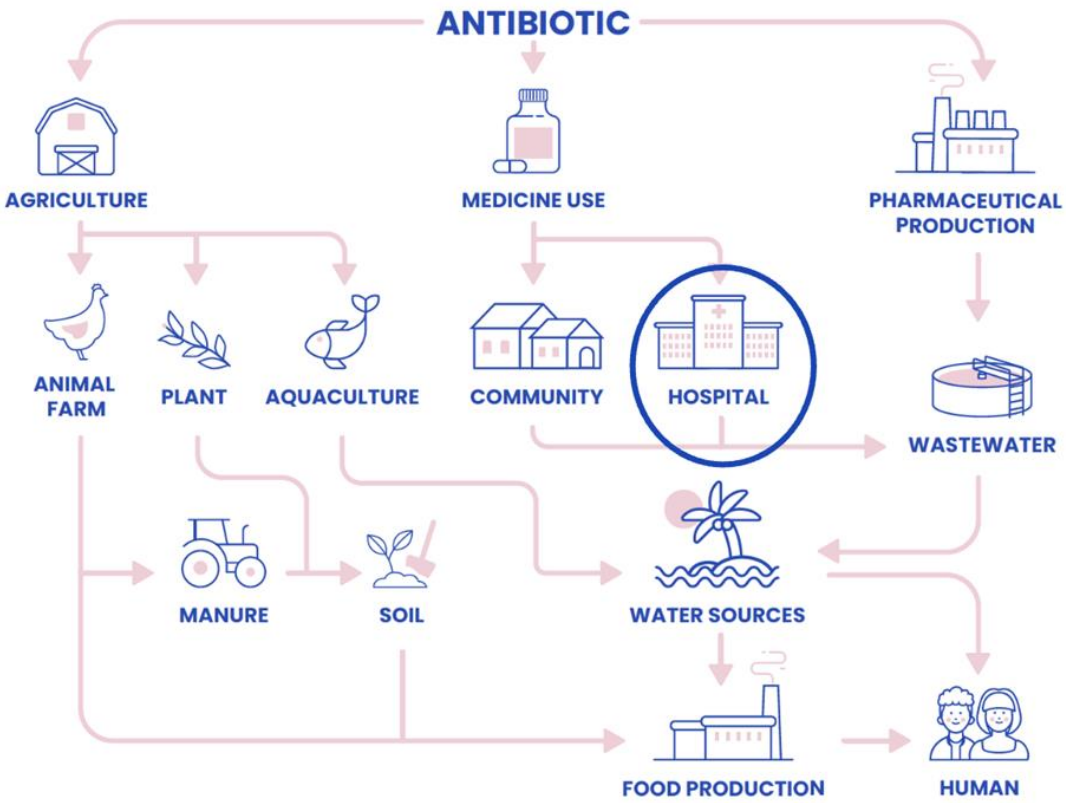
resistomap

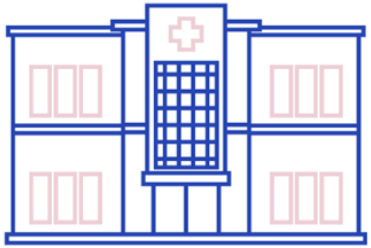
Fighting Antibiotic Resistance in the Environment



Antibiotic resistance spread in the environment

Wastewater-based monitoring of antibiotic resistance and pathogens in hospitals

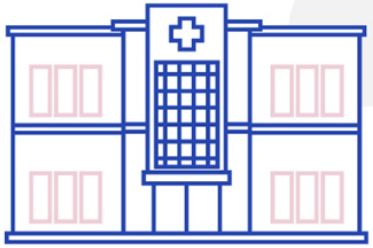
Research Objectives



Triangle Hospital (HUS1)

193
beds

2249
(DDD*/ 1000 patients)

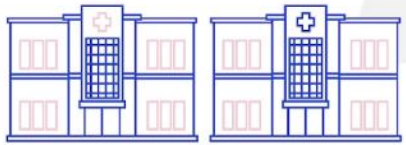


New Children's Hospital (HUS2)

125
beds

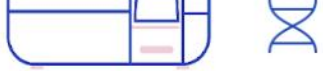
351
(DDD*/ 1000 patients)

Methodology

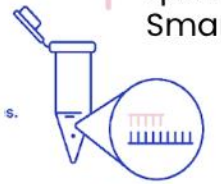


1) Wastewater sample collection

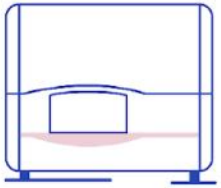
2) Sample concentration



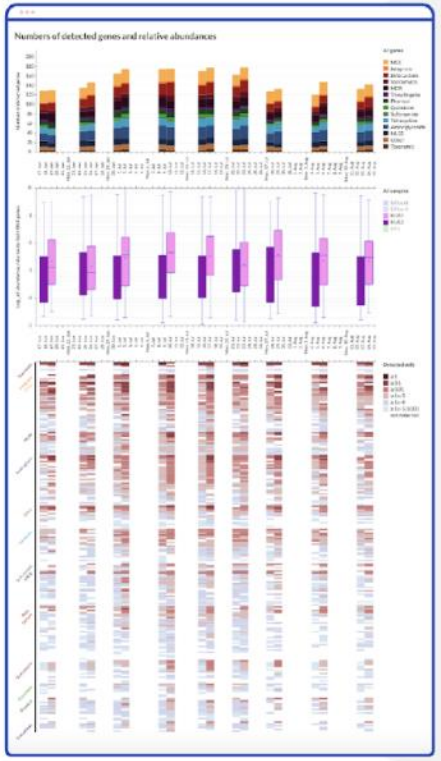
3) Environmental DNA extraction



4) 216 gene quantification - SmartChip qPCR



5) ResistApp: Data analysis and visualisation



Wastewater Sample Collection

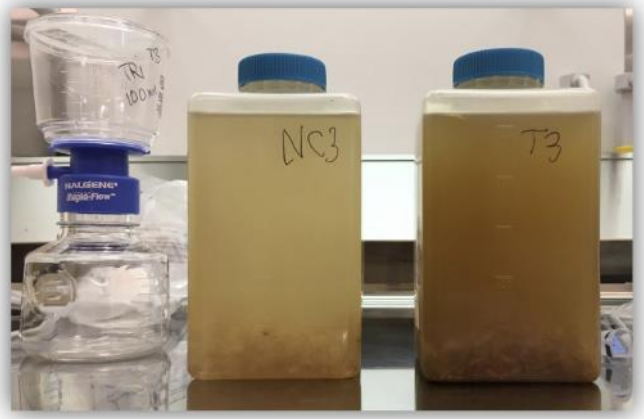
June 2020 — July 2020 — August 2020

Weekly

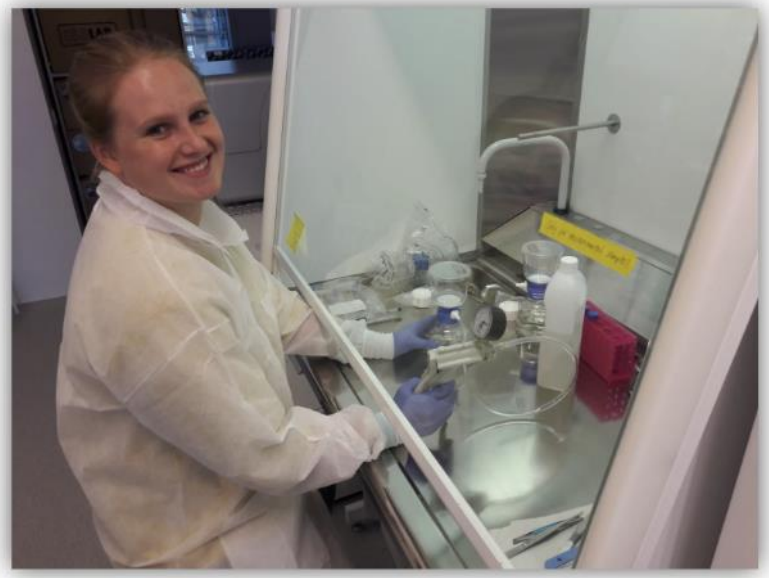
at 08:00 am

Jesse Majlander,
Operations Manager

Wastewater Sample
Concentration



100 ml of wastewater sample
Nalgene disposable filter units with
PES membrane
(pore size: **0,22 µm**, diameter: 45 mm)



Alma Seppälä,
R&D Scientist

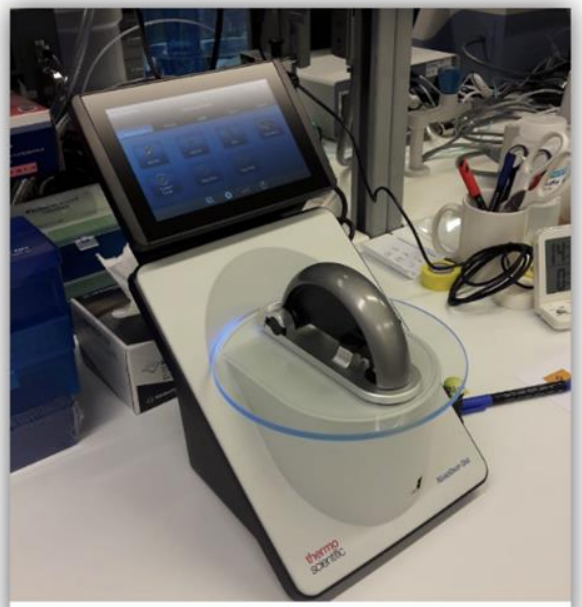
- Each filter is stored in 50 ml Falcon tube
- Store in -20°C

Environmental DNA
Extraction

DNA Isolation Kit

QIACube

DNA Measurement



NonoDrop One

Optimal measurement for the analysis

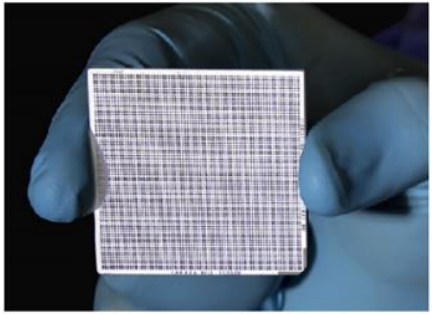
- 1.7-2.1 (DNA quality)
- 10 ng/ul (DNA concentration)
- 100 ul (DNA Volume)

Sample Metadata

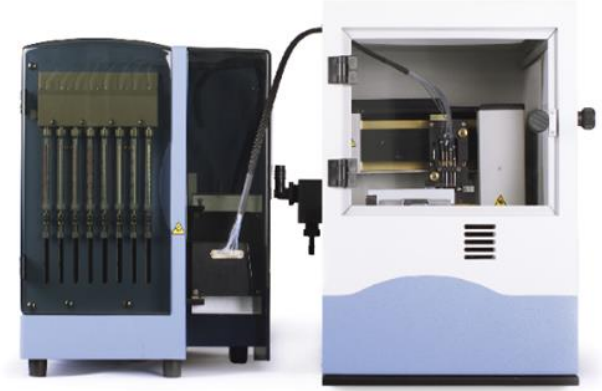
	A	B	C	D	E	F	G	H	I	J
1	* Indicates required fields.									
2										
3	Tube label									
4	Sample number	Bio replica	*Sample description	*Sample type	*DNA quality (1.8 – 2 ±0.1, 260/280 ratio)	*DNA concentration (10 ng/µl)	*Volume (100 µl)	*Sample collection date (2020-12-31)	Country	Latitude, longitude (60.170451, 24.939575)
5	49	A	HUS1	Waste water	1.85	10.00	100.00	2020-06-18 08:00	Finland	60.188819, 24.907161
6	50	A	HUS1	Waste water	1.89	10.00	100.00	2020-06-25 08:00	Finland	60.188819, 24.907161
7	51	A	HUS1	Waste water	1.88	10.00	100.00	2020-07-01 08:00	Finland	60.188819, 24.907161
8	52	A	HUS1	Waste water	1.95	10.00	100.00	2020-07-09 08:00	Finland	60.188819, 24.907161
9	53	A	HUS1	Waste water	1.84	10.00	100.00	2020-07-16 08:00	Finland	60.188819, 24.907161
10	54	A	HUS1	Waste water	1.86	10.00	100.00	2020-07-22 08:00	Finland	60.188819, 24.907161
11	55	A	HUS1	Waste water	1.85	10.00	100.00	2020-07-28 08:00	Finland	60.188819, 24.907161
12	56	A	HUS1	Waste water	1.83	10.00	100.00	2020-08-05 08:00	Finland	60.188819, 24.907161
13	57	A	HUS1	Waste water	1.86	10.00	100.00	2020-08-13 08:00	Finland	60.188819, 24.907161
14	66	A	HUS2	Waste water	1.83	10.00	100.00	2020-06-18 08:00	Finland	60.187173, 24.910124
15	67	A	HUS2	Waste water	1.90	10.00	100.00	2020-06-25 08:00	Finland	60.187173, 24.910124
16	68	A	HUS2	Waste water	1.80	10.00	100.00	2020-07-01 08:00	Finland	60.187173, 24.910124
17	69	A	HUS2	Waste water	1.86	10.00	100.00	2020-07-09 08:00	Finland	60.187173, 24.910124
18	70	A	HUS2	Waste water	1.84	10.00	100.00	2020-07-16 08:00	Finland	60.187173, 24.910124
19	71	A	HUS2	Waste water	1.88	10.00	100.00	2020-07-22 08:00	Finland	60.187173, 24.910124
20	72	A	HUS2	Waste water	1.84	10.00	100.00	2020-07-28 08:00	Finland	60.187173, 24.910124
21	73	A	HUS2	Waste water	1.87	10.00	100.00	2020-08-05 08:00	Finland	60.187173, 24.910124
22	74	A	HUS2	Waste water	1.85	10.00	100.00	2020-08-13 08:00	Finland	60.187173, 24.910124
23	104	A	NTC-A	Other	0.00	0.00	100.00	2020-06-07	Finland	
24	105	A	NTC-B	Other	0.00	0.00	100.00	2020-06-07	Finland	
25										

Sample specification | ARG selection

Gene Quantification



5,184 100-nl
reactions/chip



Sample/ Genes Assays
dispense in <1 hour

Thermal cycle and collect data
in 2.25 hours

SmartChip qPCR (Takara Bio)

Flexible Configuration

Flexibility of the SmartChip™ platform allows for high gene-content screening and high-throughput monitoring configurations

Genes	12	24	36	48	54	72	80	96	120	144	216	248	296	384
Samples	128	72	48	36	32	24	21	18	14	12	8	6	5	4

Primer Set Update

OXFORD FEMS JOURNALS investing in science

FEMS Microbiology Ecology, 94, 2018, fiy130

doi: 10.1093/femsec/fiy130
Advance Access Publication Date: 23 July 2018
Research Article

RESEARCH ARTICLE

Primer set 2.0 for highly parallel qPCR array targeting antibiotic resistance genes and mobile genetic elements

Robert D. Stedtfeld[1,†], Xueping Guo[2,3,4,†], Tiffany M. Stedtfeld[1], Hongjie Sheng[3,4,6], Maggie R. Williams[1], Kristin Hauschild[4], Santosh Gunturu[4], Leo Tift[4], Fang Wang[3,4,6], Adina Howe[5], Benli Chai[4], Daqiang Yin[2], James R. Cole[3,4], James M. Tiedje[3,4] and Syed A. Hashsham[1,3,4,*]

[1]Department of Civil and Environmental Engineering, Michigan State University, East Lansing, Michigan 48824, USA, [2]State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai, China, [3]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, Michigan 48824, USA, [4]Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824, USA, [5]Department of Agricultural and Biosystems Engineering, Iowa

Primer Sets - ARG 2.1 (2023)

- Insilico test against updated database (NCBI, CARD, ResFinder)
- NTC Analysis (false positives)
- Tm Analysis (melting curve)
- Add primer sets for group of bacteria including **three for HAI Pathogens**

Selected	Assay	Gene	Target antibiotics (major)
☐	AY473	A. baumannii	Taxanomic
☐	AY474	Bacteroidetes	Taxanomic
☐	AY475	Campylobacter	Taxanomic
☐	AY476	Enterococci	Taxanomic
☐	AY477	Firmicutes	Taxanomic
☐	AY478	K. pneumoniae	Taxanomic
☐	AY479	P. aeruginosa	Taxanomic
☐	AY480	Staphylococci	Taxanomic

Gene Selection

Target Gene by Group	Number of Primer Sets
16S rRNA gene	2
Aminoglycoside	79
Beta Lactam	106
Integrons	8
MDR	73
MGE	53
MLSB	80
Phenicol	26
Quinolone	11
Sulfonamide	12
Taxonomic	20
Tetracycline	57
Trimethoprim	19
Vancomycin	41
Other	33
Total	**620**

Chip Customisation

Target Gene by Group	Number of Primer Sets
16S rRNA gene	1
Aminoglycoside	30
Beta Lactam (Carbapenem)	32 (8)
Integrons	4
MDR	20
MGE	28
MLSB	15
Phenicol	13
Quinolone	8
Sulfonamide	4
Taxonomic (HAI Pathogens)	8 (3)
Tetracycline	20
Trimethoprim	7
Vancomycin	13
Other	13
Total	**216**

SmartChip qPCR Raw Data

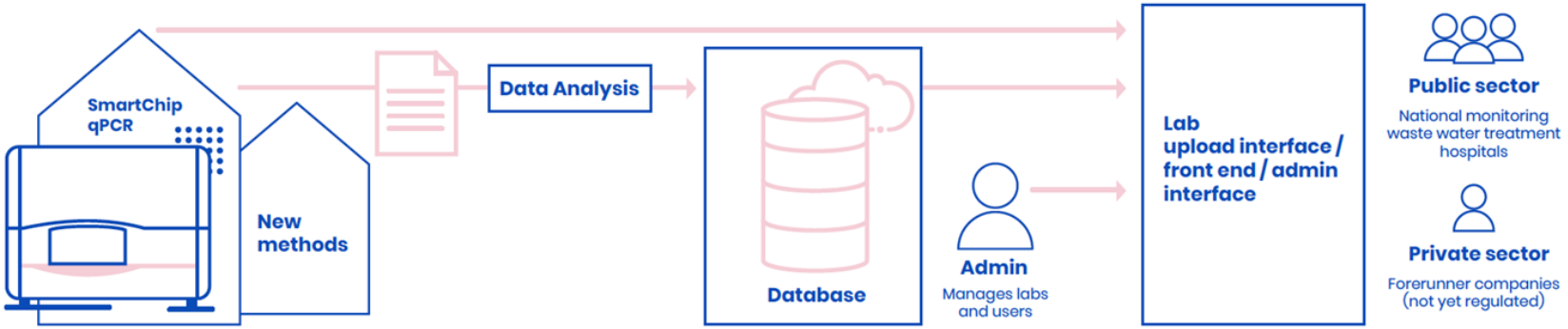
Chip1.txt

Row	Column	Assay	Sample	Conc	Ct	Tm	Efficiency	Flags
0	0	AY1	1A-rep1	-1	13.97	81.89	1.97	
1	0	AY1	2A-rep2	-1	15.05	81.98	1.98	
2	0	AY1	3A-rep3	-1	14.38	82.22	1.95	
3	0	AY1	5A-rep1	-1	13.50	82.26	1.98	
4	0	AY1	11A-rep3		-1	13.65	82.57	1.98
5	0	AY1	13A-rep1		-1	14.54	82.06	2.04
6	0	AY1	14A-rep2		-1	15.25	81.91	1.94
7	0	AY1	15A-rep3		-1	13.93	82.24	1.99
8	0	AY1	22A-rep2		-1	14.07	82.23	1.96
9	18	AY1	1A-rep2	-1	14.51	82.16	1.98	
10	18	AY1	2A-rep3	-1	15.41	82.04	1.97	
11	18	AY1	4A-rep1	-1	13.72	82.24	1.99	
12	18	AY1	5A-rep2	-1	13.68	82.18	2.00	
13	18	AY1	12A-rep1		-1	18.92	81.88	1.96
14	18	AY1	13A-rep2		-1	14.54	81.85	2.06
15	18	AY1	14A-rep3		-1	15.58	81.92	1.99
16	18	AY1	16A-rep1		-1	12.00	81.98	2.01
17	18	AY1	22A-rep3		-1	13.98	82.04	1.98
18	36	AY1	1A-rep3	-1	14.46	82.00	1.98	
19	36	AY1	3A-rep1	-1	14.37	82.01	1.97	
20	36	AY1	4A-rep2	-1	13.49	82.12	1.99	
21	36	AY1	5A-rep3	-1	13.68	82.07	2.01	
22	36	AY1	12A-rep2		-1	18.71	81.72	1.94
23	36	AY1	13A-rep3		-1	14.45	81.72	2.05
24	36	AY1	15A-rep1		-1	13.66	81.93	2.00
25	36	AY1	16A-rep2		-1	11.90	81.85	1.99
26	36	AY1	23A-rep1		-1	13.48	81.85	2.01
27	54	AY1	2A-rep1	-1	15.10	81.85	1.97	
28	54	AY1	3A-rep2	-1	14.14	81.92	1.99	

Automated Data Analysis

ResistApp
Cloud system
Python program

Data Flow


SmartChip
qPCR
New
methods
Data Analysis
Database
Admin
Manages labs
and users
Lab
upload interface /
front end / admin
interface
Public sector
National monitoring
waste water treatment
hospitals
Private sector
Forerunner companies
(not yet regulated)

Data Access

resistomap

HOME LOGIN

Email Address *

windi@resistomap.com

Password *

••••••••••••

I accept the privacy policy & anonymous usage tracking.

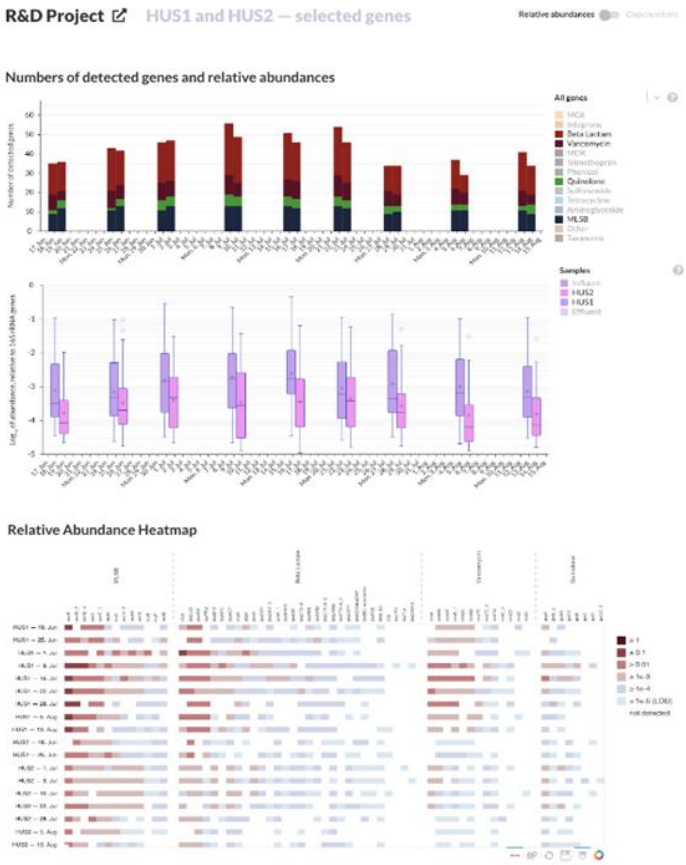
Log In

Resistomap oy
Cultivator II, Viikinkaari 4, Helsinki

Data Output

ResistApp
Interactive Web Online Report

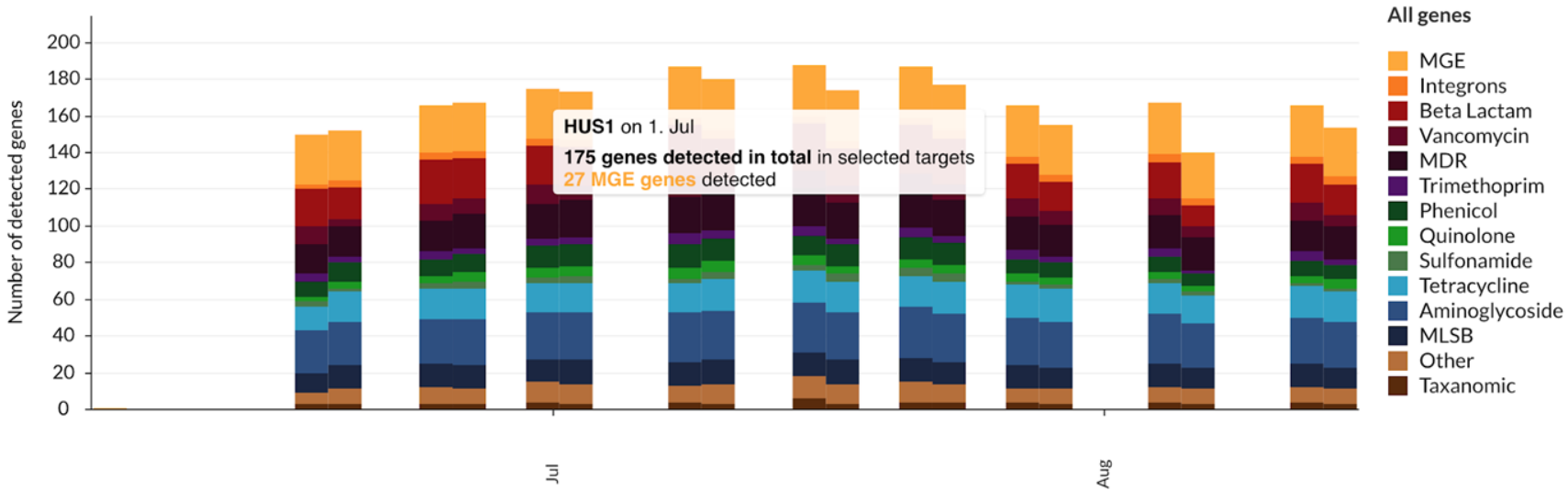
- ✔ Numbers of detected genes
- ✔ Genes relative abundances
- ✔ Genes copy numbers
- ✔ Heatmap



Antibiotic resistance monitoring in hospital wastewater

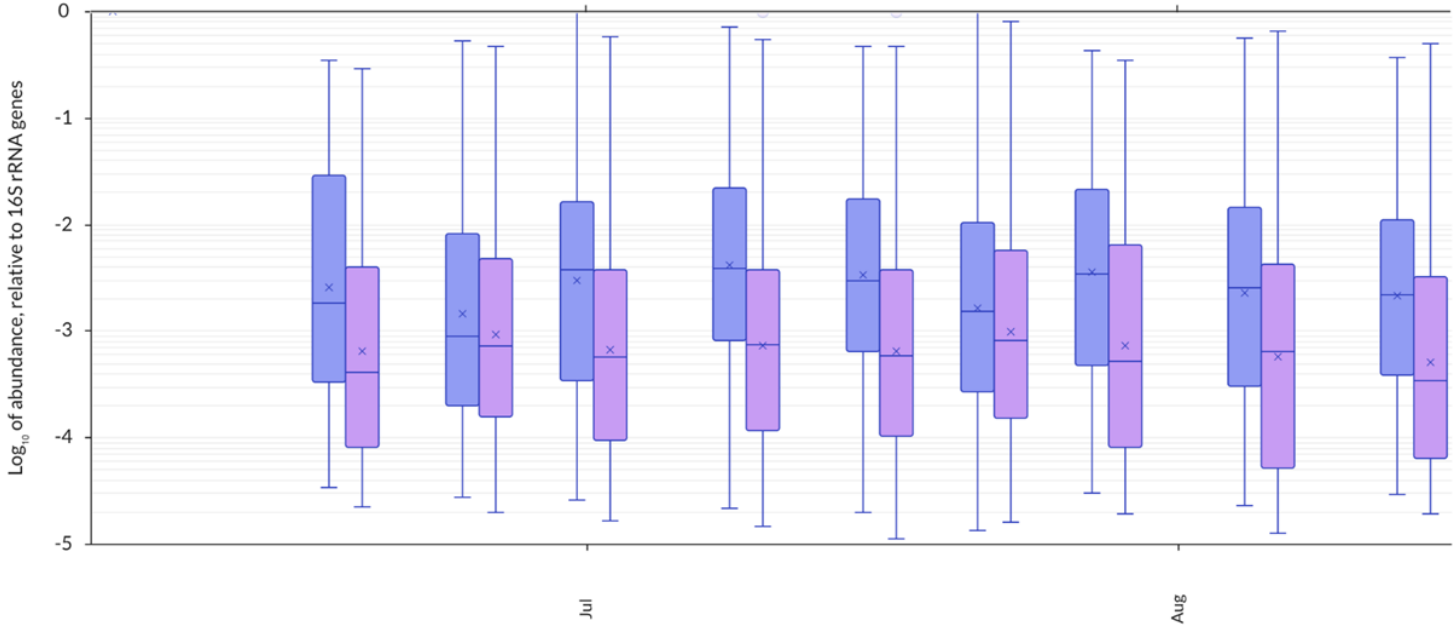
Relative abundances Copy numbers

Numbers of detected genes and relative abundances



Antibiotic resistance monitoring in hospital wastewater

Relative abundances Copy numbers

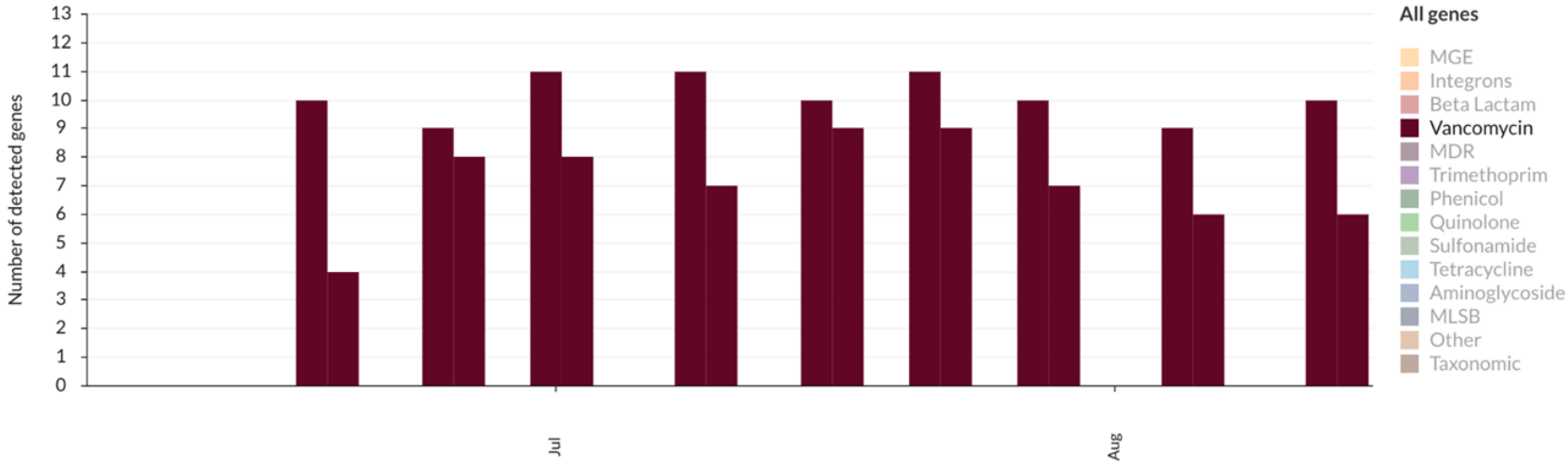


Antibiotic resistance monitoring in hospital wastewater

Vancomycin

Relative abundances | Copy numbers

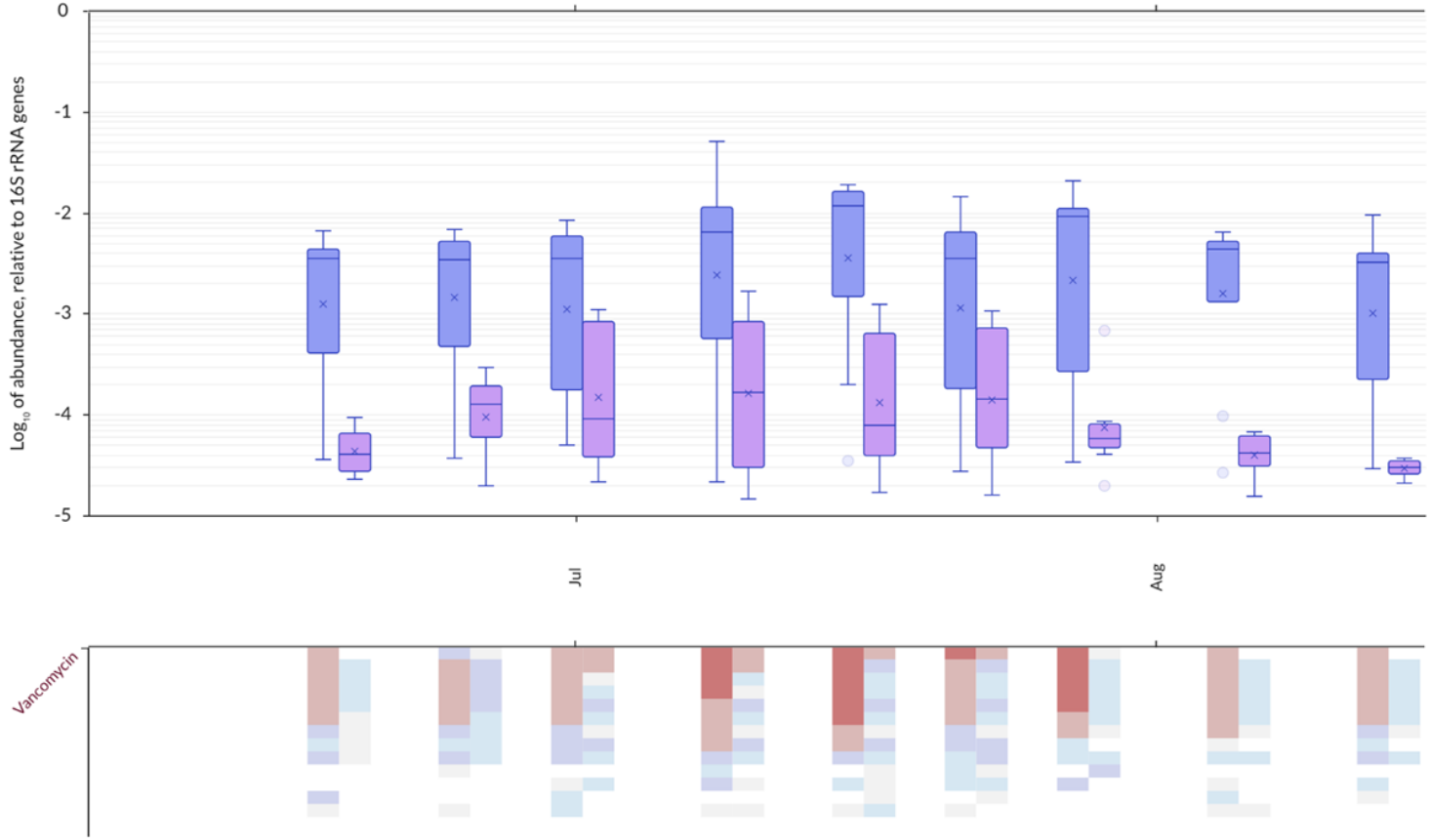
Numbers of detected genes and relative abundances



Antibiotic resistance monitoring in hospital wastewater

Vancomycin

Relative abundances | Copy numbers



All samples

- HUS1
- HUS2
- NTC-A
- NTC-B

All analyzed genes

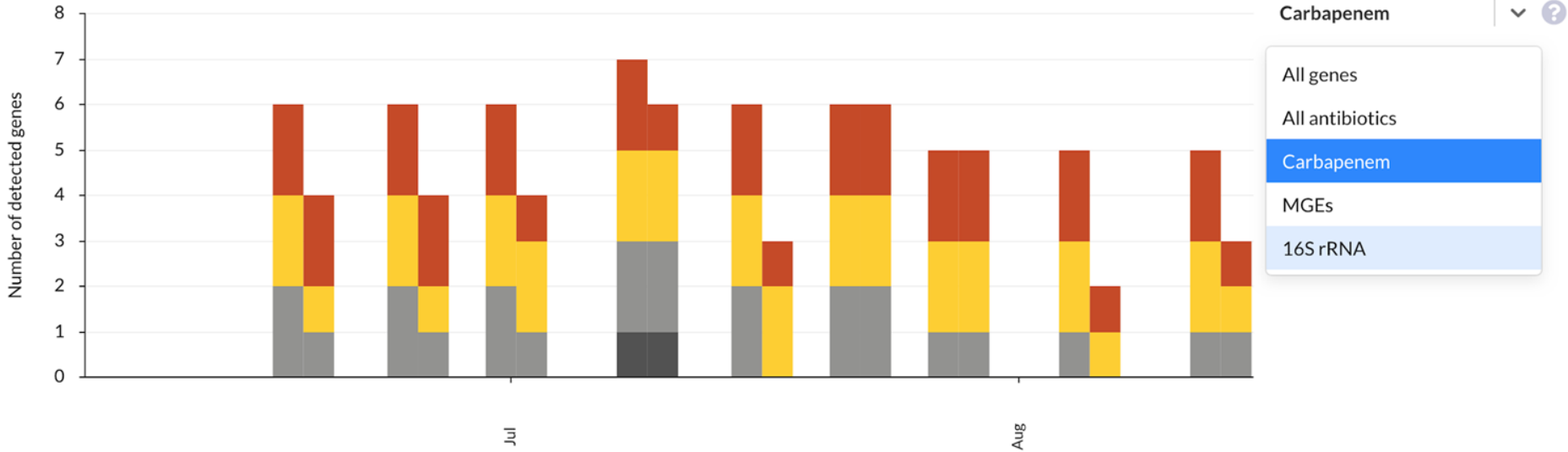
- ≥ 1
- ≥ 0.1
- ≥ 0.01
- ≥ 1e-3
- ≥ 1e-4
- ≥ 1e-5 (LOD)
- traces
- not detected

The colors in the heatmap show gene abundances relative to 16S rRNA gene.

Antibiotic resistance monitoring in hospital wastewater

Carbapenem Relative abundances Copy numbers

Numbers of detected genes and copy numbers



Antibiotic resistance monitoring in hospital wastewater

Carbapenem

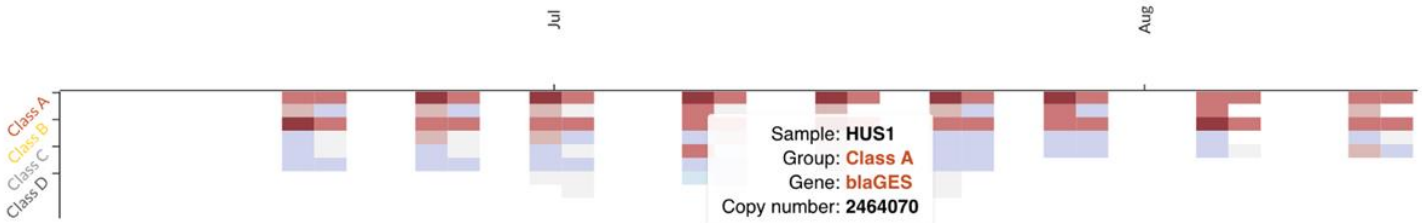
Relative abundances — Copy numbers

The colors in the heatmap show gene abundances relative to 16S rRNA gene.

Antibiotic resistance monitoring in hospital wastewater

Carbapenem

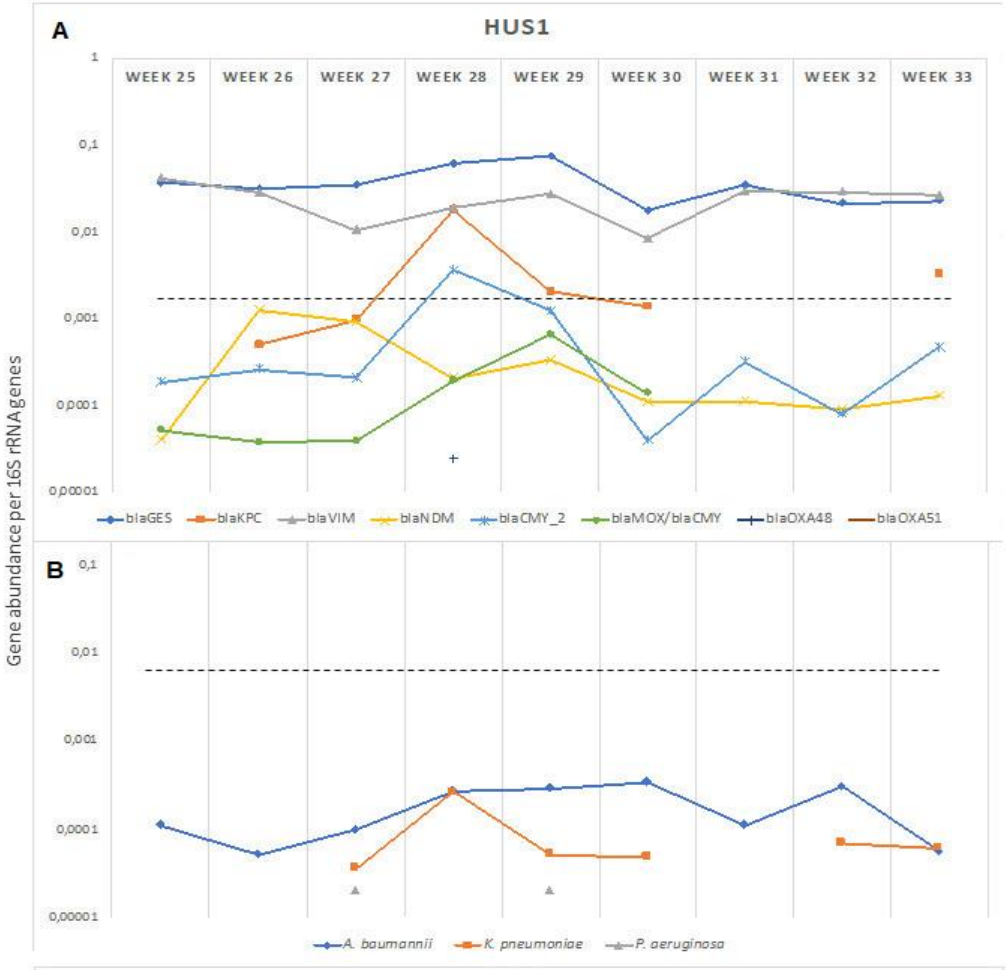
Relative abundances — Copy numbers



The colors in the heatmap show an estimation of gene copy numbers.

Carbapenem resistance gene abundances in HUS 1

- Positive correlation between ***bla*KPC and *K. pneumoniae***
- Spearman's ρ = 0.99, p-value <0.01



Data Publication

ELSEVIER

Journal of Hospital Infection

Volume 117, November 2021, Pages 157-164

Routine wastewater-based monitoring of antibiotic resistance in two Finnish hospitals: focus on carbapenem resistance genes and genes associated with bacteria causing hospital-acquired infections

J. Majlander [a], V-J. Anttila [b], W. Nurmi [a], A. Seppälä [a], J. Tiedje [c], W. Muziasari [a]

Show more

resistomap

BUSINESS FINLAND
Project No. 287/31/2020

HUS Helsinki University Hospital

FINNPARTNERSHIP

BUSINESS PARTNERSHIP SUPPORT (GRANT)

MATCHMAKING SERVICE

GLOBAL GATEWAY CAMPAIGN

Data Publication

Tropical Medicine and Infectious Disease

MDPI

Article

Prevalence and Abundance of Beta-Lactam Resistance Genes in Hospital Wastewater and Enterobacterales Wastewater Isolates

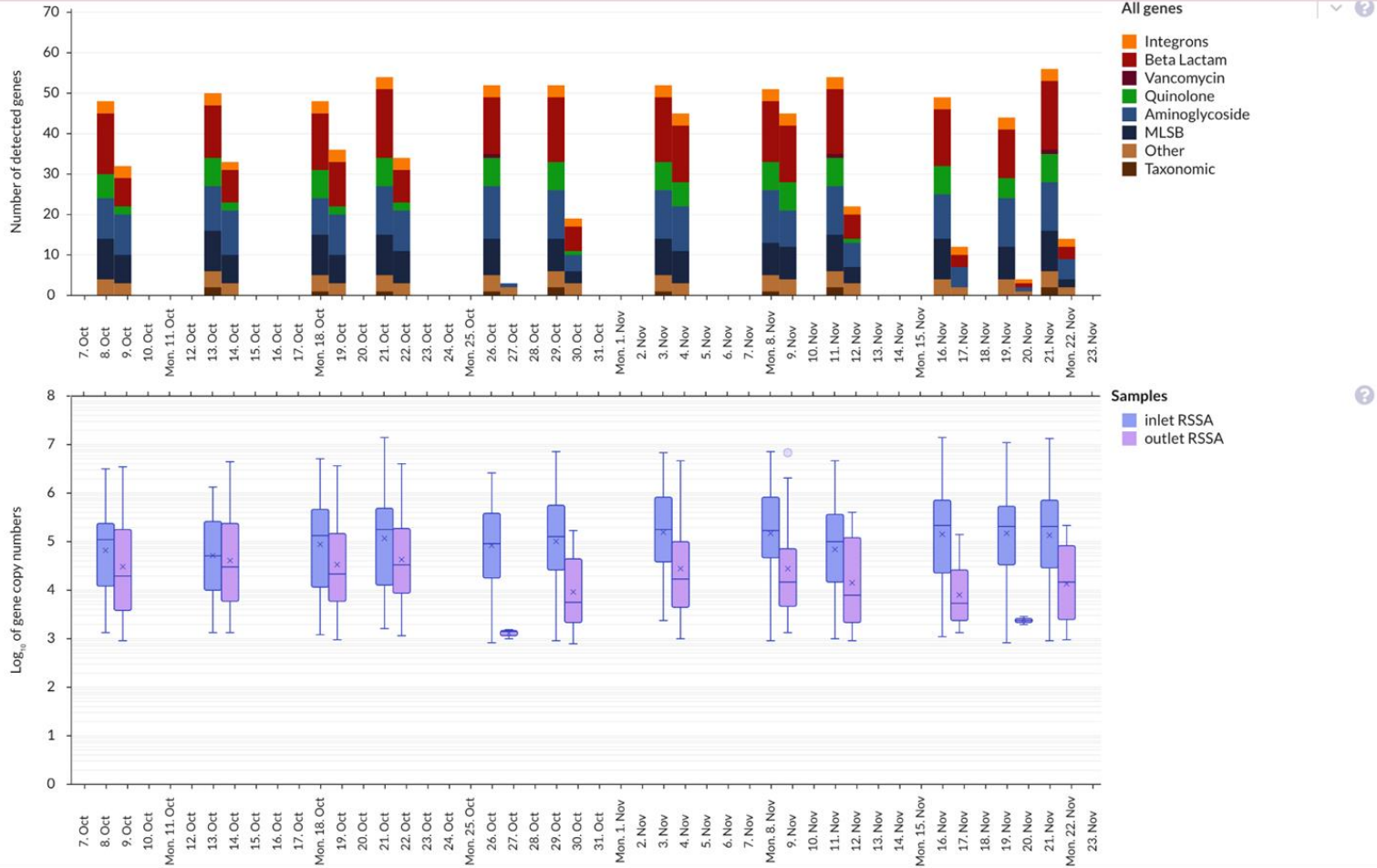
Dewi Santosaningsih [1,2,*], Aulia Putri Fadriyana [3], Nathanael Ibot David [3] and Irene Ratridewi [4,5]

1 Department of Clinical Microbiology, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia
2 Department of Clinical Microbiology, Dr. Saiful Anwar Hospital, Malang 65112, Indonesia
3 Biomedical Sciences Master Program, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia
4 Department of Pediatrics, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia
5 Department of Pediatrics, Dr. Saiful Anwar Hospital, Malang 65112, Indonesia
* Correspondence: dewi.santosa@ub.ac.id

Abstract: Antimicrobial resistance may develop in nature including in hospital wastewater through horizontal genetic transfer. Few studies were conducted on the antimicrobial resistance genes in hospital wastewater and wastewater isolates in Indonesia. The prevalence and abundance of beta-lactam

127.3 - Pilot Indonesia 2 RSSA 72 Genes

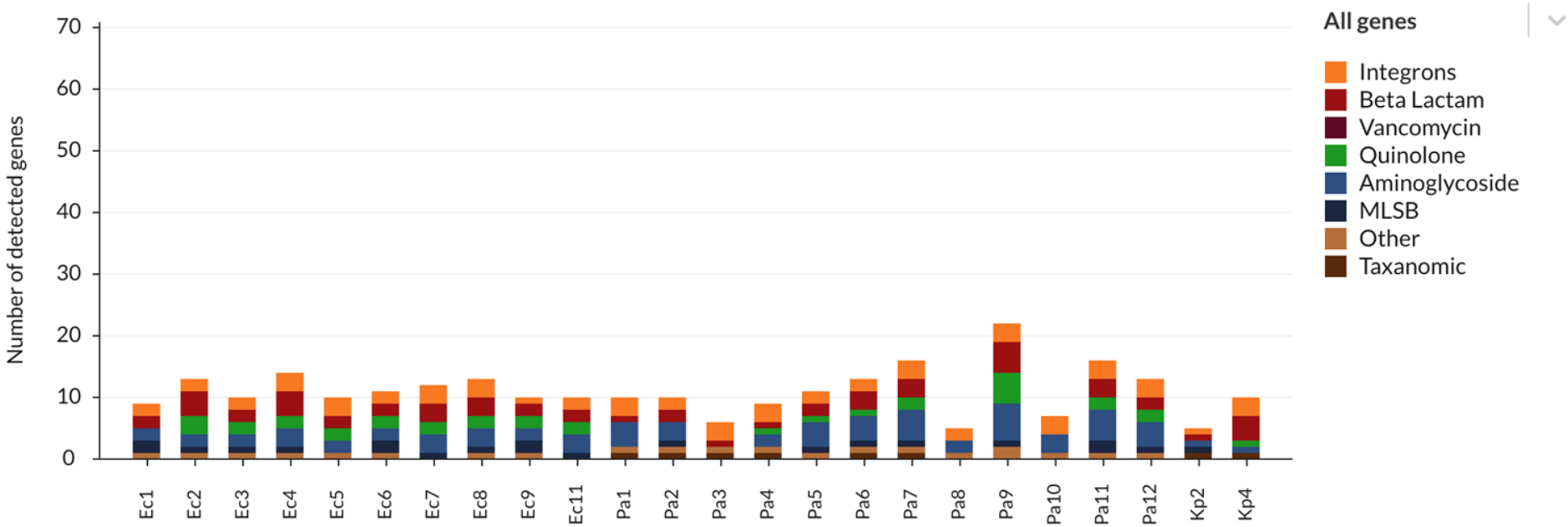
Relative abundances | Copy numbers

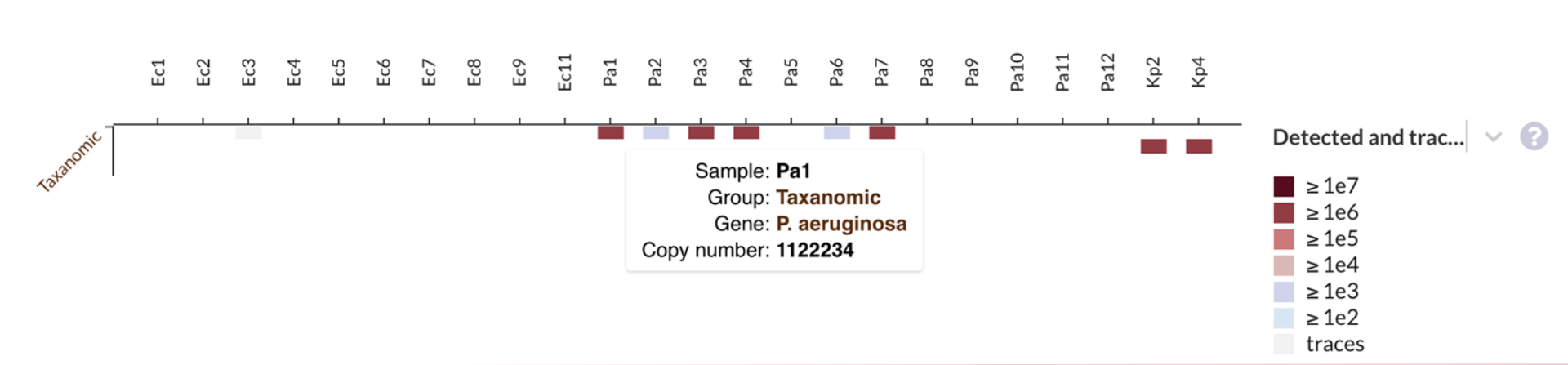


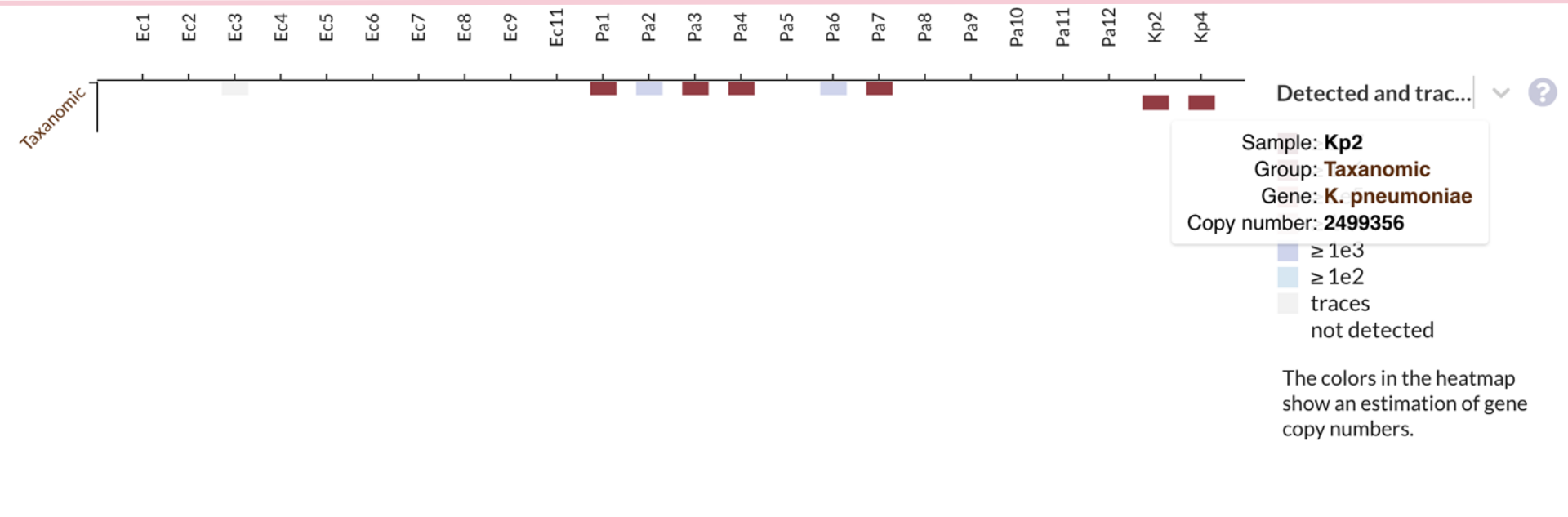
127 - RSSA 72 Genes Pathogens

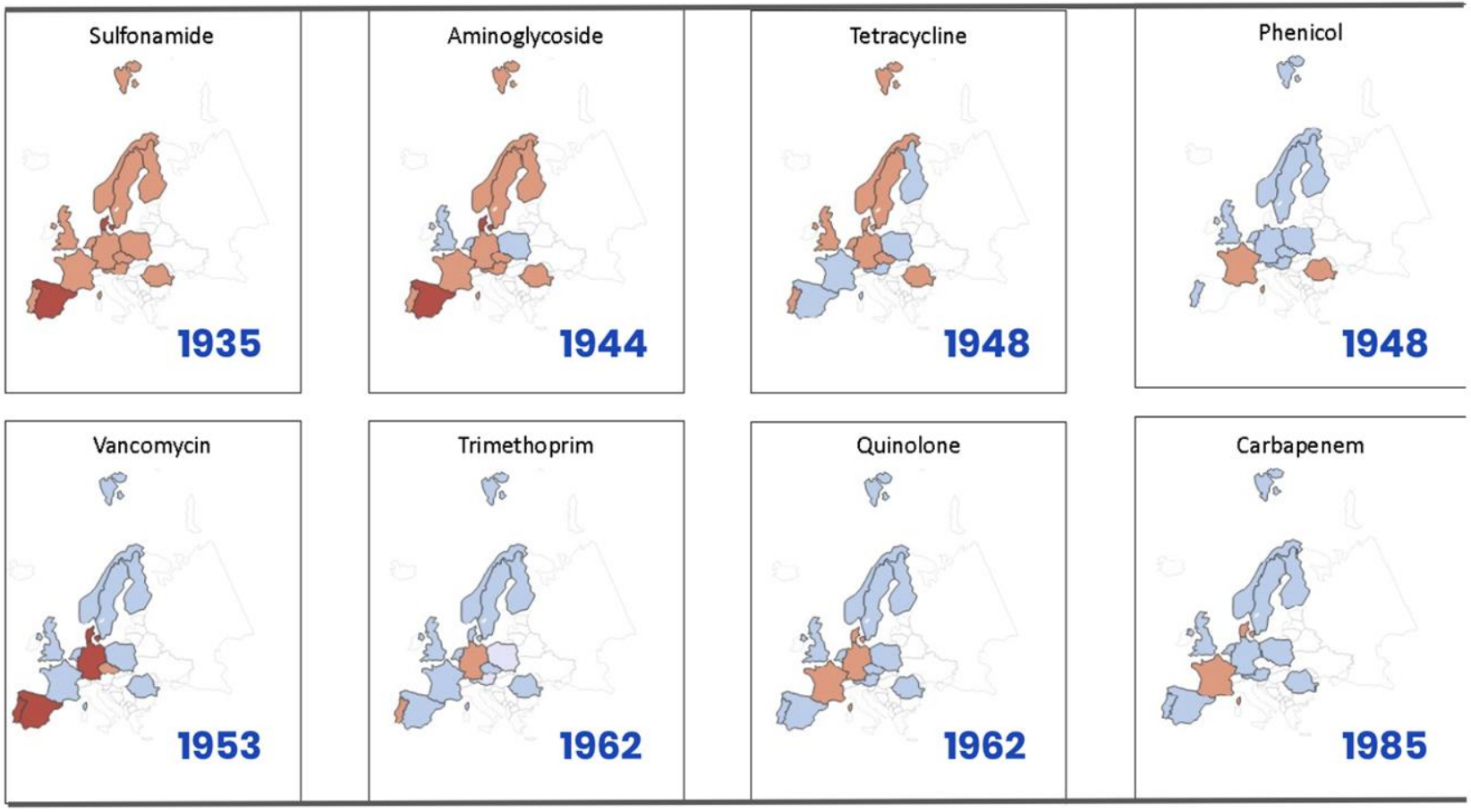
Relative abundances | Copy numbers

Numbers of detected genes and copy numbers




Ec1
Ec2
Ec3
Ec4
Ec5
Ec6
Ec7
Ec8
Ec9
Ec11
Pa1
Pa2
Pa3
Pa4
Pa5
Pa6
Pa7
Pa8
Pa9
Pa10
Pa11
Pa12
Kp2
Kp4
Taxanomic
Sample: Pa1
Group: Taxanomic
Gene: P. aeruginosa
Copy number: 1122234
Detected and trac...
≥ 1e7
≥ 1e6
≥ 1e5
≥ 1e4
≥ 1e3
≥ 1e2
traces


Ec1
Ec2
Ec3
Ec4
Ec5
Ec6
Ec7
Ec8
Ec9
Ec11
Pa1
Pa2
Pa3
Pa4
Pa5
Pa6
Pa7
Pa8
Pa9
Pa10
Pa11
Pa12
Kp2
Kp4
Taxanomic
Sample: Kp2
Group: Taxanomic
Gene: K. pneumoniae
Copy number: 2499356
Detected and trac...
≥ 1e3
≥ 1e2
traces
not detected
The colors in the heatmap show an estimation of gene copy numbers.


Sulfonamide
1935
Aminoglycoside
1944
Tetracycline
1948
Phenicol
1948
Vancomycin
1953
Trimethoprim
1962
Quinolone
1962
Carbapenem
1985
Median abundance, relative to 16S rRNA
≥ 1
≥ 0.1
≥ 0.01
≥ 1e-3
≥ 1e-4
≥ 1e-5 (LOD)
no data

What's Next?

Who will use the data?

How the data will be used?

What and where to monitor?

THANK YOU

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