Monitoring Antibiotic Resistance Gene Profiles in Hospital Wastewater – a Proof-of-Concept Study Using ResistApp

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Tiivistelmä — Referat — Abstract

The objective of the study was to demonstrate proof-of-concept for ResistApp – a newly developed digital platform for antibiotic resistance monitoring in hospital wastewater. ResistApp combines culture-independent, high throughput gene quantification with automated data analysis to synthesise and visualise monitoring data in an interactive dashboard. To do this, wastewater of two hospitals in Helsinki, Finland (HUS1 and HUS2) were monitored for over nine weeks (weeks 25-33 in 2020) for a total of 216 antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), integrons, and taxonomy of bacteria, including bacteria causing hospital acquired infections, and the 16S rRNA gene using high-throughput quantitative PCR. The data from HT-qPCR was analysed and visualised using ResistApp.

A higher number of ARGs and MGEs were detected at both hospitals in weeks 27-30 compared to other sampling weeks, with weeks 27-30 grouped separately from other sampling weeks by non-metric multidimensional scaling (NMDS)-ordination analysis. The NMDS ordination also indicated that the two hospitals, which use different amounts of antibiotics, had distinct resistance profiles. The study found that *bla*GES was the most abundant and prevalent carbapenem resistance gene in both hospitals throughout the sampling period. Low abundances of HAI-bacteria were detected in both hospitals. A correlation analysis was done, which revealed a positive association between *bla*GES and MGEs in both hospitals. Moreover, substantially more positive associations between carbapenem resistance genes and MGEs were found in HUS1 than HUS2, as well as a strong positive association between *bla*KPC and *Klebsiella pneumoni*ae in the wastewater of HUS1.

Wastewater monitoring with high-throughput qPCR is a promising tool for wastewater-based epidemiology, and it has been successfully used for the surveillance of SARS-CoV-2 -virus. Routine monitoring using ResistApp can capture both the impact of antibiotic use on resistance profiles and the dynamics of these profiles in hospital wastewater. In addition, ResistApp can simplify the analysis of HT-qPCR data considerably, compared to processing large amounts of raw data by hand.

Avainsanat — Nyckelord — Keywords

Antibiotic resistance genes, hospital wastewater, wastewater monitoring

Säilytyspaikka — Förvaringsställe — Where deposited

Muita tietoja — Övriga uppgifter — Further information

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Antibioottiresistenssigeeniprofiilien seuranta sairaaloiden jätevedessä – konseptitodistus käyttäen ResistApp-sovellusta

Työn laji — Arbetets art — Level	Aika — Datum — Month and year	Ohjaaja — Supervisor
Master's Thesis	07/2021	Professori Marko Virta

Tiivistelmä — Referat — Abstract

Tutkielman tavoitteena oli suorittaa ResistApp -alustan konseptitodistus (proof-of-concept). ResistApp on uusi digitaalinen alusta sairaaloiden jäteveden antibioottiresistenssigeenien seurantaan. ResistApp yhdistää molekulaarisen mikrobiologian ja suuritehoisen geenimäärityksen automaattiseen data-analyysiin seurantatietojen syntetisoimiseksi ja visualisoimiseksi.

Tutkimuksessa kahden Helsingin sairaalan (HUS1 ja HUS2) jätevesistä otettiin näytteitä kerran viikossa yli yhdeksän viikon ajan (viikot 25-33 vuonna 2020). Suuren kapasiteetin qPCR:n avulla analysoitiin yhteensä 216 geeniä, joihin kuului antibioottiresistenssigeenejä, liikkuvia geneettisiä elementtejä ja integroneja. Lisäksi näytteistä kvantifioitiin taksonomisia geenejä, mukaan lukien sairaaloissa infektioita aiheuttavien bakteerien geenejä, kuten *Klebsiella pneumoniae*n ja *Acinetobacter baumannii*n. Datan normalisointia varten kvantifioitiin myös 16S rRNA -geenin määrät. Suuren kapasiteetin qPCR:n raakadata analysoitiin ja visualisoitiin ResistApp-sovelluksella.

Molemmissa sairaaloissa havaittiin suurempi määrä antibioottiresistenssigeenejä ja liikkuvia geneettisiä elementtejä viikoilla 27-30 verrattuna muihin näytteenottoviikkoihin, ja kyseiset viikot ryhmiteltiin erilleen muista näytteenottoviikoista ei-metrisen moniulotteisen skaalauskoordinaatioanalyysin (non-metric multidimensional scaling analysis, NMDS) avulla. NMDS-analyysi osoitti myös, että kahdella sairaalalla, jotka käyttävät erilaisia määriä antibiootteja, oli erilaiset resistenssiprofiilit. Tutkimuksessa havaittiin *bla*GES-geenin olevan yleisin karbapeneemiresistenssigeeni molemmissa sairaaloissa koko näytteenottojakson ajan. Molemmissa sairaaloissa havaittiin vähän sairaaloissa infektioita aiheuttavien bakteerien geenejä. Korrelaatioanalyysi paljasti positiivisen yhteyden molempien *bla*GES:in ja liikkuvien geneettisten elementtien välillä molemmissa sairaaloissa. Karbapeneemiresistenssigeenien ja liikkuvien geneettisten elementtien välillä havaittiin enemmän positiivisia assosiaatioita HUS1:ssä kuin HUS2:ssa, sekä vahva positiivinen yhteys *bla*KPC:n ja *K. pneumoniaen* välillä HUS1:n jätevedessä.

Jäteveden seuranta suuren kapasiteetin qPCR:llä on lupaava työkalu jätevesipohjaiseen epidemiologiaan, ja sitä on käytetty menestyksekkäästi SARS-CoV-2 -viruksen seurannassa. Säännöllisellä seurannalla ResistApp-sovellusta apuna käyttäen voidaan havaita sekä antibioottien käytön vaikutuksen resistenssiprofiileihin että näiden profiileiden dynamiikka sairaalan jätevedessä. Lisäksi ResistApp yksinkertaistaa suuren kapasiteetin qPCR-datan analysointia huomattavasti verrattuna suurten raakadatamäärien manuaaliseen analysointiin.

Avainsanat — Nyckelord — Keywords

Antibioottiresistenssigeenit, sairaalajätevesi, jäteveden seuranta

Säilytyspaikka — Förvaringsställe — Where deposited
Muita tietoja — Övriga uppgifter — Further information
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1. Introduction

Antibiotic resistance of bacteria has been proclaimed a serious threat to human health, food security and development by the World Health Organization (WHO, 2020). Antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) might lead us to a post-antibiotic era, where the medicine we have relied on for decades wouldn't work anymore. One important aspect of antibiotic resistance is the mobility of the genes. Mobile genetic elements (MGEs) are segments of DNA coding for proteins that mediate the movement of DNA within genomes (intracellular mobility) or between bacterial cells (intercellular mobility), thus facilitating the movements of ARGs (Frost et al., 2005). To be able to understand the dynamics of antibiotic resistance, it is crucial to study mobile genetic elements as well.

Hospitals can be considered hotspots for the development and spread of antibiotic resistant bacteria and antibiotic resistance genes (ARGs) due to their high and constant use of antibiotics (Hocquet et al., 2016). In 2006, 30% of patients in European hospitals received antibiotic treatment during their stay (Ansari et al., 2009). The high use of antibiotics and even small concentrations of antibiotic residues can result in a selective pressure selecting for ARB (Sandegren, 2014; Cairns et al. 2017). Carbapenem of the beta lactam antibiotics is a broad-spectrum antibiotic and one of the most reliable medicines for treating infections caused by bacteria responsible for Hospital Acquired Infections (HAIs) such as *Acinetobacter baumannii*. A hospital in the United States recently suffered an outbreak of carbapenem-resistant *A. baumannii* during a peak in coronavirus disease 2019 (COVID-19) admissions (Perez et al., 2020). In 2015, an outbreak of New Delhi Metallo-beta-lactamase (NDM)-producing *Klebsiella pneumoniae* cost a UK hospital group €1,1 million (Otter et al., 2016). Resistant HAI-bacteria outbreaks pose a significant problem for hospitals, as these outbreaks are highly costly, and the bacteria cannot be treated using one of the last-resort antibiotics (Bonardi & Pitino, 2019). Early detection of the outbreaks would have allowed the hospitals to take preventive measures and limit the spread of infections caused by resistant bacteria. This study has a special focus on genes conferring resistance to carbapenems, because of their vital nature to human medicine.

During the SARS-CoV-2 pandemic, monitoring of wastewater with quantitative PCR (qPCR) has been used successfully to assess the amount of viral matter in community wastewater (Kitajima et al., 2020). This has been an important tool for epidemiologists and physicians

to assess the scale of the spread of the virus. High-throughput qPCR (HT-qPCR) can be used to monitor a considerable number of antibiotic resistance related genes at the same time, which gives important insights into the dynamics of the genes, such as possible correlations between ARGs and MGEs (Muurinen et al., 2017).

A cornerstone of the guidance on first line treatment is up-to-date monitoring of antibiotic resistance levels. In addition, informative monitoring can alert hospitals to the emergence of potential outbreaks as well as help prioritise interventions and evaluate their outcomes (WHO, 2015). However, there are two important limitations to how antibiotic resistance is currently monitored in hospitals: first, monitoring focuses on a limited number of pathogens, which means that ARG profiles often carried by commensal bacteria are not captured. Second, monitoring is often based on passive surveillance of pathogens isolated from patients, which leads to delayed detection of outbreaks. Wastewater monitoring using culture-independent methods can potentially provide an efficient alternative to current approaches for monitoring antibiotic resistance (Hendriksen et al., 2019). Hospital wastewater reflects inpatient activity within hospitals such as length of hospital stay (Perry et al., 2019). In addition, wastewater sampling is cost-effective and straightforward to implement, and analysis of such samples does not require informed consent from patients (Aarestrup & Woolhouse, 2020).

Here, this study seeks to demonstrate proof-of-concept for wastewater monitoring of antibiotic resistance in hospitals using a newly developed digital platform, ResistApp (Resistomap, Finland). HT-qPCR produces a lot of raw data, which can be difficult to manage and analyse if one is not used to analysing qPCR data. To answer this need, ResistApp combines culture-independent, high throughput gene quantification with the efficiency of automated data analysis to synthesise and visualise monitoring data in an interactive dashboard. The ResistApp dashboard will potentially allow researchers to compare antibiotic resistance profiles in wastewater between hospitals and over time.

2. Materials and Methods

2.1 Sampling sites and sample collection

The wastewater of two Helsinki University Hospitals (Meilahti Triangle Hospital, HUS1 and New Children's Hospital, HUS2) was monitored. A summary of antibiotic use at each hospital is shown in Table 1. HUS1 uses a higher number of antibiotic doses than HUS2. Beta lactam, macrolide-lincosamide-streptogramin B (MLSB), quinolone, and vancomycin are the most common antibiotics used in the hospitals.

Wastewater samples were collected from a sewage system containing only outgoing wastewater from each hospital. The sewage was sampled once a week from both hospitals for nine weeks between June and August 2020 (weeks 25 – 33). Samples were taken using sterilized equipment and personal protective equipment were used. Wastewater was collected as grab samples in sterile 1 litre bottles (ISOLAB, Germany) and kept cold during the 1-hour transportation to the laboratory. Each sample of 100 ml was directly concentrated through a Nalgene Rapid-Flow PES 0.2 µm filter (Thermo Fisher Scientific, MA, USA) upon arrival at the laboratory. The filters were transferred to PowerWater bead tubes (QIAGEN, Netherlands) and kept in -20 °C until DNA isolation.

Table 1. Information of the two studied hospitals that belong to Helsinki University Hospitals (HUS), Finland.

	HUS 1	HUS 2
Name of hospital	Meilahti Triangle Hospital	New Children's Hospital
Amount of antibiotic use: (DDD*/ 1000 patients) Jun 2020 Jul 2020 Aug 2020	 2 222 2 455 2 070	 333 359 362
Number of beds	193	125
Hospital speciality	Cardiology, Neurology, Infectious Diseases, Hematology, and Internal Medicine	Pediatric Medical Care

Location	60°11'19.7"N 24°54'25.8"E	60°11'14.2"N 24°54'36.2"E

*DDD : Defined Daily Dose

2.2 DNA isolation

DNA isolation was performed directly from the filters using the DNeasy PowerWater Kit (QIAGEN, Netherlands) according to the manufacturer's instructions. QIAcube Connect (QIAGEN, Netherlands) was used to automate the DNA isolation process for consistent quality. DNA was quantified and the quality was inspected using NanoDrop One (Thermo Fisher Scientific, MA, USA). The isolated DNA was stored in -20 °C until downstream processing.

2.3 High-throughput quantitative PCR analysis

Samples were pre-screened using the 384 - Primer Set 2.0 (Stedtfeld et al., 2018) to select 215 primer sets with a positive detection (Table S1). Three other validated primer sets for Class D of carbapenem resistance gene, *bla*OXA-48 (Monteiro et al., 2012) and aminoglycoside resistance genes, *armA* and *rmtB* (Hu et al., 2013) were added to complement the array of primer sets. Out of all 215 monitored genes, 32 were beta lactam resistance genes, 15 macrolide-lincosamide-streptogramin B (MLSB) resistance genes, eight quinolone resistance genes, 13 vancomycin resistance genes, 28 genes associated with MGEs, four integrase genes of integrons and eight taxonomic genes associated with clinically relevant bacteria, genus or phyla. In addition, 30 aminoglycoside-, 13 phenicol-, 4 sulfonamide-, 20 tetracycline-, 7 trimethoprim- and 20 multidrug resistance genes in addition to 13 other genes which are associated with ARGs were monitored. One primer set for the 16S rRNA gene was used to normalize the abundances of genes detected in each sample.

Gene quantification of the 216 primer sets was completed using HT-qPCR, the SmartChip Real-Time PCR system (Takara Bio, USA). The HT-qPCR SmartChip system has been widely used as a culture-independent method to profile antibiotic resistance genes from environmental samples, including wastewater (Waseem et al., 2019). Briefly, SmartChip has 5184 reaction wells with a volume of 100 nL. Each 100 nL reaction comprised of 1 × SmartChip TB Green Gene Expression Master Mix (TakaraBio, CA, USA), nuclease-free

PCR-grade water, 300 nM of each primer and a DNA template of 2 ng μL^{-1}. The chips are filled using the SmartChip Multisample Nanodispenser (Takara Bio, USA). PCR cycling conditions and initial data processing was done as previously described (Stedtfeld et al., 2018).

2.4 Data analysis and visualization in ResistApp

Data from the HT-qPCR SmartChip system was processed and analysed using ResistApp (Figure 1). The data analysis was implemented in Python as part of the ResistApp analysis. Mean of the cycle threshold (Ct) of three technical replicates in each qPCR reaction was used to calculate the ΔCt values, unless the genes were detected in only one of the three technical replicates, in which case they were removed. The $2^{-\Delta Ct}$ method (where ΔCt = Ct detected gene – Ct 16S rRNA gene) was used to calculate the relative abundances of the detected gene relative to the 16S rRNA gene in each sample (Schmittgen & Livak, 2008). The limit of detection is Ct cut-off = 27.

ResistApp stores the resulting gene abundances in an online PostgreSQL database and visualizes the results in interactive configurations of a front-end dashboard, an online user interface for exploratory analysis using Typescript, React and VX.

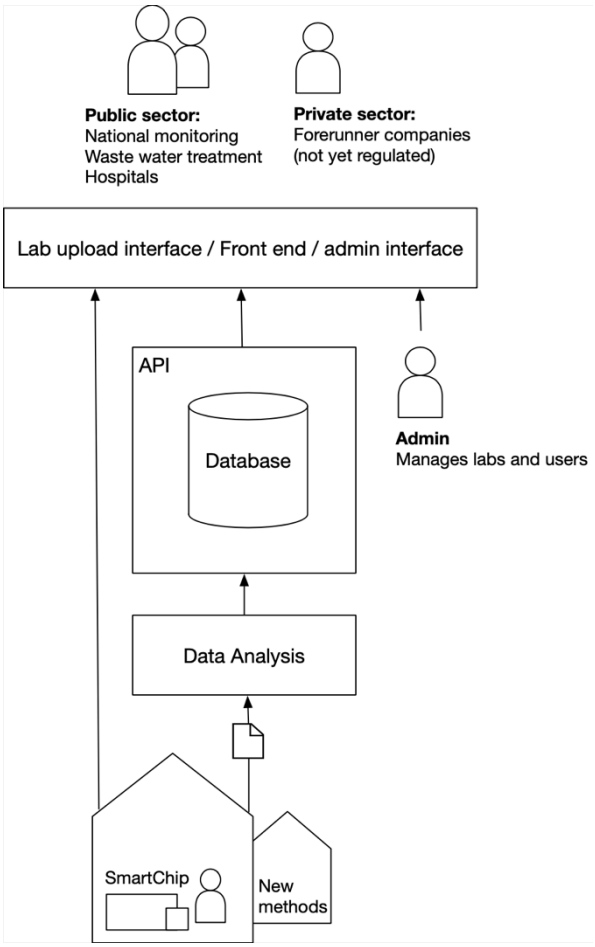

Figure 1. ResistApp, a digital platform for antibiotic resistance monitoring in wastewater; using the SmartChip qPCR for the detection and quantification of ARGs, all the resulting data is collected into an open database of ARGs and an interactive dashboard that allows users to view detailed information on the presence and abundance of ARGs in wastewater.

2.5 Statistical analysis

To examine similarities in the gene profiles of hospital wastewaters, non-metric multidimensional scaling (NMDS) ordination of detected ARGs and MGEs was performed and plotted using vegan library in RStudio Version 1.1.463. Correlations between carbapenem resistance genes, integrons, and MGEs were calculated for analysing co-occurrence in wastewater samples from each hospital. This analysis was completed using Python and only correlations with Spearman's correlation values greater than 0.8 were used (all p-values < 0.01).

3. Results

3.1 The dynamics of gene profiles in hospital wastewater

Figure 2 shows that the NMDS-ordination of detected ARG and MGE profiles in weeks 27-30 were grouped separately from other sampling times in both HUS1 and HUS2. The numbers of detected genes in weeks 27-30 were also higher in both hospitals compared to other sampling times (Table 2). The median of detected ARG abundances relative to the 16S rRNA genes in HUS1 (10^{-3}) were ten times higher compared to HUS2 (10^{-4}) (Figure 3), even though the abundances of 16S rRNA genes in wastewater from both hospitals were similar, approximately $3x10^{7}$ to $5x10^{7}$ copy numbers (Table 3). The NMDS ordination also shows that the gene profiles in HUS1 and HUS2 form distinct clusters.

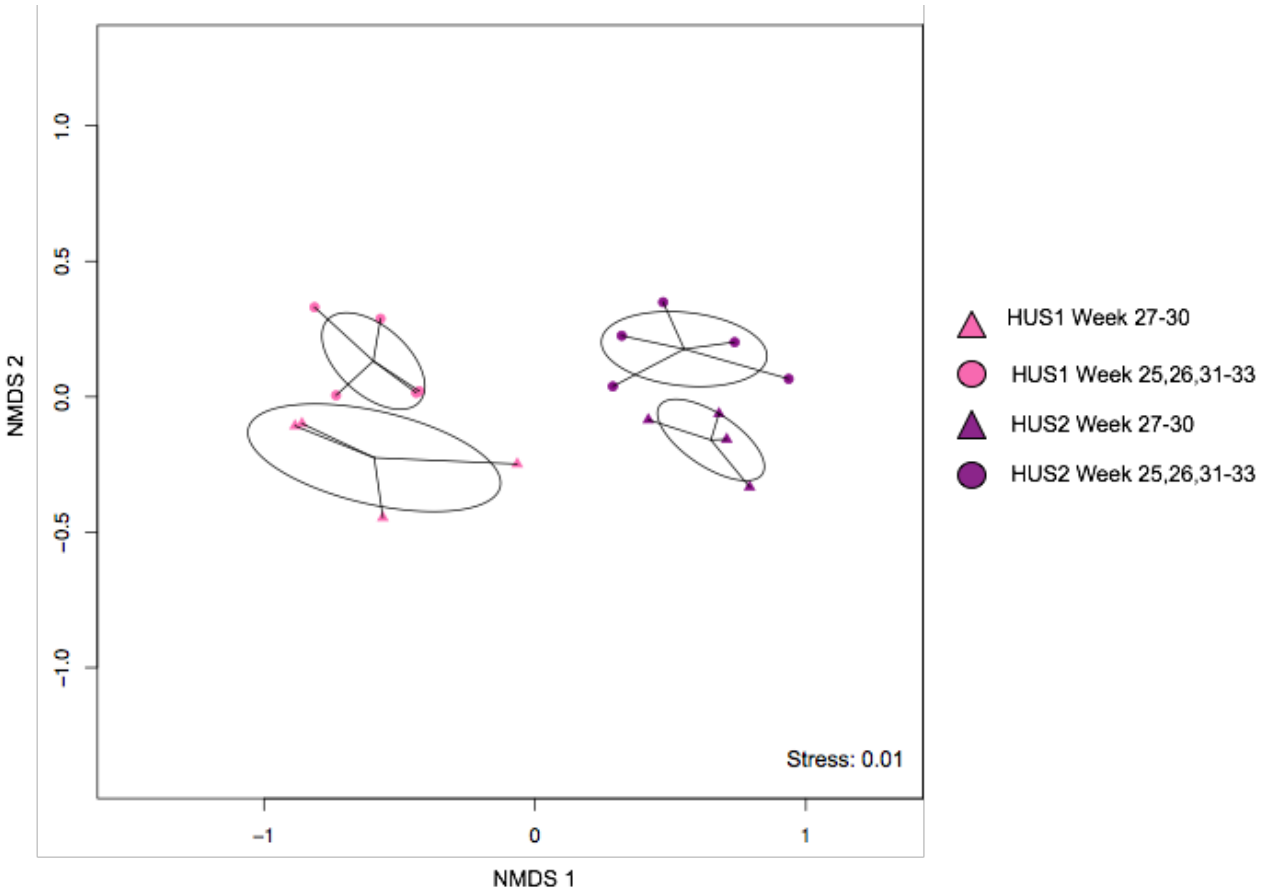

Figure 2. Non-metric multidimensional scaling (NMDS) ordination of antibiotic resistance gene and mobile genetic element profiles in wastewater from two Helsinki University hospitals, HUS1 and

HUS2 for nine weeks, week 25-33 in June, July and August 2020. The ellipse line indicates 95% confidence regions of the centers in each hospital wastewater group.

R&D ResistApp HUS1 and HUS2 — All antibiotics

Relative abundances Copy numbers

Numbers of detected genes and relative abundances

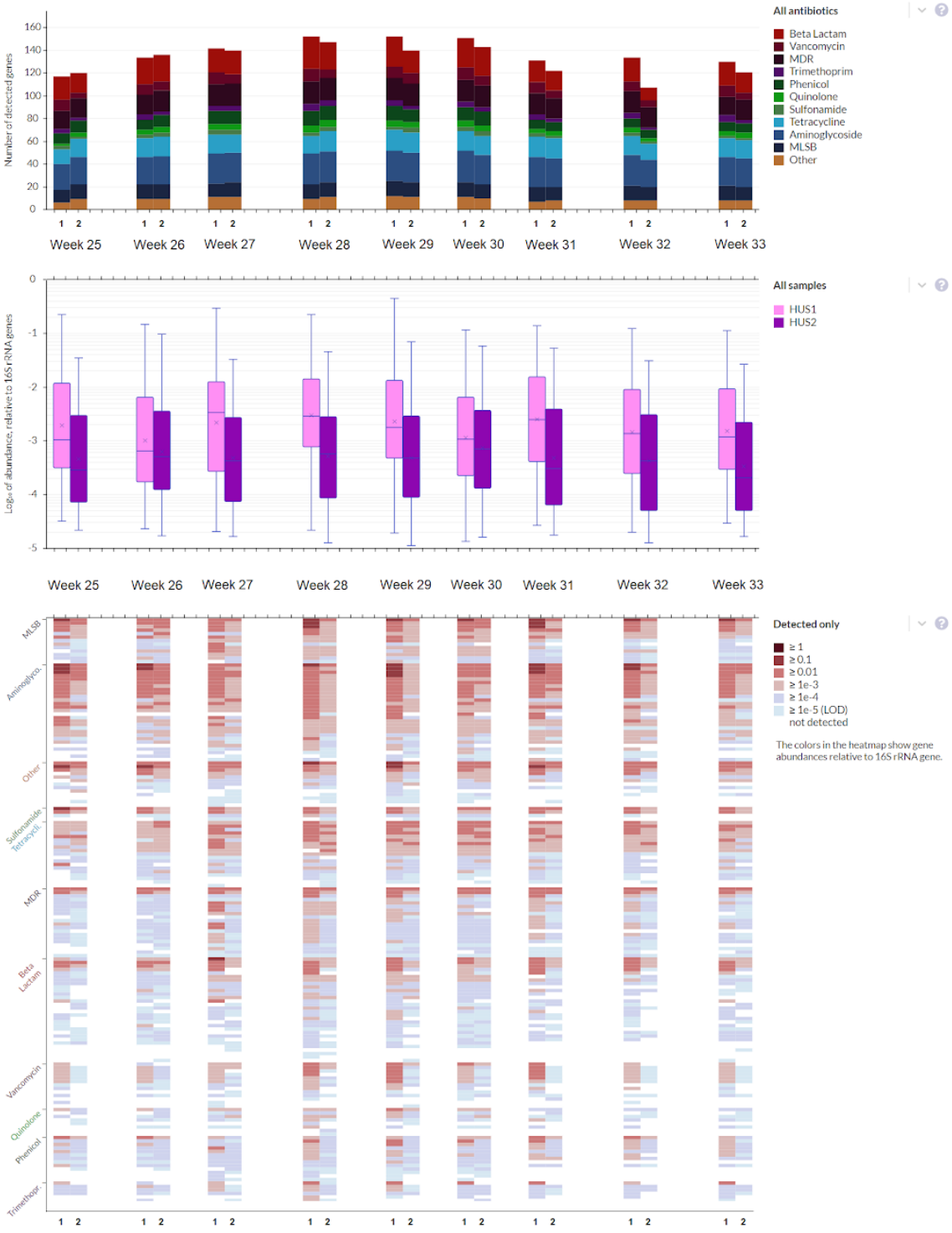

Figure 3. ResistApp dashboard for 164 out of 175 antibiotic resistance genes detected in wastewater from two Helsinki University hospitals, HUS1 and HUS2 for nine weeks, weeks 25-33 in June, July and August 2020: the bar chart shows the numbers of detected genes which are grouped by antibiotics; the box-plot chart shows the abundances of detected genes relative to the 16S rRNA genes. Each box plot represents (from top to bottom) maximum, upper-quartile, median, lower-quartile and minimum values. Within each box plot, “x” shows the average of gene abundances; A heatmap shows detected gene profiles in hospital wastewater. The colors in the heatmap show the tenfold change of gene abundances relative to the 16S rRNA gene in which red is more abundant and blue is less abundant. The limit of detection was set to Ct cut-off = 27. White means the genes were not detected.

Table 2. Numbers of genes by class of antibiotics detected in at least one wastewater sample during the sampling period in hospital wastewater.

		Numbers of detected genes																	
		Week 25		Week 26		Week 27		Week 28		Week 29		Week 30		Week 31		Week 32		Week 33	
Antibiotic Class	**Total numbers of analysed genes**	**HUS1**	**HUS2**	**HUS1**	**HUS2**	**HUS1**	**HUS2**	**HUS1**	**HUS2**	**HUS1**	**HUS2**	**HUS1**	**HUS2**	**HUS1**	**HUS2**	**HUS1**	**HUS2**	**HUS1**	**HUS2**
MLSB	15	11	13	13	13	12	13	13	13	13	13	13	12	13	12	13	12	13	12
Aminoglycoside	30	23	24	24	25	26	26	27	27	27	26	28	26	26	25	27	24	25	25
Other	13	6	9	9	9	11	11	9	11	12	11	11	10	7	8	8	8	8	8
Sulfonamide	4	3	2	3	4	4	4	3	4	3	4	4	4	3	2	3	2	2	2
Tetracycline	20	13	16	17	17	17	16	16	18	18	18	17	17	18	18	17	14	17	16
MDR	20	16	17	17	19	19	20	20	20	20	20	19	19	18	18	19	18	16	18
Beta Lactam	32	20	17	24	23	21	21	28	24	26	20	26	25	19	17	21	11	21	18
Vancomycin	13	10	5	9	8	11	8	11	7	10	9	11	9	10	7	9	6	10	6
Quinolone	8	2	4	4	5	5	5	6	6	5	5	5	5	4	4	4	3	4	5
Phenicol	13	9	10	9	10	12	12	13	12	13	11	12	12	8	8	8	7	8	8
Trimethoprim	7	4	3	5	3	4	4	6	5	5	3	5	4	5	3	5	2	6	3
Total	**175**	**117**	**120**	**134**	**136**	**142**	**140**	**152**	**147**	**152**	**140**	**151**	**143**	**131**	**122**	**134**	**107**	**130**	**121**

3.2 Presence and abundance of carbapenem resistance genes and HAI-bacteria

Eight carbapenem resistance genes were analysed: *bla*GES and *bla*KPC of Class A, *bla*VIM and *bla*NDM of Class B, *bla*CMY and *bla*MOX/CMY of Class C, and *bla*OXA48 and *bla*OXA51 of Class D in HUS1 (Figure 4A) and HUS2 (Figure 5A). The most abundant and prevalent detected carbapenem resistance gene during the sampling period in both HUS1 and HUS2 was *bla*GES. Other detected carbapenem resistance genes were more prevalent and abundant in HUS1 compared to HUS2. *Bla*OXA48 was only detected at one sampling point in both HUS1 and HUS2. *Bla*OXA51 was not detected in either hospital during the sampling period.

Three taxonomic genes of bacteria causing hospital-acquired infections (HAIs) were analysed: *A. baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Figures 4B and 5B). *A. baumannii* was prevalent in both hospitals, however, the abundances were low (10^{-5} -10^{-4} relative to the 16S rRNA genes) (Table 3). There was a noticeable peak in the prevalence of *K. pneumoniae* in HUS1 during sampling weeks 27-29.

Figure 4. A) Gene abundances relative to the 16S rRNA gene of eight carbapenem resistance genes and B) three bacteria causing hospital acquired infections in HUS1 during the 9-week sampling period, weeks 25-33 in June, July and August 2020. Black dashlane shows the median of all detected antibiotic resistance gene abundances and detected taxonomic bacterial gene abundances in hospital wastewater.

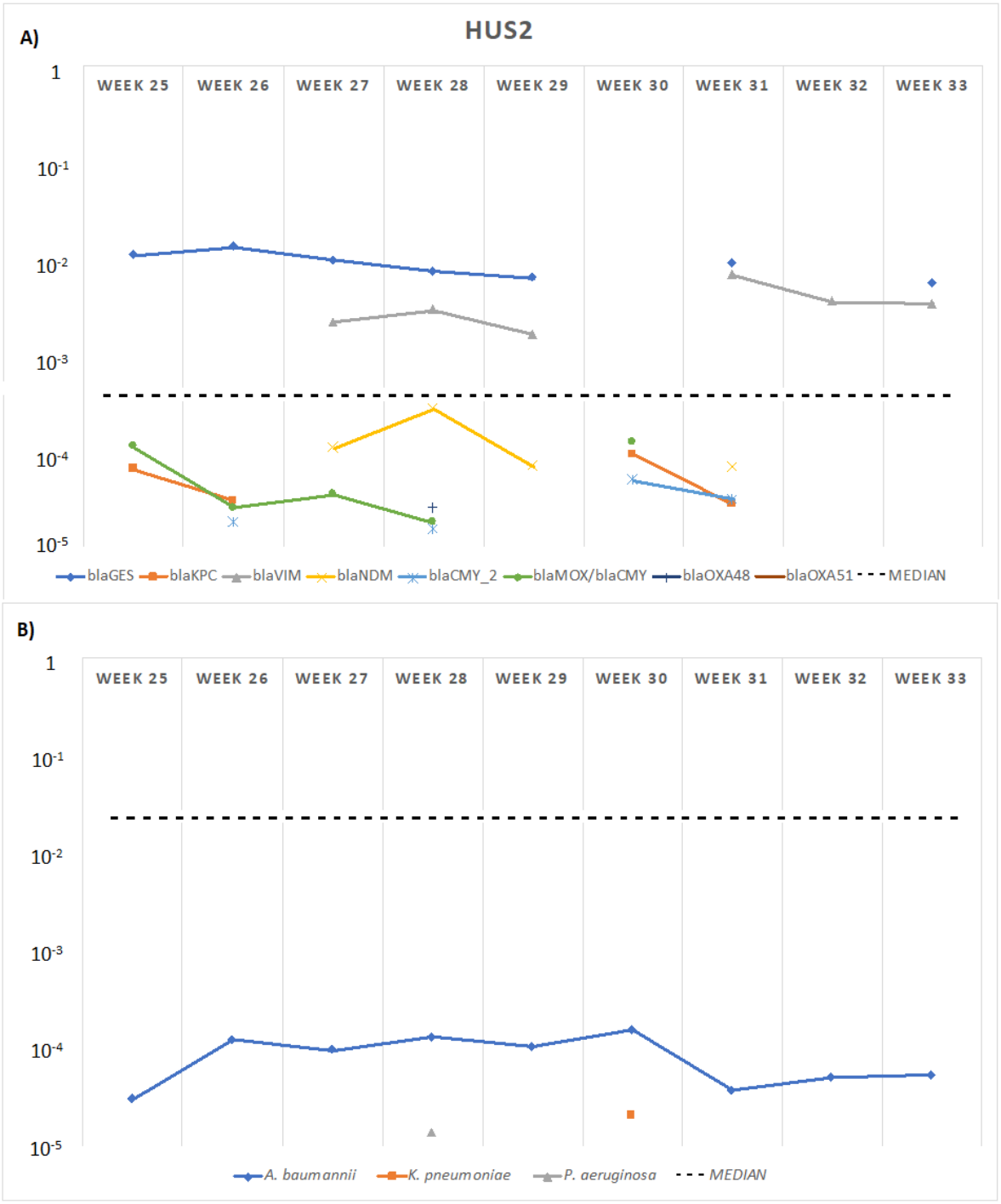

Figure 5. A) Gene abundances of eight carbapenem resistance genes B) and three bacteria causing hospital acquired infections in HUS2 during the 9-week sampling period, weeks 25-33 in June, July and August 2020. Black dashlane shows the median of all detected antibiotic resistance gene abundances and detected taxonomic bacterial gene abundances in hospital wastewater.

Table 3. Gene abundances of carbapenem resistance genes and bacteria causing Hospital Acquired Infections (HAIs) relative to the 16S rRNA genes, and copy numbers of the 16S rRNA gene in wastewater samples from two Helsinki University Hospitals (HUS1 and HUS2) in Finland for nine weeks between June and August 2020. Weeks with higher abundances of carbapenem resistance and bacterial genes have been highlighted in red.

	Gene abundances																
	HUS1									HUS2							
Carbapenem resistance genes	**W25**	**W26**	**W27**	**W28**	**W29**	**W30**	**W31**	**W32**	**W33**	**W25**	**W26**	**W27**	**W28**	**W29**	**W30**	**W31**	**W32**	**W33**
*bla*GES	$4*10^{-2}$	$3*10^{-2}$	$4*10^{-2}$	$6*10^{-2}$	$8*10^{-2}$	$2*10^{-2}$	$3*10^{-2}$	$2*10^{-2}$	$2*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	$9*10^{-3}$	$7*10^{-3}$	$6*10^{-3}$	ND	$9*10^{-3}$	ND	$5*10^{-3}$
*bla*KPC	ND	$5*10^{-4}$	$1*10^{-3}$	$2*10^{-2}$	$2*10^{-3}$	$1*10^{-3}$	ND	ND	$3*10^{-3}$	$6*10^{-5}$	$3*10^{-5}$	ND	ND	ND	$9*10^{-5}$	$3*10^{-5}$	ND	ND
*bla*VIM	$4*10^{-2}$	$3*10^{-2}$	$1*10^{-2}$	$2*10^{-2}$	$3*10^{-2}$	$8*10^{-3}$	$3*10^{-2}$	$3*10^{-2}$	$3*10^{-2}$	ND	ND	$2*10^{-3}$	$3*10^{-3}$	$2*10^{-3}$	ND	$7*10^{-3}$	$3*10^{-3}$	$3*10^{-3}$
*bla*NDM	$4*10^{-5}$	$1*10^{-3}$	$9*10^{-4}$	$2*10^{-4}$	$3*10^{-4}$	$1*10^{-4}$	$1*10^{-4}$	$9*10^{-5}$	$1*10^{-4}$	ND	ND	$1*10^{-4}$	$3*10^{-4}$	$7*10^{-5}$	ND	$7*10^{-5}$	ND	ND
*bla*CMY_2	$2*10^{-4}$	$3*10^{-4}$	$2*10^{-4}$	$4*10^{-3}$	$1*10^{-3}$	$4*10^{-5}$	$3*10^{-4}$	$8*10^{-5}$	$5*10^{-4}$	ND	$2*10^{-5}$	ND	$1*10^{-5}$	ND	$5*10^{-5}$	$3*10^{-5}$	ND	ND
*bla*MOX/CMY	$5*10^{-5}$	$4*10^{-5}$	$4*10^{-5}$	$2*10^{-4}$	$7*10^{-4}$	$1*10^{-4}$	ND	ND	ND	$1*10^{-4}$	$2*10^{-5}$	$3*10^{-5}$	$2*10^{-5}$	ND	$1*10^{-4}$	ND	ND	ND
*bla*OXA48	ND	ND	ND	$2*10^{-5}$	ND	ND	ND	ND	ND	ND	ND	ND	$3*10^{-5}$	ND	ND	ND	ND	ND
*bla*OXA51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MEDIAN of all detected ARGs	$1*10^{-3}$	$5*10^{-4}$	$3*10^{-3}$	$3*10^{-3}$	$2*10^{-3}$	$1*10^{-3}$	$2*10^{-3}$	$1*10^{-3}$	$2*10^{-3}$	$2*10^{-4}$	$5*10^{-4}$	$3*10^{-4}$	$4*10^{-4}$	$4*10^{-4}$	$5*10^{-4}$	$3*10^{-4}$	$3*10^{-4}$	$2*10^{-4}$

ND: Not detected

	Gene abundances																
	HUS1									HUS2							
HAI-bacteria	**W25**	**W26**	**W27**	**W28**	**W29**	**W30**	**W31**	**W32**	**W33**	**W25**	**W26**	**W27**	**W28**	**W29**	**W30**	**W31**	**W32**	**W33**
A. baumannii	1.10^{-4}	$5*10^{-5}$	$1*10^{-4}$	$3*10^{-4}$	$3*10^{-4}$	$3*10^{-4}$	$1*10^{-4}$	$3*10^{-4}$	$6*10^{-5}$	$3*10^{-5}$	$1*10^{-4}$	$9*10^{-5}$	$1*10^{-4}$	$1*10^{-4}$	$2*10^{-4}$	$4*10^{-5}$	$5*10^{-5}$	$5*10^{-5}$
K. pneumoniae	ND	ND	$4*10^{-5}$	$3*10^{-4}$	$5*10^{-5}$	$5*10^{-5}$	ND	$7*10^{-5}$	$6*10^{-5}$	ND	ND	ND	ND	ND	$2*10^{-5}$	ND	ND	ND
P. aeruginosa	ND	ND	$2*10^{-5}$	ND	$2*10^{-5}$	ND	ND	ND	ND	ND	ND	ND	$1*10^{-5}$	ND	ND	ND	ND	ND
MEDIAN of all detected bacterial groups	$8*10^{-2}$	$1*10^{-1}$	$2*10^{-2}$	$3*10^{-2}$	$6*10^{-3}$	$1*10^{-2}$	$1*10^{-1}$	$6*10^{-3}$	$5*10^{-3}$	$2*10^{-2}$	$1*10^{-1}$	$2*10^{-1}$	$2*10^{-4}$	$2*10^{-1}$	$4*10^{-4}$	$7*10^{-2}$	$1*10^{-1}$	$4*10^{-2}$
16S rRNA copies	$3*10^{7}$	$3*10^{7}$	$4*10^{7}$	$4*10^{7}$	$4*10^{7}$	$5*10^{7}$	$3*10^{7}$	$4*10^{7}$	$3*10^{7}$	$3*10^{7}$	$4*10^{7}$	$4*10^{7}$	$5*10^{7}$	$5*10^{7}$	$5*10^{7}$	$4*10^{7}$	$5*10^{7}$	$4*10^{7}$

ND: Not detected

3.3 Correlation between carbapenem resistance genes and MGEs

The correlation analysis revealed significant positive correlations between the most abundant carbapenem resistance gene, *bla*GES with transposase gene *tnpA_1* of the IS6 group in HUS1 and with ISPps in HUS2 (Table 4). In HUS1, a strong positive association was observed between *bla*KPC and *Klebsiella pneumoniae* (Spearman correlation = 0,99). More significant positive correlations were found between carbapenem resistance genes and MGEs in HUS 1 compared to HUS 2.

Table 4. Correlations between carbapenem resistance genes and mobile genetic elements in wastewater from HUS1 and HUS2 hospitals. Only correlations with Spearman's correlation values greater than 0,8 were used (all *p*-values < 0,01).

Class	**Gene**	**HUS1**									**HUS2**				
		intI1_2	*tnpA_1*	*tnpA_2*	*tnpA_3*	IS6100	Tn3	trbC	*intI2_2*	Tn5403	*tnpA_3*	ISPps	IS1247_2	*intI3*	orf37-IS26
Class A	*blaGES*		0,9									0,83			
Class A	*blaKPC*						0,85	0,87	0,87	0,90	0,82		0,88		
Class B	*blaVIM*	0,83		0,95	0,88	0,93									
Class B	*blaNDM*													0,86	0,83
Class C	*blaCMY_2*														
Class C	*blaMOX/blaCMY*														
Class D	*blaOXA48*														

4. Discussion

The NMDS ordination analysis showed that samples from week 27-30 were grouped separately from other samples and the gene profiles in HUS1 were grouped separately from those of HUS2. The median of detected ARG abundances was higher in HUS1 than HUS2. Besides *bla*GES, other detected carbapenem resistance genes were more abundant and prevalent in HUS1 compared to HUS2.

Carbapenems are used to treat patients that suffer from severe infections. As HUS1 specialises in infectious, hematological and internal diseases, the number of patients treated with carbapenems is likely to be higher at HUS1 than HUS2 (Luyt et al., 2014). As shown in Table 1, the amount of antibiotics given to patients in HUS1 was six times higher than in HUS2. This may lead to a larger selection pressure when the antibiotics are secreted into wastewater, thus increasing the selection and prevalence of ARGs in HUS1 wastewater (Hocquet et al., 2016). The selection of ARGs could also occur in patients' gut microbiomes before being released into wastewater (Kim et al., 2017). The findings of this study indicate that there were changes in detected gene profiles in hospital wastewater over time and that the two hospitals, which use different amounts of antibiotic, have distinct resistance profiles. However, there might be other factors that contribute to the differences in the genetic profiles between the two hospitals, such as the age-related differences in patients' microbiomes, different diets and other treatments.

Both integrons and MGEs are known to facilitate the mobility of ARGs (Frost et al., 2005). As shown in the correlation analysis, the most abundant and prevalent carbapenem resistance gene, *bla*GES was positively associated with MGEs in both hospitals. These correlations may show co-occurrence between the genes, which in turn might explain the abundance and prevalence of *bla*GES in hospital wastewater. There were also more positive associations between carbapenem resistance genes and MGEs in HUS1, suggesting the risk of carbapenem resistance gene transfer and spread in HUS1 could be higher than in HUS2. The correlation analysis also showed a strong positive association between carbapenem resistance gene, *bla*KPC and one HAI-bacteria, *K. pneumoniae* in HUS1 wastewater. *Klebsiella pneumoniae* carbapenemases (KPCs) were first found in 1996 and are currently globally spread and often carried by *K. pneumoniae* (Munoz-Price et al., 2013). Thus, the presence of *bla*KPC, which was notably observed in weeks 27-30 in HUS1, may be explained by the prevalence of *K. pneumoniae*. It was also observed that the amount

of antibiotics used in July (weeks 27-30) was higher compared to June and August in HUS1, however, I do not have information on the specific types of antibiotics used. This study demonstrates that in addition to comprehensive, routine monitoring of ARGs, MGEs, integrons, and HAI-bacteria in hospital wastewater, detailed information on antibiotic use is needed to better understand the abundance and prevalence of ARGs.

Previous studies have examined antibiotic resistance in hospital wastewater, although not from the hospital's perspective of monitoring. Other studies have reported the abundances of several ARGs using culture independent qPCR (Narciso-da-Rocha et al., 2014; Rodriguez-Mozaz et al., 2015; Le et al., 2016), analysed the presence of 84 genes using HT-qPCR (Khan et al., 2019) and conducted a comprehensive ARG profiling using sequencing analysis (Perry et al., 2019). Importantly, none of the previous studies examined ARG profiles over time. As a result, we still know little about the dynamics of ARG profiles in hospital wastewater. To my knowledge, this is the first study to report comprehensive and routine wastewater monitoring for 175 antibiotic resistance genes as well as the application of a digital monitoring platform. However, it is also important to note potential limitations of the present study: first, grab samples were used, which are only reflective of the time at which they are collected. Composite samples collected for a 24-hour time period would give more representative information about the profiles of ARGs in wastewater. Second, even though a wide range of ARGs and MGEs were monitored using HT-qPCR, qPCR methods only target known ARGs and MGEs as qPCR relies on primer sets from known gene sequences in available databases. Thus, we may miss other variations or new resistance mechanisms in the samples.

In recent years, data digitalisation in clinical microbiology and infectious disease has made significant progress (Egli, 2020). Digitalisation and automated analysis also provide opportunities to improve the monitoring of antibiotic resistance. For effective monitoring, hospitals need to be able to obtain and compare timely, reliable and standardised data. As demonstrated in this proof-of-concept study, ResistApp can provide hospitals in-depth information on ARG and HAI-bacteria levels in wastewater and allow comparison of resistance data between hospitals and over time. With ResistApp's interactive dashboard, ARG monitoring data can also be visualised according to genes of interest, which could possibly help hospitals monitor clinically relevant ARGs, such as carbapenem resistance genes or specific bacteria. When combined with routine wastewater monitoring, ResistApp

can potentially be used as an early warning system to detect emerging outbreaks of resistant bacteria in hospitals.

However, I recognise that antibiotic use in Finland is low (WHO, 2018) and, as shown in the results, the most prevalent of HAI-bacteria *A. baumannii* abundances were low in all samples. Further research in regions with higher antibiotic use and more frequent outbreaks is required to further evaluate the capabilities and proof-of-value of ResistApp. In summary, this is the first study to report comprehensive routine wastewater monitoring for antibiotic resistance, as well as the application of a digital monitoring solution. Digitalisation and methods such as HT-qPCR make large-scale monitoring more efficient, but at the same time large amounts of data is produced. ResistApp could potentially help answer this need by simplifying the analysis and visualisation processes.

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Supplementary materials

Table S1. 216 primer sets, their assay numbers, genes they code, target antibiotics and primer sequences used in the study.

Assay	Target Gene	Target antibiotics (major)	Forward Primer	Reverse Primer
AY1	16S rRNA	16S rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG
AY2	aacC2	Aminoglycoside	ACGGCATTCTCGATTGCTTT	CCGAGCTTCACGTAAGCATTT
AY4	aacA/aphD	Aminoglycoside	AGAGCCTTGGGAAGATGAAGTTT	TTGATCCATACCATAGACTATCTCATCA
AY6	aac(6')-II	Aminoglycoside	CGACCCGACTCCGAACAA	GCACGAATCCTGCCTTCTCA
AY7	aphA3_1	Aminoglycoside	AAAAGCCCGAAGAGGAACTTG	CATCTTTCACAAAGATGTTGCTGTCT
AY8	aac(6')-Ib_1	Aminoglycoside	CGTCGCCGAGCAACTTG	CGGTACCTTGCCTCTCAAACC
AY9	aadA2_1	Aminoglycoside	ACGGCTCCGCAGTGGAT	GGCCACAGTAACCAACAAATCA
AY10	aadA_1	Aminoglycoside	GTTGTGCACGACGACATCATT	GGCTCGAAGATACCTGCAAGAA
AY13	aadD	Aminoglycoside	CCGACAACATTTCTACCATCCTT	ACCGAAGCGCTCGTCGTATA
AY17	aphA1/7	Aminoglycoside	TGAACAAGTCTGGAAAGAAATGCA	CCTATTAATTTCCCCTCGTCAAAAA

AY21	aadE	Aminoglycoside	TACCTTATTGCCCTTGGAAGAGTTA	GGAACTATGTCCCTTTTAATTCTACAATCT
AY22	str	Aminoglycoside	AATGAGTTTTGGAGTGTCTCAACGTA	AATCAAAACCCCTATTAAAGCCAAT
AY24	strB	Aminoglycoside	GCTCGGTCGTGAGAACAATCT	CAATTTCGGTCGCCTGGTAGT
AY328	aadA5_2	Aminoglycoside	ATCACGATCTTGCGATTTTGCT	CTGCGGATGGGCCTAGAAG
AY331	aadA2_3	Aminoglycoside	CAATGACATTCTTGCGGGTATC	GACCTACCAAGGCAACGCTATG
AY393	aac(3)-iid_iia	Aminoglycoside	CGATGGTCGCGGTTGGTC	TCGGCGTAGTGCAATGCG
AY399	aac(6)-im	Aminoglycoside	CGTGAGCATTATACAGAGCAATGG	CCATTTCCGTTCGTAGATATTGGC
AY406	aac6-aph2	Aminoglycoside	CCAAGAGCAATAAGGGCATACCAA	GCCACACTATCATAACCACTACCG
AY408	aadA10	Aminoglycoside	ACAGGCACTCAACGTCATCG	CGCGGAGAACTCTGCTTTGA
AY409	aadA16	Aminoglycoside	ACGGTGGCCTGAAGCC	GAATTGCAGTTCCCGTCTGG
AY410	aadA1_2	Aminoglycoside	TGTACGGCTCCGCAGTG	CACGGAATGATGTCGTCGTG
AY411	aadA6	Aminoglycoside	CCATCGAGCGTCATCTGGAA	CCCGTCTGGCCGGATAAC
AY412	aadA7	Aminoglycoside	CACTCCGCGCCTTGGA	TGTGGCGGGCTCGAAG
AY413	aadB	Aminoglycoside	CCTGCTTGGTGGGCAGAC	CGGCACGCAAGACCTCAA
AY415	ant6-ia	Aminoglycoside	TCGCCATGAGCTGCTGA	CCTATCATACTCCGGATAGGCATA

AY416	ant6-ib	Aminoglycoside	AGAACATCCGACAGCACGTTC	CCAACCTTCCATGAAATCATTCGC
AY418	aph(3")-ia	Aminoglycoside	TAACAGCGATCGCGTATTTCG	TCCGACTCGTCCAACATCAATA
AY420	aph3-iii	Aminoglycoside	CAGAAGGCAATGTCATACCACTTG	GACAGCCGCTTAGCCGAA
AY424	aph4-ib	Aminoglycoside	GGGAACACCGTGCTCACC	GTTGGTCCCGTGCAGGTC
AY97	cfiA	Beta Lactam	GCAGCGTTGCTGGACACA	GTTCGGGATAAACGTGGTGACT
AY101	blaMOX/blaCMY	Beta Lactam	CTATGTCAATGTGCCGAAGCA	GGCTTGTCCTCTTTCGAATAGC
AY105	blaVEB	Beta Lactam	CCCGATGCAAAGCGTTATG	GAAAGATTCCCTTTATCTATCTCAGACAA
AY108	blaOXY	Beta Lactam	CGTTCAGGCGGCAGGTT	GCCGCGATATAAGATTTGAGAATT
AY109	blaPSE	Beta Lactam	TTGTGACCTATTCCCCTGTAATAGAA	TGCGAAGCACGCATCATC
AY111	cphA_1	Beta Lactam	GCGAGCTGCACAAGCTGAT	CGGCCCAGTCGCTCTTC
AY114	cfxA	Beta Lactam	TCATTCCTCGTTCAAGTTTTCAGA	TGCAGCACCAAGAGGAGATGT
AY115	cepA	Beta Lactam	AGTTGCGCAGAACAGTCCTCTT	TCGTATCTTGCCCGTCGATAAT
AY125	blaGES	Beta Lactam	GCAATGTGCTCAACGTTCAAG	GTGCCTGAGTCAATTCTTTCAAAG
AY126	blaSFO	Beta Lactam	CCGCCGCCATCCAGTA	GGGCCGCCAAGATGCT
AY127	blaTLA	Beta Lactam	ACACTTTGCCATTGCTGTTTATGT	TGCAAATTTCGGCAATAATCTTT

AY129	blaVIM	Beta Lactam	GCACTTCTCGCGGAGATTG	CGACGGTGATGCGTACGTT
AY131	pbp5	Beta Lactam	GGCGAACTTCTAATTAATCCTATCCA	CGCCGATGACATTCTTCTTATCTT
AY134	blaCTX-M_5	Beta Lactam	GCGATAACGTGGCGATGAAT	GTCGAGACGGAACGTTTCGT
AY138	penA	Beta Lactam	AGACGGTAACGTATAACTTTTTGAAAGA	GCGTGTAGCCGGCAATG
AY147	blaCTX-M_8	Beta Lactam	CGTCACGCTGTTGTTAGGAA	CGCTCATCAGCACGATAAAG
AY152	blaNDM	Beta Lactam	GGCCACACCAGTGACAATATCA	CAGGCAGCCACCAAAAGC
AY339	blaCMY_2	Beta Lactam	AAAGCCTCAT GGGTGCATAAA	ATAGCTTTTGTTTGCCAGCATCA
AY432	blaCTX-M	Beta Lactam	CGTACCGAGCCGACGTTAA	CAACCCAGGAAGCAGGCA
AY433	blaFOX	Beta Lactam	CCTACGGCTATTCGAAGGAAGATAAG	CCGGATTGGCCTGGAAGC
AY435	blaOXA51	Beta Lactam	CGACCGAGTATGTACCTGCTTC	TCAAGTCCAATACGACGAGCTA
AY436	blaOXY1	Beta Lactam	AAAGGTGACCGCATTCGC	CCAGCGTCAGCTTGCG
AY438	blaSHV11	Beta Lactam	TTGACCGCTGGGAAACGG	TCCGGTCTTATCGGCGATAAAC
AY439	blaTEM	Beta Lactam	CGCCGCATACACTATTCTCAG	GCTTCATTCAGCTCCGGTTC
AY440	blaKPC	Beta Lactam	GCCGCCAATTTGTTGCTGAA	GCCGGTCGTGTTTCCCTTT
AY443	beta_B2	Beta Lactam	GTAACGCCTACTGGAAGTCCA	CAGCTTCTCCTTGAGAATGCAG

AY444	blaACT	Beta Lactam	AAGCCGCTCAAGCTGGA	GCCATATCCTGCACGTTGG
AY446	blaCARB	Beta Lactam	TGATTTGAGGGATACGACAACTCC	CTGTAATACTCCGAGCACCAA
AY451	blaLEN	Beta Lactam	TGTTCGCCTGTGTGTTATCTCC	GCAGCACTTTAAAGGTGCTCAC
AY452	blaMIR	Beta Lactam	CGGTCTGCCGTTACAGGTG	AAAGACCCGCGTCGTCATG
AY453	blaBEL-nonmobile	Beta Lactam	ATGTCCATGGCACAGACTGTG	CCTGTCTTGTCACCCGTTACC
AY289	intI1_2	Integrons	CGAAGTCGAGGCATTTCTGTC	GCCTTCCAGAAAACCGAGGA
AY293	intI1_1	Integrons	CGAACGAGTGGCGGAGGGTG	TACCCGAGAGCTTGGCACCCA
AY500	intI3	Integrons	CAGGTGCTGGGCATGGA	CCTGGGCAGCATCACCA
AY199	acrB_1	MDR	AGTCGGTGTTCGCCGTTAAC	CAAGGAAACGAACGCAATACC
AY201	acrF	MDR	GCGGCCAGGCACAAAA	TACGCTCTTCCCACGGTTTC
AY202	adeA	MDR	CAGTTCGAGCGCCTATTTCTG	CGCCCTGACCGACCAAT
AY207	acrA_1	MDR	GGTCTATCACCCTACGCGCTATC	GCGCGCACGAACATACC
AY208	emrD_1	MDR	CTCAGCAGTATGGTGGTAAGCATT	ACCAGGCGCCGAAGAAC
AY211	mdtE	MDR	CGTCGGCGCACTCGTT	TCCAGACGTTGTACGGTAACCA
AY224	oprD	MDR	ATGAAGTGGAGCGCCATTG	GGCCACGGCGAACTGA
AY226	ttgA	MDR	ACGCCAATGCCAAACGATT	GTCACGGCGCAGCTTGA

AY228	mexE	MDR	GGTCAGCACCGACAAGGTCTAC	AGCTCGACGTACTTGAGGAACAC
AY353	tolC_2	MDR	CAGGCAGAGAACCTGATGCA	CGCAATTCCGGGTTGCT
AY484	bexA/norM	MDR	TCGGGCATCCCGTTTATGATC	GTAGGCTGCGCATAATACCCA
AY485	mdtA	MDR	ACAAGCCCAGGGCCAAC	CCTTAATGGTGCCTTCGGTTTC
AY486	mdtH	MDR-chromo	ATGCTGGCTGTACAAGTGATG	CACTCCAGCGGGCGATA
AY487	cefa_qaceltA	MDR-mobile	TAGTTGGCGAAGTAATCGCAAC	TGCGATGCCATAACCGATTATG
AY489	qacF/H	MDR-mobile	CTGAAGTCTAGCCATGGATTCACTAG	CAAGCAATAGCTGCCACAAGC
AY490	arsA	MDR-mobile	CAGGTCAGCCGCATCAACC	GCCTGAAACACGGCAATTTCTTC
AY491	cadC	MDR-mobile	CGCTCTGTGTCAGGATGAAGAG	CTTTCTTATGTGCTAGGGCGATCA
AY495	pcoA	MDR-mobile	TGGCGTATGGAGTTTCAATGC	GAATAATGCCGTGCCAGTGAA
AY497	sugE	MDR-mobile	CTTAGTTATTGCTGGTCTGCTGGA	GCATCGGGTTAGCGGACTC
AY498	tcrB	MDR-mobile	GTGCCGGAACTCAAGTAGCA	GCACCGACTGCTGGACTTAA
AY297	Tp614	MGE	GGAAATCAACGGCATCCAGTT	CATCCATGCGCTTTTGTCTCT
AY298	IS613	MGE	AGGTTCGGACTCAATGCAACA	TTCAGCACATACCGCCTTGAT
AY299	tnpA_1	MGE	GCCGCACTGTCGATTTTTATC	GCGGGATCTGCCACTTCTT

AY300	tnpA_2	MGE	CCGATCACGGAAAGCTCAAG	GGCTCGCATGACTTCGAATC
AY301	tnpA_3	MGE	GGGCGGGTCGATTGAAA	GTGGGCGGGATCTGCTT
AY302	tnpA_4	MGE	CATCATCGGACGGACAGAATT	GTCGGAGATGTGGGTGTAGAAAGT
AY303	tnpA_5	MGE	GAAACCGATGCTACAATATCCAATTT	CAGCACCGTTTGCAGTGTAAG
AY304	tnpA_6	MGE	TGCAGATGGTTTAACCTTGGATATTT	TCGGTTCATCAAACTGCTTCAC
AY307	orf37-IS26	MGE	GCCGGGTTGTGCAAATAGAC	TGGCAATCTGTCGCTGCTG
AY309	ISPps	MGE	CACACTGCAAAAACGCATCCT	TGTCTTTGGCGTCACAGTTCTC
AY310	IS1247_2	MGE	TGGATCGACCGGTTCCAT	GCTGACCGAGCTGTCCATGT
AY311	ISAba3	MGE	TCAGAGGCAGCGGTATACGA	GGTTGATTCAGTTAAAGTACGTAAAACTTT
AY312	ISEfm1	MGE	AGGTGTCCATGACGTGAAAGTG	TCCTTTGTCCCCTAGGATATTGG
AY315	Tn5	MGE	TCAGAGGCAGCGGTATACGA	GGTTGATTCAGTTAAAGTACGTAAAACTTT
AY316	IncN_rep	MGE	AGTTCACCACCTACTCGCTCCG	CAAGTTCTTCTGTTGGGATTCCG
AY318	IncP_oriT	MGE	CAGCCTCGCAGAGCAGGAT	CAGCCGGGCAGGATAGGTGAAGT
AY506	IS1247_1	MGE	CGGCCGTCACTGACCAA	TCGGCAGGTTGGTGACG
AY509	IS200_2	MGE	GCACACCCGATGGAACTGTAAA	TCGGCGGGATCTCCAGAAG

AY510	IS21-ISAs29	MGE	GGTCCGTCAGGCACAAGTC	GGGATCGTATCGGCAAGCC
AY511	IS256	MGE	CTTGCGCATCATTGGATGATGG	AAGAACGGCTCCAATTAAGCGA
AY512	IS26_1	MGE	ATGGATGAAACCTACGTGAAGGTC	CGGTACTTAATCTGTCGGTGTTCA
AY513	IS3	MGE	CGGTCTGAGCTTCGGGAA	AGAACTGTCACTCCGGTCTG
AY516	IS6100	MGE	CGCACCGGCTTGATCAGTA	CTGCCACGCTCAATACCGA
AY519	ISCR1	MGE	ATGGTTTCATGCGGGTT	CTGAGGGTGTGAGCGAG
AY520	ISEcp1	MGE	CATGCTCTGCGGTCACTTC	GACGCACCTTCTTGATGACC
AY523	Tn3	MGE	GCTGAGGTGTTCAGCTACATCC	GCTGAGGTAGTCACAGGCATTC
AY524	Tn5403	MGE	AAGCGAATGGCGCGAAC	CGCGCAGGGTAAACTGC
AY527	trbC	MGE	CGGYATWCCGSCSACRCTGCG	GCCACCTGYSBGCAGTCMCC
AY46	ermF_1	MLSB	CAGCTTTGGTTGAACATTTACGAA	AAATTCCTAAAATCACAACCGACAA
AY528	ereA	MLSB	GATAATTCTGCTGGCGCACA	GCAGGCGTGGTCACAAC
AY533	ermB_2	MLSB	GAACACTAGGGTTGTTCTTGCA	CTGGAACATCTGTGGTATGGC
AY536	lnuB	MLSB	GGATCGTTTACCAAAGGAGAAGG	AGCATAGCCTTCGTATCAGGAA
AY537	lnuC	MLSB	GGGTGTAGATGCTCTTCTTGGA	CTTTACCCGAAAGAGTTTCTACCG

AY538	mefA	MLSB	TAATTATCGCAGCAGCTGGTTC	GTTCCCAAACGGAGTATAAGAGTG
AY539	mphA	MLSB	TCAGCGGGATGATCGACTG	GAGGGCGTAGAGGGCGTA
AY544	ermE	MLSB	GTCACGCAGCTGGAGTTCG	CGGTGAAGCACAGCTCGAC
AY546	ermX_2	MLSB	TGATGACGGCTCAGTGG	GTGCACCAGCGCCTGA
AY547	ermB_3	MLSB	TGAAAGCCATGCGTCTGAC	TTCAGCTGGCAGCTTAAGC
AY549	lnuF	MLSB	ATACCGGTCATTTCCACTTGGC	GCATCAGGCTGATGAGGTTCAA
AY551	mefB	MLSB	CCGATAGGCTTACTTGTTGCAG	AGTCCACTTGCGGTTTCATTG
AY553	msrE	MLSB	CGGCAGATGGTCTGAGCTTAAA	CGCACTCTTCCTGCATAAAGGA
AY142	ttgB	Other	TCGCCCTGGATGTACACCTT	ACCATTGCCGACATCAACAAC
AY186	nisB_1	Other	GGGAGAGTTGCCGATGTTGTA	AGCCACTCGTTAAAGGGCAAT
AY188	nimE	Other	TGCGCCAAGATAGGGCATA	GTCGTGAATTCGGCAGGTTTA
AY191	merA	Other	GTGCCGTCCAAGATCATG	GGTGGAAGTCCAGTAGGGTGA
AY197	crAss56	Other	CAGAAGTACAAACTCCTAAAAAACGTAGAG	GATGACCAATAAACAAGCCATTAGC
AY198	crAss64	Other	TGTATAGATGCTGCTGCAACTGTACTC	CGTTGTTTTCATCTTTATCTTGTCCAT
AY204	sat4	Other	GAATGGGCAAAGCATAAAAACTTG	CCGATTTTGAAACCACAATTATGATA

AY218	qacEΔ1_1	Other	TCGCAACATCCGCATTAAAA	ATGGATTTCAGAACCAGAGAAAGAAA
AY236	qacEΔ1_3	Other	GTCGGTGTTGCTTATGCAGTCT	CAACCAGGCAATGGCTGTAA
AY465	bacA	Other	ATCCGCGGCACCCTGA	CCTGCTTGATGGACTTGATGAAGA
AY466	mcr1	Other	CACATCGACGGCGTATTCTG	CAACGAGCATACCGACATCG
AY471	arr2	Other	TTGGCGATTGGTGACTTGCTAA	ATCGTCTTCGAACGGTCCTG
AY472	fabK	Other	CAGGAGCAGGAAATCCAAGC	CCAGCTTCCATTCCTTCTGC
AY29	catB3	Phenicol	GCACTCGATGCCTTCCAAAA	AGAGCCGATCCAAACGTCAT
AY30	catB8	Phenicol	CACTCGACGCCTTCCAAAG	CCGAGCCTATCCAGACATCATT
AY31	ceoA	Phenicol	ATCAACACGGACCAGGACAAG	GGAAAGTCCGCTCACGATGA
AY35	cmlA_2	Phenicol	TAGGAAGCATCGGAACGTTGAT	CAGACCGAGCACGACTGTTG
AY37	cmxA	Phenicol	GCGATCGCCATCCTCTGT	TCGACACGGAGCCTTGGT
AY38	catA1	Phenicol	GGGTGAGTTTCACCAGTTTTGATT	CACCTTGTCGCCTTGCGTATA
AY559	catB2	Phenicol	GCTACTATTCCGGCTATTACCATG	GGGCTCCTCGTTCATGTAGA
AY561	catP	Phenicol	CCTTTGGACTGAGTGTAAGTCTGA	TAAAGCCATCGAAGGTTGACCA
AY562	catQ	Phenicol	AGGTGCACTTACAGTATGACTGC	AACGTGGGAAGTTCTCGTCATAC

AY563	cmlV	Phenicol	GCCCTCATCACCGTCTTCG	GGACGTTGGCGATGGAGAG
AY566	floR	Phenicol	AACCCGCCCTCTGGATCA	GCCGTCGAGAAGAAGACGAA
AY95	qnrA	Quinolone	AGGATTTCTCACGCCAGGATT	CCGCTTTCAATGAAACTGCAA
AY96	qnrB	Quinolone	GCGACGTTCAGTGGTTCAGA	GCTGCTCGCCAGTCGAA
AY456	qepA	Quinolone	GGGCATCGCGCTGTTC	GCGCATCGGTGAAGCC
AY457	qnrB4	Quinolone	TCACCACCCGCACCTG	GGATATCTAAATCGCCCAGTTCC
AY458	qnrB_2	Quinolone	CGACGTTCAGTGGTTCAGATCTC	GCCAAGCCGCTCCATGAG
AY459	qnrD	Quinolone	CGCTGGAATGGCACTGTGA	GCTCTCCATCCAACTTCACTCC
AY461	qnrS2	Quinolone	TCCCGAGCAAACTTTGCCAA	GGTGAGTCCCTATCCAGCGA
AY463	qnrVC_2	Quinolone	TTCCTTTAAACGGGCAAACCTC	CGATACCTGATTCATGAAGCTAGC
AY241	sul4	Sulfonamide	TCAACGTCACTCCAGACAGC	TGGAAATAACGACGTCCACA
AY245	sul1_2	Sulfonamide	GCCGATGAGATCAGACGTATTG	CGCATAGCGCTGGGTTTC
AY365	sul2_2	Sulfonamide	TCATCTGCCAAACTCGTCGTTA	GTCAAAGAACGCCGCAATGT
AY249	tet36_1	Tetracycline	AGAATACTCAGCAGAGGTCAGTTCCT	TGGTAGGTCGATAACCCGAAAAT
AY250	tet32	Tetracycline	CCATTACTTCGGACAACGGTAGA	CAATCTCTGTGAGGGCATTTAACA

AY254	tetA_2	Tetracycline	CTCACCAGCCTGACCTCGAT	CACGTTGTTATAGAAGCCGCATAG
AY255	tetA/B_1	Tetracycline	AGTGCGCTTTGGATGCTGTA	AGCCCCAGTAGCTCCTGTGA
AY259	tetQ	Tetracycline	CGCCTCAGAAGTAAGTTCATACACTAAG	TCGTTCATGCGGATATTATCAGAAT
AY260	tetH	Tetracycline	TTTGGGTCATCTTACCAGCATTAA	TTGCGCATTATCATCGACAGA
AY263	tetW	Tetracycline	ATGAACATTCCCACCGTTATCTTT	ATATCGGCGGAGAGCTTATCC
AY264	tetO_2	Tetracycline	CAACATTAACGGAAAGTTTATTGTATACCA	TTGACGCTCCAAATTCATTGTATC
AY267	tetX	Tetracycline	AAATTTGTTACCGACACGGAAGTT	CATAGCTGAAAAAATCCAGGACAGTT
AY268	tetC_2	Tetracycline	ACTGGTAAGGTAAACGCCATTGTC	ATGCATAAACCAGCCATTGAGTAAG
AY269	tetS	Tetracycline	TTAAGGACAAACTTTCTGACGACATC	TGTCTCCCATTGTTCTGGTTCA
AY273	tetE	Tetracycline	TTGGCGCTGTATGCAATGAT	CGACGACCTATGCGATCTGA
AY276	tetT	Tetracycline	CCATATAGAGGTTCCACCAAATCC	TGACCCTATTGGTAGTGGTTCTATTG
AY367	tetL_2	Tetracycline	ATGGTTGTAGTTGCGCGCTATAT	ATCGCTGGACCGACTCCTT
AY568	tet39	Tetracycline	TATAGCGGGTCCGGTAATAGGTG	CCATAACGATCCTGCCCATAGATAAC
AY571	tetD	Tetracycline	AATTGCACTGCCTGCATTGC	GACAGATTGCCAGCAGCAGA
AY572	tetG	Tetracycline	TCGCGTTCCTGCTTGCC	CCGCGAGCGACAAACCA

AY574	tetM	Tetracycline	GGAGCGATTACAGAATTAGGAAGC	TCCATATGTCCTGGCGTGTC
AY576	tet44	Tetracycline	CTCATGTAGATGCAGGAAAGACG	GTAACTGCTGCCTGAATTGTGA
AY577	tetR	Tetracycline	CCGTCAATGCGCTGATGAC	GCCAATCCATCGACAATCACC
AY284	dfrA1_1	Trimethoprim	GGAATGGCCCTGATATTCCA	AGTCTTGCGTCCAACCAACAG
AY285	dfrA12	Trimethoprim	CCTCTACCGAACCGTCACACA	GCGACAGCGTTGAAACAACTAC
AY580	dfrA15	Trimethoprim	AGGCCGAAAGACTTTCGAGTC	TCACCTTCTGGCTCAATGTCG
AY581	dfra17	Trimethoprim	CGGGAACGGCCCTGATATTCC	CGTGTTGCGACCGCATACTTTC
AY586	dfrA27	Trimethoprim	GCCGCTCAGGATCGGTA	GTCGAGATATGTAGCGTGTCG
AY590	dfrAB4	Trimethoprim	CGGTTCGCATTCCCATCAAA	CGCAGTCATGGGATAAATCTGG
AY593	dfrG	Trimethoprim	TCAATCGGAAGAGCCTTACCTGA	TGGGCAAATACCTCATTCCATTCC
AY156	vanC_2	Vancomycin	CCTGCCACAATCGATCGTT	CGGCTTCATTCGGCTTGATA
AY159	vanB_1	Vancomycin	TTGTCGGCGAAGTGGATCA	AGCCTTTTTCCGGCTCGTT
AY161	vanD	Vancomycin	CAGAGGAACATAATGTTTCGATAAAATCT	GCCGGATTTTGTGATTCCAA
AY163	vanHB	Vancomycin	GAGGTTTCCGAGGCGACAA	CTCTCGGCGGCAGTCGTAT
AY170	vanXB	Vancomycin	AGGCACAAAATCGAAGATGCTT	GGGTATGGCTCATCAATCAACTT

AY174	vanRB	Vancomycin	GCCCTGTCGGATGACGAA	TTACATAGTCGTCTGCCTC TGCAT
AY181	vanTG	Vancomycin	CGTGTAGCCGTTCCGTTCT T	CGGCATTACAGGTATATCT GGAAA
AY182	vanYB	Vancomycin	GGCTAAAGCGGAAGCAGA AA	GATATCCACAGCAAGACCA AGCT
AY381	vanTC_2	Vancomycin	ACAGTTGCCGCTGGTGAA G	CGTGGCTGGTCGATCAAAA
AY595	vanA	Vancomycin	GGGCTGTGAGGTCGGTTG	TTCAGTACAATGCGGCCGT TA
AY596	vanC2	Vancomycin	TGACTGTCGGTGCTTGTGA	GATAGAGCAGCTGAGCTTG TTC
AY597	vanG	Vancomycin	TGTTTCGCAGAACCGTGTC AA	CCCTGCACTGTTCCATCTT CTC
AY599	vanSB	Vancomycin	GAAGATAAAGAGGGAAGC GTACTC	CCGAATTGTCAGCCCTTGA TAA
AY294	intI2_2	Integrons	TGCTTTTCCCACCCTTACC	GACGGCTACCCTCTGTTAT CTC
AY43	ermD_1	MLSB	GGACTCGGCAATGGTCAG AA	CCCCGAAACGCAATATAAT GTT
AY71	vgaA_1	MLSB	CGAGTATTGTGGAAAGCA GCTAGTT	CCCGTACCGTTAGAGCCGA TA
AY33	yidY/mdtL	Phenicol	GCAGTTGCATATCGCCTTC TC	CTTCCCGGCAAACAGCAT
AY34	mdtL	Phenicol	TGCTGATCGGGATTCTGAT TG	CAGGCGCGACGAACATAAT
AY244	sul3_1	Sulfonamide	TCCGTTCAGCGAATTGGTG CAG	TTCGTTCACGCCTTACACC AGC
AY473	A. baumannii	Taxanomic	TCTTGGTGGTCACTTGAAG C	ACTCTTGTGGTTGTGGAGC A

AY474	Bacteroidetes	Taxanomic	GGARCATGTGGTTTAATTCGATGAT	AGCTGACGACAACCATGCAG
AY475	Campylobacter	Taxanomic	CTGCTTAACACAAGTTGAGTAGG	TTCCTTAGGTACCGTCAGAA
AY476	Enterococci	Taxanomic	AGAAATTCCAAACGAACTTG	CAGTGCTCTACCTCCATCATT
AY477	Firmicutes	Taxanomic	GGAGYATGTGGTTTAATTCGAAGCA	AGCTGACGACAACCATGCAC
AY478	K. pneumoniae	Taxanomic	ACGGCCGAATATGACGAATTC	AGAGTGATCTGCTCATGAA
AY479	P. aeruginosa	Taxanomic	AGCGTTCGTCCTGCACAAGT	TCCACCATGCTCAGGGAGAT
AY480	Staphylococci	Taxanomic	CGCAACGTTCAATTTAATTTTGTTAA	TGGTCTTTCTGCATTCCTGGA
AY601	blaOXA48	Beta Lactam	TGTTTTTGGTGGCATCGAT	GTAAMRATGCTTGGTTCGC
AY602	armA	Aminoglycoside	TGCATCAAATATGGGGGTCT	TGAAGCCACAACCAAAATCT
AY603	rmtB	Aminoglycoside	GCTGTGATATCCACCAGGGA	AAGCTTAAAAATCAGCGCCA