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Assessing the risk of antimicrobial resistance and potential environmental harm through national-scale surveillance of antimicrobials in hospital and community wastewater

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ABSTRACT

Antimicrobial resistance (AMR) is a major public health concern. Antimicrobial residues enter wastewater where their continued presence can lead to an increased risk of AMR while also causing environmental harm when untreated wastewater is discharged into the environment. This research presents the antimicrobial residue surveillance results of a national-scale wastewater sampling campaign across Wales. Wastewater from 15 sites—effluent from 7 hospitals and influent from 8 community wastewater treatment plants—was collected for 5 consecutive days monthly from May–July 2023. This campaign captured more than 30 % of the Welsh population and over 30 % of the population receiving care as hospital inpatients. Using a quantitative approach, over 175 unique wastewater samples were analyzed by ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) for more than 40 antimicrobials including: β-lactams, macrolides, quinolones, sulfonamides, a veterinary antibiotic, an antifungal, antivirals, and numerous metabolites. The most prevalent antimicrobials were clarithromycin, its metabolite N-desmethyl clarithromycin, fluconazole, and trimethoprim as they were detected in all samples. Sulfamethoxazole concentrations ranged from 724–28031 ng/L and trimethoprim ranged from 785–44539 ng/L in hospital effluent—concentrations significantly higher than those reported in published literature. In hospital wastewater, many antimicrobials were present at concentrations which were orders of magnitude higher than their respective predicted no-effect concentrations (PNECs) for antibiotic resistance selection (e.g., metronidazole, trimethoprim). These concentrations create a selective pressure which can drive AMR emergence. Furthermore, some antimicrobials remained at high-risk concentrations even after dilution in community wastewater (e.g., ciprofloxacin, clarithromycin). Environmental risk assessments also identified clarithromycin and ciprofloxacin as agents of concern while vancomycin posed the highest environmental risk (concentrations ca. 1000–38000-fold > PNEC in hospital effluent) should this wastewater enter the environment untreated (e.g., combined sewer overflows). Instances of direct disposal of antimicrobials were clearly identified in hospital wastewater. These results demonstrate the importance of regular monitoring of AMR and potential environmental risk posed by antimicrobials in wastewater, while demonstrating the need for comprehensive national action (e.g., treatment of hospital wastewater on-site, tertiary/quaternary treatment of community wastewater, tailored stewardship programs, focussed control efforts on high-risk antimicrobials) to minimize risks to public health and the environment.

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1. Introduction

Antimicrobial resistance (AMR) is a continuing major global health concern. In 2019, it is estimated that AMR caused 1.27 million deaths globally (Murray et al., 2022), and an estimated 13.6 % of deaths worldwide in 2019 could be attributed to 33 bacterial organisms (Ikuta et al., 2022). The same year, the World Health Organization identified AMR as one of the top 10 greatest threats to global health (World Health Organization, 2019). The cause of AMR is multifaceted. Excessive use of antibiotics from inappropriate prescribing (e.g., empiric antibiotic treatment, nominal treatment durations), premature antimicrobial therapy cessation, over-the-counter access, limited antibiotic selection on the drug market, suboptimal vaccination rates, travel, agricultural applications, growing population, and ability of microorganisms to adapt are a limited selection of factors propelling the current crisis (Abushaheen et al., 2020; Holmes et al., 2016; Michael et al., 2014).

Wastewater-based epidemiology (WBE) has been firmly established as a valuable and sustainable approach to assess both population health, and the exposure and fate of chemicals in the environment. From a public health perspective, wastewater is an ideal sample matrix as it provides anonymized, community-wide data, and to date, a variety of target analytes have been evaluated including: drugs of abuse (Bijlsma et al., 2016; Ceolotto et al., 2024), prescription pharmaceuticals (Ceolotto et al., 2024; Styszko et al., 2021), lifestyle markers (e.g., nicotine, caffeine, alcohol) (Ceolotto et al., 2024; Kasprzyk-Hordern et al., 2023; Styszko et al., 2021), industrial chemicals and personal care products (e.g., pesticides, bisphenols, phthalates) (Kasprzyk-Hordern et al., 2023; Rousis et al., 2017; Senta et al., 2022), and pathogens (Ahmed et al., 2020; Barber et al., 2023; Jagadeesan et al., 2024; Kasprzyk-Hordern et al., 2023). This approach is widely accessible in both high- and low-income countries. WBE can comprehensively assess antimicrobial usage in near real-time allowing the impact of current population or environmental stressors to be promptly assessed. With this data, new policies and interventions can be established to target the antimicrobials at highest risk of propagating AMR, and prevention strategies can be employed. WBE is also advantageous as it facilitates a One Health approach. In the environment, wastewater serves as a natural reservoir containing antimicrobials derived from human consumption, and industrial and agricultural processes. Previous studies have identified and quantified numerous antimicrobials and their metabolites in wastewater across a wide range of antimicrobial classes: antibiotics, such as β-lactams (e.g., amoxicillin, cephalexin, meropenem), macrolides (e.g., clarithromycin, erythromycin), quinolones (e.g., ciprofloxacin, ofloxacin), sulfonamides (e.g., sulfamethoxazole), and tetracyclines (e.g., doxycycline, tetracycline); antiretrovirals, such as emtricitabine and lamivudine; and antifungals, such as fluconazole (Holton and Kasprzyk-Hordern, 2021; Holton et al., 2022; Xu et al., 2022). Spatial and temporal trends of antimicrobial usage can be defined, while public consumption estimates and instances of industrial emissions can be elucidated (Holton and Kasprzyk-Hordern, 2021; Holton et al., 2022; Xu et al., 2022). This approach can help provide relevant insights to antimicrobial stewardship.

Antimicrobials present in wastewater pose risks to both public health and the health of the environment. Microorganisms are continuously exposed to naturally produced and human-derived antimicrobials present in wastewater. Worryingly, the regular exposure of bacteria to these agents, even at low concentrations, has been shown to exert a selective pressure on bacteria leading to increased antibacterial resistance (Gullberg et al., 2014; Gullberg et al., 2011; Hayes et al., 2022; Kristiansson et al., 2011; Stanton et al., 2020). This selective pressure can be concentration-dependent, as higher antibiotic concentrations can lead to greater abundance of antimicrobial resistant genes and mobile genetic elements, consequently promoting the development of antibacterial resistant organisms (Rodriguez-Mozaz et al., 2015). Hospital effluent wastewater is of particular concern. It can contain significantly higher concentrations of antibiotics and antimicrobial resistant genes than influent wastewater (Perry et al., 2021; Rodriguez-Mozaz et al., 2015; Zhang et al., 2020) while also harbouring a variety of multi-drug resistant organisms (Amaya et al., 2012; Cahill et al., 2019; Conte et al., 2017; Gündoğdu et al., 2013; Iweriebor et al., 2015; Qin et al., 2019; Spindler et al., 2012; Yousfi et al., 2019). Recent literature has highlighted the importance of understanding the prevalence and distribution of antimicrobial residues within sewer catchment systems due to the presence of biofilms. Unique differences between hospital wastewater biofilms and community wastewater biofilms have been identified (Buelow et al., 2023). Hospital wastewater biofilms have a higher risk of inducing AMR (e.g., greater baseline expression of antibiotic resistance genes)—a risk which increased upon exposure to ciprofloxacin, a quinolone antibiotic frequently seen at high concentrations in hospital effluent (Buelow et al., 2023; Diwan et al., 2010; Rodriguez-Mozaz et al., 2015; Santos et al., 2013; Verlicchi et al., 2012). As the development of AMR can directly affect the population health of a community, elucidation of the risk of AMR is crucial from a public health, and One Health perspective.

The presence of antibiotic residues in wastewater can also pose ecotoxicological risks should untreated wastewater enter the aquatic environment (e.g., via combined sewer overflows) (Perry et al., 2024). Clarithromycin, erythromycin, ofloxacin and sulfamethoxazole antibiotics in the hospital wastewater effluent from an Italian hospital were found to pose a high risk to aquatic life (Verlicchi et al., 2012). In Portugal, the concentrations of azithromycin, clarithromycin, sulfamethoxazole, ciprofloxacin, and ofloxacin from the wastewater of four hospital sites posed an ecotoxicological risk to algae, while the latter two antibiotics also posed a high risk to daphnids and fish (Santos et al., 2013). Similarly, an analysis of hospital wastewater from 16 hospitals in Türkiye concluded that azithromycin and clarithromycin concentrations presented a high risk to algae, but azithromycin concentrations were additionally found to pose a high risk to fish (Aydin et al., 2019). Unfortunately, many antibiotics are inefficiently removed during wastewater treatment and enter the aquatic environment (Aydin et al., 2019; Rodriguez-Mozaz et al., 2015; Santos et al., 2013; Verlicchi et al., 2012). A multi-year pan-European study of wastewater treatment plant (WWTP) effluent found that concentrations of ciprofloxacin, azithromycin, and cefalexin posed a risk of environmental harm in many countries, further highlighting the necessity of adopting a One Health approach (Rodriguez-Mozaz et al., 2020).

Hospital effluent is often considered a hotspot for AMR (Gwenzi et al., 2020; Rozman et al., 2020). Past works have evaluated antimicrobial residues in hospital effluent and their influence on community wastewater with a focus on the environmental risks (Al-Maadheed et al., 2019; Aydin et al., 2019; Dinh et al., 2017; Rodriguez-Mozaz et al., 2015; Santos et al., 2013; Sims et al., 2023b; Thomas et al., 2007; Verlicchi et al., 2012). However, the risk of these antimicrobial residues on inducing AMR is often neglected. A comprehensive assessment of antimicrobial use across a whole population is possible through WBE. Influent wastewater provides a collective sample from the community and is reflective of both primary and secondary care prescribing, with primary care having a larger contribution. On the contrary, hospital wastewater provides a pooled sample from all patients in secondary care. From a public health perspective, comparison of community wastewater treatment plant (WWTP) influent wastewater and hospital effluent wastewater can reveal differences between primary and secondary care prescribing of antimicrobials, and can elucidate the true usage of antimicrobials within a catchment—usage which may not be adequately captured by prescription data. This study aims to address the limitations present in many published works including: the use of wastewater grab sampling (Ahmad et al., 2012; Al-Maadheed et al., 2019; Brown et al., 2006; Diwan et al., 2010), use of a limited selection of antibiotics (Ahmad et al., 2012; Al-Maadheed et al., 2019; Ashfaq et al., 2016; Diwan et al., 2010; Lien et al., 2016), and sampling from a limited number of hospitals (Ahmad et al., 2012; Al-Maadheed et al., 2019; Ashfaq et al., 2016; Azuma et al., 2024; Diwan et al., 2010; Lien

et al., 2016; Riaz ul Haq et al., 2012; Rodriguez-Mozaz et al., 2015; Verlicchi et al., 2012). This work will elucidate potential environmental risks but also the risk of AMR—a growing issue which can have a significant impact on public health.

This study presents a novel, comprehensive national-scale antimicrobial surveillance campaign in Wales, uniquely assessing paired hospital and community wastewater sites. Over a 3-month period in 2023, wastewater effluent from seven large municipal hospitals (131–635 average occupied beds), and influent wastewater from eight urban WWTPs (10,184–612,002 population equivalents) were sampled to obtain an in-depth spatial analysis of antimicrobial presence in wastewater (StatsWales, 2023). This comprehensive work captures over 30 % of the total Welsh population and over 30 % of inpatients in Welsh hospitals (StatsWales, 2023; The Office for National Statistics, 2022). Using ultra-performance liquid chromatography tandem mass spectrometry, over 175 wastewater samples (24 h composite) were assessed for more than 40 antimicrobials in quadruplicate, resulting in the generation of over 28,000 individual measurements throughout the course of this research. In addition to assessing the risk of AMR, the potential environmental risk posed by antimicrobials in Welsh wastewater was explored through comparison to established predicted no-effect concentrations. The inclusion of antibiotic metabolites allows for the identification of possible direct disposal events and can show potential industrial contribution to antimicrobial prevalence. This research will be used to gain insight into the challenges of managing antimicrobial pollution and resistance across different healthcare settings. The observed variations in antimicrobial concentrations across different sites and antimicrobial classes call for tailored stewardship programs, enhanced wastewater monitoring, and focussed control efforts on high-risk antimicrobials—all of which should be incorporated into national AMR action plans.

2. Experimental

2.1. Materials, reagents, and chemicals

Antimicrobials used in this multi-residue method are presented in Table 1. Supplementary Material Table S1 lists the analytical standards with their chemical formula, CAS number, log P values, and respective suppliers: LGC / Toronto Research Chemicals (Teddington, UK), MedChem Express (Cambridge, UK), or Merck Group / Sigma-Aldrich (Gillingham, UK). Ultrapure LC–MS water (H_2O; Sigma-Aldrich) and LC–MS grade methanol (MeOH; Sigma-Aldrich) were used as solvents and to prepare mobile phase. Formic acid (> 98 %; Sigma-Aldrich) was used as a mobile phase additive. Wastewater samples were filtered using Whatman™ GF/F 0.7 µm filter paper (Fisher Scientific, Loughborough, UK), while Oasis HLB solid phase extraction cartridges (60 mg, 3 cc; Waters, Manchester, UK), silanized glass tubes (Waters), and polypropylene LC vials (Waters) were used during the sample preparation process.

2.2. Wastewater sample collection

Wastewater samples were collected during a 3-month sampling period from May to July 2023, sampling 5 consecutive days each month. Fifteen sites were selected across Wales to provide geographical coverage of the main urban centres (see Fig. 1): influent wastewater was collected from eight community WWTPs, and hospital effluent from seven sites (the population equivalents served by the WWTPs and the average hospital bed occupancies are presented in Supplementary Material Table S2, while the wastewater sample availability throughout this campaign is shown in Table S3). In Fig. 1, Hospital 1 through to Hospital 6 are located in the same wastewater catchment as WWTP 1 through to WWTP 6, respectively, therefore the hospital wastewater will merge and dilute with the community wastewater. Wastewater samples were 24 h composites (samples taken every 20 min using refrigerated autosamplers) and were frozen immediately after collection to preserve analyte stability.

Table 1
Antimicrobials included in method organized by antimicrobial class.

Antimicrobial Class	Antimicrobial	Abbreviation
Amphenicol	Chloramphenicol	CHL
Antifungal	Fluconazole	FCZ
Antiretroviral	Emtricitabine	FTC
	Lamivudine	3TC
Antitubercular Agent	Ethambutol	EMB
	Rifampicin	RMP
β-Lactam	Amoxicillin	AMX
	Amoxicilloic acid	AMXa
	Cefalexin	LEX
	Cefazolin	CFZ
	Mecillinam	MEC
	Meropenem	MEM
	Penicillin V	PenV
	Penicilloic G acid	PenGa
	Piperacillin	PIP
Glycopeptide	Vancomycin	VAN
Macrolide & Lincomycin	Clarithromycin	CLR
	Clarithromycin, N-desmethyl-	dmCLR
	Clindamycin	CLI
	Clindamycin, N-desmethyl-	dmCLI
	Erythromycin	ERY
	Erythromycin, N-desmethyl-	dmERY
	Roxithromycin	ROX
Nitrofuran	Nitrofurantoin	NIT
	1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione	NP-AHD
Nitroimidazole	Metronidazole	MTZ
	Metronidazole, Hydroxy-	hMTZ
Oxazolidinone	Linezolid	LZD
Pleuromutilin	Tiamulin	TIA
Quinolone	Ciprofloxacin	CIP
	Ciprofloxacin, Desethylene-	deCIP
	Ofloxacin	OFX
	Ofloxacin, Desmethyl-	dmOFX
	Ofloxacin N-oxide	OFXo
Sulfonamide & Trimethoprim	Sulfadiazine	SDZ
	Sulfadiazine, N-acetyl-	aSDZ
	Sulfamethoxazole	SMX
	Sulfamethoxazole, N-acetyl-	aSMX
	Sulfapyridine	SPY
	Sulfapyridine, N-acetyl-	aSPY
	Sulfasalazine	SLZ
	Trimethoprim	TMP
Tetracycline	Tetracycline	TET

Italicized antimicrobials are metabolites.

2.3. Standards and sample preparation

As the sample preparation method has been previously described elsewhere (Holton and Kasprzyk-Hordern, 2021), a brief overview will be presented. A 1 µg/mL internal standard mix was prepared in methanol using isotopically-labelled standards while analyte standard mixes were made in methanol at 10 µg/mL and 1 µg/mL. To ensure reproducible extraction, the pH of wastewater samples was adjusted to 7.5 (± 0.5) with either 0.1 M HCl or 0.1 M NaOH. Samples were split into two aliquots of 50 mL, spiked with 50 µL of internal standard mix, gently shaken, and set to equilibrate for 30 min. SPE cartridges were conditioned with 2 mL MeOH followed by 2 mL H_2O, both occurring under gravity. After sample homogenization, samples were filtered then loaded onto SPE cartridges under vacuum (at approximately 5 mL/min) until dry, and stored at –20 °C. Target analytes were eluted from thawed cartridges into silanized glass tubes using 4 mL MeOH under gravity. The eluant was dried in an evaporator at 40 °C with nitrogen (99.998 % purity), reconstituted with 500 µL H_2O:MeOH 80:20 and transferred to LC vials for analysis.

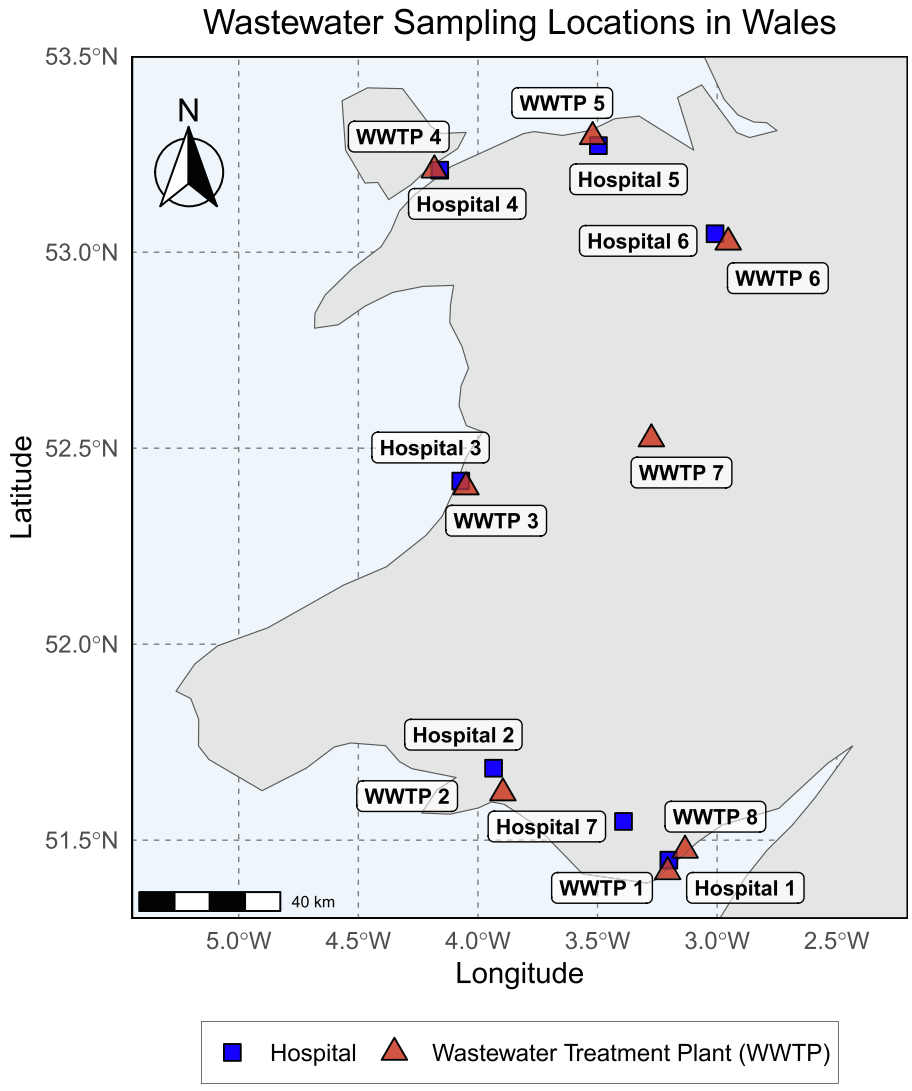


Fig. 1. Map of wastewater sampling locations in Wales.

2.4. Instrumentation and data analysis

The instrumental parameters have been adapted from those previously published by Holton and Kasprzyk-Hordern (2021). Samples were analyzed using a Waters ACQUITY UPLC™ with a Xevo TQD mass spectrometer operating in multiple reaction monitoring mode (ion transitions, cone voltages, and collision energies are listed in Supplementary Material Table S4). A 20 µL sample volume was injected onto a BEH C18 column (50 × 2.1 mm, 1.7 µm) with a pre-filter (2.1 mm, 0.2 µm; Waters), and the mobile phase flow rate was set to 0.2 mL/min. Each wastewater sample replicate was injected twice. Mobile phase A comprised of H_2O:MeOH 95:5 with formic acid 0.1 % and mobile phase B was 100 % MeOH operating under the following gradient: 0 % B (hold 1 min), then increase to 40 % B over 8.5 min, then increase to 100 % B over 3.5 min (hold 3 min), finally return to 0 % B (hold 0.5 min) with a 2.5 min re-equilibration period. The MS was operated in ESI+ mode. The MS was set to collect 20 points-per-peak for a 17 s peak width ensuring at least 10 points-per-peak across all peaks. Additional LC and MS parameters are available in the original publication. Data was processed using Waters MassLynx software (V4.1 SCN854).

2.5. Instrument and method performance

Calibration curves were made for the included analytes using over 20 calibration points across the 0–5000 ng/mL range with six independent replicates. Instrumental performance parameters and method performance parameters are listed in Supplementary Material Table S5. For instrumental performance, retention time and relative retention time were determined at three concentration levels: 25, 200, and 500 ng/mL (n = 6). Intraday accuracy (**Eq. (1)**) and precision (**Eq. (2)**) were calculated from triplicate results at the 500 ng/mL level obtained in a single day, while interday accuracy and precision reflected the mean of these calculations over three consecutive days.

$$Accuracy\ (\%) = \frac{C_S}{C_T} * 100 \tag{1}$$

$$Precision\ (\%) = \frac{\sqrt{\frac{\sum (C_S - C_T)^2}{(n-1)}}}{C_{S,mean}} * 100 \tag{2}$$

where C_S is the experimental spiked concentration, $C_{S,mean}$ is the mean of experimental spiked concentrations for n replicates, C_T is the theoretical concentration, and n is the number of replicates. Reduced accuracy and precision were noted closer to the limits of quantification. The instrument quantification limit (IQL) for each analyte was determined from the calibration curves and represents the concentration at which the quantitative transition ion had a signal-to-noise ratio consistently ≥ 10 and qualitative transition ion had a signal-to-noise ratio consistently ≥ 3. The

instrument detection limit (IDL) was derived from the IQL using **Eq. (3)**.

$$IDL = \frac{IQL}{10} * 3.3 \quad (3)$$

Ion ratios were determined across the range of 10–1000 ng/mL to aid in analyte identification. The IQL was used for analytes which had an IQL > 10 ng/mL, and the concentration range maximum was used if this maximum was < 1000 ng/mL.

For method performance parameters, retention time and relative retention time were calculated from wastewater matrix spiked at 20, 200 and 500 ng/mL (n = 6). Matrix recovery was determined at these same concentrations using six replicates according to **Eq. (4)**.

$$Matrix\ Recovery\ (\%) = \frac{C_S - C_{US}}{C_T} * 100 \quad (4)$$

where C_S is the experimental concentration in spiked matrix, C_{US} is the experimental concentration in unspiked matrix, and C_T is the theoretical concentration. The method detection limit (MDL) and method quantification limit (MQL) were calculated from the IDL and IQL, respectively, using **Eq. (5)** and **Eq. (6)**.

$$MDL = \frac{IDL * 100}{Matrix\ Recovery\ (\%) * C_f} \quad (5)$$

$$MQL = \frac{IQL * 100}{Matrix\ Recovery\ (\%) * C_f} \quad (6)$$

where C_f is the concentration factor from the solid phase extraction process (here, wastewater samples were concentrated by a factor of 100). Method accuracy and precision were calculated from six matrix replicates spiked at the 500 ng/mL concentration level. As this research aims to quantify and assess the risk of AMR and risk of ecotoxicity, only antimicrobial residues which were present above the method quantification limit were reported.

2.6. Quality assurance

Quality control samples at 20, 200 and 500 ng/mL were prepared in 80:20 H_2O: MeOH and analyzed with each batch of samples to ensure method performance. Quality control samples were also prepared in influent wastewater matrix at the same concentration levels. A wastewater matrix blank was included to account for any antimicrobials present in the matrix prior to spiking. To ensure analyte integrity, new quality control samples were prepared daily. Welsh wastewater samples were analyzed in batches organized by sampling date, and quality control samples were assessed multiple times throughout each analysis.

2.7. Risk assessments

2.7.1. Antimicrobial resistance risk assessment

To assess the risk of AMR, predicted no-effect concentrations (PNECs) are required. In 2016, Bengtsson-Palme and Larsson developed a comprehensive list of PNECs for AMR selection (PNEC-RS) for over 110 antibiotics using the lowest observed, or predicted, minimum inhibitory concentrations and an assessment factor of 10 (full details of the methodology are available in the original publication; PNEC-RS are listed in Supplementary Material Table S6) (Bengtsson-Palme and Larsson, 2016). When a PNEC-RS was unavailable and the analyte has antibacterial activity (not all antimicrobial metabolites are active compounds), a default PNEC of 50 ng/L was used (Vestel et al., 2022). Using measured antibiotic concentrations and the PNEC-RS, the degree of risk for AMR selection can be quantified using the following equation:

$$Risk\ Quotient\ (RQ) = \frac{Measured\ Antimicrobial\ Concentration\ in\ Wastewater\ (ng/L)}{Predicted\ No\ Effect\ Concentration\ (ng/L)} \quad (7)$$

where RQ < 0.1 is low risk, 0.1 ≤ RQ < 1 is moderate risk, and RQ ≥ 1 is high risk. As there were frequently order of magnitude differences between risk quotients in the high risk category, a two-tiered system (1 ≤ RQ < 10, RQ ≥ 10) was arbitrarily implemented to highlight these differences.

2.7.2. Environmental risk assessment

Antimicrobials in wastewater can pose ecotoxicological risks. The NORMAN Network publishes predicted no-effect concentrations for numerous chemicals of concern, including antimicrobials, in the NORMAN Ecotoxicology Database (see Supplementary Material Table S6) (The NORMAN Network, 2024). To quantify the risk to the aquatic environment, the measured antimicrobial concentrations in wastewater were compared to the lowest PNEC in freshwater ($PNEC_{fw}$) from the NORMAN Ecotoxicology Database (see Eq. (7)). Again, a default value of 50 ng/L was employed when PNEC values were unavailable (Vestel et al., 2022).

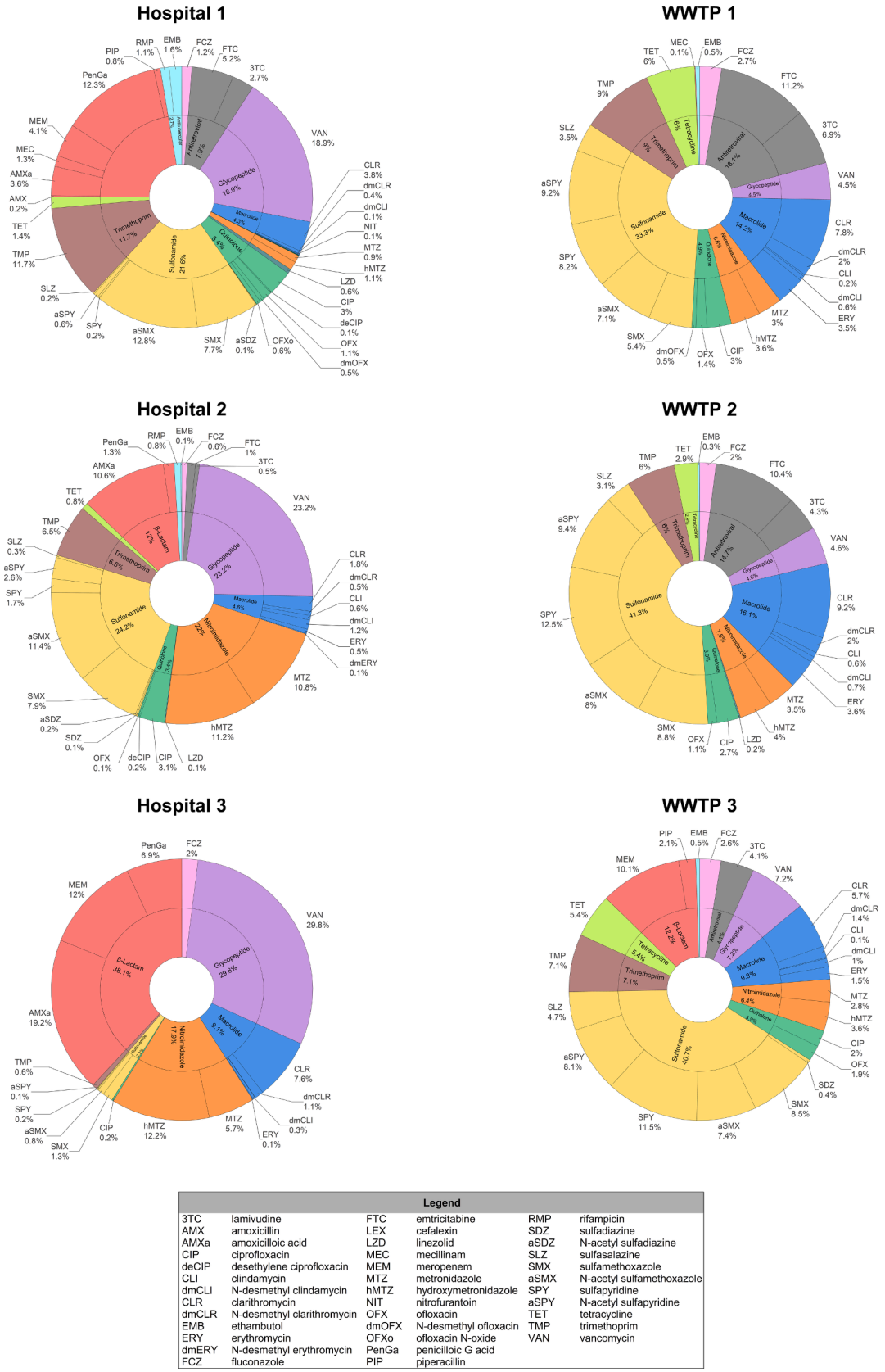
3. Results and discussion

In this study, antimicrobials were selected to create a comprehensive method that builds upon the breadth and depth of previous works and allows for both elucidation of antimicrobial trends while accurately capturing the degree of risk. Antimicrobials with a demonstrated risk of environmental harm (e.g., clarithromycin, ciprofloxacin) were included, while an effort was made to include agents from a variety of antimicrobial classes—even those which have predominantly hospital use (e.g., oxazolidinones, glycopeptides). Metabolites were added to confirm human consumption and possibly allow for both the identification of direct disposal events and the influence of industry on the antimicrobial load in wastewater. Antivirals (emtricitabine, lamivudine), an antifungal (fluconazole), and a veterinary medicine (tiamulin) were added to expand the scope of this work and provide a more complete picture of the potential environmental risk and risk of AMR from antimicrobials in wastewater.

3.1. Relative distribution of antimicrobials by site

As a qualitative assessment of antimicrobial usage patterns, the relative distribution of antimicrobials across all 15 sites are presented in Figs. 2 and 3, and S1**.** Across all WWTPs, sulfonamides were the most prevalent antimicrobial class representing approximately a third of antimicrobial residues quantified. The relative proportion of macrolides, nitroimidazoles, and trimethoprim were fairly consistent representing approximately 10–15 %, 5–10 %, and 5 % of the total, respectively, while β-lactams showed significant variability. The relative distribution of antimicrobial classes among hospital sites was heterogeneous. Unlike community wastewater, β-lactams comprised of around 10–40 % of the residues measured. β-lactams exhibit poor stability (Hirsch et al., 1999) and their lack of detection in wastewater has been previously documented (Rodriguez-Mozaz et al., 2015). The short in-sewer residence time prior to wastewater collection for hospital sites, and possible increased use of β-lactams in hospitals could explain this difference. Similarly, the tetracycline family of antibiotics is also known to have poor stability in wastewater likely owing to its ability to bind with polyvalent cations (e.g., Ca^{2+}, Mg^{2+}) (Hirsch et al., 1999). The increased proportion of tetracycline in community wastewater could indicate more prevalent community use. Vancomycin, a glycopeptide, was generally found in greater proportions in hospital wastewater. Its use as an antibiotic of "last resort" and its intravenous administration limit its use primarily to hospital settings.

An interesting series of observations was noted for the β-lactam, meropenem. At Hospital 3 and 4, meropenem comprised a large portion of the β-lactams quantified (12 % and 15.8 %, respectively), and similarly, it was detected in their respective community wastewaters (10.1 %



Legend					
3TC	lamivudine	FTC	emtricitabine	RMP	rifampicin
AMX	amoxicillin	LEX	cefalexin	SDZ	sulfadiazine
AMXa	amoxicilloic acid	LZD	linezolid	aSDZ	N-acetyl sulfadiazine
CIP	ciprofloxacin	MEC	mecillinam	SLZ	sulfasalazine
deCIP	desethylene ciprofloxacin	MEM	meropenem	SMX	sulfamethoxazole
CLI	clindamycin	MTZ	metronidazole	aSMX	N-acetyl sulfamethoxazole
dmCLI	N-desmethyl clindamycin	hMTZ	hydroxymetronidazole	SPY	sulfapyridine
CLR	clarithromycin	NIT	nitrofurantoin	aSPY	N-acetyl sulfapyridine
dmCLR	N-desmethyl clarithromycin	OFX	ofloxacin	TET	tetracycline
EMB	ethambutol	dmOFX	N-desmethyl ofloxacin	TMP	trimethoprim
ERY	erythromycin	OFXo	ofloxacin N-oxide	VAN	vancomycin
dmERY	N-desmethyl erythromycin	PenGa	penicilloic G acid		
FCZ	fluconazole	PIP	piperacillin		

Fig. 2. Relative distribution of antimicrobials in wastewater relative to total antimicrobial concentration over a 3-month sampling campaign for Hospitals 1–3 (left), and their corresponding wastewater treatment plants (WWTPs) 1–3 (right).

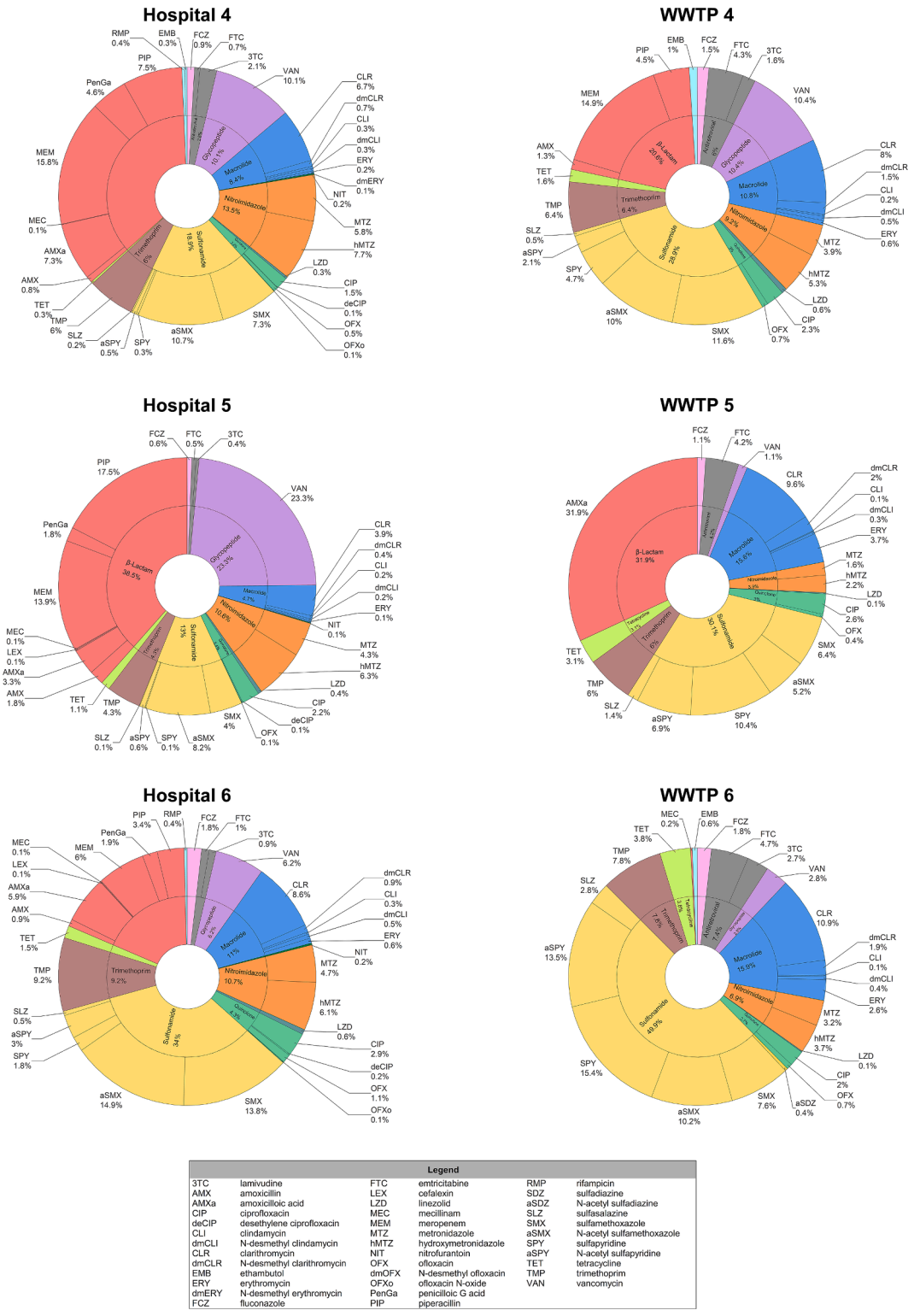


Fig. 3. Relative distribution of antimicrobials in wastewater relative to total antimicrobial concentration over a 3-month sampling campaign for Hospitals 4–6 (left), and their corresponding wastewater treatment plants (WWTPs) 4–6 (right).

and 14.9 %, respectively). In this work, the flow rate of hospital wastewater was unavailable; however, to permit a qualitative comparison, an assumption can be made that the hospital wastewater flow rate was constant and proportional to the number of occupied beds at the hospital sites. With this assumption, WWTP 3 and 4 service the two communities with the highest hospital bed density (beds per 10,000 inhabitants, see Table S2), thereby explaining the greater influence of hospital wastewater on the antimicrobial proportions in the community wastewater. However, Hospital 5 has a similar hospital bed density as Hospital 3 and similar proportion of meropenem (13.9 %), yet no meropenem was detected in the community wastewater. This suggests that bed density is only one factor among many (e.g., analyte stability, wastewater flow rates) which may influence antimicrobial proportions in community wastewater.

Geographical differences in antimicrobial use were also evident. The use of emtricitabine and lamivudine, two antivirals typically used for treating human immunodeficiency virus (HIV), were generally more prevalent in the southern region of Wales. These antivirals comprise of approximately 10 % and 15 % of antimicrobial load at WWTP 8 and WWTP 2, respectively, with the highest proportion for hospital and community use coming from Hospital 1 (~8%) and WWTP 1 (~18 %). WWTP 1, 2, and 8 service the most populated communities of the sampled sites. Interestingly, antiviral residues were detected in a high proportion at WWTP 7 (~15 %) while this site services the community with the smallest population in this study. These antivirals make up 5–20 % of community wastewater while they contribute < 5 % to hospital wastewater. This coincides with HIV infection being a chronic medical condition where most patients receive treatment as outpatients rather than in an acute patient care setting.

These results from detection of antivirals highlight an important

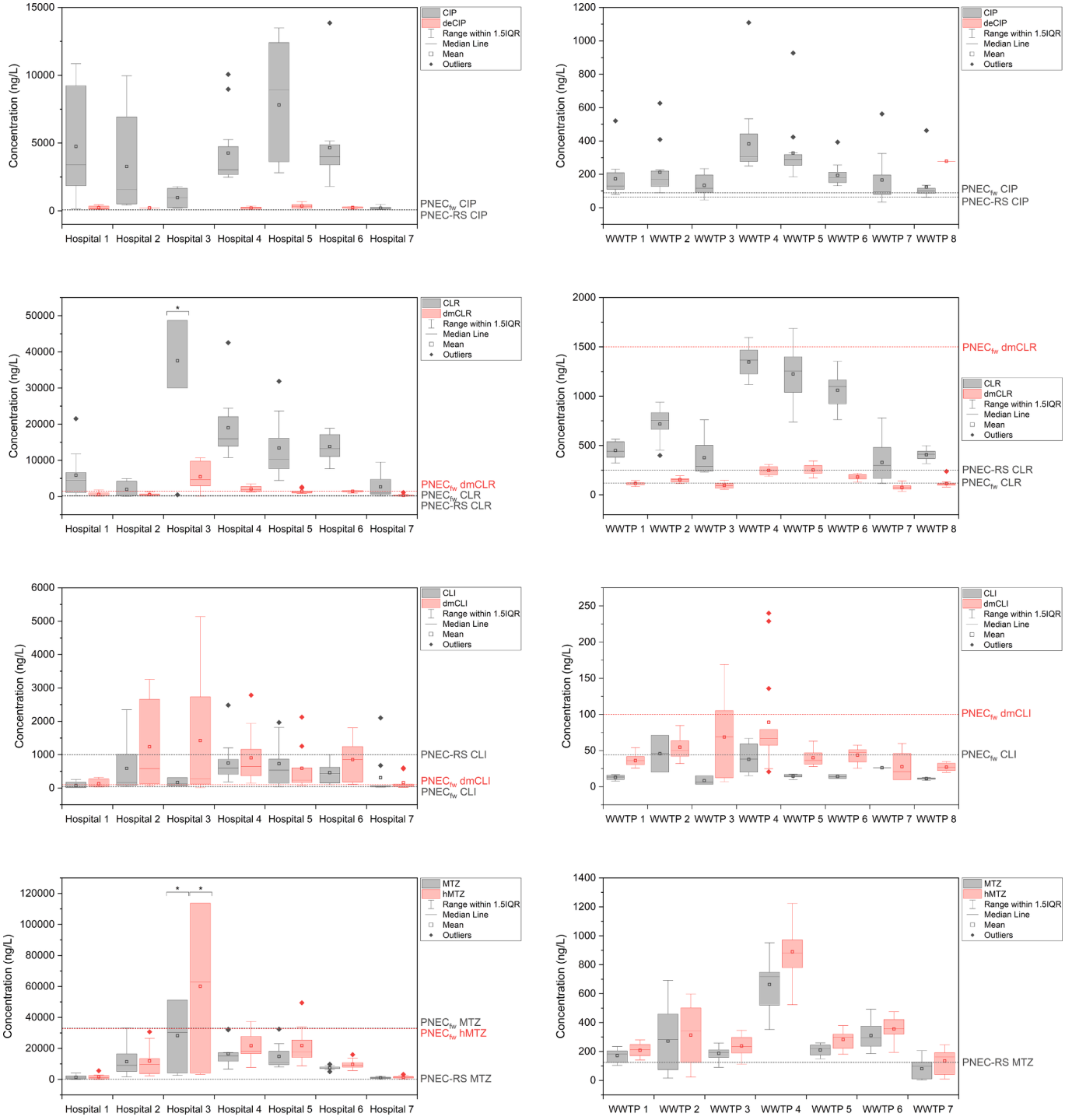


Fig. 4. Box plots of antimicrobial concentrations in hospital wastewater (left) and community wastewater (right). Asterisk (*) above bars indicates that the antimicrobial concentration exceeded the upper end of the calibration curve and were limited to the maximal value. Abbreviations: CIP, ciprofloxacin; CLI, clindamycin; CLR, clarithromycin; deCIP, desethylene ciprofloxacin; dmCLI, N-desmethyl clindamycin; dmCLR, N-desmethyl clarithromycin; hMTZ, hydroxymetronidazole; IQR, interquartile range; MTZ, metronidazole; PNEC$_{fw}$, lowest predicted no-effect concentration in freshwater; PNEC-RS, predicted no-effect concentration for resistance selection; WWTP, wastewater treatment plant.

difference between hospital and community wastewater. Community wastewater reflects a population's use of antimicrobials as shaped by primary care prescribing. Given the treatment of chronic diseases and acute infections with less complex clinical management, community wastewater may be more homogenous in the selection and prevalence of antimicrobial use (e.g., limited use of parenteral antimicrobials, more limited use of broad-spectrum agents like aminoglycosides and carbapenems). By contrast, hospital wastewater can be more heterogenous due to the variety and relatively higher levels of potential pathogens in patients (community-acquired and nosocomial infections) and their more complex antimicrobial treatment regimes. A hospital's treatment specialties will also influence antimicrobial use. The assessment of both AMR risk and potential environmental risk in hospital wastewater will therefore be highly site-specific. This needs to be considered in One Health actions as appropriate interventions (e.g., public health regulations, addition of wastewater treatment technologies, etc.) may vary from site to site.

3.2. Antimicrobial concentrations across sampling period

In this study, > 40 antimicrobials were quantified in wastewater from seven hospitals and eight communities across Wales during the May to July 2023 sampling campaign. Figs. 4, S2 and S3 present box plots of measured concentrations with relevant PNEC values while Supplementary Material Table S7 presents the minimum, maximum, mean and median concentrations as well as the detection frequency for the tested antimicrobials at each of the 15 sites. Clarithromycin, N-desmethyl clarithromycin, fluconazole, and trimethoprim were found in all samples, while cefazolin, chloramphenicol, 1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione (a nitrofurantoin metabolite), penicillin V, roxithromycin, and tiamulin were not present above the method quantification limit (MQL).

Quinolones are frequently identified in high concentrations in hospital effluent (Dinh et al., 2017; Diwan et al., 2010; Lindberg et al., 2004; Thomas et al., 2007), with a few studies listing quinolones as the most prevalent antibiotics quantified (Rodriguez-Mozaz et al., 2015; Santos et al., 2013) (see Supplementary Material Table S8 for mean antimicrobial concentrations and concentration ranges in hospital wastewater reported in published literature). In this study, ciprofloxacin ranged in concentration from 96 ng/L to a maximum of 13,856 ng/L, and ofloxacin concentrations from < MQL to 14,153 ng/L. These results fall within the concentration ranges reported in literature (Ashfaq et al., 2016; Dinh et al., 2017; Rodriguez-Mozaz et al., 2015; Santos et al., 2013; Verlicchi et al., 2012); however, there is a large range of reported concentrations (e.g., ciprofloxacin: 3.20–417 ng/L (Aydin et al., 2019) vs. 2,200–218,300 ng/L (Diwan et al., 2010); ofloxacin: 10–110 ng/L (Sims et al., 2023b) vs. 6,800–66,000 ng/L (Ashfaq et al., 2016)). Ciprofloxacin and ofloxacin are both highly prevalent in hospital and community wastewater, and they were detected in nearly all samples.

Sulfamethoxazole and its metabolite N-acetyl sulfamethoxazole were the two most prevalent sulfonamides quantified. At three hospital sites, sulfamethoxazole concentrations exceeded the upper end of the calibration range (>28,031 ng/L), while its metabolite, N-acetyl sulfamethoxazole, exceeded the calibration range (>31,262 ng/L) at five hospital sites. The maximum concentrations of sulfamethoxazole identified at Hospitals 1–6 are greater than the highest concentrations found in numerous previous studies; however, Hospital 4–6 have mean sulfamethoxazole concentrations that exceed many of these reported maxima (Aydin et al., 2019; Brown et al., 2006; Chang et al., 2010; Dinh et al., 2017; Lindberg et al., 2004; Ngigi et al., 2020; Rodriguez-Mozaz et al., 2015; Santos et al., 2013; Sims et al., 2023b; Szekeres et al., 2017; Thomas et al., 2007; Verlicchi et al., 2012). Dinh et al. (2017) included N-acetyl sulfamethoxazole in their analysis and found a mean concentration of 7,100 ng/L (range: 280–21,300 ng/L). Mean N-acetyl sulfamethoxazole concentrations for Welsh hospitals were generally two to threefold higher. In Wales, sulfamethoxazole is available in a combination product with trimethoprim (in a 5:1 ratio) due to their synergistic antibacterial effects (Joint Formulary Committee, 2025). Like sulfamethoxazole, the mean concentrations of trimethoprim in hospital wastewater are higher than the maximum concentrations found in many previous studies (Aydin et al., 2019; Chang et al., 2010; Dinh et al., 2017; Rodriguez-Mozaz et al., 2015; Verlicchi et al., 2012).

The concentrations identified in comparative literature may not reflect current prescribing practices in Wales since many studies were done in other countries (e.g., China (Chang et al., 2010), France (Dinh et al., 2017), Italy (Verlicchi et al., 2012), Kenya (Ngigi et al., 2020), Norway (Thomas et al., 2007), Portugal (Santos et al., 2013), Romania (Szekeres et al., 2017), Spain (Rodriguez-Mozaz et al., 2015), Sweden (Lindberg et al., 2004), United States (Brown et al., 2006)) and most were completed prior to 2019. Differences in antibiotic availability between countries, current clinical practice guidelines, local infection rates, antibiotic cost and drug coverage, and local antibiotic resistance rates may influence the preferential prescribing of sulfonamides over alternative treatments in Wales. These results likely reflect systemic influences on prescribing habits given the consistently high reported concentrations, the geographic spread of the studied hospital sites, and the variation in hospital size.

Another example indicating spatial differences in the use of antimicrobials is with sulfadiazine and its metabolite, N-acetyl sulfadiazine. In Hospital 2, these antimicrobials were at quantifiable concentrations in about half of all wastewater samples and it had the highest mean concentrations of these antimicrobials during the 3-month study (Table S7). Sulfadiazine comes in tablet form (indicated for prevention of rheumatic fever recurrence), but also as a topically applied cream in the form of silver sulfadiazine (Joint Formulary Committee, 2025). Interestingly, silver sulfadiazine is indicated to prevent or treat infection in burn wounds, and Hospital 2 is the only hospital in Wales with a burn centre (Joint Formulary Committee, 2025). Systemic absorption of silver sulfadiazine is possible with application to large treatment areas and this could explain the presence of N-acetyl sulfadiazine in wastewater (Joint Formulary Committee, 2025).

Clarithromycin is the most commonly prescribed macrolide antibiotic in hospitals with a concentration ranging from 40 to > 48,705 ng/L. This maximum concentration is higher than the maximum concentration recently reported in a study of hospital wastewater (24,441 ng/L (Azuma et al., 2024)), although the mean concentration they reported (10,907 ± 11,315 ng/L) is in concordance with those found here. Erythromycin was detected in much lower concentrations than clarithromycin in hospital wastewater (174–3,061 ng/L), and these results are consistent with two more recent works (Al-Maadheed et al., 2019; Dinh et al., 2017).

The antimicrobials detected at the highest concentrations in wastewater in this study were: amoxicilloic acid (252,132 ng/L), vancomycin (>183,942 ng/L), meropenem (>177,105 ng/L), and piperacillin (>175,218 ng/L). Notably, amoxicilloic acid, meropenem and piperacillin are β-lactams, while vancomycin, meropenem, and piperacillin are intravenously administered antibiotics.

3.3. Antimicrobial resistance risk assessment

Risk quotients for the development of AMR are presented in Table 2 (hospital wastewater) and Table 3 (community wastewater) using the mean risk quotient across the 3-month sampling campaign. In hospital wastewater, ciprofloxacin, clarithromycin, metronidazole, and trimethoprim were present in concentrations which could induce AMR at all hospital sites across Wales, while amoxicillin, meropenem, piperacillin, and vancomycin were high risk at most sites. Worryingly, these high-risk antimicrobials span multiple antimicrobial classes, each with a different mechanism of action, and many are broad-spectrum agents (e.g., ciprofloxacin, piperacillin). Ciprofloxacin and trimethoprim are agents of concern as all concentration measurements across hospital sites were above the PNEC-RS, while clarithromycin and

Table 2
Risk quotients for development of antibiotic resistance in hospital wastewater effluent using mean risk quotient across sampling campaign, where RQ < 0.1 is low risk (green), 0.1 ≤ RQ < 1 is moderate risk (yellow), 1 ≤ RQ < 10 is high risk (red), and RQ ≥ 10 is high risk (dark red). The percent of concentration measurements above the predicted no-effect concentration for resistance selection (PNEC-RS) are noted in brackets after the risk quotient.

Antimicrobial	Site						
	Hospital 1	Hospital 2	Hospital 3	Hospital 4	Hospital 5	Hospital 6	Hospital 7
Amoxicillin	1 ± 0.3 (10%)			8.7 ± 11.9 (66.7%)	25.4 ± 29.2 (88.9%)	5.6 ± 4.4 (58.3%)	9.3 ± 14 (33.3%)
Cefalexin					0.1 (0%)	0.02 (0%)	
Cefazolin							
Chloramphenicol							
Ciprofloxacin	74.2 ± 60 (100%)	51 ± 53.6 (100%)	15.1 ± 12.2 (100%)	66.6 ± 40.5 (100%)	122 ± 70.8 (100%)	72.8 ± 47.7 (100%)	3.1 ± 1.8 (100%)
Ciprofloxacin, Desethylene-	4.5 ± 2.8 (60%)	4.2 (9.1%)		4.1 ± 1.9 (58.3%)	7.1 ± 3.5 (88.9%)	4.9 ± 1.4 (66.7%)	
Clarithromycin	23.5 ± 26 (90%)	7.8 ± 7.3 (81.8%)	> 150.2 ± >78.6 (100%)	76.1 ± 34.2 (100%)	53.6 ± 36.4 (100%)	55.2 ± 14.5 (100%)	10.7 ± 12.3 (91.7%)
Clindamycin	0.08 ± 0.1 (0%)	0.6 ± 0.8 (27.3%)	0.2 ± 0.1 (0%)	0.8 ± 0.6 (16.7%)	0.7 ± 0.7 (22%)	0.5 ± 0.3 (0%)	0.3 ± 0.7 (8.3%)
Clindamycin, N-desmethyl-	2.6 ± 2.4 (40%)	24.7 ± 25.0 (100%)	28.5 ± 49.6 (50%)	18.1 ± 15.4 (100%)	11.9 ± 13.6 (100%)	17.0 ± 11.4 (91.7%)	3.2 ± 4.3 (58.3%)
Erythromycin	0.07 ± 0.1 (0%)	0.5 ± 0.5 (9.1%)	0.4 ± 0.5 (0%)	0.7 ± 1.1 (8.3%)	0.3 ± 0.2 (0%)	1 ± 0.6 (25%)	0.3 ± 0.6 (8.3%)
Ethambutol	1.2 ± 1.8 (30%)	0.08 (0%)		0.4 ± 0.3 (0%)			
Fluconazole	7.6 ± 8.9 (80%)	2.8 ± 3 (63.6%)	> 39.3 ± > 39.6 (83.3%)	10.3 ± 9 (100%)	8.1 ± 10.1 (100%)	11.5 ± 7.1 (100%)	0.6 ± 0.8 (16.7%)
Linezolid	0.1 ± 0.1 (0%)	0.01 (0%)		0.09 ± 0.1 (0%)	0.2 ± 0.2 (0%)	0.1 ± 0.1 (0%)	0.005 ± 0.004 (0%)
Mecillinam	2.1 ± 2.8 (10%)			0.2 (0%)	0.3 ± 0.5 (0%)	0.2 ± 0.1 (0%)	
Meropenem	99.7 ± 97.9 (30%)		922.7 ± 927.5 (33.3%)	699.5 ± 707.8 (75%)	754.8 ± 892.9 (88.9%)	149.9 ± 121 (83.3%)	31.7 (8.3%)
Metronidazole	10.9 ± 11.4 (80%)	91.5 ± 70.6 (100%)	> 226.2 ± > 201.2 (100%)	132 ± 64.1 (100%)	118.7 ± 66 (100%)	59.8 ± 9.6 (100%)	8.3 ± 4.5 (100%)
Metronidazole, Hydroxy-	32.9 ± 33.9 (90%)	239.0 ± 182.0 (100%)	> 1202.1 ± > 1153.8 (100%)	434.8 ± 179.8 (100%)	435.9 ± 258.5 (100%)	193.3 ± 59.2 (100%)	29.8 ± 17.3 (100%)
Nitrofurantoin	0.002 ± 0.0009 (0%)			0.007 ± 0.005 (0%)	0.005 ± 0.002 (0%)	0.006 ± 0.002 (0%)	
NP-AHD							
Ofloxacin	3.4 ± 8.8 (20%)	0.2 ± 0.2 (0%)	0.07 ± 0.02 (0%)	2.9 ± 5.3 (58.3%)	0.4 ± 0.4 (11.1%)	3.4 ± 2.4 (66.7%)	0.08 ± 0.1 (0%)
Ofloxacin, Desmethyl-	16.4 (10%)					1.5 ± 0.7 (16.7%)	
Penicillin V							
Piperacillin	2.5 (10%)			42.2 ± 31.2 (41.7%)	> 121.4 ± > 123.3 (66.7%)	10.8 ± 11.1 (33.3%)	15.4 ± 14.7 (33.3%)
Rifampicin	26.7 ± 6 (30%)	13.6 (9.1%)		16.6 (8.3%)		9.7 ± 3.4 (16.7%)	
Roxithromycin							
Sulfadiazine	0.7 (0%)	3.0 ± 2.0 (54.5%)					
Sulfamethoxazole	0.8 ± 0.6 (30%)	0.5 ± 0.4 (27.3%)	0.4 ± 0.4 (16.7%)	> 1.3 ± > 0.4 (75%)	> 0.9 ± > 0.5 (33.3%)	> 1.1 ± > 0.6 (50%)	0.2 ± 0.1 (0%)
Sulfapyridine	7.4 ± 10.1 (70%)	36.3 ± 33.9 (100%)	15.4 ± 31.1 (50%)	17.6 ± 19.3 (91.7%)	8.3 ± 12.0 (88.9%)	58.6 ± 46.5 (100%)	4.0 ± 3.5 (83.3%)
Sulfasalazine	4.7 ± 4.8 (30%)	6.5 ± 8.1 (81.8%)	0.8 (0%)	9.3 ± 10.5 (41.7%)	4.8 ± 6.9 (66.7%)	17.5 ± 10.5 (100%)	1.7 ± 0.7 (66.7%)
Tetracycline	2.2 ± 1.9 (50%)	0.9 ± 0.3 (18.2%)		0.8 ± 0.3 (8.3%)	3.9 ± 7.7 (66.7%)	2.5 ± 1.1 (83.3%)	
Tiamulin							
Trimethoprim	36.6 ± 26.9 (100%)	13.8 ± 7.8 (100%)	6.1 ± 5.6 (100%)	33.7 ± 10.7 (100%)	30 ± 11.2 (100%)	29.5 ± 15 (100%)	6.4 ± 5.2 (100%)
Vancomycin	3.7 ± 3.4 (90%)	3.1 ± 1.8 (90.9%)	> 18.4 ± > 8.6 (100%)	3.6 ± 2.9 (100%)	> 10.1 ± > 7.3 (100%)	1.2 ± 0.7 (50%)	0.5 ± 1.1 (8.3%)

Note: Where a risk quotient is not specified for antimicrobials with low risk, the antimicrobial concentrations were consistently less than the method quantification limit across the sampling campaign.
Abbreviations: NP-AHD, 1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione.

metronidazole concentrations exceeded the PNEC-RS in the majority of samples (Table 2). The magnitude of risks identified here are also notable. For example, the mean risk quotients for meropenem ranged from about 32 to 923, indicating that the concentrations in wastewater were approximately one to two orders of magnitude higher than the PNEC-RS. Similarly, metronidazole had mean risk quotients ranging from approximately 8 to > 225. As the results presented in Table 2 are mean risk quotients across the sampling period, it is evident there is a persistently high risk for the development of AMR for numerous antimicrobials in hospital wastewater. Low antibiotic concentrations create a selective pressure that allows moderately resistant bacteria to survive and reproduce (Andersson and Hughes, 2014). Very low, sub-inhibitory concentrations of antibiotics can actually promote mutations and gene transfer, accelerating the development of resistance without killing sensitive strains (Andersson and Hughes, 2014). These results confirm that hospital wastewater is a potential hot spot for the evolution of AMR organisms.

A previous study examining antimicrobial concentrations in effluent from 16 hospitals found seasonal differences in antimicrobial use with ciprofloxacin and clarithromycin concentrations being approximately 35-fold and 85-fold higher in the winter months, respectively (Aydin et al., 2019). Trends of increased antimicrobial use in winter months were also noted by Verlicchi et al. (2012) who reported that concentrations were approximately 13-fold higher for ciprofloxacin, 30-fold higher for metronidazole, and 190-fold higher for clarithromycin at the hospital site studied. In light of the findings presented here from this summer sampling campaign, it is reasonable to assume the concentrations of select antimicrobials could be higher in winter months.

Hospital specific trends were observed in this study, with Hospital 7 generally showing the lowest risk among the hospitals and antimicrobials studied. Conversely, wastewater from Hospital 3 had the highest risk with ciprofloxacin, clarithromycin, fluconazole, meropenem, metronidazole, and vancomycin having the highest mean concentrations observed in the study. Interestingly, Hospital 3 has on average 131 beds occupied, which is the lowest capacity of the seven sites, while Hospital 7 has 352, a figure comparable to Hospitals 1, 4, and 5 (Table S2). Again, this suggests that the number of hospital beds does not appear to be the sole determinant of antimicrobial concentrations in hospital wastewater. It would be reasonable to suggest that local infection rates, antibiotic susceptibility rates, hospital specialties, and the unique clinical presentation of patients during the tested timeframe likely contribute to antimicrobial usage patterns.

As previously noted, wastewater from Hospitals 1 through 6 merges with community wastewater treated by WWTP 1 through 6, respectively. The risk of AMR in community wastewater presented in Table 3 shows that the dilution effect generally reduces the risk of resistance for many antimicrobials which were a moderate or high risk in hospital effluent (e.g., amoxicillin, clindamycin, fluconazole, piperacillin, rifampicin, vancomycin). Ciprofloxacin and clarithromycin were again detected at high risk concentrations across all sites, while metronidazole and trimethoprim remain high risk at most sites. The overall reduction from high to low risk at most sites for piperacillin, rifampicin, and vancomycin could be attributable to the limited use of these agents within the community, while conversely, the increased risk posed by sulfapyridine indicates greater use of its parent drug sulfasalazine in the community. Amoxicillin displayed an overall risk reduction in community wastewater; however, this is likely a consequence of β-lactam instability, as discussed earlier, rather than indicative of limited community use. Interestingly, the sulfonamide sulfamethoxazole, which was normally the most prevalent antimicrobial across sites (Figs. 2 and 3, S1), exhibited a moderate risk for AMR in hospital wastewater and low risk in community wastewater. This highlights how PNEC-RS are highly compound specific.

Table 3
Risk quotients for development of antibiotic resistance in community wastewater using mean risk quotient across sampling campaign, where RQ < 0.1 is low risk (green), 0.1 ≤ RQ < 1 is moderate risk (yellow), 1 ≤ RQ < 10 is high risk (red), and RQ ≥ 10 is high risk (dark red). The percent of concentration measurements above the predicted no-effect concentration for resistance selection (PNEC-RS) are noted in brackets after the risk quotient.

Antimicrobial	Site							
	WWTP 1	WWTP 2	WWTP 3	WWTP 4	WWTP 5	WWTP 6	WWTP 7	WWTP 8
Amoxicillin				0.8 (0%)				
Cefalexin								
Cefazolin								
Chloramphenicol								
Ciprofloxacin	2.7 ± 1.7 (100%)	3.3 ± 2.3 (100%)	2.1 ± 1 (80%)	6 ± 3.4 (100%)	5.1 ± 2.7 (100%)	3 ± 1 (100%)	2.6 ± 2.4 (66.7%)	1.9 ± 1.6 (92.9%)
Ciprofloxacin, Desethylene-								5.6 (7.1%)
Clarithromycin	1.8 ± 0.3 (100%)	2.9 ± 0.7 (100%)	1.5 ± 0.7 (73.3%)	5.4 ± 0.6 (100%)	4.9 ± 1.0 (100%)	4.2 ± 0.7 (100%)	1.3 ± 0.7 (60%)	1.6 ± 0.2 (100%)
Clindamycin	0.01 ± 0.004 (0%)	0.05 ± 0.04 (0%)	0.008 ± 0.006 (0%)	0.04 ± 0.02 (0%)	0.01 ± 0.003 (0%)	0.01 ± 0.004 (0%)	0.03 ± 0.0002 (0%)	0.01 ± 0.002 (0%)
Clindamycin, N-desmethyl-	0.7 ± 0.2 (7.1%)	1.1 ± 0.3 (46.2%)	1.4 ± 1.1 (33%)	1.8 ± 1.4 (66.7%)	0.8 ± 0.2 (13.3%)	0.9 ± 0.2 (20%)	0.6 ± 0.5 (6.7%)	0.5 ± 0.1 (0%)
Erythromycin	0.2 ± 0.04 (0%)	0.3 ± 0.05 (0%)	0.1 ± 0.04 (0%)	0.1 ± 0.05 (0%)	0.5 ± 0.4 (6.7%)	0.3 ± 0.04 (0%)	0.2 ± 0.09 (0%)	0.2 ± 0.04 (0%)
Ethambutol	0.02 ± 0.006 (0%)	0.01 ± 0.003 (0%)	0.02 ± 0.01 (0%)	0.09 ± 0.04 (0%)		0.03 ± 0.01 (0%)		0.03 ± 0.05 (0%)
Fluconazole	0.6 ± 0.2 (0%)	0.6 ± 0.2 (0%)	0.7 ± 0.3 (20%)	1 ± 0.3 (46.7%)	0.5 ± 0.1 (0%)	0.7 ± 0.2 (6.7%)	0.3 ± 0.2 (0%)	0.6 ± 0.3 (7.1%)
Linezolid		0.002 (0%)		0.01 ± 0.008 (0%)	0.002 ± 0.0006 (0%)	0.001 ± 0.0002 (0%)		
Mecillinam	0.005 (0%)					0.02 ± 0.004 (0%)		
Meropenem			10.5 (6.7%)	39.1 ± 20.2 (53.3%)				
Metronidazole	1.4 ± 0.4 (71.4%)	2.2 ± 1.8 (53.8%)	1.5 ± 0.4 (86.7%)	5.3 ± 1.3 (100%)	1.7 ± 0.3 (100%)	2.5 ± 0.7 (100%)	0.6 ± 0.5 (26.7%)	1.5 ± 0.3 (92.9%)
Metronidazole, Hydroxy-	4.2 ± 0.9 (100%)	6.3 ± 4.2 (76.9%)	4.8 ± 1.3 (100%)	17.8 ± 3.5 (100%)	5.7 ± 1.2 (100%)	7.1 ± 1.6 (100%)	2.7 ± 1.6 (46.7%)	4.9 ± 0.6 (100%)
Nitrofurantoin								
NP-AHD								
Ofloxacin	0.2 ± 0.04 (0%)	0.2 ± 0.07 (0%)	0.3 ± 0.1 (0%)	0.3 ± 0.1 (0%)	0.1 ± 0.06 (0%)	0.1 ± 0.03 (0%)	0.03 ± 0.01 (0%)	0.1 ± 0.03 (0%)
Ofloxacin, Desmethyl-	0.6 (0%)							4.7 (7.1%)
Penicillin V								
Piperacillin			0.3 (0%)	1.5 ± 0.8 (26.7%)				
Rifampicin								
Roxithromycin								
Sulfadiazine			0.6 (0%)				0.5 (0%)	
Sulfamethoxazole	0.02 ± 0.008 (0%)	0.04 ± 0.02 (0%)	0.04 ± 0.01 (0%)	0.1 ± 0.05 (0%)	0.05 ± 0.008 (0%)	0.05 ± 0.02 (0%)	0.02 ± 0.01 (0%)	0.02 ± 0.01 (0%)
Sulfapyridine	9.4 ± 4.1 (100%)	19.3 ± 8.2 (100%)	15.2 ± 5.3 (100%)	15.7 ± 4.0 (100%)	26.7 ± 6.3 (100%)	30.0 ± 6.8 (100%)	20.7 ± 16.9 (100%)	15.0 ± 15.2 (100%)
Sulfasalazine	4.0 ± 2.6 (100%)	4.8 ± 2.5 (69.2%)	6.3 ± 2.6 (100%)	1.6 ± 0.5 (66.7%)	3.5 ± 1.0 (100%)	5.4 ± 2.4 (100%)	1.9 ± 0.7 (73.3%)	3.6 ± 1.5 (100%)
Tetracycline	0.3 ± 0.1 (0%)	0.2 ± 0.08 (0%)	0.4 ± 0.09 (0%)	0.3 ± 0.3 (0%)	0.4 ± 0.1 (0%)	0.4 ± 0.1 (0%)	0.5 ± 0.2 (0%)	
Tiamulin								
Trimethoprim	1.03 ± 0.2 (57.1%)	0.9 ± 0.3 (38.5%)	0.9 ± 0.2 (40%)	2.1 ± 0.7 (100%)	1.5 ± 0.2 (100%)	1.5 ± 0.3 (100%)	1.4 ± 0.6 (66.7%)	0.9 ± 0.2 (14.3%)
Vancomycin	0.03 ± 0.01 (0%)	0.04 ± 0.02 (0%)	0.06 ± 0.02 (0%)	0.2 ± 0.2 (0%)	0.02 ± 0.01 (0%)	0.03 ± 0.01 (0%)		

Note: Where a risk quotient is not specified for antimicrobials with low risk, the antimicrobial concentrations were consistently less than the method quantification limit across the sampling campaign.
Abbreviations: NP-AHD, 1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione; WWTP, wastewater treatment plant.

3.4. Environmental risk assessments

Risk quotients for ecotoxicological endpoints are presented in Table 4 (hospital wastewater) and Table 5 (community wastewater). As wastewater is usually subject to treatment, the risks presented here would only apply when untreated wastewater is discharged into the environment, such as during combined sewer overflow (CSO) events or in low-resource settings where there is no wastewater treatment infrastructure. An additional consideration is that untreated wastewater would likely be subject to dilution which would make the risk less pronounced than what is indicated in Tables 4 and 5. Based on this risk assessment, hospital wastewater presents a significant risk to the aquatic environment. The at-risk chemicals are not limited to a specific antimicrobial class, but rather are persistent compounds from many of the classes monitored in this study: β-lactams (e.g., amoxicillin, meropenem), glycopeptides (e.g., vancomycin), lincomycins (e.g., clindamycin), macrolides (e.g., clarithromycin), quinolones (e.g., ciprofloxacin, ofloxacin), sulfonamides (e.g., sulfamethoxazole, sulfasalazine), and antifungals (e.g., fluconazole). While some antimicrobials were present in high-risk concentrations at all sites (e.g. clarithromycin, clindamycin, sulfamethoxazole), others displayed significant site-dependence. For example, neither piperacillin nor rifampicin were detected above the method quantification limit in Hospital 3, yet piperacillin had a mean risk quotient of > 813 at Hospital 5 and rifampicin has a mean risk quotient of 331 at Hospital 1. Of the included antimicrobials, vancomycin is the most likely to cause ecotoxicity with mean risk quotients ranging from about 1,128 to > 38,320. As seen previously, the dilution of hospital effluent with community wastewater can significantly reduce risk (Table 5). For example, meropenem was a high-risk antimicrobial at 5/7 hospital sites, while after dilution with community wastewater, meropenem posed a moderate risk only at two sites, and low risk at the others. It is important to note that the risk of environmental harm and risk of AMR are not always congruent. For example, in community wastewater, ciprofloxacin and clarithromycin were at high risk for both development of AMR and environmental toxicity, while metronidazole and trimethoprim only exhibited a high risk for AMR, and vancomycin only presented a high risk for ecotoxicity. This highlights the importance for considering both the potential clinical and environmental impact when conducting risk assessments.

While the environmental risk assessments consider the risk of each antimicrobial individually, in reality, antimicrobials are present in wastewater as mixtures. The risk posed by binary antimicrobial mixtures are not always additive, as synergistic and antagonistic effects have been documented (Fang et al., 2016; González-Pleiter et al., 2013; Long et al., 2016; Magdaleno et al., 2015; Marx et al., 2015; Qin et al., 2024; Yamagishi et al., 2017). For example, erythromycin and tetracycline display a synergistic effect on risk towards green algae as do the combination of levofloxacin (the levorotatory isomer of ofloxacin) and tetracycline on cyanobacterium (González-Pleiter et al., 2013). Overall, research suggests that antimicrobial mixtures predominantly cause synergistic effects on environmental risk (González-Pleiter et al., 2013; Magdaleno et al., 2015; Marx et al., 2015; Qin et al., 2024). Therefore, special attention is required when interpreting the risks identified here as they may likely be underestimations of the true environmental risk.

3.5. Assessment of direct disposal events and potential of industrial contributions to antimicrobial concentrations in wastewater

This study considered several antimicrobial metabolites as their presence is evidence of antimicrobial consumption. Direct disposal events can be inferred when the ratio of parent compound to metabolite are higher than the parent to metabolite excretion ratios as derived through a systematic literature review by Holton et al. (2022). The mean parent to metabolite ratios with their statistical significance are

Table 4
Risk quotients for aquatic toxicity (lowest predicted no-effect concentration in freshwater) in hospital wastewater using the mean risk quotient across sampling campaign, where RQ < 0.1 is low risk (green), 0.1 ≤ RQ < 1 is moderate risk (yellow), 1 ≤ RQ < 10 is high risk (red), and RQ ≥ 10 is high risk (dark red). The percent of concentration measurements above the lowest predicted no-effect concentration in freshwater ($PNEC_{fw}$) are noted in brackets after the risk quotient.

Antimicrobial	Site						
	Hospital 1	Hospital 2	Hospital 3	Hospital 4	Hospital 5	Hospital 6	Hospital 7
Amoxicillin	3.2 ± 0.9 (20%)			28 ± 38.2 (66.7%)	81.5 ± 93.7 (88.9%)	18 ± 14.2 (58.3%)	29.7 ± 44.7 (50%)
Amoxicilloic Acid	111.9 ± 42.8 (30%)	226.3 ± 41.5 (18.2%)	1899.6 ± 2722.1 (50%)	412.1 ± 506.7 (83.3%)	226.5 ± 190.6 (55.6%)	187.5 ± 68.6 (100%)	125.3 ± 84.5 (41.7%)
Cefalexin					0.1 (0%)	0.02 (0%)	
Cefazolin							
Chloramphenicol							
Ciprofloxacin	53.3 ± 43.1 (100%)	36.7 ± 38.6 (100%)	10.9 ± 8.8 (100%)	47.9 ± 29.2 (100%)	87.8 ± 50.9 (100%)	52.4 ± 34.3 (100%)	2.2 ± 1.3 (100%)
Ciprofloxacin, Desethylene-	4.5 ± 2.8 (60%)	4.2 (9.1%)		4.1 ± 1.9 (58.3%)	7.1 ± 3.5 (88.9%)	4.9 ± 1.4 (66.7%)	
Clarithromycin	49 ± 54.2 (100%)	16.2 ± 15.3 (90.9%)	> 312.9 ± > 163.7 (100%)	158.5 ± 71.3 (100%)	111.8 ± 75.9 (100%)	115 ± 30.1 (100%)	22.3 ± 25.5 (91.7%)
Clarithromycin, N-desmethyl-	0.4 ± 0.4 (10%)	0.4 ± 0.2 (0%)	3.6 ± 2.7 (83.3%)	1.4 ± 0.5 (58.3%)	0.9 ± 0.4 (33.3%)	1 ± 0.2 (33.3%)	0.2 ± 0.2 (0%)
Clindamycin	1.8 ± 2.2 (20%)	13.4 ± 18 (90.9%)	3.8 ± 2.9 (50%)	17.1 ± 14.1 (100%)	16.6 ± 16.4 (88.9%)	10.5 ± 7.2 (75%)	7.1 ± 15 (33.3%)
Clindamycin, N-desmethyl-	1.3 ± 1.2 (30%)	12.4 ± 12.5 (81.8%)	14.2 ± 24.8 (50%)	9 ± 7.7 (100%)	5.9 ± 6.8 (88.9%)	8.5 ± 5.7 (91.7%)	1.6 ± 2.1 (41.7%)
Emtricitabine	> 0.3 ± > 0.4 (0%)	0.05 ± 0.02 (0%)		0.08 ± 0.06 (0%)	0.08 ± 0.06 (0%)	0.06 ± 0.03 (0%)	0.02 (0%)
Erythromycin	0.2 ± 0.3 (0%)	1.7 ± 1.7 (36.4%)	1.5 ± 1.6 (16.7%)	2.2 ± 3.7 (16.7%)	1 ± 0.8 (33.3%)	3.3 ± 2.1 (33.3%)	1.1 ± 2 (16.7%)
Erythromycin, N-desmethyl-		2.5 (9.1%)		5.0 (8.3%)			
Ethambutol	0.07 ± 0.1 (0%)	0.004 (0%)		0.02 ± 0.02 (0%)			
Fluconazole	1.8 ± 2.1 (50%)	0.7 ± 0.7 (27.3%)	> 9.4 ± > 9.5 (66.7%)	2.5 ± 2.2 (75%)	1.9 ± 2.4 (44.4%)	2.8 ± 1.7 (91.7%)	0.1 ± 0.2 (0%)
Lamivudine	0.2 ± 0.3 (0%)	0.03 ± 0.02 (0%)		0.3 ± 0.3 (0%)	0.07 ± 0.1 (0%)	0.07 ± 0.04 (0%)	
Linezolid	0.2 ± 0.3 (0%)	0.03 (0%)		0.2 ± 0.3 (0%)	0.4 ± 0.4 (0%)	0.3 ± 0.3 (0%)	0.01 ± 0.01 (0%)
Mecillinam	0.4 ± 0.5 (0%)			0.03 (0%)	0.07 ± 0.09 (0%)	0.03 ± 0.02 (0%)	
Meropenem	1.2 ± 1.2 (10%)		11.3 ± 11.4 (33.3%)	8.6 ± 8.7 (58.3%)	9.3 ± 11 (77.8%)	1.8 ± 1.5 (50%)	0.4 (0%)
Metronidazole	0.04 ± 0.04 (0%)	0.3 ± 0.3 (9.1%)	> 0.9 ± > 0.8 (50%)	0.5 ± 0.2 (0%)	0.4 ± 0.2 (0%)	0.2 ± 0.04 (0%)	0.03 ± 0.02 (0%)
Metronidazole, Hydroxy-	0.05 ± 0.05 (0%)	0.4 ± 0.3 (0%)	> 1.8 ± > 1.8 (50%)	0.7 ± 0.3 (16.7%)	0.7 ± 0.4 (22.2%)	0.3 ± 0.09 (0%)	0.05 ± 0.03 (0%)
Nitrofurantoin	0.02 ± 0.01 (0%)			0.09 ± 0.06 (0%)	0.06 ± 0.03 (0%)	0.07 ± 0.03 (0%)	
NP-AHD							
Ofloxacin	1.2 ± 3.2 (20%)	0.08 ± 0.06(0%)	0.03 ± 0.006 (0%)	1.1 ± 1.9 (25%)	0.2 ± 0.1 (0%)	1.2 ± 0.9 (58.3%)	0.03 ± 0.04 (0%)
Ofloxacin, Desmethyl-	0.5 (0%)					0.04 ± 0.02 (0%)	
Ofloxacin N-oxide	17.3 ± 23.0 (20%)			3.5 ± 4.4 (16.7%)		2.6 ± 1.4 (33.3%)	
Penicillin V							
Penicilloic G acid	> 3.6 ± > 4.7 (10%)	0.3 ± 0.07 (0%)	> 7 (16.7%)	> 2.7 ± > 2.2 (66.7%)	1.2 ± 1.4 (44.4%)	0.6 ± 0.4 (16.7%)	0.2 ± 0.2 (0%)
Piperacillin	16.5 (10%)			282.8 ± 208.7 (41.7%)	> 812.8 ± > 825.8 (66.7%)	72.1 ± 74.5 (33.3%)	103 ± 98.1 (41.7%)
Rifampicin	330.9 ± 74.7 (30%)	168.1 (9.1%)		205.9 (8.3%)		120.5 ± 42.7 (16.7%)	
Roxithromycin							
Sulfadiazine	0.04 (0%)	0.2 ± 0.1 (0%)					
Sulfadiazine, N-acetyl-	0.1 ± 0.04 (0%)	0.2 ± 0.2 (0%)					
Sulfamethoxazole	20.1 ± 14.8 (100%)	14 ± 11.7 (100%)	10.4 ± 10.6 (100%)	> 34.2 ± > 9.6 (100%)	> 23.3 ± > 14.5 (100%)	> 29.9 ± > 15 (100%)	4.2 ± 3.5 (100%)
Sulfamethoxazole, N-acetyl-	> 8.4 ± > 6.2 (70%)	> 5.1 ± > 4.1 (100%)	1.7 ± 1.4 (66.7%)	> 12.8 ± > 0.9 (100%)	> 11.9 ± > 2.1 (100%)	> 10 ± > 2.9 (100%)	3.8 ± 3.5 (91.7%)
Sulfapyridine	0.8 ± 1.1 (20%)	3.9 ± 3.7 (63.6%)	1.7 ± 3.4 (16.7%)	1.9 ± 2.1 (50%)	0.9 ± 1.3 (22.2%)	6.4 ± 5.1 (100%)	0.4 ± 0.4 (8.3%)
Sulfapyridine, N-acetyl-	0.5 ± 0.9 (10%)	1.8 ± 2.1 (45.5%)	0.3 ± 0.5 (16.7%)	0.9 ± 0.8 (25%)	1.4 ± 2.6 (22.2%)	3 ± 1.7 (83.3%)	0.4 ± 0.3 (0%)
Sulfasalazine	9.1 ± 9.2 (30%)	12.5 ± 15.6 (81.8%)	1.5 (16.7%)	17.9 ± 20.2 (50%)	9.3 ± 13.4 (77.8%)	33.8 ± 20.3 (100%)	3.3 ± 1.4 (75%)
Tetracycline	24.5 ± 21.3 (70%)	9.7 ± 3 (63.6%)		8.4 ± 3 (41.7%)	43.7 ± 85.7 (100%)	27.3 ± 11.9 (91.7%)	
Tiamulin							
Trimethoprim	0.2 ± 0.1 (0%)	0.06 ± 0.03 (0%)	0.03 ± 0.02 (0%)	0.1 ± 0.04 (0%)	0.1 ± 0.05 (0%)	0.1 ± 0.06 (0%)	0.03 ± 0.02 (0%)
Vancomycin	7666.6 ± 7127.1 (100%)	6419.3 ± 3779.8 (100%)	> 38319.5 ± > 17970.3 (100%)	7456.2 ± 6096.1 (100%)	21103.9 ± 15236.3 (100%)	2561.9 ± 1411.4 (100%)	1127.7 ± 2279.4 (100%)

Note: Where a risk quotient is not specified for antimicrobials with low risk, the antimicrobial concentrations were consistently less than the method quantification limit across the sampling campaign.
Abbreviations: NP-AHD, 1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione.

presented in Supplementary Material Table S9, and the variation in parent to metabolite ratios over the 3-month period are presented in Fig. 5 and Supplementary Material Fig. S4.

Parent to metabolite ratios for ciprofloxacin/desethylene ciprofloxacin (CIP/deCIP) were only available for a subset of hospital sites. Due to its low excretion rate of 1.41 %, desethylene ciprofloxacin was infrequently quantified in this study, and its presence was limited to hospital sites (Holton et al., 2022). At three of the hospitals, the CIP/deCIP ratios were significantly lower than the excretion ratio, but the low stability of desethylene ciprofloxacin in both refrigerated and ambient temperatures likely contributed to these findings (Holton et al., 2022).

The ratios of clindamycin/N-desmethyl clindamycin (CLI/dmCLI) were lower than the excretion ratio—a finding which was statistically significant at almost all sites. However, when assessing evidence of direct disposal, the relative stability of the analytes throughout the analytical workflow, such as during freeze–thaw cycles, and the influence of topical administration, where systemic absorption and thus metabolism may be limited, must be considered. Previous findings concluded that the β-lactam family (including amoxicillin and amoxicilloic acid), clindamycin, and its metabolite N-desmethyl clindamycin are all unstable during storage at –20 °C with losses between 33–66 % of the initial concentration (Xu and Kasprzyk-Hordern, 2023). Therefore, direct disposal of clindamycin cannot be reliably detected using this analysis alone.

The mean ratio for clarithromycin and its metabolite N-desmethyl clarithromycin (CLR/dmCLR) ranged from 3.76 to 5.88 in community wastewaters. These ratios were generally lower than the excretion ratio, although, they were relatively consistent across WWTPs. A similarly low finding was also found in Hospital 2 wastewater. A year-long longitudinal study from two communities in southwestern England determined the CLR/dmCLR ratios in wastewater to be around 2.75 (Sims et al., 2023a). Given the demonstrated stability of clarithromycin and N-desmethyl clarithromycin, these results could suggest alternative processes are affecting the clarithromycin concentrations (e.g. adsorption) (Holton et al., 2022; Sims et al., 2023a; Xu and Kasprzyk-Hordern, 2023). Notably, significantly higher ratios were found in wastewaters from Hospitals 4, 5, and 6 ranging from around 9.3 to 9.5. Topical administration of antimicrobials could increase the concentration of parent drug in wastewater; however, clarithromycin is only available as an oral formulation (Joint Formulary Committee, 2025). The concordance of these values and the fact these hospitals are all located in North Wales could suggest a systematic influence on the results rather than evidence of direct disposal events. By contrast, the mean CLR/dmCLR ratio at Hospital 1 was not statistically different from the excretion ratio, although there was a great range of measured ratios. As there were numerous instances where the CLR/dmCLR ratio was significantly higher than the excretion ratio of 6.12 in Hospital 1, multiple direct disposal events are suspected (Fig. 5).

The ratios of metronidazole/hydroxymetronidazole (MTZ/hMTZ) were consistently at or below the excretion ratio in wastewater across hospital and WWTP sites. Previous research has found elevated MTZ/hMTZ ratios in community wastewater, attributed to use of topical metronidazole products (Sims et al., 2023a). In our study, the use of topical products likely had limited influence on metronidazole concentrations. In regard to stability, metronidazole and hydroxymetronidazole were found to be stable when stored at –20 °C (<12 %

Table 5
Risk quotients for aquatic toxicity (lowest predicted no-effect concentration in freshwater) in community wastewater using the mean risk quotient across sampling campaign, where RQ < 0.1 is low risk (green), 0.1 ≤ RQ < 1 is moderate risk (yellow), 1 ≤ RQ < 10 is high risk (red), and RQ ≥ 10 is high risk (dark red). The percent of concentration measurements above the lowest predicted no-effect concentration in freshwater ($PNEC_{fw}$) are noted in brackets after the risk quotient.

Antimicrobial	Site							
	WWTP 1	WWTP 2	WWTP 3	WWTP 4	WWTP 5	WWTP 6	WWTP 7	WWTP 8
Amoxicillin				2.7 (6.7%)				
Amoxicilloic Acid					81.7 ± 1.9 (13.3%)			
Cefalexin								
Cefazolin								
Chloramphenicol								
Ciprofloxacin	1.9 ± 1.2 (92.9%)	2.4 ± 1.7 (100%)	1.5 ± 0.7 (80%)	4.3 ± 2.4 (100%)	3.7 ± 2 (100%)	2.2 ± 0.7 (100%)	1.9 ± 1.7 (46.7%)	1.4 ± 1.1 (71.4%)
Ciprofloxacin, Desethylene-								5.6 (7.1%)
Clarithromycin	3.7 ± 0.7 (100%)	6 ± 1.4 (100%)	3.1 ± 1.5 (100%)	11.2 ± 1.3 (100%)	10.2 ± 2.1 (100%)	8.8 ± 1.5 (100%)	2.7 ± 1.5 (93.3%)	3.4 ± 0.4 (100%)
Clarithromycin, N-desmethyl-	0.08 ± 0.01 (0%)	0.1 ± 0.02 (0%)	0.06 ± 0.02 (0%)	0.2 ± 0.03 (0%)	0.2 ± 0.03 (0%)	0.1 ± 0.02 (0%)	0.05 ± 0.02 (0%)	0.08 ± 0.03 (0%)
Clindamycin	0.3 ± 0.08 (0%)	1 ± 0.8 (7.7%)	0.2 ± 0.1 (0%)	0.9 ± 0.4 (26.7%)	0.3 ± 0.07 (0%)	0.3 ± 0.09 (0%)	0.6 ± 0.004 (0%)	0.3 ± 0.04 (0%)
Clindamycin, N-desmethyl-	0.4 ± 0.08 (0%)	0.5 ± 0.1 (0%)	0.7 ± 0.6 (20%)	0.9 ± 0.7 (20%)	0.4 ± 0.1 (0%)	0.4 ± 0.1 (0%)	0.3 ± 0.2 (0%)	0.3 ± 0.05 (0%)
Emtricitabine	0.03 ± 0.008 (0%)	0.03 ± 0.01 (0%)		0.03 ± 0.006 (0%)	0.02 ± 0.005 (0%)	0.02 ± 0.004 (0%)	0.04 ± 0.05 (0%)	0.01 ± 0.00006 (0%)
Erythromycin	0.7 ± 0.1 (0%)	0.9 ± 0.2 (23.1%)	0.3 ± 0.1 (0%)	0.3 ± 0.2 (0%)	1.6 ± 1.3 (73.3%)	0.8 ± 0.1 (6.7%)	0.7 ± 0.3 (20%)	0.6 ± 0.1 (0%)
Ethambutol	0.0008 ± 0.0003 (0%)	0.0006 ± 0.0002 (0%)	0.0009 ± 0.0006 (0%)	0.005 ± 0.002 (0%)		0.002 ± 0.0007 (0%)		0.002 ± 0.003 (0%)
Fluconazole	0.1 ± 0.04 (0%)	0.1 ± 0.04 (0%)	0.2 ± 0.08 (0%)	0.2 ± 0.07 (0%)	0.1 ± 0.03 (0%)	0.2 ± 0.06 (0%)	0.07 ± 0.05 (0%)	0.1 ± 0.08 (0%)
Lamivudine	0.02 ± 0.003 (0%)	0.02 ± 0.004 (0%)	0.01 (0%)	0.01 ± 0.002 (0%)		0.01 (0%)		0.01 ± 0.001 (0%)
Linezolid		0.004 (0%)		0.03 ± 0.02 (0%)	0.004 ± 0.001 (0%)	0.003 ± 0.0005 (0%)		
Mecillinam	0.001 (0%)					0.004 ± 0.0008 (0%)		
Meropenem			0.1 (0%)	0.5 ± 0.2 (6.7%)				
Metronidazole	0.005 ± 0.001 (0%)	0.008 ± 0.007 (0%)	0.006 ± 0.001 (0%)	0.02 ± 0.005 (0%)	0.006 ± 0.001 (0%)	0.009 ± 0.003 (0%)	0.002 ± 0.002 (0%)	0.006 ± 0.001 (0%)
Metronidazole, Hydroxy-	0.006 ± 0.001 (0%)	0.01 ± 0.006 (0%)	0.007 ± 0.002 (0%)	0.03 ± 0.005 (0%)	0.009 ± 0.002 (0%)	0.01 ± 0.002 (0%)	0.004 ± 0.002 (0%)	0.007 ± 0.001 (0%)
Nitrofurantoin								
NP-AHD								
Ofloxacin	0.06 ± 0.01 (0%)	0.06 ± 0.03 (0%)	0.09 ± 0.04 (0%)	0.09 ± 0.05 (0%)	0.04 ± 0.02 (0%)	0.05 ± 0.01 (0%)	0.01 ± 0.004 (0%)	0.04 ± 0.01 (0%)
Ofloxacin, Desmethyl-	0.02 (0%)							0.1 (0%)
Ofloxacin N-oxide								3.1 (7.1%)
Penicilloic G acid				0.2 ± 0.1 (0%)				
Piperacillin			1.9 (6.7%)	10.2 ± 5.5 (33.3%)				
Rifampicin								
Roxithromycin								
Sulfadiazine			0.03 (0%)					
Sulfadiazine, N-acetyl-						0.04 ± 0.005 (0%)		
Sulfamethoxazole	0.5 ± 0.2 (0%)	1.1 ± 0.4 (69.2%)	0.9 ± 0.3 (40%)	3.3 ± 1.3 (100%)	1.4 ± 0.2 (93.3%)	1.2 ± 0.5 (60%)	0.5 ± 0.3 (6.7%)	0.6 ± 0.3 (14.3%)
Sulfamethoxazole, N-acetyl-	0.2 ± 0.05 (0%)	0.3 ± 0.1 (0%)	0.2 ± 0.07 (0%)	0.7 ± 0.3 (20%)	0.3 ± 0.06 (0%)	0.4 ± 0.1 (0%)	0.2 ± 0.1 (0%)	0.1 ± 0.03 (0%)
Sulfapyridine	1 ± 0.4 (100%)	2.1 ± 0.9 (84.6%)	1.7 ± 0.6 (86.7%)	1.7 ± 0.4 (100%)	2.9 ± 0.7 (100%)	3.3 ± 0.7 (100%)	2.3 ± 1.8 (86.7%)	1.6 ± 1.7 (71.4%)
Sulfapyridine, N-acetyl-	0.3 ± 0.09 (0%)	0.5 ± 0.1 (0%)	0.3 ± 0.08 (0%)	0.2 ± 0.07 (0%)	0.5 ± 0.2 (0%)	0.8 ± 0.2 (20%)	0.5 ± 0.2 (0%)	0.3 ± 0.2 (0%)
Sulfasalazine	7.7 ± 5.1 (100%)	9.3 ± 4.9 (69.2%)	12.1 ± 5 (100%)	3.1 ± 0.9 (73.3%)	6.7 ± 1.9 (100%)	10.3 ± 4.7 (100%)	3.7 ± 1.4 (73.3%)	7 ± 2.8 (100%)
Tetracycline	3.9 ± 1.6 (35.7%)	2.5 ± 0.8 (23.1%)	4 ± 1 (46.7%)	2.9 ± 3.2 (33.3%)	4.4 ± 1.5 (53.3%)	4.1 ± 1.6 (26.7%)	5 ± 2.3 (53.3%)	
Tiamulin								
Trimethoprim	0.004 ± 0.0009 (0%)	0.004 ± 0.001 (0%)	0.004 ± 0.0008 (0%)	0.009 ± 0.003 (0%)	0.006 ± 0.001 (0%)	0.006 ± 0.001 (0%)	0.006 ± 0.002 (0%)	0.004 ± 0.001 (0%)
Vancomycin	67.9 ± 27.2 (14.3%)	93.4 ± 34.5 (38.5%)	124.7 ± 33.3 (40%)	457 ± 313.6 (93.3%)	37 ± 26.5 (13.3%)	72.1 ± 29 (53.3%)		

Note: Where a risk quotient is not specified for antimicrobials with low risk, the antimicrobial concentrations were consistently less than the method quantification limit across the sampling campaign.
Abbreviations: NP-AHD, 1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione; WWTP, wastewater treatment plant.

degradation), but metronidazole displayed more degradation in refrigerated conditions (8–14 °C) after 24 h than its metabolite (79.3 % ± 1.7 % vs. 84.0 % ± 8.5 %) (Holton et al., 2022). Autosamplers operated at 4 °C were employed in this study to collect wastewater over a 24 h period. During this timeframe, aliquots of collected wastewater are stored in refrigerated conditions prior to the removal of the composite sample. Given the lower stability of metronidazole and the greater variability in stability for hydroxymetronidazole in refrigerated conditions, it is plausible this could account for the low MTZ/hMTZ ratios observed here (Holton et al., 2022).

The mean ratios of sulfamethoxazole/N-acetyl sulfamethoxazole (SMX/aSMX) were above the excretion ratio at all WWTPs—a finding which was consistently statistically significant. This could be attributed to the stability of sulfamethoxazole. Although both parent and metabolite are stable in frozen and refrigerated conditions, after 24 h at ambient temperature (18–21 °C) a loss of about 26 % was noted for N-acetyl sulfamethoxazole compared to only about 3 % with sulfamethoxazole (Holton et al., 2022; Xu and Kasprzyk-Hordern, 2023). During this summer sampling campaign, influent wastewater can be exposed to these ambient temperatures thereby promoting degradation. The effect of summer temperatures on the parent to metabolite ratio can be noted in the work by Sims et al. (2023a) where the reported ratios were consistently higher in the summer months (June–September 2019) relative to the rest of the year in the two communities sampled. At the hospital sites, assessment of SMX/aSMX was limited. Except for Hospitals 3 and 7, sulfamethoxazole and/or N-acetyl sulfamethoxazole concentrations had exceeded the upper end of the calibration curve and could not be accurately quantified above the maxima, potentially skewing the true ratio. There was significant variability in ratios determined for Hospital 3, with two high ratio measurements (approximately 6 and 140) skewing the mean. These high SMX/aSMX ratios likely indicate direct disposal events or unaccounted for sources of usage as shorter in-sewer residence time for hospital wastewater would limit the extent of degradation due to temperature. The mean SMX/aSMX ratio determined for Hospital 7 was not statistically different than the excretion ratio.

In Wales, sulfamethoxazole and trimethoprim are often prescribed together as they are available in a combination tablet with these two antimicrobials present in a 5:1 ratio (Joint Formulary Committee, 2025). Trimethoprim is also available and prescribed independently, therefore the finding of the mean sulfamethoxazole/trimethoprim ratio being less than 5 at all sites was an expected result (see Supplementary Material, Fig. S4) (Joint Formulary Committee, 2025).

Sulfasalazine, a sulfonamide prescribed to treat inflammatory bowel disease and rheumatoid arthritis (Joint Formulary Committee, 2025), is metabolized to sulfapyridine, an antibiotic, which is then subsequently metabolized to N-acetyl sulfapyridine. Although these three antimicrobials are stable in refrigerated conditions (8–14 °C), at ambient temperature (18–21 °C) losses of approximately 27 %, 7 %, and 25 % were observed for sulfasalazine, sulfapyridine, and N-acetyl sulfapyridine, respectively (Holton et al., 2022). By comparison, the maximum loss for these antimicrobials after freezing (–20 °C) for 3 months was 10 % (Xu and Kasprzyk-Hordern, 2023). The mean ratios of sulfasalazine/sulfapyridine were statistically lower than expected across all sites, which can be attributed to the ambient temperature instability of sulfasalazine. In general, the mean ratios of sulfasalazine/N-acetyl sulfapyridine were

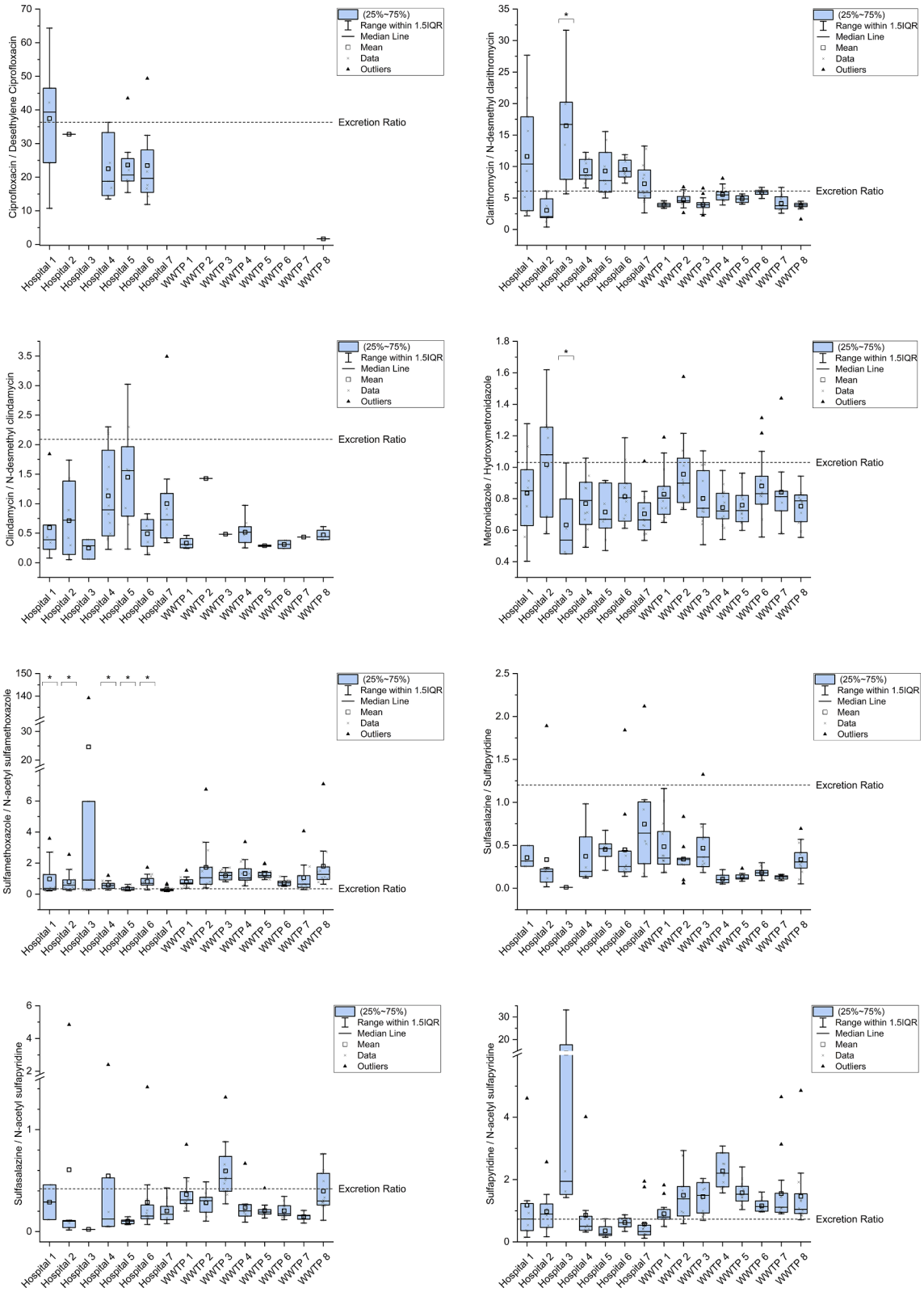


Fig. 5. Box plots of antimicrobial parent to metabolite ratios across sampling campaign. Parent to metabolite excretion ratios are from Holton et al. (2022). Asterisk (*) above bars indicates that the parent and/or metabolite concentration exceeded the upper end of the calibration curve and were limited to the maximal value. Abbreviations: IQR, interquartile range; WWTP, wastewater treatment plant.

similar or lower than the excretion ratio which is also in line with their respective instability. The sulfapyridine/N-acetyl sulfapyridine ratios determined in the majority of hospital wastewater samples were in concordance with the excretion ratio, while the ratios in community wastewater were at or above the expected level. This difference between patient and community wastewater could be attributable to the in-sewer residence time, as initially noted by Sims et al. (2023a), as shorter exposure to the environmental temperature prior to sampling for the

hospital sites in this work could have resulted in less N-acetyl sulfapyridine loss. Sulfapyridine is not listed in the British National Formulary nor are any sulfapyridine products licensed for use by the United Kingdom's Medicines and Healthcare Products Regulatory Agency; however, it does have potential uses in veterinary medicine (Joint Formulary Committee, 2025; Medicines & Healthcare Products Regulatory Agency, 2025). A review of the Product Information Database published by the United Kingdom's Veterinary Medicines Directorate shows that sulfasalazine and sulfapyridine are not authorized for veterinary use in the UK (Veterinary Medicines Directorate, 2024). This implies that the results for the sulfapyridine/N-acetyl sulfapyridine ratios are secondary to antimicrobial instability rather than community wastewater contamination from agricultural runoff. The relative instability of sulfasalazine and sulfapyridine prevented the conclusive identification of direct disposal events in this study.

3.6. Considerations and future work

The environmental risk assessments presented here were conducted in untreated wastewater and therefore represents the worst-case scenario. Subsequent wastewater treatment and dilution upon release into the receiving aquatic environment would reduce the environmental risk. The European Medicines Agency (2024) and the European Chemicals Agency (2016) recommend using a dilution factor of 10 when predicting environmental concentrations of contaminants entering receiving waters from effluent wastewater. The validity of a universal dilution factor has been contested, and the use of the recommended ten-fold dilution can underestimate the true environmental risk (Di Marcantonio et al., 2023; Ehalt MacEdo et al., 2022; Link et al., 2017). A median dilution factor of approximately 37 (interquartile range: ca. 6 to 186) has been estimated for the United Kingdom; however, there is significant variability in this result (Keller et al., 2014). If the hospital wastewater studied here were to enter the environment, for example through CSOs, the environmental risk could remain high for many antimicrobials owing to the magnitude of risk identified here. Future work will focus on predicting antimicrobial concentrations in effluent wastewater, and will re-assess both the risk of AMR and environmental harm.

4. Conclusions

This study provides insights into the current levels of risk, regarding both AMR and environmental harm, posed by antimicrobials in hospital and community wastewater throughout Wales. As this work quantified antimicrobials in untreated wastewater, the environmental risks presented here demonstrate the worst-case scenario. With the data capturing > 30 % of the Welsh population, it establishes a comprehensive baseline for future risk assessments within Wales. The following conclusions can be drawn:

- Risk assessments for the development of AMR should be routinely conducted when environmental risk is assessed. Antimicrobials which carry significant environmental risks (e.g. sulfamethoxazole, vancomycin) are not always the same antimicrobials which pose a high risk for inducing AMR (e.g. metronidazole, trimethoprim). Environmental harm and development of AMR are intricately linked: the development of AMR can increase use of more environmentally toxic antimicrobials. A One Health approach is recommended—both the public health and environmental implications of antimicrobials in aquatic systems should be considered in tandem.
- Hospitals are a significant source of antimicrobials, and these compounds and their metabolites can readily enter the aquatic environment (e.g., via CSOs). As well as containing an intrinsic load of AMR pathogens, hospital wastewater also represents a hot spot for the further development of AMR organisms within the sewer network (e.g., in biofilms). It is also likely to carry a high intrinsic load of AMR human pathogens. Therefore, treatment of wastewater at source prior to entering the sewer system would be instrumental in reducing this risk (e.g., on-site membrane bioreactors or advanced oxidation processes, activated carbon filtration systems, electrochemical treatment).
- All management strategies should target high-risk antimicrobials which are persistent across sites and carry significant risks (e.g. clarithromycin, ciprofloxacin, vancomycin). Additional interventions for management of antimicrobial residues should be customized according to the antimicrobial usage at each hospital site (e.g., implementing stricter prescribing guidelines, mandatory antibiotic stewardship programs, advanced wastewater treatment technologies, and regular monitoring as part of a national surveillance program).
- The potential identification of direct disposal events, specifically in hospitals, warrants implementation or addition of supplementary training for hospital staff to ensure the safe disposal of antimicrobials.

CRediT authorship contribution statement

Neil Andrew Byrnes: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Reshma Silvester:** Writing – review & editing, Investigation, Conceptualization. **Gareth Cross:** Writing – review & editing, Resources, Conceptualization. **Andrew J. Weightman:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization. **Davey L. Jones:** Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Barbara Kasprzyk-Hordern:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109606.

Data availability

Data will be made available on request.

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