International Journal of Scientific Research and Modern Education (IJSRME)
International Peer Reviewed - Refereed Research Journal, Website: www.crystalpen.in
Impact Factor: 7.137, ISSN (Online): 2455 - 5630, Volume 10, Issue 2, July - December, 2025

PHARMACEUTICAL POLLUTION PROFILES AND ANTIBIOTIC RESISTANCE GENE DETECTION PATTERNS IN WASTEWATER EFFLUENTS ACROSS INTERNATIONAL BASINS

Kannan Vishwanath* & Lloyd Zulu**
* Garwood Innovation Fellow, Garwood Center for Corporate Innovation, Haas School of Business, Berkeley
** World Christian University, Lusaka, Zambia

Cite This Article: Kannan Vishwanath & Lloyd Zulu, "Pharmaceutical Pollution Profiles and Antibiotic Resistance Gene Detection Patterns in Wastewater Effluents Across International Basins", International Journal of Scientific Research and Modern Education, Volume 10, Issue 2, July - December, Page Number 187-194, 2025.
Copy Right: © Crystal Pen Publication, 2025 (All Rights Reserved). This is an Open Access Article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
DOI: https://doi.org/10.5281/zenodo.24050568

Abstract:

This analysis examines how antibiotic concentration distributions shape comparisons with reported resistance gene detection, addressing the interpretation of international wastewater surveillance. Published measurements from 12 treatment plants across nine countries in the Danube basin during 2017 were analysed using descriptive group comparisons and exploratory sensitivity analysis. Effluents with reported qnrS detection had higher median clarithromycin, sulfamethoxazole and ofloxacin concentrations, with median ratios of 4.68, 1.49 and 2.62, respectively. Mean comparisons diverged for clarithromycin and ofloxacin because an extreme observation occurred without reported detection. Removing that plant reversed both mean contrasts, whereas median differences remained positive after every individual plant removal. Sulfamethoxazole retained positive mean and median contrasts throughout the sensitivity analysis. These findings distinguish typical concentration differences from comparisons driven by exceptional contamination without establishing biological selection or transmission. The empirical contribution qualifies interpretations that treat greater chemical exposure as a uniform indicator of molecular detection. International monitoring protocols should report means alongside medians, retain individual observations and assess sensitivity before using concentration rankings to characterize resistance patterns.

Key Words: Pharmaceutical Pollution; Antibiotic Resistance; Wastewater Surveillance; Distributional Analysis; Sensitivity Analysis

1. Introduction:

Wastewater monitoring can mischaracterize the relationship between antibiotic pollution and resistance when exceptional concentrations dominate comparisons. Chemical residues and resistance determinants coexist in treatment plant effluents, but their simultaneous occurrence does not establish a direct exposure response relationship. Larsson and Flach in 2022 distinguish environmental resistance selection, transmission and the introduction of bacteria already carrying resistance genes. These processes have different implications for surveillance. Monitoring must therefore establish what measured concentrations and molecular outcomes reveal together before using either to characterize the other. Evolutionary selection provides a biological rationale for examining antibiotics alongside resistance determinants. Exposure can favour resistant organisms when it changes their competitive position relative to susceptible populations. However, measured effluent concentrations also reflect wastewater composition and treatment processes, while gene detection depends on the organisms and genetic material present in the sample. Karkman et al. in 2019 show that fecal contamination can explain resistance gene abundance in many environments affected by human activity. Ju et al. in 2019 further identify bacterial community composition and genetic exchange as determinants of wastewater resistomes. Together, these findings establish competing explanations for chemical and molecular cooccurrence and caution against interpreting concentration differences as evidence of selection alone.

Clarithromycin, sulfamethoxazole and ofloxacin provide distinct exposure measures within this problem. They represent macrolide, sulfonamide and quinolone antibiotics, respectively. Ofloxacin has the closest biological correspondence with qnrS because Qnr proteins protect bacterial target enzymes against quinolone activity, as Hooper and Jacoby explain in 2015. Associations involving clarithromycin or sulfamethoxazole may instead reflect shared wastewater inputs or other relationships among resistance determinants. The analysis treats these possibilities as theoretical explanations rather than measured pathways. Its outcome is reported qnrS detection, which identifies a molecular classification rather than gene abundance, expression, bacterial viability or clinical resistance. The closest empirical foundation is the integrated Danube investigation published by Alygizakis et al. in 2019. That investigation combined chemical screening, bioassays and resistance gene measurements across wastewater treatment plants in nine countries. The present work is a secondary analysis of its published measurements, with a narrower question concerning the interpretation of concentration distributions. Specifically, it examines whether comparisons between effluents with and without reported qnrS detection convey the same pattern when summarized by means and medians, and whether individual plants determine those comparisons. This focus complements research on treatment performance. Pallares Vega et al. in 2019 assess resistance presence and removal across 62 Dutch treatment plants using influent and effluent measurements. Here, the quantity of interest is variation among effluents rather than removal efficiency.

This study aims to characterize how clarithromycin, sulfamethoxazole and ofloxacin concentrations differ between effluents with and without reported qnrS detection. Three specific objectives compare each antibiotic across the two detection groups. The analysis uses 12 matched plant records from the Danube sampling campaign conducted during August and September 2017. Each plant contributes one composite effluent observation. Descriptive summaries retain the original concentration units, distinguish typical values from extreme observations and preserve overlap between groups. An exploratory sensitivity analysis removes each plant individually to assess the stability of the median contrasts. The design supports transparent comparisons within the observed sample rather than causal identification or populationwide estimation. The completed analysis contributes by

showing that the interpretation of exposure differences depends on their distribution. Median concentrations are higher in the detection group for all three antibiotics, whereas mean concentrations are higher only for sulfamethoxazole. Every median contrast remains positive after individual plants are removed, while the clarithromycin and ofloxacin mean comparisons are sensitive to an exceptional effluent. The contribution is therefore a bounded empirical demonstration of how typical exposure patterns and extreme concentrations can support different readings of the same molecular classification.

This distinction matters for international surveillance because monitoring systems must communicate both routine conditions and exceptional contamination without conflating them. Larsson, Flach and Laxminarayan in 2023 identify opportunities and interpretive challenges in sewage resistance surveillance. The present analysis offers a transferable reporting approach: examine concentration distributions alongside molecular detection, retain individual observations and disclose sensitivity to influential records. The observed magnitudes remain specific to the sampled plants and campaign, but the distinction between typical concentrations, extreme values and reported gene detection applies more broadly to the interpretation of wastewater monitoring evidence.

2. Theory, Conceptual Framework and Research Questions Development:

This analysis adopts an evolutionary ecology perspective in which antibiotic residues and resistance determinants coexist within wastewater systems. Antibiotic exposure can alter the relative fitness of susceptible and resistant bacteria, while wastewater inputs introduce both pharmaceutical compounds and microorganisms carrying resistance genes. Davies and Davies in 2010 establish that resistance reflects interactions between microbial evolution, environmental reservoirs and antibiotic exposure. This perspective distinguishes the biological plausibility of selection from an observed association between a chemical concentration and gene detection. The empirical framework connects three measured antibiotic concentrations with reported qnrS detection across 12 wastewater treatment plants in nine countries within the Danube basin during 2017. Pharmaceutical pollution is represented by clarithromycin, sulfamethoxazole and ofloxacin concentrations, measured in nanograms per litre. The outcome records whether qnrS was reported detected in the corresponding effluent sample. These are observed measures rather than indicators of a constructed latent index. Alygizakis et al. in 2019 provide the matched chemical and molecular measurements underlying these comparisons. The framework therefore concerns variation among sampled effluents within one international basin. Clarithromycin represents macrolide exposure within the pharmaceutical mixture. Antibiotic concentrations below those that inhibit bacterial growth can influence microbial competition and resistance maintenance. Andersson and Hughes in 2014 explain that sublethal exposure can affect bacterial physiology and the selection of resistant populations. However, this mechanism does not establish a direct relationship between clarithromycin and qnrS, which concerns quinolone resistance. Any association between these measures could reflect their joint occurrence in wastewater rather than resistance selected specifically by clarithromycin. The appropriate empirical question is consequently exploratory.

RQ1: How does the distribution of clarithromycin concentrations differ between effluents with and without reported qnrS detection?

Sulfamethoxazole represents sulfonamide exposure and introduces a distinct theoretical pathway. Selection acting on one resistance determinant can maintain other determinants when they occur within the same genetic background. Gullberg et al. in 2014 demonstrate that sublethal antibiotic exposure can maintain a multidrug resistance plasmid, supporting the general plausibility of selection across linked resistance traits. The available measurements do not establish genetic linkage between sulfonamide resistance and qnrS. Sulfamethoxazole concentration is therefore treated as a measured exposure whose relationship with qnrS detection requires empirical description, without assuming a positive direction.

RQ2: How does the distribution of sulfamethoxazole concentrations differ between effluents with and without reported qnrS detection?

Ofloxacin provides the closest mechanistic correspondence with the measured outcome because both concern quinolones. Qnr proteins protect bacterial target enzymes from quinolone activity, providing a biological basis for examining ofloxacin alongside qnrS detection. Hooper and Jacoby in 2015 describe this target protection mechanism and distinguish it from other pathways of quinolone resistance. This correspondence strengthens the conceptual relevance of the comparison but does not establish that the measured ofloxacin concentration caused the observed detection pattern. Experimental evidence also shows why concentration and detection need not follow a simple proportional relationship. Gullberg et al. in 2011 demonstrate that resistant bacteria can gain a selective advantage at antibiotic concentrations substantially below the inhibitory concentration of susceptible bacteria. Consequently, lower measured concentrations cannot automatically be interpreted as an absence of selective pressure. A concentration measured in a composite effluent sample also differs from the exposure history experienced by the microbial population. These boundary conditions support an open comparison rather than a predetermined directional hypothesis.

RQ3: How does the distribution of ofloxacin concentrations differ between effluents with and without reported qnrS detection?

The conceptual framework comprises three parallel descriptive comparisons between antibiotic concentration and reported qnrS detection. It does not combine the antibiotics into a single score or assign causal meaning to their relationships with the outcome. Each question can be resolved by comparing the observed concentration distributions across the two detection groups. Nondetection remains an analytical classification rather than proof of biological absence. The questions permit higher, lower or overlapping concentrations and retain the same formulation regardless of the observed pattern.

3. Data:

This section establishes data provenance, documents sample construction and defines the observed measures used in the analysis. It aligns chemical concentrations with reported gene detection and provides the checks needed to reproduce the descriptive comparisons.

3.1 Data Sources, Population, and Sample Construction:

The analysis uses the published supplementary dataset accompanying Alygizakis et al. in 2019. The source combines chemical screening and molecular measurements from wastewater treatment plants in the Danube River Basin. Its contributing institutions include the Environmental Institute, the National and Kapodistrian University of Athens and KWR Watercycle Research Institute. Table S1.1 provides sampling metadata, Table S4.2 provides chemical concentrations, and Figure S6.1A

provides the reported qnrS detection pattern. The publication DOI identifies the source release, which has no separate numbered dataset version. The article and supplement are available under the Creative Commons Attribution 4.0 licence. The analytical workbook retains the extraction date separately from the observation dates. The unit of analysis is one averaged composite effluent sample from each treatment plant. Sampling occurred from 15 August to 2 September 2017. The resulting dataset is a cross section of 12 plants in Austria, Croatia, Czechia, Germany, Hungary, Romania, Serbia, Slovakia and Slovenia. Chemical measurements represent concentrations in composite samples rather than discharge loads or annual averages. This distinction matters because treatment processes influence antibiotic persistence, as Michael et al. in 2013 demonstrate, while wastewater plants can release both antibiotics and resistance determinants, as Rizzo et al. in 2013 explain.

The target population comprises wastewater treatment plant effluents entering international river basins. The accessible sampling frame consists of the 12 plants in the published Danube campaign. The original investigators selected plants to represent predominant treatment technologies and different plant sizes rather than through probability sampling. The source does not enumerate the complete target population. The secondary analysis retains all accessible plants with matched measurements for the three antibiotics and reported qnrS status. No selection changes were introduced after examining the descriptive comparisons. The empirical coverage supports comparisons among sampled effluents within one international basin. It does not provide repeated observations of individual plants. Coutu et al. in 2013 demonstrate temporal variation in antibiotic inputs, reinforcing the distinction between campaign measurements and persistent exposure conditions. Karkman et al. in 2018 identify wastewater as a setting where antibiotics, bacteria and resistance genes from different sources meet, supporting the relevance of matched chemical and molecular observations.

Table 1: Sample construction

Stage	Plants	Observations	Reason or Rule
Accessible published frame	12	12	Plants included in the Danube sampling campaign
Plants excluded for missing focal chemical measurements	0	0	All three selected antibiotic concentrations available
Plants excluded for unavailable reported qnrS status	0	0	Status recoverable from the published figure
Duplicate plant records removed	0	0	All plant identifiers unique
Matched and checked sample	12	12	Chemical and molecular records aligned by plant
Final descriptive sample	12	12	Common sample for all three research questions

3.2 Data Integration, Cleaning, and Quality Assurance:

Chemical concentrations, sampling metadata and reported gene detection were integrated through plant identifiers. Harmonization reconciled Cluj with Cluj Napoca, Varazdin with Varaždin, Zilina with Žilina, and Vipap with Vipap Krško. Each plant contributes one analytical record. The rectangular structure separates plant identifiers, measured values and explanatory metadata, consistent with the spreadsheet organization principles described by Broman and Woo in 2018. The integration produced 12 unique records without unmatched plants or an increase in observations. The 36 chemical values in the analytical sheet agree with the retained source records. The 12 binary outcome values also agree with their documented figure classifications. Preserving source entries separately from analytical representations provides an audit trail consistent with Sandve et al. in 2013. Automated checks confirmed positive chemical concentrations, binary outcome coding and consistency between the analytical and source sheets, following the emphasis on explicit computational checks in Wilson et al. in 2014. All four focal variables have complete coverage across the 12 plants. No chemical concentration required imputation, interpolation or substitution for a censored value. For qnrS, one denotes a coloured cell in the published detection figure and zero denotes a grey cell. These zeros preserve the reported classification and do not replace missing measurements. Source values and derived representations remain distinguishable, consistent with the data management recommendations of Wilson et al. in 2017. Exploratory screening identified concentrations above the upper quartile plus 1.5 times the interquartile range. These comprised clarithromycin and ofloxacin at Ljubljana and sulfamethoxazole at Sabac and Brno. The flagged values agreed with the retained source records and were preserved. Screening therefore changed neither sample composition nor recorded concentrations. The retained records allow these decisions to be checked independently, consistent with the reproducibility principle articulated by Peng in 2011.

3.3 Variable Measurement, Construction, and Validation:

Pharmaceutical pollution is operationalized through the measured concentrations of clarithromycin, sulfamethoxazole and ofloxacin. The compounds remain separate because their biological activities differ. Andersson and Hughes in 2014 explain that antibiotic effects depend on exposure conditions and microbial responses. Gullberg et al. in 2011 further demonstrate that selection can occur below concentrations that inhibit susceptible bacteria. These findings justify examining measured exposure while avoiding the assumption that concentration alone establishes resistance selection. Reported qnrS detection is the observed molecular outcome. Hooper and Jacoby in 2015 describe the role of Qnr proteins in protecting bacterial target enzymes against quinolones. The binary measure identifies reported detection rather than gene abundance, expression or phenotypic resistance. Chemical concentrations are likewise treated as exposure measurements rather than classifications of selective risk. Bengtsson Palme and Larsson in 2016 distinguish concentration measurements from thresholds intended to evaluate resistance selection.

Table 2: Variable dictionary

Variable or Indicator	Analytical Role	Definition and Construction	Source and Identifier	Unit and Frequency	Transformation and Direction	Missing Value Rule	Coverage And Limitation
Clarithromycin concentration	Exposure measure	Published concentration in composite effluent	Supplement, Table S4.2	ng/L; one campaign observation per plant	Original units; higher values indicate greater measured concentration	No missing values or substitutions	12 plants; campaign exposure

Variable or Indicator	Analytical Role	Definition and Construction	Source and Identifier	Unit and Frequency	Transformation and Direction	Missing Value Rule	Coverage And Limitation
Sulfamethoxazole concentration	Exposure measure	Published concentration in composite effluent	Supplement, Table S4.2	ng/L; one campaign observation per plant	Original units; higher values indicate greater measured concentration	No missing values or substitutions	12 plants; campaign exposure
Ofloxacin concentration	Exposure measure	Published concentration in composite effluent	Supplement, Table S4.2	ng/L; one campaign observation per plant	Original units; higher values indicate greater measured concentration	No missing values or substitutions	12 plants; campaign exposure
Reported qnrS detection	Outcome	One for a coloured cell and zero for a grey cell in the source figure	Supplement, Figure S6.1A	Binary; one campaign observation per plant	One indicates reported detection	Grey cells retain zero classification	12 plants; does not measure abundance
Plant identifier	Record linkage	Harmonized plant name	Supplement, Table S1.1	Categorical; plant level	No numerical interpretation	Complete	Unique within the accessible frame

Validation concerns transcription consistency, measurement units and coding rather than latent construct reliability. All selected concentrations exceed their reported chemical detection limits of 0.4 ng/L for clarithromycin, 0.1 ng/L for sulfamethoxazole and 1.6 ng/L for ofloxacin. These limits describe chemical measurement and do not define the detection threshold for qnrS. The outcome classification also cannot distinguish selection within treatment systems from the introduction of bacteria already carrying resistance determinants. Karkman et al. in 2019 demonstrate why such distinctions matter when interpreting resistance measurements in wastewater affected environments.

Table 3: Descriptive statistics for the focal variables

Variable	Valid Observations	Mean	Sample Standard Deviation	Median	Minimum	Maximum
Clarithromycin, ng/L	12	168.09	197.13	111.30	3.00	706.80
Sulfamethoxazole, ng/L	12	11.46	14.60	5.95	1.50	43.00
Ofloxacin, ng/L	12	367.43	855.81	73.55	15.50	3050.70
Reported qnrS detection	12	0.583	0.515	1	0	1

The outcome comprises seven reported detections and five records without reported detection. Its mean corresponds to a detection proportion of 58.33 percent. Concentration means exceed medians for all three antibiotics, with the largest difference for ofloxacin. Reporting medians and ranges alongside means preserves the influence of uneven concentration distributions without removing valid extreme observations.

3.4 Analytical Framework, Identification, and Reproducibility:

The implemented analysis is descriptive. It summarizes each antibiotic concentration across all plants and separately within the two reported qnrS groups. Means, medians and ranges characterize differences in location and spread, while individual records retain the observed overlap between groups. This emphasis on magnitude and distribution follows the estimation focused reasoning presented by Cumming in 2014. It also avoids treating a significance threshold as a substitute for scientific interpretation, consistent with Wasserstein and Lazar in 2016. The comparisons describe the accessible plants rather than estimate a causal effect or a basinwide detection probability. Treatment conditions, wastewater composition and bacterial inputs can influence both chemical concentrations and gene detection. The sampling design does not isolate these influences. Greenland et al. in 2016 emphasize that inferential conclusions depend on assumptions about data generation, while Wasserstein et al. in 2019 recommend interpreting evidence through its design and substantive context. Accordingly, higher, lower or overlapping concentrations remain descriptive outcomes rather than confirmation or rejection of a biological mechanism. Amrhein et al. in 2019 support retaining this distinction rather than equating an inconclusive comparison with an absence of a relationship. Reproducibility rests on the analytical workbook, retained source records, documented identifier mappings and explicit outcome coding. The same 12 observations support every descriptive comparison. Chemical concentrations retain their original units and source precision, while the molecular outcome retains its reported binary classification. This alignment permits reproduction of the summaries without introducing synthetic observations, selective exclusions or assumptions about unobserved exposure histories.

4. Methods:

4.1 Research Design and Analytical Scope:

This analysis uses a descriptive cross sectional design to examine antibiotic concentration distributions across effluents classified by reported qnrS detection. The unit of analysis is one composite effluent observation per wastewater treatment plant. The analytical sample comprises 12 plants sampled during August and September 2017. Table 1 documents sample construction, while Table 2 defines the focal measurements. The comparisons address three research questions concerning clarithromycin, sulfamethoxazole and ofloxacin. For each compound, the analytical target is the observed difference in concentration between plants with and without reported qnrS detection. Useful variation arises from differences among plants within the sampling campaign. These comparisons describe the accessible sample rather than estimate a treatment effect or a populationwide relationship. Each plant receives equal analytical weight. This choice preserves the plant as the comparison unit rather than weighting observations by population served or wastewater discharge. Chemical concentrations and molecular classifications are matched within the same campaign. Their temporal alignment supports descriptive comparison but does not establish that measured exposure preceded gene occurrence. Interpretation therefore distinguishes observed concentration differences from causal explanations, consistent with Greenland et al. in 2016.

4.2 Descriptive Specification and Comparative Measures:

The analysis retains concentrations in nanograms per litre. For each antibiotic, it calculates the valid count, arithmetic mean, sample standard deviation, median, minimum and maximum. These summaries are calculated for the complete sample and separately for each reported detection group. Sample standard deviations use the group count minus one as the denominator. The group mean is defined by Equation 1.

$$\bar{x}_{jg} = \frac{1}{n_g} \sum_{i \in S_g} x_{ij}$$

Here, $\bar{x}_{jg}$ is the mean concentration of antibiotic j in group g, S_g is the set of plants belonging to that group, n_g is its plant count, and x_{ij} is the concentration of antibiotic j at plant i. Group $g = 1$ denotes reported qnrS detection, while $g = 0$ denotes the comparison classification. The principal contrasts are defined by Equation 2.

$$\Delta_j^{mean} = \bar{x}_{j1} - \bar{x}_{j0}, \qquad \Delta_j^{median} = m_{j1} - m_{j0}, \qquad R_j = \frac{m_{j1}}{m_{j0}}$$

Here, Δ_j^{mean} is the mean concentration difference, Δ_j^{median} is the median concentration difference, m_{jg} is the median concentration in group g, and R_j is the ratio of the detection group median to the comparison group median. Positive differences indicate higher concentrations in the detection group. The ratio expresses a concentration comparison rather than a change in detection probability. For groups containing an even number of observations, the median is the arithmetic average of the two central ordered values. Means and medians are interpreted together to distinguish typical concentration differences from sensitivity to extreme observations. Reporting magnitudes in their original units follows the emphasis on interpretable estimates described by Cumming in 2014.

4.3 Measurement Alignment, Processing and Quality Checks:

Processing begins with the matched workbook records described in Tables 1 and 2. Plant identifiers are reconciled across chemical measurements, sampling metadata and molecular classifications. Each identifier must correspond to one analytical row. This organization preserves the distinction between identifiers, observations and calculated outputs, consistent with Broman and Woo in 2018. The reported qnrS classification is coded as one for a coloured cell and zero for a grey cell in the published source figure. Zero preserves that classification and is not interpreted as proof of biological absence. Chemical concentrations remain numeric source values. All 12 plants have complete focal measurements, so the same observations enter every full sample comparison. Quality checks examine identifier uniqueness, focal variable completeness, positive chemical concentrations and binary outcome coding. Analytical values are compared with the retained source records to identify transcription or alignment discrepancies. The calculations use the original concentration values rather than the logarithmic columns retained in the workbook. Exploratory screening flags concentrations exceeding the upper quartile plus 1.5 times the interquartile range. Quartiles are calculated using linear interpolation. Flagged values are checked against the retained source records and preserved when they agree. This screening identifies unusual observations without treating extremity as evidence of error. Calculations use Python 3.12.14 and NumPy 2.3.5. Workbook cells are read through the underlying spreadsheet XML using lxml 6.1.1. Numeric precision is retained during calculation, with rounding applied only to reported outputs.

4.4 Sensitivity Analysis, Interpretation and Replicability:

An exploratory sensitivity analysis evaluates whether individual plants determine the direction or magnitude of the comparisons. Each plant is removed once, and the group summaries are recalculated from the remaining 11 observations. Removing a detected record leaves six detected and five comparison records. Removing a comparison record leaves seven detected and four comparison records. The recalculated median contrast is defined by Equation 3.

$$\Delta_{j,\backslash i}^{median} = m_{j1,\backslash i} - m_{j0,\backslash i}$$

Here, $\Delta_{j,\backslash i}^{median}$ is the median contrast after plant i is removed, and $m_{jg,\backslash i}$ is the corresponding group median. Equation 3 is evaluated for every plant and antibiotic. The analysis records the smallest and largest resulting contrasts and counts how often their direction remains positive. Mean contrasts are recalculated using Equation 2 on each reduced sample. This procedure addresses sensitivity to individual observations. Its ranges describe variation across deliberate sample removals and are not confidence intervals. Comparisons between full and reduced samples identify influential records without replacing the full sample results. Table 4 reports the full sample group summaries, while Table 5 reports the median sensitivity results. Reproducibility rests on the analytical workbook, retained source records, documented identifier mappings, classification rules and explicit calculation definitions. Separating source entries from derived summaries provides an auditable sequence consistent with Sandve et al. in 2013. The method answers the research questions through observed distributions and their sensitivity to sample composition, with conclusions confined to the measured campaign.

5. Findings:

This analysis examines how clarithromycin, sulfamethoxazole and ofloxacin concentrations differ between effluents with and without reported qnrS detection.The findings resolve the three research questions through concentration distributions and observed group contrasts.An exploratory sensitivity analysis assesses whether individual plants determine the direction of the median comparisons. The analytical sample contains 12 wastewater treatment plants across nine countries in the Danube basin, sampled during August and September 2017. Each plant contributes one composite effluent observation. All three antibiotic concentrations and the reported qnrS classification are available for every plant, producing a common analytical sample without exclusions for missing focal measurements. Seven plants have reported qnrS detection and five do not. The measurements originate from the campaign published by Alygizakis et al. in 2019. Table 4 presents concentrations in their original units. Reported qnrS detection is a binary classification derived from the published molecular results. A record without reported detection does not establish biological absence. Means and medians are presented together because extreme concentrations can change the apparent direction of a group comparison. The emphasis remains on observed magnitudes rather than a significance threshold, consistent with Cumming in 2014.

Table 4: Antibiotic concentrations by reported qnrS detection status

Antibiotic	Reported qnrS status	Plants	Mean, ng/L	Standard deviation, ng/L	Median, ng/L	Minimum, ng/L	Maximum, ng/L
Clarithromycin	Detected	7	163.44	106.08	166.00	25.40	353.20
Clarithromycin	Not detected	5	174.60	299.82	35.50	3.00	706.80
Sulfamethoxazole	Detected	7	16.41	17.87	6.40	2.40	43.00
Sulfamethoxazole	Not detected	5	4.52	2.10	4.30	1.50	7.00
Ofloxacin	Detected	7	166.46	165.09	162.40	15.50	448.50
Ofloxacin	Not detected	5	648.78	1342.98	62.10	17.60	3050.70

For RQ1, clarithromycin concentrations show a higher median in effluents with reported qnrS detection. The median difference is 130.50 ng/L, and the detection group median is 4.68 times the comparison group median. However, the mean difference runs in the opposite direction: the detection group mean is 11.16 ng/L lower. Ljubljana accounts for the highest clarithromycin concentration, 706.80 ng/L, despite having no reported qnrS detection. RQ1 therefore resolves into a distribution dependent pattern. Typical concentrations are higher in the detection group, but the arithmetic mean is influenced by an extreme observation in the comparison group. The overlapping ranges preclude treating clarithromycin concentration as a separating indicator of reported detection. For RQ2, sulfamethoxazole produces consistent mean and median contrasts. The detection group median exceeds the comparison group median by 2.10 ng/L, corresponding to a ratio of 1.49. Its mean is also higher by 11.89 ng/L. The larger mean contrast reflects concentrations of 43.00 ng/L at Sabac and 41.60 ng/L at Brno, both with reported qnrS detection. Nevertheless, the detection group range extends down to 2.40 ng/L, within the comparison group range. RQ2 is answered by higher central concentrations among detected records, accompanied by substantial variation within that group. This coexistence does not identify selection as its cause. Karkman et al. in 2019 demonstrate that wastewater associated resistance patterns can also reflect the introduction of bacteria carrying resistance determinants.

For RQ3, ofloxacin has a higher median in the detection group but a lower mean. The median difference is 100.30 ng/L, and the median ratio is 2.62. In contrast, the detection group mean is 482.32 ng/L lower. This reversal reflects the concentration of 3050.70 ng/L at Ljubljana, which exceeds every other sampled concentration while occurring without reported qnrS detection. The biological correspondence between quinolones and Qnr target protection, described by Hooper and Jacoby in 2015, therefore does not translate into a uniform concentration ordering across these effluents. RQ3 resolves into higher typical exposure among detected records alongside a contradictory extreme observation. Table 5 reports an exploratory sensitivity analysis that removes each plant in turn and recalculates the median contrast. Each calculation retains 11 plants, with the contrast defined as the detection group median minus the comparison group median.

Table 5: Sensitivity of median contrasts to removal of individual plants

Antibiotic	Full Sample Median Contrast, ng/L	Lowest Contrast After One Removal, ng/L	Highest Contrast After One Removal, ng/L	Removals Retaining a Positive Contrast
Clarithromycin	130.50	96.95	141.40	12 of 12
Sulfamethoxazole	2.10	1.30	4.30	12 of 12
Ofloxacin	100.30	30.95	117.10	12 of 12

All three median contrasts remain positive after every individual removal. Their direction is therefore not determined by a single plant. Their magnitude remains sensitive to sample composition, particularly for ofloxacin, whose contrast ranges from 30.95 to 117.10 ng/L. Mean comparisons are less stable. Removing Ljubljana changes the clarithromycin mean contrast from negative 11.16 to positive 121.89 ng/L and the ofloxacin mean contrast from negative 482.32 to positive 118.16 ng/L. Sulfamethoxazole retains a positive mean contrast after every individual removal. The evidence answers all three research questions at the descriptive level. Effluents with reported qnrS detection have higher median concentrations of each antibiotic, while only sulfamethoxazole also maintains a consistently higher mean. The sensitivity analysis supports the direction of the median comparisons within the observed sample, but not a causal interpretation or a populationwide relationship. This distinction follows Greenland et al. in 2016, who emphasize that inferential claims depend on assumptions about sampling and data generation. The central empirical finding is the contrast between stable median ordering and sensitivity of the means to extreme concentrations.

6. Discussion:

Higher average antibiotic contamination does not necessarily coincide with reported resistance gene detection. Table 4 reveals this distinction: all three antibiotics have higher median concentrations among effluents with reported qnrS detection, yet clarithromycin and ofloxacin have lower means in that group. The contribution of this analysis is the separation of typical concentration differences from patterns driven by extreme observations. This distinction qualifies a simple interpretation in which increasing pharmaceutical concentration corresponds uniformly to increasing resistance detection. Larsson and Flach in 2022 describe environmental resistance as an interaction among exposure, bacterial populations and genetic determinants. The observed contrasts add a descriptive qualification to that framework: even the direction of an exposure comparison can depend on how its distribution is summarized. The ofloxacin comparison illustrates why biological correspondence should guide interpretation without predetermining the empirical result. Although qnrS concerns quinolone resistance, Table 4 places the highest ofloxacin concentration in an effluent without reported detection. This observation prevents concentration alone from serving as a reliable indicator of the recorded molecular outcome. Ju et al. in 2019 identify bacterial community composition and genetic exchange as determinants of wastewater resistomes. Those findings provide plausible explanations for heterogeneous concentration and detection patterns, but the present measurements do not distinguish among them. The conceptual refinement is therefore specific:

chemical exposure and molecular detection provide complementary information, and neither should substitute for the other when interpreting individual effluents.

Sulfamethoxazole differs from the other compounds because both its mean and median are higher in the detection group, as Table 4 shows. This consistency makes its descriptive relationship less dependent on the choice of central tendency. It does not establish sulfamethoxazole as a dominant biological pathway, because the observed concentrations neither measure genetic linkage nor partition resistance mechanisms. Manaia in 2017 distinguishes environmental resistance abundance from transmission risk, emphasizing that their relationship is not directly proportional. The present comparison extends that interpretive caution to chemical concentration and binary detection. A consistent exposure contrast identifies an empirical pattern worth characterizing, while its biological and public health meaning remains distinct from the concentration difference itself. The sensitivity results identify a practical challenge for environmental comparisons: an individual effluent can reverse a mean contrast without determining the direction of the median contrast. Table 5 shows that every median difference remains positive when plants are removed individually, whereas removing Ljubljana reverses the clarithromycin and ofloxacin mean comparisons. This finding supports reporting both typical conditions and exceptional concentrations rather than selecting one summary as universally representative. In their analysis of 62 Dutch treatment plants, Pallares Vega et al. in 2019 examine resistance removal using matched influent and effluent measurements. The present analysis addresses a different quantity, variation among effluents, and cannot translate that variation into treatment performance. The distinction changes how plant comparisons should inform decisions: observed effluent concentrations identify monitoring priorities, while removal efficiency requires measurements across treatment stages.

The international relevance lies in an analytical lesson rather than a claim that these plants represent all river basins. Tables 4 and 5 demonstrate that concentration rankings, typical exposure differences and reported gene detection can convey different information within the same monitoring campaign. Larsson, Flach and Laxminarayan in 2023 emphasize both the opportunities and challenges of sewage resistance surveillance. The present evidence gives that concern a concrete implication: monitoring reports should retain individual observations, present means alongside medians and distinguish molecular detection from chemical exposure. Across surveillance settings, this approach can prevent exceptional concentrations from being mistaken for a general relationship while preserving their operational importance. The resulting contribution is a transparent interpretation of heterogeneous measurements, with conclusions bounded by what each measure records.

7. Conclusion and Implications:

Environmental surveillance can tell conflicting stories when exceptional contamination obscures typical exposure patterns. This analysis shows that three measured antibiotic exposures share higher typical concentrations among effluents with reported resistance detection, while their average concentrations do not convey a uniform relationship. Its contribution is an empirical distinction between persistent descriptive ordering and sensitivity to extreme observations. This distinction qualifies frameworks that interpret greater chemical contamination as a straightforward indicator of resistance occurrence. Chemical exposure and molecular detection provide complementary evidence, and their relationship requires attention to distributional structure rather than reliance on a single summary. For monitoring managers, the findings support reporting typical concentrations alongside exceptional values and retaining plant specific records during comparative assessment. Resource allocation should distinguish investigation of contamination extremes from surveillance of recurring molecular detections, because these priorities need not identify the same locations. Policy reporting protocols can strengthen comparability by requiring transparent concentration summaries, explicit detection definitions and sensitivity checks. Operationally, these practices can reduce misleading rankings without discarding important contamination events. The wider social value lies in more defensible environmental reporting and better-informed monitoring priorities. The transferable contribution concerns how evidence is interpreted across surveillance systems, rather than a universal biological relationship or demonstrated reduction in health risk.

The sampling design creates opportunities for targeted extension. Repeated observations across seasons and additional international basins would establish whether the descriptive ordering persists across time and settings. Quantitative molecular measurements, documented detection thresholds and bacterial community information would sharpen interpretation beyond binary classifications. Matched observations before and after treatment would distinguish effluent differences from removal performance. Designs incorporating wastewater composition and treatment conditions could examine competing explanations for chemical and molecular cooccurrence. Independent replication with prespecified comparisons would assess transferability, while longitudinal and experimental approaches could investigate the causal processes that the present descriptive evidence cannot identify.

References:

1. Alygizakis, N. A., Besselink, H., Paulus, G. K., Oswald, P., Hornstra, L. M., Oswaldova, M., Medema, G., Thomaidis, N. S., Behnisch, P. A., & Slobodnik, J. 2019. Characterization of wastewater effluents in the Danube River Basin with chemical screening, in vitro bioassays and antibiotic resistant genes analysis. Environment International, 127, 420-429. https://doi.org/10.1016/j.envint.2019.03.060
2. Amrhein, V., Greenland, S., & McShane, B. 2019. Scientists rise up against statistical significance. Nature, 567, issue 7748, 305-307. https://doi.org/10.1038/d41586-019-00857-9
3. Andersson, D. I., & Hughes, D. 2014. Microbiological effects of sublethal levels of antibiotics. Nature Reviews Microbiology, 12, issue 7, 465-478. https://doi.org/10.1038/nrmicro3270
4. Bengtsson-Palme, J., & Larsson, D. G. J. 2016. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International, 86, 140-149. https://doi.org/10.1016/j.envint.2015.10.015
5. Broman, K. W., & Woo, K. H. 2018. Data organization in spreadsheets. The American Statistician, 72, issue 1, 2-10. https://doi.org/10.1080/00031305.2017.1375989
6. Coutu, S., Rossi, L., Barry, D. A., Rudaz, S., &Vernaz, N. 2013. Temporal variability of antibiotics fluxes in wastewater and contribution from hospitals. PLOS ONE, 8, issue 1, Article e53592. https://doi.org/10.1371/journal.pone.0053592

7. Cumming, G. 2014. The new statistics: Why and how. Psychological Science, 25, issue 1, 7-29. https://doi.org/10.1177/0956797613504966
8. Davies, J., & Davies, D. 2010. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74, issue 3, 417-433. https://doi.org/10.1128/MMBR.00016-10
9. Greenland, S., Senn, S. J., Rothman, K. J., Carlin, J. B., Poole, C., Goodman, S. N., & Altman, D. G. 2016. Statistical tests, P values, confidence intervals, and power: A guide to misinterpretations. European Journal of Epidemiology, 31, issue 4, 337-350. https://doi.org/10.1007/s10654-016-0149-3
10. Gullberg, E., Albrecht, L. M., Karlsson, C., Sandegren, L., & Andersson, D. I. 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. mBio, 5, issue 5, Article e01918-14. https://doi.org/10.1128/mBio.01918-14
11. Gullberg, E., Cao, S., Berg, O. G., Ilbäck, C., Sandegren, L., Hughes, D., & Andersson, D. I. 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLOS Pathogens, 7, issue 7, Article e1002158. https://doi.org/10.1371/journal.ppat.1002158
12. Hooper, D. C., & Jacoby, G. A. 2015. Mechanisms of drug resistance: Quinolone resistance. Annals of the New York Academy of Sciences, 1354, issue 1, 12-31. https://doi.org/10.1111/nyas.12830
13. Ju, F., Beck, K., Yin, X., Maccagnan, A., McArdell, C. S., Singer, H. P., Johnson, D. R., Zhang, T., &Bürgmann, H. 2019. Wastewater treatment plant resistomes are shaped by bacterial composition, genetic exchange, and upregulated expression in the effluent microbiomes. The ISME Journal, 13, issue 2, 346-360. https://doi.org/10.1038/s41396-018-0277-8
14. Karkman, A., Do, T. T., Walsh, F., & Virta, M. P. J. 2018. Antibiotic-resistance genes in waste water. Trends in Microbiology, 26, issue 3, 220-228. https://doi.org/10.1016/j.tim.2017.09.005
15. Karkman, A., Pärnänen, K., & Larsson, D. G. J. 2019. Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nature Communications, 10, Article 80. https://doi.org/10.1038/s41467-018-07992-3
16. Larsson, D. G. J., & Flach, C. F. 2022. Antibiotic resistance in the environment. Nature Reviews Microbiology, 20, issue 5, 257-269. https://doi.org/10.1038/s41579-021-00649-x
17. Larsson, D. G. J., Flach, C. F., & Laxminarayan, R. 2023. Sewage surveillance of antibiotic resistance holds both opportunities and challenges. Nature Reviews Microbiology, 21, issue 4, 213-214. https://doi.org/10.1038/s41579-022-00835-5
18. Manaia, C. M. 2017. Assessing the risk of antibiotic resistance transmission from the environment to humans: Non-direct proportionality between abundance and risk. Trends in Microbiology, 25, issue 3, 173-181. https://doi.org/10.1016/j.tim.2016.11.014
19. Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C., & Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. Water Research, 47, issue 3, 957-995. https://doi.org/10.1016/j.watres.2012.11.027
20. Pallares-Vega, R., Blaak, H., van der Plaats, R., de Roda Husman, A. M., Hernandez Leal, L., van Loosdrecht, M. C. M., Weissbrodt, D. G., & Schmitt, H. 2019. Determinants of presence and removal of antibiotic resistance genes during WWTP treatment: A cross-sectional study. Water Research, 161, 319-328. https://doi.org/10.1016/j.watres.2019.05.100
21. Peng, R. D. 2011. Reproducible research in computational science. Science, 334, issue 6060, 1226-1227. https://doi.org/10.1126/science.1213847
22. Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I., & Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Science of the Total Environment, 447, 345-360. https://doi.org/10.1016/j.scitotenv.2013.01.032
23. Sandve, G. K., Nekrutenko, A., Taylor, J., & Hovig, E. 2013. Ten simple rules for reproducible computational research. PLOS Computational Biology, 9, issue 10, Article e1003285. https://doi.org/10.1371/journal.pcbi.1003285
24. Wasserstein, R. L., & Lazar, N. A. 2016. The ASA's statement on p-values: Context, process, and purpose. The American Statistician, 70, issue 2, 129-133. https://doi.org/10.1080/00031305.2016.1154108
25. Wasserstein, R. L., Schirm, A. L., & Lazar, N. A. 2019. Moving to a world beyond $p < 0.05$. The American Statistician, 73, supplement 1, 1-19. https://doi.org/10.1080/00031305.2019.1583913
26. Wilson, G., Aruliah, D. A., Brown, C. T., Chue Hong, N. P., Davis, M., Guy, R. T., Haddock, S. H. D., Huff, K. D., Mitchell, I. M., Plumbley, M. D., Waugh, B., White, E. P., & Wilson, P. 2014. Best practices for scientific computing. PLOS Biology, 12, issue 1, Article e1001745. https://doi.org/10.1371/journal.pbio.1001745
27. Wilson, G., Bryan, J., Cranston, K., Kitzes, J., Nederbragt, L., & Teal, T. K. 2017. Good enough practices in scientific computing. PLOS Computational Biology, 13, issue 6, Article e1005510. https://doi.org/10.1371/journal.pcbi.1005510