Clinical prevalence of collateral sensitivity: a systematic exploration of multicentre antimicrobial surveillance data

Tandar, S.T.; Zwep, L.B; Woudt, S.H.S.; Schoffelen, A.F.; Smits, W.K.; Aulin, L.B.S.; ... ; Zoetigheid, R.E.

Clinical prevalence of collateral sensitivity: a systematic exploration of multicentre antimicrobial surveillance data

*Sebastian T Tandar, Laura B Zwep, Sjoukje H S Woudt, Annelot F Schoffelen, Wiep Klaas Smits, Linda B S Aulin, Apostolos Liakopoulos, J G Coen van Hasselt, Infectious Diseases Surveillance Information System–Antimicrobial Resistance Study Group**

Summary

Background Collateral effects arise when resistance to one antibiotic alters the susceptibility of a bacterial strain to another antibiotic, resulting in either increased (collateral sensitivity) or decreased (collateral resistance) susceptibility. Collateral sensitivity-based antibiotic treatment offers a promising strategy against antibiotic resistance. To date, the clinical occurrence of collateral sensitivity between bacterial strains and species remains to be further evaluated. Our study aims to evaluate the occurrence patterns of collateral sensitivity in clinical settings.

Methods For this systematic exploration of multicentre antimicrobial surveillance data, we analysed large-scale antimicrobial resistance surveillance data from three datasets, covering over 5 million minimum inhibitory concentration (MIC) measurements across 86 antibiotics and 30 pathogen species, to identify collateral effect interactions. Pairwise and three-way collateral effects were quantified to assess species-wide trends in both collateral sensitivity and collateral resistance within individual pathogen species. Additionally, we compared the prevalence of collateral sensitivity between and within antibiotic classes. By comparing collateral sensitivity occurrence across species, we identified collateral sensitivity interactions conserved across several pathogens.

Findings We found a low occurrence of collateral sensitivity in clinical strains, with 364 of 12 024 species-antibiotic pairs (3·0%) affected, compared to 5044 cases (42·0%) of collateral resistance. Most collateral sensitivity interactions involved antibiotics from different classes, except for β-lactams, which showed 41 (34·2%) of 120 occurrences of intraclass collateral sensitivity. We identified six collateral sensitivity pairs that were conserved across four bacterial species, including several highly virulent pathogens belonging to the ESKAPEE group. Three of these conserved collateral sensitivity pairs were associated with a higher MIC towards colistin. Only one three-way collateral sensitivity interaction was shared across four pathogen species. The collateral effect network generated in this study is available via a web application, enabling further data exploration and supporting future research on antibiotic collateral effects.

Interpretation Several collateral sensitivity interactions were conserved across several clinically relevant pathogens. The identified collateral sensitivity pairs can be considered for the development and application of collateral sensitivity-based antibiotic therapies to prevent and reverse antimicrobial resistance.

Funding The Longfonds foundation and the Dutch Ministry of Health, Welfare, and Sport.

Lancet Microbe 2026; 7: 101274

Published Online March 5, 2026 https://doi.org/10.1016/j.lanmic.2025.101274

*Members listed at the end of the Article

Leiden Academic Centre for Drug Research, Leiden University, Leiden, Netherlands (S T Tandar MSc, L B Zwep PhD, L B S Aulin PhD, Prof J G C van Hasselt PhD); **Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, Netherlands** (S H S Woudt MSc, A F Schoffelen PhD); **Leiden University Center for Infectious Diseases, Leiden University Medical Centre, Leiden, Netherlands** (W K Smits PhD); **Microbiology, Department of Biology, Utrecht University, Utrecht, Netherlands** (A Liakopoulos PhD); **Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, Netherlands** (A Liakopoulos)

Correspondence to: Prof J G Coen van Hasselt, Leiden Academic Centre for Drug Research, Leiden University, Leiden University, 2333 Leiden, Netherlands **coen.vanhasselt@lacdr.leidenuniv.nl**

Introduction

Antimicrobial resistance (AMR) in bacterial pathogens poses a substantial threat to human health.[1] The evolution of AMR can be attributed to several underlying cellular mechanisms. AMR mechanisms offer not only a survival strategy against a specific antibiotic, but can also alter the susceptibility of a cell to other antibiotics as evolutionary trade-off.[2] The effect of resistance to one antibiotic on susceptibility to another is known as a collateral effect. This effect can appear as decreased susceptibility to another antibiotic, termed collateral resistance, often seen between antibiotics sharing a common resistance mechanism, such as efflux pump overexpression.[3] Resistance to one antibiotic might also lead to increased susceptibility to another antibiotic, which is referred to as collateral sensitivity.[2] Although the precise mechanisms behind collateral sensitivity often remain elusive, past studies have provided potential mechanistic explanations for its occurrence.[2] For example, *trkH* mutations in *Escherichia coli* were found to mediate resistance to aminoglycosides by reducing proton motive force, hampering aminoglycoside uptake but simultaneously impairing the function of the AcrAB-TolC efflux pump, causing *trkH* mutant *E coli* to be more susceptible to other antibiotics.[4]

Exploiting the occurrence of collateral sensitivity phenotypes in antibiotic treatment regimens has been suggested as a promising strategy to prevent and reverse AMR.[5–7] The design of a collateral sensitivity-based combination therapy involves selecting and combining drugs such that resistance to one increases susceptibility to the other to overcome antibiotic resistance.[2,6–8] Choosing an effective collateral sensitivity antibiotic pair therefore requires information on

Research in context

Evidence before this study

We conducted a PubMed search for studies published between January, 2015 and September, 2025 using the terms "collateral sensitivity", "disjoint resistance", "antibiotics", "clinic", and "retrospective analysis", without language restrictions. Of the 117 publications identified, only two specifically focused on evaluating the clinical occurrence of collateral sensitivity. The first study used antibiotic susceptibility surveillance data to investigate minimum inhibitory concentration (MIC) patterns, but was limited to a single-centre dataset and did not explore collateral sensitivity across different pathogenic species, highlighting a gap in our understanding of the clinical occurrence of collateral sensitivity. The second study, done by our group, introduced a tool for analysing large-scale population surveillance data to systematically identify collateral effects linked to antibiotic resistance.

Added value of this study

The current study provides a comprehensive overview of the occurrence of collateral sensitivity across a wide range of antibiotics and pathogens through analysis of large-scale antibiotic surveillance data. The collateral effect relationships identified in our study are made accessible through a web application to enable further exploration of the data by clinicians and the scientific community, thereby supporting future studies aiming to better understand collateral antibiotic effects.

Implications of all the available evidence

The comprehensive analysis of collateral sensitivity in diverse clinical environments presents evidence of consistent collateral sensitivity in clinical settings and suggests the potential for the implementation of collateral sensitivity-based therapies. Our findings can serve as a starting point for subsequent efforts to develop collateral sensitivity-based therapeutic strategies against antibiotic resistance, in particular for ESKAPEE pathogens. By demonstrating the conservation of collateral sensitivity effects across multiple pathogens, our findings support further studies towards the clinical evaluation of collateral sensitivity-based combination therapies that could potentially thwart or reverse the evolution of resistance.

the reciprocity and magnitude of the observed collateral effect.[8] Despite the promising potential of collateral sensitivity-based therapies, questions remain about their occurrence and applicability in clinical settings,[2] largely because most collateral sensitivity studies have been done in controlled laboratory environments. Although several collateral sensitivity pairs, such as fluoroquinolone-β-lactam[9] and cefazolin–meropenem,[10] have been identified in clinical *E coli* isolates, the consistency of collateral sensitivity across different pathogens remains unclear. This consistency is essential to ensure the broader applicability of a particular collateral sensitivity pair for treating a wide range of infections.[11] Therefore, an analysis evaluating the occurrence, magnitude, reciprocity, and consistency of collateral effects across diverse drug pairs and pathogen species is needed.

In the current study we use a pooled, large-scale multicentre antimicrobial surveillance dataset that includes the antibiotic susceptibility profiles of 30 pathogens, including the ESKAPEE group pathogens (ie, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp, and *E coli*).[12,13] Using a previously developed statistical inference technique to identify collateral sensitivity,[10] we explore the ubiquity of antibiotic pairs demonstrating collateral sensitivity across different species, supporting the potential application of collateral sensitivity-based therapies as a generic treatment in cases where the main pathogen causing the infection is unknown. Additionally, we investigated whether reduced susceptibility to several antibiotics better predicts collateral sensitivity to another antibiotic. By investigating the collateral effect network in this dataset, our aim is to advance understanding of the clinical prevalence of collateral sensitivity and to identify collateral sensitivity interactions that might consistently emerge in clinical settings.

See Online for appendix

Methods

Data source and preprocessing

For this systematic exploration of multicentre antimicrobial surveillance data, clinical isolate minimum inhibitory concentration (MIC) data were acquired and pooled from three datasets, including the National Institutes of Health (NIH;[14] access date Feb 19, 2021), the Infectious Diseases Surveillance Information System Antimicrobial Resistance (ISIS-AR;[15] access date March 3, 2021), and the Antimicrobial Testing Leadership and Surveillance (ATLAS;[16] access date Nov 3, 2023). Each source provided MIC values for several pathogens, encompassing a wide range of antibiotic classes. Censored MIC values were imputed as follows: less-than-or-equal-to values were directly used as the imputed MIC; and greater-than values were imputed by doubling the reported value. Rounded MIC values were imputed to the nearest power of 2. MIC obtained from different measurement methods (appendix p 1) were assumed to be equivalent. Data from the NIH database were filtered to exclude non-clinical bacterial isolates. Potentially overlapping entries between datasets were removed on the basis of the reported measurement or strain ID. For each species, the dataset was further curated by excluding antibiotics with fewer than 100 observations, and isolates which were only tested for one antibiotic. For the current analysis, entries on the *Acinetobacter baumannii-calcoaceticus* complex were pooled together with that of *Acinetobacter baumannii*. MIC breakpoint values for each antibiotic-species set were obtained from the AMR

package[17] in R, using the latest published data from European Committee on Antimicrobial Susceptibility Testing or Clinical and Laboratory Standards Institute. If the corresponding breakpoint for the exact species was unavailable, the closest available breakpoint value at the next higher taxonomic level was used for analysis.

Pairwise collateral effect quantification

Collateral effect quantification was done to assess population-wide trends in both collateral sensitivity and collateral resistance within specific pathogen species. This analysis was performed using the *collatRal* package.[10] The pairwise collateral effect quantification involved two drugs denoted as test antibiotic A and focal antibiotic B. Here, a focal antibiotic refers to the primary drug of interest for which resistance characteristics are being examined, whereas test antibiotic refers to the secondary drug for which potential collateral effect was assessed in relation to resistance to the focal antibiotic. Analysis was done separately for each species. For each species-antibiotic pair set, MIC data were dichotomised into groups with higher (group $B_{High\text{-}MIC}$) and lower (group $B_{Low\text{-}MIC}$) MIC towards the focal antibiotic B (figure 1). The dichotomisation threshold (τ) was set at the median MIC for antibiotic B to ensure roughly equal group sizes with a maximum allowed imbalance of 9:1. Collateral effect was quantified as the mean difference of log-MIC value between the groups $B_{High\text{-}MIC}$ and $B_{Low\text{-}MIC}$.

$$\phi_{A|B_{High}} = \overline{\log_2(MIC_{A|B_{High}})} - \overline{\log_2(MIC_{A|B_{Low}})}$$

The identification of collateral effect was determined by hypothesis testing with a null hypothesis of $\phi=0$. The alternative hypotheses were $\phi<0$ for collateral sensitivity and $\phi>0$ for collateral resistance. A false discovery rate (FDR)-adjusted p value threshold of 0·05 was used to determine the statistical significance of the tested collateral effect interaction.[10] A minimum absolute collateral effect size $|\phi|$ of 0·5 was used to ensure the identified collateral effects reflect a relevant change in antibiotic susceptibility (figure 1). All possible antibiotic pairs were tested bidirectionally. The prevalence of collateral sensitivity interaction between antibiotic classes was evaluated on the basis of the occurrence rate of collateral sensitivity between each two antibiotic classes.

In this analysis, collateral effects were assessed solely from antibiotic susceptibility profiles without determining underlying mechanisms. The primary focus of this analysis is to identify collateral sensitivity, defined as cases in which a higher MIC for the focal antibiotic corresponds to a lower MIC for the test antibiotic. Conversely, the term collateral resistance is used to describe cases in which a higher MIC for the focal antibiotic is associated with a higher MIC for the test antibiotic without distinguishing collateral resistance and co-resistance. Thus, we used the term collateral resistance in this study as a general term for both events.

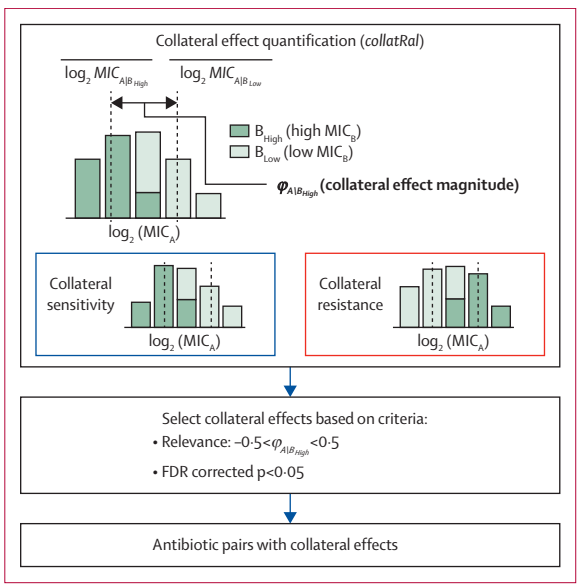

Figure 1: **Collateral effect quantification workflow**
Collateral effect quantification was done separately for each species and antibiotic pair (test antibiotic A and focal antibiotic B). The top panel shows a sample MIC distribution towards test antibiotic A between strains that have a high or low MIC towards the focal antibiotic B. This dichotomisation into high or low MIC for the focal antibiotic was based on the median MIC observed for that antibiotic within the tested species. Collateral effect magnitude ϕ was calculated as log2 ratio of the mean MIC value towards antibiotic A observed in groups with a higher MIC (lower susceptibility) and a lower MIC (higher susceptibility) to the focal antibiotic B. Relevant collateral effect pairs were selected based on the magnitude of the collateral effect (ϕ) and statistical significance of the observed MIC difference (FDR-corrected p value). FDR=false discovery rate. MIC=minimum inhibitory concentration.

Quantification of three-way collateral sensitivity effects

The current analysis aims to quantify collateral effects involving an antibiotic triplet. For each set of test antibiotic A and focal antibiotics B and C, the strains were grouped into strains with a higher MIC to both B and C ($B_{High}\&C_{High}$) and strains with a lower MIC to either B or C ($B_{Low}|C_{Low}$). Three-way collateral effect of B and C resistance to A (ψ) was quantified by the mean difference of log-MIC value between groups $B_{High}\&C_{High}$ and $B_{Low}|C_{Low}$.

$$\psi_{A|(B_{High}\&C_{High})} = \overline{\log_2(MIC_{A|(B_{High}\&C_{High})})} - \overline{\log_2(MIC_{A|(B_{Low}|C_{Low})})}$$

A minimum absolute three-way collateral effect size $|\psi|$ of 0·5 was used to identify physiologically relevant effects.

Software

Data (pre-)processing, analysis, and visualisation were conducted in R (version 4.3.2). Standardisation of antibiotic name, abbreviation, and classification was done using the AMR package[17] in R (appendix pp 11–12).

For more on **the collatRal package** see https://github.com/vanhasseltlab/collatRal

Role of the funding source
The role of the funders of this study is limited to data collection. The funder was not involved in data analysis, data interpretation, or writing of the report.

Results

The analysis was performed on a MIC dataset from 5·10 million isolates pooled from the NIH (2050 isolates [0·04%]), ISIS-AR (4·5 million isolates [88·75%]), and ATLAS (0·6 million isolates [11·21%]) datasets. The ISIS-AR dataset provides extensive information for six ESKAPEE pathogens (appendix p 2). The NIH and ATLAS databases were incorporated to increase the diversity of antibiotic compounds and pathogen species included in the analysis. The pooled dataset included MIC measurements for 86 antibiotics from human isolates measured in 30 pathogenic species (figure 2A–C; appendix pp 2, 11–12), from 73 countries across several continents, with most entries originating from the Americas and Europe (appendix p 2). The dataset was not equally distributed across all species and antibiotics. Species with the highest number of MIC measurements (n) and different antibiotics (d) were *E coli* (n=$2{\cdot}82\times10^6$; d=58), *S aureus* (n=$1{\cdot}05\times10^6$; d=51), *K pneumoniae* (n=$4{\cdot}97\times10^5$; d=53), *P aeruginosa* (n=$4{\cdot}63\times10^5$; d=44), and *Streptococcus pneumoniae* (n=$6{\cdot}61\times10^4$; d=45), which altogether accounted for $4{\cdot}89\times10^6$ (95·42%) isolates in the pooled dataset. The combined dataset included 3630 antibiotic pairs (r), which when stratified by species resulted in 12 024 species-antibiotic pairs.

We identified collateral effects in 5408 (45·0%) of the 12 024 tested species-antibiotic pairs. Despite the general prevalence of collateral effects (figure 3A–B), collateral sensitivity was found to be significantly less prominent than collateral resistance (5044 [41·9%] of 12 024; figure 3B), occurring in only 364 (3·03%) of the 12 024 observed species-antibiotic pairs (figures 3B–C). This finding aligns with previous reports that also found collateral sensitivity to be less frequent than collateral resistance.[10,18] Some collateral sensitivity interactions are specific to either Gram-positive or Gram-negative bacteria, which might reflect the use of certain antibiotics according to Gram-staining classification, because of different bacterial properties between both groups (appendix p 3).

High MICs to colistin and chloramphenicol showed the highest frequency of relevant collateral sensitivity hits (figure 3C). Among pairs with collateral sensitivity effects for these antibiotics, reciprocal collateral sensitivity was found in six (3·3%) of 182 pairs for colistin and three (1·4%) of 218 pairs for chloramphenicol (appendix p 13). Inverse proportionality between the MICs of the focal and test antibiotics within a collateral sensitivity pair was not associated with collateral effect magnitude and occurred mainly among pairs with reciprocal collateral sensitivity (appendix p 4).

Collateral effect networks for each antibiotic pair and species evaluated in this study are accessible through a web application dashboard. This application enables both general and species-specific and antibiotic-specific explorations of collateral effects through interactive visualisation tools. An example exploration of the specific collateral sensitivity network for *Haemophilus influenzae* is shown in the appendix (p 5).

For more on **the Collateral effects network** see https://collateralviz.lacdr.leidenuniv.nl

Although we applied a cutoff collateral effect size $|\phi|$ of 0·5 to select for a collateral sensitivity with a more relevant effect on test antibiotics MIC, 43·0% of all identified collateral sensitivity was associated with a two-fold reduction of test antibiotics MIC (equivalent to $\phi \leq -1{\cdot}0$; appendix p 6). 14·2% of the identified collateral sensitivity interactions with known MIC breakpoint value for the corresponding species was associated with a decrease in MIC value that recategorised the mean population from above to below the corresponding MIC breakpoint for susceptible isolates (appendix pp 6–7).

The current analysis evaluated the prevalence of interclass (between-antibiotic classes) and intraclass (within-antibiotic classes) collateral sensitivity among the identified collateral effect network by comparing the pooled occurrence rates of collateral sensitivity within and between antibiotic classes. The analysis showed that interclass collateral sensitivity interactions were generally more common than intraclass collateral sensitivity, with the exception of the β-lactam group. Within this group, intraclass collateral sensitivity accounted for up to 41 (34·2%) of 120 of the identified collateral sensitivity interactions (figure 4). Further division of the β-lactam group into penicillin, cephalosporin, and carbapenem subclasses revealed that the prevalence of intraclass collateral sensitivity in the β-lactam group was mainly observed within the cephalosporin subclass, in which 14 (21·9%) of 64 cephalosporin-resistant isolates showed collateral sensitivity to another cephalosporin (appendix p 8). Other intraclass β-lactam collateral sensitivity interactions involving penicillin and carbapenem were associated with increased cephalosporin susceptibility. Antibiotics categorised under other antibacterials also exhibited intraclass collateral sensitivity, probably because of the integration of several smaller antibiotic classes into this group.

To assess the consistency of collateral sensitivity occurrence across different pathogen species, we screened the collateral effect network to identify collateral sensitivity interactions that consistently emerged across multiple species. The majority of detected collateral sensitivity occurrences were found to be unique for one (r=220 pairs) or shared across two (r=45 pairs) species. For example, a strong and reciprocal collateral sensitivity between imipenem and chloramphenicol was observed in *S pneumoniae* strains but not in any of the other pathogenic species (appendix p 9). Six antibiotic pairs showed consistent collateral sensitivity across four pathogens (necessarily the same four pathogens). This included azithromycinin isolates with a high linezolid MIC, cefuroxime in isolates with high MIC to tobramycin, amoxicillin–clavulanate in isolates with high MIC to chloramphenicol, as well as cefazolin, ampicillin, and levofloxacin in isolates with high MIC

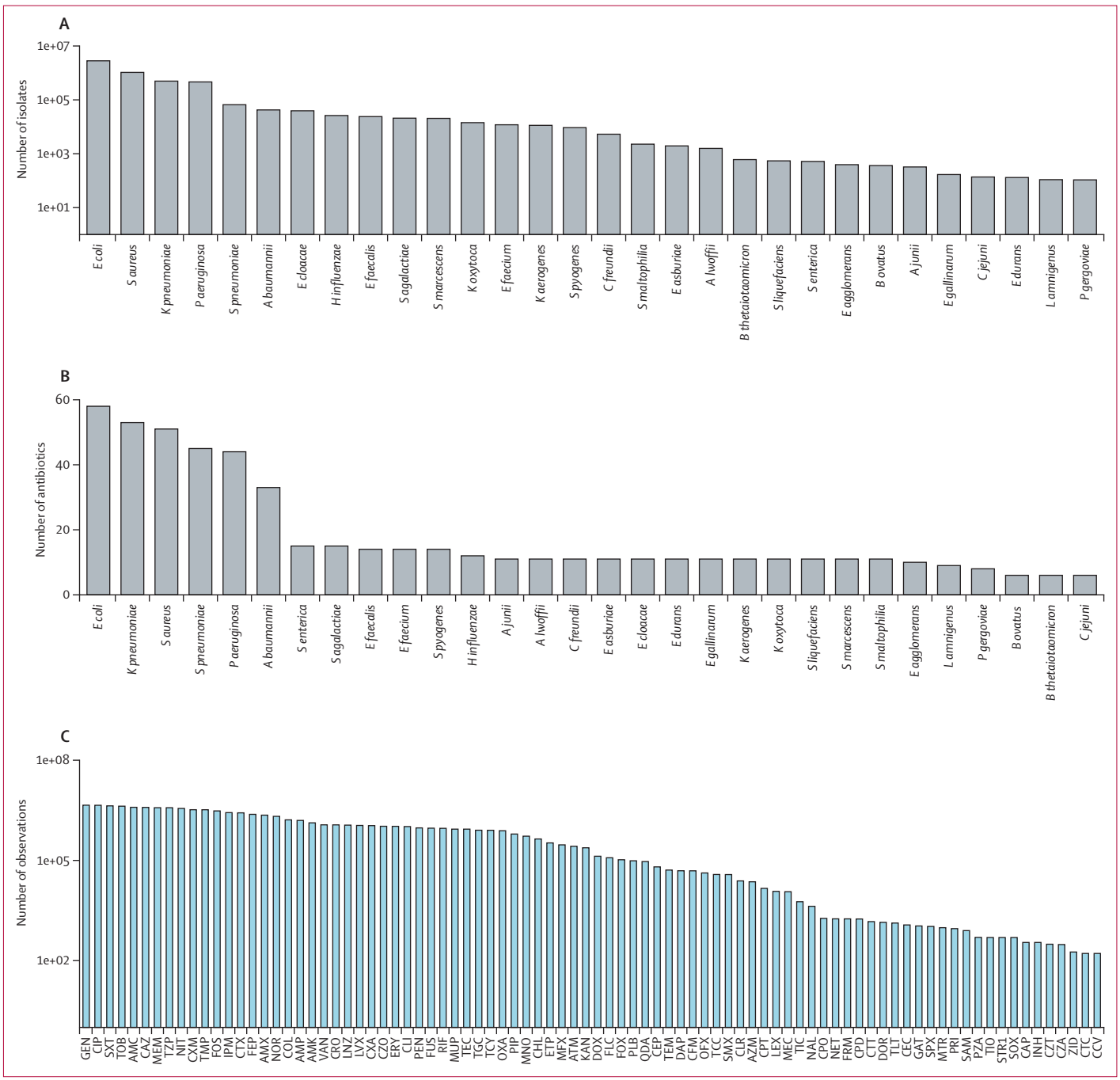

Figure 2: **Characteristics of the pooled antimicrobial resistance surveillance data**

Data was pooled from National Institutes of Health, ISIS-AR, and ATLAS datasets. (A) Number of isolates (observation sets) for each species included in the dataset. Each isolate can be associated with several MIC measurements for different antibiotics. (B) Number of antibiotics tested on each species included in the dataset. (C) Number of MIC measurements identified for each antibiotic included in the dataset. Antibiotics name abbreviations are listed in the appendix (appendix pp 11–12). MIC=minimum inhibitory concentration. Species included: *Acinetobacter baumanii*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Campylobacter jejuni*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lelliottia amnigenus*, *Pluralibacter gergoviae*, *Pseudomonas aeruginosa*, *Serratia liquefaciens*, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*.

to colistin (figure 5A; appendix p 9). Notably, all three occurrences of collateral sensitivity in isolates showing a high MIC to colistin were conserved across four ESKAPEE pathogens, namely *A baumannii*, *E coli*, *K pneumoniae*, and *P aeruginosa*. Among these six pairs, reciprocal collateral sensitivity was observed on colistin–cefazolin pairs in

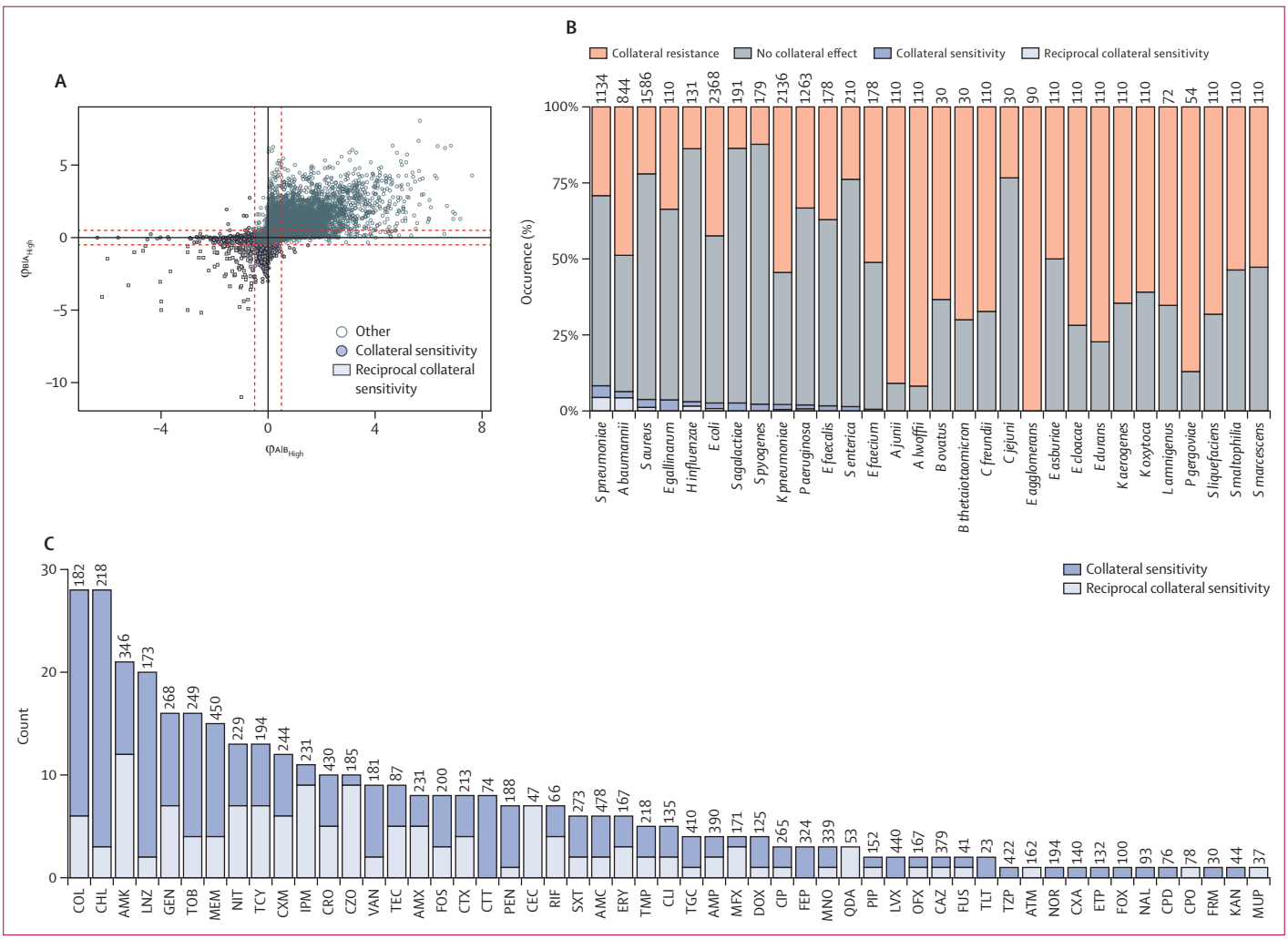

Figure 3: **Clinical occurrence of collateral effects**

(A) Collateral effect size for each antibiotic pair (using $|\phi| \geq 0{\cdot}5$ as threshold for relevant collateral effects, denoted by red dashed lines). All tested pairs were categorised on the basis of the direction and reciprocity of the interaction, namely reciprocal collateral sensitivity, collateral sensitivity, and other (unidirectional and reciprocal collateral resistance as well as no collateral effect). (B) Occurrence percentages of collateral resistance, collateral sensitivity, reciprocal collateral sensitivity, and no collateral effect across different species. The total number of antibiotic pairs tested for each species was shown above the corresponding bars. (C) Number of collateral sensitivity pairs identified for each antibiotic. The number of species–antibiotic pairs tested for the antibiotic of interest (including no collateral effects and collateral resistance) was shown above the bars. Antibiotics with no identified collateral sensitivity interaction were excluded. Antibiotics name abbreviations are listed in the appendix (pp 11–12). Species included: *Acinetobacter baumanii*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Campylobacter jejuni*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lelliottia amnigenus*, *Pluralibacter gergoviae*, *Pseudomonas aeruginosa*, *Serratia liquefaciens*, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*.

A baumanii and *E coli* (appendix p 9). This finding suggests the species specificity of reciprocal collateral sensitivity. None of the observed collateral sensitivity pairs were shared across more than four species.

We did a three-way collateral sensitivity to investigate the potential occurrence of complex collateral sensitivity phenotypes involving antibiotic triplets. The analysis identified 3186 (4·24% of 75 056 species-antibiotic triplet sets) three-way collateral sensitivity interactions. For comparison, pairwise collateral sensitivity evaluation covered 3630 pairs, with 281 (7·74%) pairwise collateral sensitivity interactions identified (figure 5B). Only one antibiotic triplet showed consistent collateral sensitivity across four species: azithromycin collateral sensitivity was observed in *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus agalactiae*, and *S pneumoniae* isolates with increased MICs to linezolid and meropenem (appendix p 10).

Discussion

This study aimed to explore the collateral effect network and identify collateral sensitivity antibiotic pairs using a large, multicentre clinical surveillance dataset. Although collateral sensitivity is typically assessed by comparing MICs between parental and mutant strains,[6,19] such comparisons

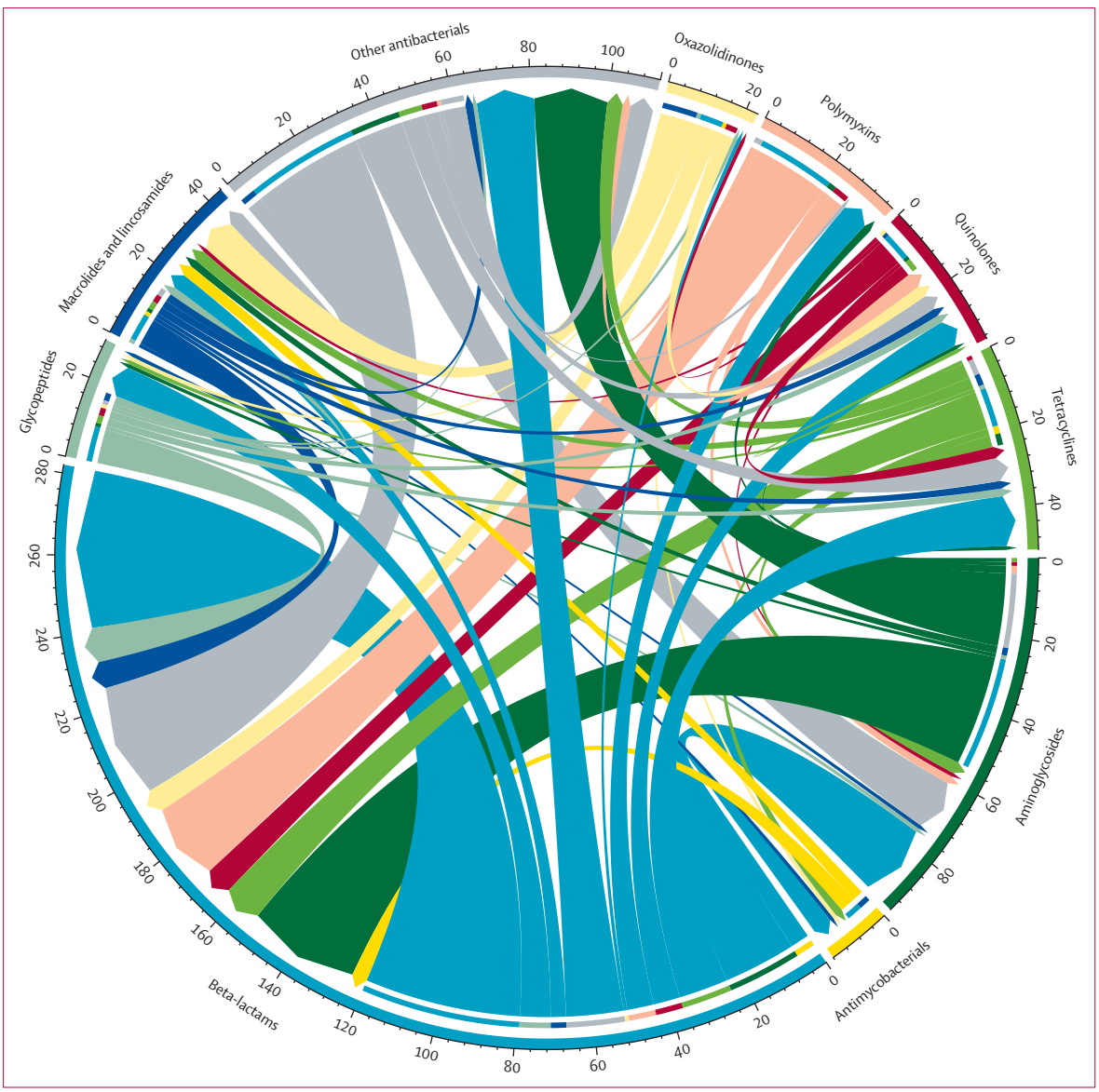

Figure 4: **Prevalence of interclass and intraclass occurrence of collateral sensitivity**

Frequency and direction of collateral sensitivity interactions between different antibiotic classes. Arrows (chords) originate from the focal antibiotic class (where higher MIC was observed) and point toward the test antibiotic class (where collateral sensitivity effects were detected). The colour of the inner arc at the base of each arrow corresponds to the antibiotic class of the test antibiotic (the class at which the arrow ends), whereas the colour of the outer arc represents the antibiotic class at each segment of the circle. The width of each arrow chord is proportional to the number of detected collateral sensitivity interactions between the focal and test antibiotic classes. Numbers at the outer arcs indicate the total number of detected collateral sensitivity interactions either originating from (start) or received by (end) each antibiotic class. Penicillins, cephalosporins, and carbapenems were grouped as β-lactams; no observed collateral sensitivity involved monobactams. The class of other antibacterials includes chloramphenicol, daptomycin, fosfomycin, fusidic acid, metronidazole, mupirocin, nitrofurantoin, sulfamethoxazole, sulfisoxazole, trimethoprim, trimethoprim and sulfamethoxazole, and zidebactam.

are challenging in clinical surveillance datasets because of the frequent scarcity of isolate lineage information. To address this gap, we focused on species-wide trends. Here, collateral sensitivity was identified from consistent patterns in which a lower MIC to the test antibiotic was associated with a higher MIC to the focal antibiotic across more than 100 isolates of the same species (figure 1). Such a pattern indicates an underlying trade-off in antibiotic susceptibility, which by definition constitutes collateral sensitivity. Conversely, if the two MICs varied independently, no statistically significant association would be expected in the aggregate data. By systematically identifying antibiotic pairs with consistent collateral sensitivity, our analysis pinpoints those most likely to produce reproducible collateral sensitivity in clinical settings, a crucial consideration for collateral sensitivity-guided therapies.[5]

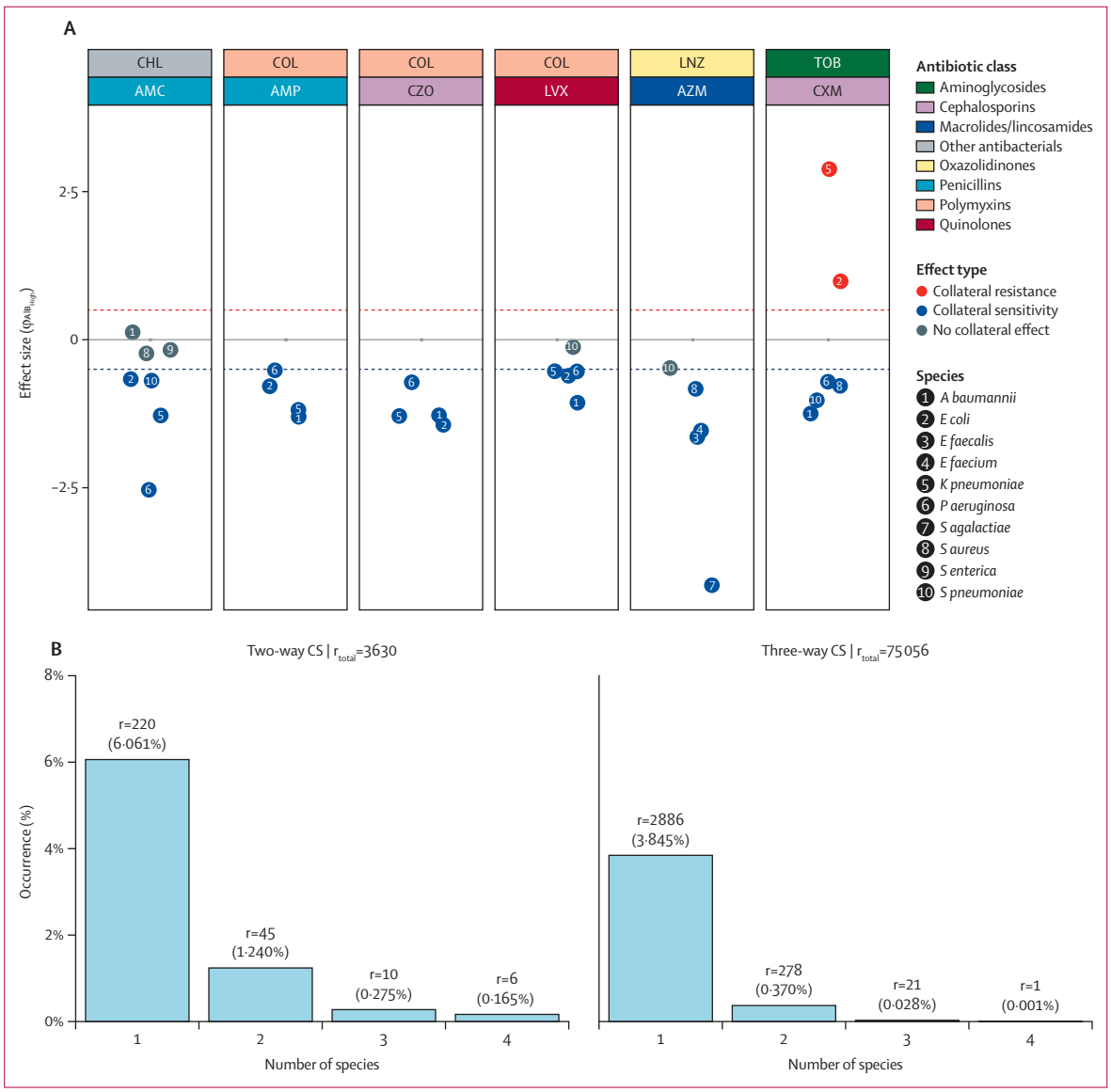

Figure 5: **Magnitude and bacterial species distribution of consistent collateral sensitivity pairs**
(A) Consistency of two-antibiotics collateral sensitivity. The collateral sensitivity interactions were considered consistent if the effect was observed in at least four different species. A threshold collateral effect size of $|\phi| \geq 0{\cdot}5$ was applied (dashed red and blue lines). Drug label colours denote the different antibiotic classes between which collateral sensitivity effects were detected. The number of species shown for each antibiotic pair might vary on the basis of data availability, given that species with fewer than 100 MIC measurements for a given pair were excluded from the analysis. (B) Occurrence rate between consistent two-way collateral sensitivity across all tested antibiotic pairs and three-way collateral sensitivity across all tested triplets. The number of antibiotic pairs and triplets is denoted by *r*. Two-way collateral sensitivity constituted of collateral sensitivity interactions involving two antibiotics, and three-way collateral sensitivity constituted of collateral sensitivity interactions involving three antibiotics. Two-way collateral sensitivity was examined in 3630 antibiotic pairs and three-way collateral sensitivity in 75 056 antibiotic triplets. The y-axis represents the occurrence as a percentage of the total number of antibiotic pairs and triplets that showed shared collateral sensitivity across one, two, three, and four species. Antibiotic name abbreviations are listed in the appendix (pp 11–12).

Our analysis does not capture all collateral sensitivity phenotypes within a pathogen species. Instead, it identifies only those that consistently emerge across isolates of each tested species. Thus, our approach may only flag collateral sensitivity phenotypes driven by either a single, highly prevalent resistance mechanism, or by several mechanisms that lead to the same phenotype in clinical settings. Consequently, this approach might limit the current analysis in identifying collateral sensitivity pairs that can be reliably evolved in the laboratory but are less prevalent in clinical settings, probably because of the more diverse and uncontrolled evolutionary trajectories found in clinical environments.[20,21] For example, aminoglycoside collateral sensitivity consistently emerged in ciprofloxacin-resistant

P aeruginosa during adaptive laboratory evolution,[19,22] but this interaction was not detected in our clinical dataset, suggesting it might not be as consistent in real-world settings. Adaptive laboratory evolution studies linked aminoglycoside collateral sensitivity in ciprofloxacin-resistant strains to mutations in *gyrA–B*, *mexS* (mexEF-oprN) and *nfxB* (mexCD-oprJ).[19] This absence might be due to the presence of alternative ciprofloxacin resistance mechanisms that do not confer aminoglycoside collateral sensitivity, the occurrence of escape mutations that negate aminoglycoside collateral sensitivity in ciprofloxacin-resistant strains, or epistatic interactions between the genetic background and specific mutations that resulted in pleiotropic effects. Together, these factors highlight the complexity of translating laboratory collateral sensitivity observations to clinical contexts and help explain the rarity of collateral sensitivity in clinical settings, where environmental conditions drive variable evolutionary trajectories.

Seven consistent collateral sensitivity pairs (six pairwise and one three-way collateral sensitivity) were identified. These collateral sensitivity pairs were conserved across four pathogenic species. The rarity of conserved collateral sensitivity, particularly across species, might partly reflect interspecies differences in pathogen physiology and genetic background,[12,13,23] which can in turn influence the development of pleiotropic effects, including collateral sensitivity. Given the high prevalence of ESKAPEE pathogens in clinical settings, these consistent collateral sensitivity interactions may offer a strategic advantage in developing collateral sensitivity-based therapies.

Elevated MICs for colistin were consistently associated with reduced MICs toward ampicillin, cefazolin, and levofloxacin across all Gram-negative pathogens in which these pairs were tested (figure 5A). Notably, the association between colistin resistance and increased susceptibility to other antibiotics has also been highlighted in previous studies.[22,24] One study pinpointed the mechanism of this collateral sensitivity phenotype in colistin-resistant Gram-negative bacteria as loss of the lipopolysaccharide layer, which increases membrane permeability and susceptibility to other antibiotics.[24] These findings suggest a shared collateral sensitivity mechanism across colistin-resistant Gram-negative pathogens. The alignment between previous laboratory studies and current clinical observations underscores these collateral sensitivity pairs as both mechanistically characterised and clinically prevalent. Therefore, future studies are encouraged to explore the clinical potential of these collateral sensitivity pairs in combination therapies and to further investigate the occurrence of similar collateral sensitivity phenotypes in other clinically relevant Gram-negative pathogens.

A high number of collateral sensitivity interactions were identified in pathogens showing elevated MIC towards chloramphenicol (figure 3C). This includes three reciprocal collateral sensitivity interactions between chloramphenicol and three β-lactam antibiotics, including amoxicillin–clavulanate and imipenem in *S pneumoniae* as well as cefpirome in *K pneumoniae* (appendix p 13). This finding is in line with a previous finding of chloramphenicol collateral sensitivity in several pathogen species,[25] and the mechanistically characterised chloramphenicol resensitisation observed in extended-spectrum β-lactamase-producing pathogens.[26] Thus, future studies are encouraged to further characterise the underlying mechanisms and pharmacodynamics collateral sensitivity pairs involving chloramphenicol and β-lactams.

Three-way collateral sensitivity interaction was uncommon in this dataset (figure 5B). The low number of identified interactions could partly reflect the limited measurements available for each antibiotic triplet and the stricter penalties introduced by FDR correction compared with pairwise analyses. One three-way collateral sensitivity, azithromycin collateral sensitivity in isolates with elevated linezolid and meropenem MICs, was conserved across four species. Although this triplet shows potential as a candidate for three-way collateral sensitivity-based therapy, further investigation is needed to assess its clinical utility.

Collateral sensitivity is often assumed to occur primarily between antibiotic pairs from different classes.[27] Consistent with this expectation, our analysis found that collateral sensitivity interactions were predominantly identified between antibiotics of different classes, with the notable exception of β-lactams (figure 4). Within the β-lactam class, we observed a higher frequency of intraclass collateral sensitivity interactions, particularly among cephalosporins (appendix p 8). The prevalence of intraclass collateral sensitivity within β-lactams may be related to the evolutionary constraints imposed by β-lactamase evolution.[28] Further research is needed to clarify the mechanisms underlying the apparent exclusivity of intraclass collateral sensitivity interactions among β-lactams.

This analysis used pooled MIC data from NIH, ISIS-AR, and ATLAS datasets. The ISIS-AR dataset provides dense information on six ESKAPEE pathogens in the Netherlands, whereas the NIH and ATLAS dataset contribute data on a broader range of species, antibiotics, and geographic regions (appendix p 2). Nevertheless, uneven coverage across antibiotics and pathogens remains in the pooled dataset, limiting our ability to identify collateral sensitivity interactions for under-represented species and antibiotics. Moreover, the much larger size of the ISIS-AR dataset means that data for certain pathogens are dominated by measurements from the Netherlands, especially for *S aureus* (89·4%) and *E coli* (97·2%). As such, the outcomes for these two species may predominantly reflect phenotypes common in Dutch clinical settings. Future studies incorporating more regionally diverse datasets could further assess the generalisability of the currently identified collateral effect profiles for these two pathogens.

The reliance of the current analysis on MIC values may introduce several limitations. The standard two-fold dilution used in MIC assays, combined with the imputation of MIC values required when results are reported as greater-than or less-than-or-equal values, can lead to

overestimation or underestimation of actual antibiotic susceptibility. Consequently, this study may only approximate, and not precisely capture, the magnitude of collateral effects. Nonetheless, MIC remains the most widely available and practical metric for large-scale surveillance and for identifying collateral effects in clinical settings. Subsequent studies aiming to further characterise selected collateral sensitivity pairs identified in the current analysis may benefit from alternative metrics that offer higher resolution and accuracy in measuring antibiotic susceptibility, such as the concentration to inhibit bacterial growth by 50% or the bactericidal effect concentration to achieve half-maximal bactericidal effect.

This analysis focused on identifying antibiotic pairs with conserved collateral sensitivity across several pathogens. However, the dataset also supports targeted investigations of the collateral effect network. To facilitate this, the collateral effect network is available online for further exploration of pathogen-specific or antibiotic-specific patterns.

For more on **the collateral effect network** see https://collateralviz.lacdr.leidenuniv.nl

In summary, this large-scale analysis contributes to our understanding of collateral effects by evaluating their occurrence in clinical settings. By highlighting collateral sensitivity interactions conserved across several pathogens, our study supports further efforts for developing collateral sensitivity-based therapies that can be effective across a broader range of pathogens. Altogether, these findings support ongoing efforts to translate collateral sensitivity-based strategies into clinical practice to help combat antibiotic resistance.

ISIS-AR study group

J W T Cohen Stuart (Department of Medical Microbiology, Noordwest Ziekenhuisgroep, Alkmaar), D C Melles (Department of Medical Microbiology, Meander Medical Centre, Amersfoort), K van Dijk (Department of Medical Microbiology and Infection Prevention, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Center (UMC), University of Amsterdam, Amsterdam), A Alzubaidy (Department of Medical Microbiology, Atalmedial, Amsterdam), M Scholing (Department of Medical Microbiology, OLVG Lab BV, Amsterdam), S D Kuil (Public Health Laboratory, Public Health Service, Amsterdam), G J Blaauw (Department of Medical Microbiology and Infection Prevention, Gelre Hospitals, Apeldoorn), W van den Bijllaardt (Laboratory for Microbiology and Infection Control, Microvida Amphia, Breda), E M Kraan (Department of Medical Microbiology, IJsselland Hospital, Capelle aan de IJsel), M B Haeseker (Department of Medical Microbiology, Reinier de Graaf Group, Delft), J M da Silva (Department of Medical Microbiology, Deventer Hospital, Deventer), E de Jong (Department of Medical Microbiology, Slingeland Hospital, Doetinchem), B Maraha (Department of Medical Microbiology, Albert Schweitzer Hospital, Dordrecht), A J van Griethuysen (Department of Medical Microbiology, Gelderse Vallei Hospital, Ede), B B Wintermans (Department of Medical Microbiology, Admiraal De Ruyter Hospital, Goes), M J C A van Trijp (Department of Medical Microbiology and Infection Prevention, Groene Hart Hospital, Gouda), A E Muller (Department of Medical Microbiology, Haaglanden, 's-Gravenhage), M Wong (Department of Medical Microbiology, Haga Hospital, 's-Gravenhage), A Ott (Certe, Medical Microbiology Groningen|Drenthe, Groningen), E Bathoorn (Department of Medical Microbiology, University Medical Center, University of Groningen, Groningen), M Lokate (Department of Medical Microbiology, University Medical Center, University of Groningen, Groningen), J Sinnige (Regional Public Health Laboratory Haarlem, Haarlem), D C Melles (Department of Medical Microbiology, St Jansdal Hospital, Harderwijk), L Bank (Department of Medical Microbiology, St Jansdal Hospital, Harderwijk), F W Sebens (Labmicta, Hengelo), N H Renders (Department of Medical Microbiology and Infection Control, Jeroen Bosch Hospital, 's-Hertogenbosch), J W Dorigo-Zetsma (Department of Medical Microbiology, Centraal Bacteriologisch en Serologisch Laboratorium, Tergooi, Hilversum), L J Bakker (Department of Medical Microbiology, Centraal Bacteriologisch en Serologisch Laboratorium), Tergooi, Hilversum), W Ang (Department of Medical Microbiology, Comicro, Hoorn), K Waar (Medical Microbiology Friesland|NOP, Leeuwarden), M T van der Beek (Department of Medical Microbiology, Leiden University Medical Center, Leiden), M A Leversteijn-van Hall (Department of Medical Microbiology, Eurofins Clinical Diagnostics, Leiden-Leiderdorp), S P van Mens (Department of Medical Microbiology, Infectious Diseases, and Infection Prevention, Maastricht University Medical Centre, Maastricht), E Schaftenaar (Department of Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein), M H Nabuurs-Franssen (Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen), I Maat (Department of Medical Microbiology, Radboud University Medical Center, Nijmegen), P D J Sturm (Department of Medical Microbiology, Laurentius Hospital, Roermond), B M W Diederen (Department of Medical Microbiology, Bravis Hospital, Roosendaal), L G M Bode (Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam), D S Y Ong (Department of Medical Microbiology and Infection Control, Franciscus Gasthuis and Vlietland, Rotterdam), M van Rijn (Department of Medical Microbiology, Ikazia Hospital, Rotterdam), S Dinant (Department of Medical Microbiology, Maasstad Hospital, Rotterdam), M den Reijer (Star-SHL, Rotterdam), D W van Dam (Department of Medical Microbiology and Infection Control, Zuyderland Medical Centre, Sittard-Geleen), E I G B de Brauwer (Department of Medical Microbiology and Infection Control, Zuyderland Medical Centre, Sittard-Geleen), R G Bentvelsen (Department of Medical Microbiology, Microvida ZorgSaam, Terneuzen), J J J M Stohr (Department of Medical Microbiology, Microvida Elisabeth-Tweesteden Hospital, Tilburg), A L M Vlek (Department of Medical Microbiology and Immunology, Diakonessenhuis, Utrecht), M de Graaf (Department of Medical Microbiology, Saltro Diagnostic Centre, Utrecht), A Troelstra (Department of Medical Microbiology, University Medical Center Utrecht, Utrecht), K B Gast (Department of Medical Microbiology, Eurofins-PAMM, Veldhoven), M P A van Meer (Laboratory for Medical Microbiology and Immunology, Rijnstate Hospital, Velp), J de Vries (Department of Medical Microbiology, VieCuri Medical Center, Venlo), S B Debast (Laboratory of Medical Microbiology and Infectious Diseases, Isala Hospital, Zwolle), and W Altorf van der Kuil, S M Bierman, S C de Greeff, S R Groenendijk, R Hertroys, L Kruithof, I M Nauta, D W Notermans, J Polman, W J van den Reek, A F Schoffelen, F Velthuis, C C H Wielders, B J de Wit, and R E Zoetigheid (Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Veldhoven).

Contributors

LBZ and STT conducted the data analysis and drafted the manuscript. JGCvH conceptualised the study design. SHSW and AFS facilitated data collection with assistance from the Infectious Diseases Surveillance Information System–Antimicrobial Resistance (ISIS-AR) study group. Data collection from the ATLAS and US National Institutes of Health databases was done by LBZ and STT. LBZ and STT verified the combined dataset. LBSA, AL, WKS, and JGCvH provided oversight and contributed to the manuscript writing. All authors had full access to all the data in the study, participated in the reviewing process of the final manuscript, and accepted responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The collateral effect network analysed in this study will be available for further exploration and analysis at collateralviz.lacdr.leidenuniv.nl from July 9, 2025.

Acknowledgments
This study received funding from the Longfonds foundation grant (11.1.20.028). The Dutch national antimicrobial resistance surveillance system (ISIS-AR) is supported by the Dutch Ministry of Health, Welfare and Sport. The authors express their gratitude to the ISIS-AR study group for their contribution to the collection of the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu) dataset. Additionally, large language model (LLM)-assisted tools (Grammarly and GPT-4o) were used to improve the readability of the manuscript. Authors verified the proposed changes made by the aforementioned LLM tools and take full responsibility for the final content.

References

1 Oliphant CM, Eroschenko K. Antibiotic resistance, part 2: Gram-negative pathogens. *J Nurse Pract* 2015; **11:** 79–86.
2 Roemhild R, Andersson DI. Mechanisms and therapeutic potential of collateral sensitivity to antibiotics. *PLoS Pathog* 2021; **17:** e1009172.
3 Fernandes P, Ferreira BS, Cabral JMS. Solvent tolerance in bacteria: role of efflux pumps and cross-resistance with antibiotics. *Int J Antimicrob Agents* 2003; **22:** 211–16.
4 Lázár V. Bacterial evolution of antibiotic hypersensitivity. *Mol Syst Biol* 2013; **9:** 700.
5 Yekani M. Collateral sensitivity: an evolutionary trade-off between antibiotic resistance mechanisms, attractive for dealing with drug-resistance crisis. *Health Sci Rep* 2023; **6:** e1418.
6 Sakenova N. Systematic mapping of antibiotic cross-resistance and collateral sensitivity with chemical genetics. *Nat Microbiol* 2025; **10:** 202–16.
7 Barbosa C, Beardmore R, Schulenburg H, Jansen G. Antibiotic combination efficacy (ACE) networks for a Pseudomonas aeruginosa model. *PLOS Biol* 2018; **16:** e2004356.
8 Aulin LBS, Liakopoulos A, van der Graaf PH, Rozen DE, van Hasselt JGC. Design principles of collateral sensitivity-based dosing strategies. *Nat Commun* 2021; **12:** 5691.
9 Beckley AM, Wright ES. Identification of antibiotic pairs that evade concurrent resistance via a retrospective analysis of antimicrobial susceptibility test results. *Lancet Microbe* 2021; **2:** e545–54.
10 Zwep LB. Identification of antibiotic collateral sensitivity and resistance interactions in population surveillance data. *JAC Antimicrob Resist* 2021; **3:** dlab175.
11 Mahmud HA, Wakeman CA. Navigating collateral sensitivity: insights into the mechanisms and applications of antibiotic resistance trade-offs. *Front Microbiol* 2024; **15:** 1478789.
12 Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. *Expert Rev Anti Infect Ther* 2013; **11:** 297–308.
13 Ruekit S. Molecular characterization of multidrug-resistant ESKAPEE pathogens from clinical samples in Chonburi, Thailand (2017–2018). *BMC Infect Dis* 2022; **22:** 695.
14 Sayers EW. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 2021; **49:** D10–17.
15 Altorf-van der Kuil W. National laboratory-based surveillance system for antimicrobial resistance: a successful tool to support the control of antimicrobial resistance in the Netherlands. *Euro Surveill* 2017; **22:** 17–62.
16 Catalán P. Seeking patterns of antibiotic resistance in ATLAS, an open, raw MIC database with patient metadata. *Nat Commun* 2022; **13:** 2917.
17 Berends MS. AMR: an R package for working with antimicrobial resistance data. *J Stat Softw* 2022; **104:** 1–31.
18 Nichol D. Antibiotic collateral sensitivity is contingent on the repeatability of evolution. *Nat Commun* 2019; **10:** 334.
19 Hernando-Amado S, Sanz-García F, Martínez JL. Rapid and robust evolution of collateral sensitivity in *Pseudomonas aeruginosa* antibiotic-resistant mutants. *Sci Adv* 2020; **6:** eaba5493.
20 Obolski U, Dellus-Gur E, Stein GY, Hadany L. Antibiotic cross-resistance in the lab and resistance co-occurrence in the clinic: discrepancies and implications in *E coli*. *Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis* 2016; **40:** 155–61.
21 Allen RC, Pfrunder-Cardozo KR, Hall AR. Collateral sensitivity interactions between antibiotics depend on local abiotic conditions. *mSystems* 2021; **6:** e0105521.
22 Imamovic L. Drug-driven phenotypic convergence supports rational treatment strategies of chronic infections. *Cell* 2018; **172:** 121–34.
23 Liakopoulos A. Allele-specific collateral and fitness effects determine the dynamics of fluoroquinolone resistance evolution. *Proc Natl Acad Sci USA* 2022; **119:** e2121768119.
24 García-Quintanilla M. Lipopolysaccharide loss produces partial colistin dependence and collateral sensitivity to azithromycin, rifampicin and vancomycin in *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2015; **46:** 696–702.
25 Wang X. Collateral sensitivity and cross-resistance in six species of bacteria exposed to six classes of antibiotics. *Microbiol Spectr* 2025; **1:** e0098325.
26 Graf FE. Molecular mechanisms of re-emerging chloramphenicol susceptibility in extended-spectrum beta-lactamase-producing Enterobacterales. *Nat Commun* 2024; **15:** 9019.
27 Pál C, Papp B, Lázár V. Collateral sensitivity of antibiotic-resistant microbes. *Trends Microbiol* 2015; **23:** 401–07.
28 Rosenkilde CEH. Collateral sensitivity constrains resistance evolution of the CTX-M-15 β-lactamase. *Nat Commun* 2019; **10:** 618.