Statistical learning for complex data to enable precision medicine strategies

Zwep, L.B.

Chapter 6

Inference of collateral sensitivity effects in large scale antimicrobial resistance surveillance data

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Abstract

Collateral sensitivity (CS) occurs when bacteria develop resistance to one antibiotic leading to an increase in antibiotic sensitivity towards a second antibiotic. The phenomenon of CS has been proposed to be of interest for design of antibiotic treatment strategies to prevent emergence of resistance. Limited knowledge is available concerning the occurrence of CS across species and strains. Here, we aimed to comprehensively characterize the occurrence of CS in large scale population surveillance data of antimicrobial resistance for multiple species, strains, and antibiotics.

We combined multiple databases with minimal inhibitory concentration (MIC) values for >2.7 million MIC measurements for 20 bacterial species and 94 antibiotics. We applied a novel methodology to infer CS effect size. The similarity of CS effects between species and patterns over different antibiotic classes and for higher order collateral effects were explored.

A large set of CS effects was identified, with CS effects identified in the majority of species studied. We find that most antibiotic-specific CS effects are species-specific. CS effects were more commonly observed between different antibiotic classes, than within. Finally we find that specific combinations of resistance towards to antibiotics can be associated with specific CS effect towards a third antibiotic. All identified CS relationships have been incorporated in a web-application to allow in-depth exploration of our findings.

Our systematic analysis highlights the occurrence of CS across a large panel of strains. These results can help prioritize the selection of CS-based antibiotic treatment strategies for further experimental and clinical studies investigating antibiotic combination strategies to suppress resistance.

6.1 Introduction

Antimicrobial resistance (AMR) is an increasing global health threat, rendering commonly used antibiotics increasingly infective to treat bacterial infections. To this end, strategies which can reduce the risk for emergence of AMR are urgently needed. The occurrence of collateral sensitivity (CS) of AMR has been proposed as one strategy to support this goal (Maltas & Wood, 2019; Pál et al., 2015). CS occurs when bacteria develop resistance to one antibiotic which leads as a collateral effect to increased antibiotic sensitivity towards a second antibiotic. The phenomenon of CS may be used to design combinatory antibiotics strategies which can reduce the risk of developing AMR during treatment (Baym et al., 2016).

The potential clinical application of CS-based treatments is currently limited because of insufficient knowledge regarding its occurrence across bacterial species and strains. CS has so far mainly been studied in well controlled in vitro experiments, mainly conducted using a limited selection of bacterial species, strains, and antibiotics (Imamovic et al., 2018; Lázár et al., 2018; Liakopoulos et al., 2022). The majority of experimental studies have focused on a limited number of laboratory strains. Little

is known to what extent CS effects for specific antibiotics or their classes may be conserved or vary between different bacterial species but also how CS effects may be conserved across strains for specific species, and which of these CS are reciprocal. In addition, so far, studies have focused on situations where only the collateral effects for a single type of resistance. In clinical reality however, strains may develop resistance against multiple antibiotics. It is unclear if such higher-order resistance patterns affect the observed collateral responses towards other antibiotics.

Population surveillance of antibiotic susceptibility and resistance in clinically isolated pathogens represents a crucially important tool to monitor AMR in health care across countries, and such data is increasingly available for research purpose (van der Kuil et al., 2017; Johnson, 2015) Typically, such surveillance data consists of minimal inhibitory concentrations (MICs) for different antibiotics. Recently, we developed a method to infer CS effect sizes from individual-level MIC data, and is very suitable to profile for CS effects in MIC-based antimicrobial population surveillance data (Zwep et al., 2021).

In this study we apply our method to infer CS from large scale MIC datasets obtained through population surveillance and other public resources to provide a comprehensive overview of the occurrence of CS affects for clinically used antibiotics and commonly occurring bacterial strains and species for pairwise as well as higher-order CS effects.

6.2 Methods

6.2.1 Data resources

Individual-level MIC data for bacterial pathogens were acquired from four large resources: (i) publicly available MIC data deposited in National Center for Biotechnology Information (NCBI) (Sayers et al., 2020) and (ii) the Pathosystems Resource Integration Center (PATRIC) (Davis et al., 2019), (iii) proprietary MIC data from ARESdb data provided by the company OpGen (OpGen, 2022), and (iv) population surveillance data from the Dutch National Institute for Public Health and the Environment (RIVM) (van der Kuil et al., 2017). All four sources included minimal inhibitory concentrations (MICs) for different bacterial strains, over a wide range of antibiotics and antimicrobial resistance profiles.

6.2.2 Data preparation

The NCBI and PATRIC resources contained overlapping data and were merged to prevent duplicate inclusion of strains. Antibiotics with less than 100 observations for RIVM and NIH & PATRIC were removed, as well as strains with MICs for less than two antibiotics. The ARESdb data were complete, so no strains or antibiotics were removed. The data from the four sources were merged into a single data set, which was used in further analysis.

6.2.3 Collateral effect quantification and testing

All pairs of two antibiotics (antibiotic pairs) were tested for collateral effects using a previously described method developed by our group which quantifies empirical fold change ($\log_2$ FC) and tests the statistical significance of this outcome (Zwep et al., 2021). This method uses MICs to calculate the collateral effect by dichotomizing the MIC data on one antibiotic (B) to a group with high resistance B_r and a group with low resistance or sensitivity $\neg B_r$, and testing the difference between the means of the MIC distributions of a second antibiotic (A), such that

$$\log_2 \text{ FC}_{A|B_r} = \overline{\log_2 \ (\text{MIC}_{A|B_r})} - \overline{\log_2 \ (\text{MIC}_{A|\neg B_r})}$$

where B is the dichotomizing antibiotic and A is the antibiotic of which the MIC is evaluated. The bar denotes the sample mean. The following hypotheses were tested:

$$H_0 : \mu_{A|B_r} = \mu_{A|\neg B_r}$$

$$H_{CS} : \mu_{A|B_r} < \mu_{A|\neg B_r}$$

$$H_{CR} : \mu_{A|B_r} > \mu_{A|\neg B_r}$$

where $\mu_{A|B_r} = \overline{\log_2 \ (\text{MIC}_{A|B_r})}$.

All combinations of antibiotics were tested in two directions, i.e., the effect of an antibiotic A on the antibiotic B and the effect of antibiotic B on antibiotic A. The dichotomization based on the first antibiotic was done so both groups were as close to equal sizes, by choosing the median as dichotomization criterion.

6.2.4 Multiple testing correction

After quantification and testing the collateral effects, the p-values were adjusted for multiple testing using the Benjamini-Yekutieli correction for false discovery rate (Benjamini & Yekutieli, 2001). In the context of collateral sensitivity, an important factor to take into account is the effect size. A small significant collateral effect, might be clinically irrelevant, so clinical relevance was also taken into account when deciding on which collateral effects are of interest. Clinical relevance was defined here by two factors: the size of the estimated fold change and the equality of the group sizes. Small effect sizes ($-0.5 < \log 2\text{FC} < 0.5$) were excluded and effects were only considered relevant if both groups consisted of at least 5% of the total number of strains. Lastly, only antibiotic pairs with more than 100 complete pairwise observations were evaluated (Figure 6.1).

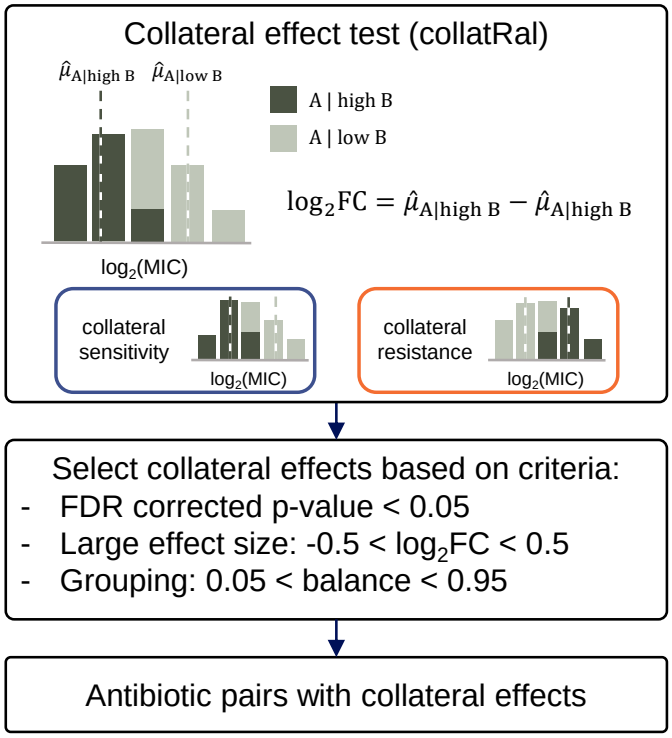

Figure 6.1: Data analysis workflow. First, a collateral effect test was used for each pair of antibiotics (antibiotic A and antibiotic B), within each species. The difference between the means of the $\log_2$-transformed MIC distributions is the estimated $\log_2$ FC. Next, results were filtered based on three criteria, for statistical significance and clinical relevance. Finally resulting in a set of antibiotic pairs with collateral effect.

6.2.5 Collateral effect networks

To evaluate all found collateral effects, a shiny dashboard was developed to explore subsets of species and antibiotics with network mapping of all collateral effect. The antibiotics were shown as nodes and the antibiotic effects as arrows, indicating the directionality, with options to adjust the thresholds for the clinical relevance: the minimal absolute effect size and the minimal balance.

6.2.6 Similarity between species and antibiotics

To evaluate the similarity in CS effects between the different species, the Euclidean distances between the CS responses were mapped using a proximity mapping program (Heiser et al., 2020). The Euclidean distances were calculated between each pair of species, based on the collateral effect size. Only the antibiotic pairs where at least one of the two species showed a negative collateral effect were considered, to remove the influence of collateral resistance effects. Proximity mapping was used to project the distances in two dimensions. We also explored which CS effects were

prevalent over multiple species.

We evaluated if collateral effects between certain antibiotic classes were more prevalent, by comparing CS between antibiotic pairs with different antibiotic classes. Antibiotics within the same antibiotic class were expected to appear less often, than between different antibiotic classes. This was evaluated both over all species and within each species.

6.2.7 Three-way CS interactions

To assess more complex interactions, we analyzed a three-way collateral effects. The strains were dichotomized based on a group with resistance against two different antibiotics, a dual resistance, and a group with sensitivity to at least one of the antibiotics. For each set of three antibiotics (A, B and C), the MICs of antibiotic A were compared between the group with resistance against both antibiotic B and antibiotic C (B_r&C_r) and the group without dual resistance ($\neg(B_r\&C_r)$). The collateral effects were calculated in the same way as for the pairwise collateral effects, by comparing the mean $\log_2$ (MIC between the two groups and testing the hypotheses

$$H_0 : \mu_{A|B_r\&C_r} = \mu_{A|\neg(B_r\&C_r)})$$

$$H_{CS} : \mu_{A|B_r\&C_r} < \mu_{A|\neg(B_r\&C_r)})$$

We compared the results of these three-way collateral effects $\log_2$ $\text{FC}_{A|B_r\&C_r}$ to the two corresponding pairwise collateral effects ($\log_2$ $\text{FC}_{A|B_r}$ and $\log_2$ $\text{FC}_{A|C_r}$) to assess the interaction effect of B&C on A.

6.3 Results

6.3.1 Database characteristics

A pooled dataset of individual MIC data derived from multiple databases consisting of up to 2.7 million measurements for 20 bacterial species and 94 antibiotics, was used for the final analysis (Figure 6.2A. A total of 2066 unique antibiotic pairs was tested, where some were tested in multiple species and almost all were tested in two directions. The species with the largest number of strains (n) and antibiotics (d) are *E. coli* ($n = 2,740,266$, $d = 63$), *K. pneumoniae* ($n = 435,987$, $d = 54$), P. aeruginosa ($n = 403,095$, $d = 42$) and S. aureus ($n = 934,659$, $d = 49$). Not all strains contain measurements for every antibiotic. Within a species, each antibiotic contained a discreet distribution of MICs, such as for example for *C. coli* (Figure 6.2B). All used abbreviations for antibiotics can be found in Table S6.1.

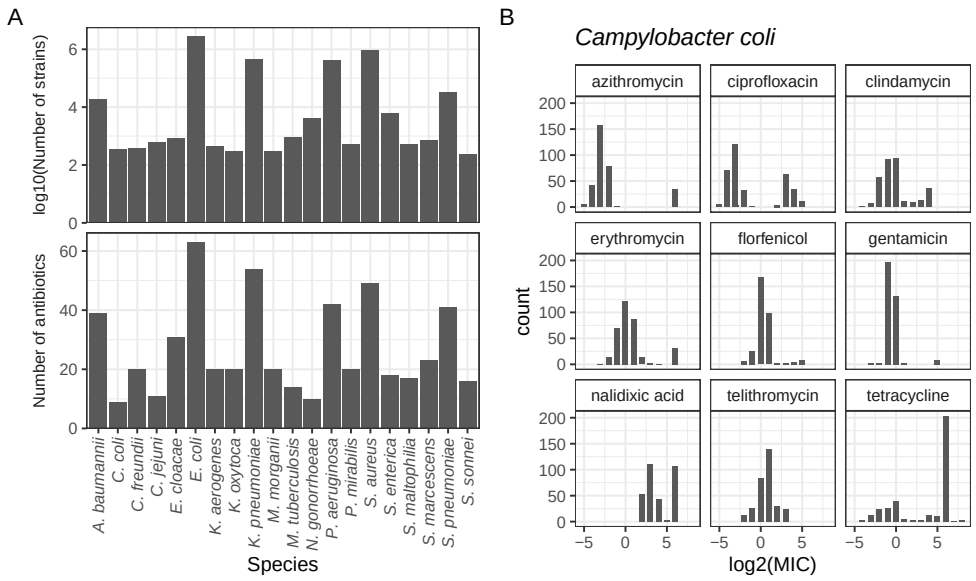

Figure 6.2: Description of the MIC data used for the estimation of collateral effects. A) The number of strains per species (top) and the number of unique antibiotics per species (bottom). B) Example of the MIC data as log transformed MIC distributions for the 9 different antibiotics available for *Campylobacter coli*.

6.3.2 Collateral sensitivity quantification

After testing all combinations, a total of 385 antibiotic pairs with collateral sensitivity effects was detected over the different species. Of these, 120 showed reciprocal CS (Figure 6.3). Most of the non-reciprocal effects showed negative effect sizes, but did not meet the threshold of minimal effect size (< 0.5), balance (< 0.1) or statistical significance (q-value < 0.05) (Figure 6.3A). CS was found for 15 out of 20 studied species. No CS effects were not found in *K. oxytoca*, *M. morganii*, *P. mirabilis*, *S. enterica* and *S. maltophilia* (Figure 6.3B). Overall, CS effects were found across all studied antibiotic classes, and for the majority of antibiotics (Figure 6.3C). The collateral effect networks for each antibiotic pair and species can be explored in the developed Shiny dashboard at `collateralviz.lacdr.leidenuniv.nl` (Figure 6.4).

6.3.3 Differences and similarities in CS between species

With a large number of species and antibiotic pairs tested, we compared how similar the CS effects were between the different species across all antibiotic pairs. Because of the large number of collateral effects tested, the differences between the collateral effects were used to map the species in a two-dimensional plain, using proximity mapping (Figure 6.5). Some species were very dissimilar such as *S. pneumonia* and *K. oxycota*, which are at opposite sides of the map.

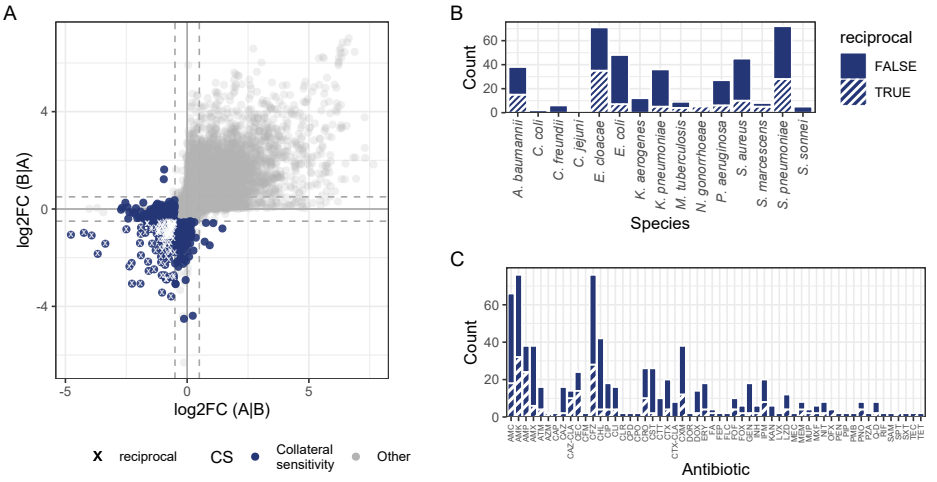

Figure 6.3: Overview of estimated collateral effects and reciprocality across species. A) Estimated effect sizes ($\log_2$ fold change (FC) of the MIC) of reciprocal and non-reciprocal collateral sensitivity responses and other responses, no collateral effect and collateral resistance. Antibiotic A and B represent an antibiotic pair, for which the effect size in both directions is estimated, A given dichotomization on B $(A|B)$ and, B given dichotomization on A $(B|A)$. Dashed lines denote the threshold of relevant effect sizes (abs($\log_2$ FC $= 0.5$). B) Number of collateral sensitivity effects for each species and whether they are reciprocal (striped) or not (blue). C) Number of collateral sensitivity effects for each antibiotic. Note that every collateral sensitivity effect contributes to two antibiotics.

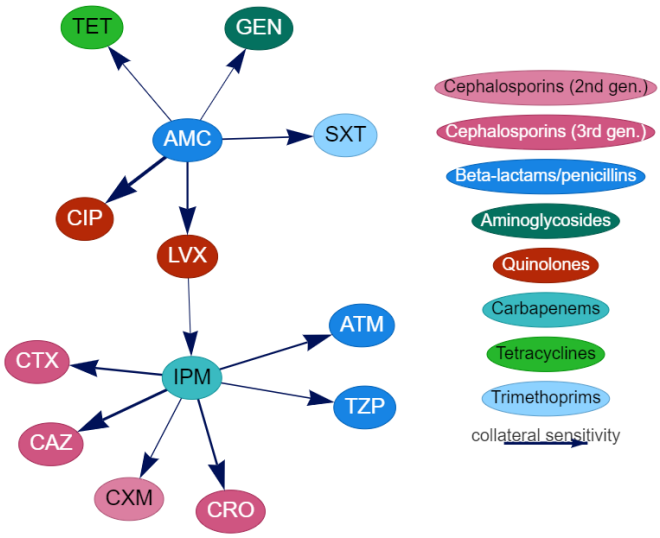

Figure 6.4: Collateral sensitivity network, example for *Klebsiella aerogenes*. Arrows denote the CS effect and direction, colors denote the different antibiotic classes (from Shiny dashboard).

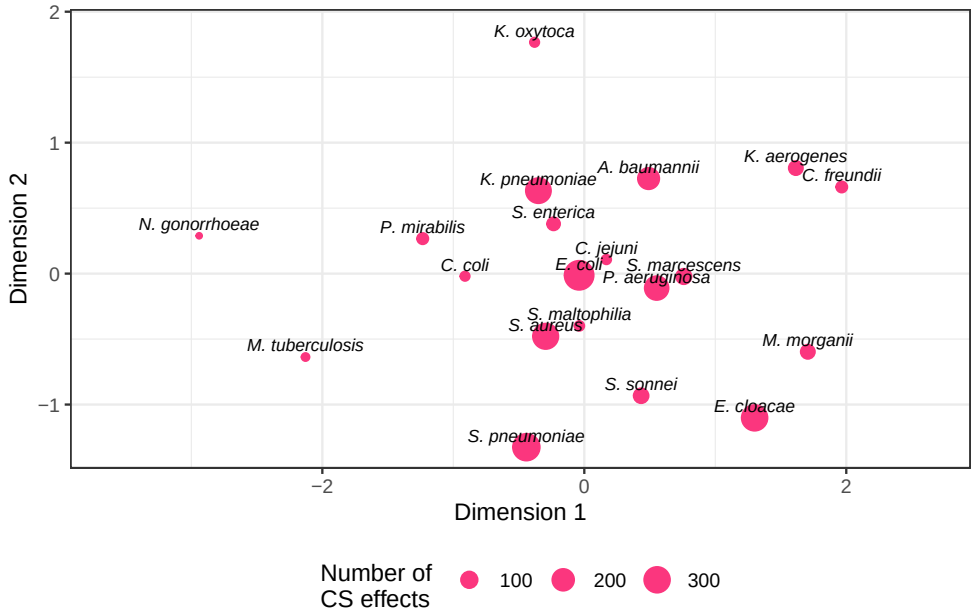

Figure 6.5: Similarity in CS responses between different species. Proximity mapping of different species based on collateral sensitivity effect estimates. Species with similar CS estimates are mapped in close proximity, while species with very different collateral sensitivity are mapped far apart. The size of the nodes shows on how many CS effects the distance was based.

When we focus on similarities in collateral effects at the level of specific antibiotic pairs, we find that certain pairs are more commonly associated with CS effects (Figure 6.6). In total, 22 antibiotic pairs were found for which consistent CS was identified over at least three species. In some pairs we only detected CS (e.g., MEM on LZD), while for most pairs the type of the collateral effect varied depending on the species. Interestingly, all these more consistent CS effects were found between different antibiotic classes.

6.3.4 Antibiotic classes with more CS effects

We explored how CS effects were distributed within- and between antibiotic classes. For the CS responses that were detected in multiple species (Figure 6.6), cephalosporins (CFZ, CXM, CTX and CRO) mostly showed CS with aminoglycosides (AMK, TOB and GEN) and carbapenems (MEM and IMP), and penicillins (AMP, AMX) had more CS with colistin (CST) and the class 'other antibacterials' (SXT, CHL). There were no CS effects consistent over at least three species (Figure 6.6) within the same antibiotic class, although there were CS effects between the different classes of beta-lactams: carbapenems, cephalosporins and penicillins.

Overall, across all species, no distinct patterns of CS were seen (Figure S6.1). When

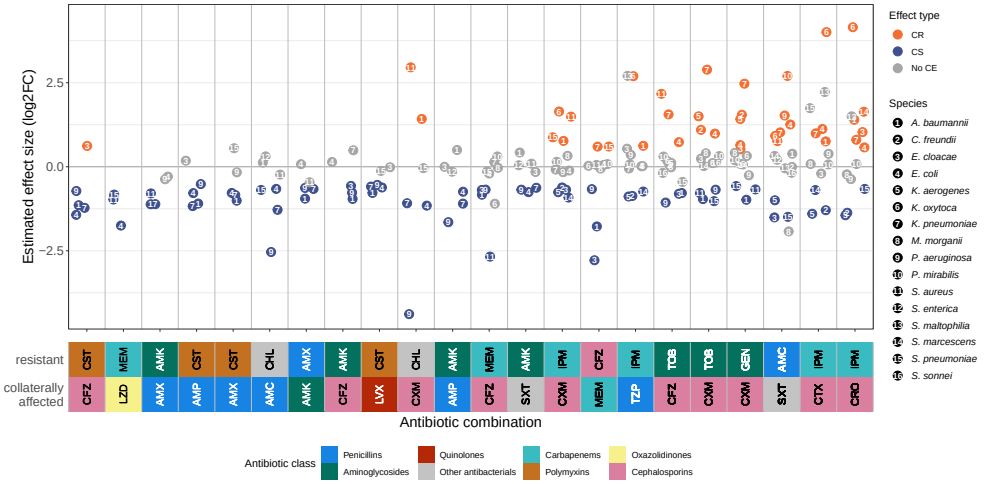

Figure 6.6: Effect sizes of antibiotic pairs with consistent collateral sensitivity (CS) effects over different species. Collateral sensitivity was considered 'consistent' if it was detected in at least three species. The x-axis label colors denote the different antibiotic classes between which these consistent CS effects were detected.

looking at differences between and within antibiotic classes, as expected, CS within the same class was either not found at all (quinolones, tetracyclines, aminoglycosides, glycopeptides) or found in a lower rate than between-class CS (penicillins, macrolides) (Figure 6.7, Figure S6.2). For cephalosporins the proportion of CS between- and within-classes was similar. Specific inspection of this class indicates that such within-class CS occurred mostly between different generations of cephalosporin antibiotics.

Within each species, different patterns of CS could be seen. For example in *S. pneumonia*, most CS effects are from the cephalosporins to other classes, such as the quinolones (Figure 6.8). There are also many CS effects between penicillins and other antibiotic classes.

6.3.5 Three-way interaction CS effects

To evaluate the effect of three-way interaction effects of antibiotics B&C on antibiotic A were estimated. In general, a large number of such CS effects was found, but the proportion of CS effects found did not differ between the antibiotic pairs and the sets of three antibiotics, with dual resistances (Figure S6.3). As expected, we did find an increased proportion of collateral resistance effects for most species, associated with the occurrence of multidrug resistance. Some combinations of dual resistances led to identification of additional antibiotics that show CS compared to the CS associated antibiotics conditioned on only single-antibiotic resistance. Some dual resistances (B&C) led to CS for at least 50% of the tested antibiotics (A) (Figure 6.9). Of these dual resistances, especially the antibiotic pairs gentamycin and imipenem (GEN & IMP) and ampicillin and imipenem (AMP & IMP) were interesting. For *K. aerogenes*,

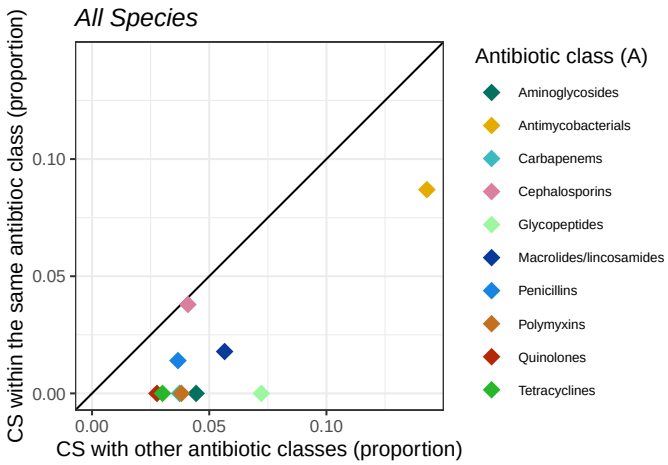

Figure 6.7: Collateral sensitivity (CS) between antibiotic classes. Over all species, the proportion of CS effects detected within and between antibiotic classes. On the x-axis the proportion of detected CS effects with other antibiotic classes. On the y-axis the proportion of detected CS effects within the same antibiotic class. The line denotes no difference between CS detected within and between antibiotic classes. All antibiotic classes in the lower right triangle more often show CS with other classes than within their own antibiotic class.

these dual resistances led to collateral sensitivity effects that were larger than the effects of the individual antibiotics on the third antibiotic (A). The difference between the two-way CS effects and the three-way effects indicate whether there is an interaction effect of the two conditioning antibiotics on the tested antibiotic.

6.4 Discussion

In this study we explored collateral sensitivity (CS) effects in clinical strains and quantified these effects in a wide variety of antibiotics and bacterial species. The combined data from the four databases used enabled comprehensive inference of expected CS effects, identifying a large number of reciprocal and unidirectional CS effects across species and antibiotics.

The databases used for this research contain a large set of different strains and MIC measurement techniques. The strains in ARESdb were very homogeneously measured and contains only clinical strains and the database is more specifically focused on strains with AMR profiles. PATRIC and NIH contained both laboratory and clinical strains and multiple techniques, such as the E-test and a disk diffusion assay. These techniques can yield different results, but due to the large numbers of strains, and since the MIC techniques were the same within a strain, this is expected to be averaged out. The RIVM database only contains Dutch samples, which can skew the

analysis to more commonly detected AMR and CS in the Netherlands.

From the detected collateral effects, some combinations were also found in experimental studies, such as amikacin (AMK) and ampicillin (AMP) in *E. coli* (Imamovic & Sommer, 2013) and small CS effect between ciprofloxacin (CIP) and gentamicin (GEN) in *S. pneumoniae* (Liakopoulos et al., 2022), but other findings from experimental studies were not detected in our screening, for example the CS between nitrofurantoin (NIT) and tigecycline (TGC) (Roemhild et al., 2020). It is not strange to find different results between experimental studies and this large clinical screening, since results from lab strains and environments are not directly translatable to the clinical setting.

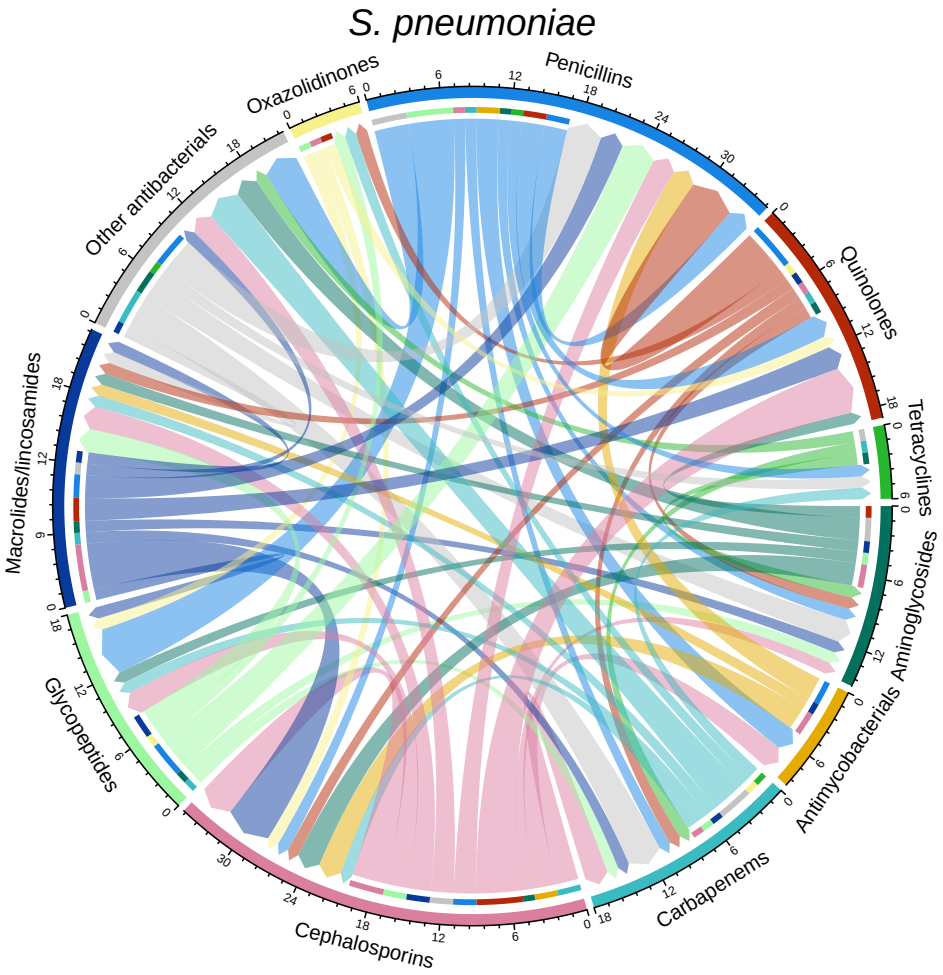

Figure 6.8: Number of CS effects between different classes of antibiotics. The arrows point towards the antibiotic classes where antibiotic sensitivity was increased.

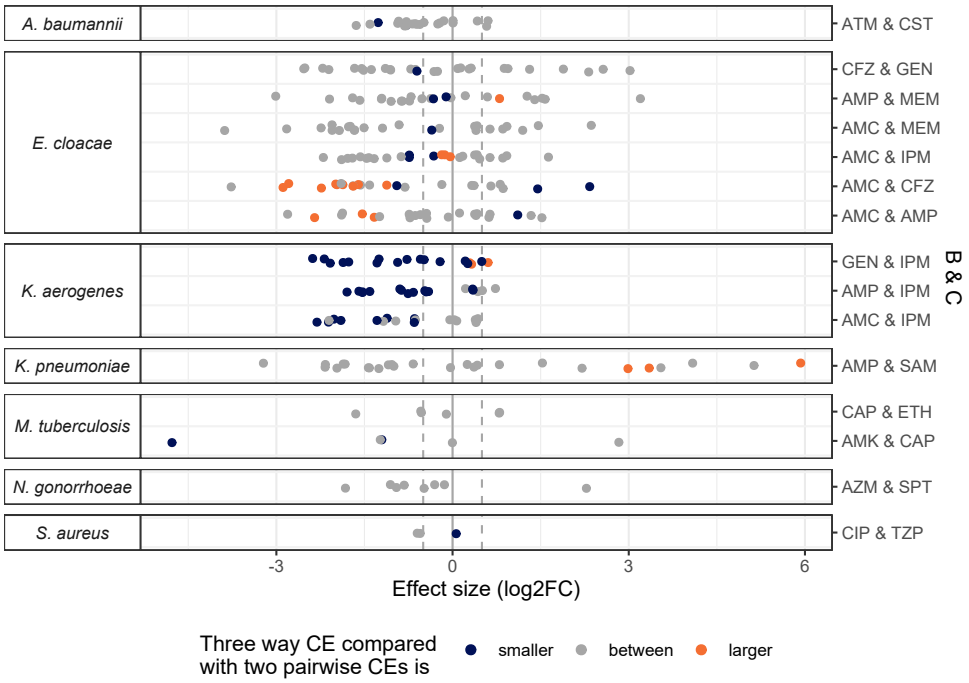

Figure 6.9: Collateral effects for the 15 three-way interactions with most collateral sensitivity responses, where resistance to a pair of antibiotic (B&C) increases the sensitivity to a third antibiotic (A). The color shows whether the three-way effect is smaller than ($A|B\&C < A|B$ & $A|B\&C < A|C$, blue), in between (grey) or larger ($A|B\&C > A|B$ & $A|B\&C > A|C$, orange) than the individual two-way collateral effects. With a smaller effect (blue) indicating an interaction between two antibiotics that makes the third antibiotic even more sensitive, such as for GEN & IMP and AMP & IMP in *K. aerogenes*.

Although many collateral sensitivity effects were found in most species, only few of them were found to be consistent over multiple species, showing the large diversity between species and indicating a need for species specific research for CS effects. We did show more similarity between certain species, such as *S. maltophilia* and *S. aureus*, which may allow for translation of CS effects between these species. Further apart from most species, as maybe expected, was for example *M. tuberculosis*, which is a less typical species, physiologically different from most other studied pathogens (Gagneux et al., 2006).

Most CS effects were detected between different antibiotic classes, however, no apparent patterns could be distinguished on which antibiotic classes more often show CS between each other, which is expected due to their different mechanisms of action (Lázár et al., 2013). There were, however, multiple CS responses found between different types of beta-lactams, but this has been shown before to occur also in experimental setting (Rosenkilde et al., 2019). Among the more consistent CS effects, a few trends could be spotted, like more CS between cephalosporins, aminoglycosides and carbapenems, and penicillins showed CS with colistin (CST) and trimetho-

prim/sulfamethoxazole and chloramphenicol, however these were not more prevalent over all detected CS effects. Especially aminoglycosides have been previously been identified as CS inducing antibiotics (Lázár et al., 2013).

Many CS effects showed no reciprocality, meaning there was only an effect of one antibiotic on another and not of the other on the one. This is also what is often found in experimental research (Imamovic & Sommer, 2013). Non-reciprocal CS effect have been shown not to necessarily hamper the usefulness of drug cycling therapies (Aulin et al., 2021). This indicates the relevance of discovering not only reciprocal, but also non-reciprocal CS effects. One study recently studied observational collateral effects in a large database with clinical strains, where the authors identified apparent CS effects in different species, based on a measure of mutual information (Beckley & Wright, 2021). This mutual information criterium can indicate whether there is a negative (CS) or positive (collateral resistance) association found between two antibiotics, but cannot take directionality in account, where the method used in our study, does estimate an effect size for each direction (Zwep et al., 2021).

The statistical method used in this study is not able to detect causal relations between antibiotics, so all found results are associative, rather than causal collateral effects, which also makes it impossible to distinguish between multidrug resistance and collateral resistance (Zwep et al., 2021). Next to this, the collateral effect estimate can depend on the dichotomization criterion chosen, which in this study was chosen to create the most equal group sizes of B_r and $\neg B_r$. Collateral sensitivity seems prevalent in clinical strains, but in order to translate these findings to the clinic, experimental validation is needed, both to validate these findings and to better understand the mechanisms behind CS responses. A better understanding of the mechanisms can facilitate discovery of CS responses in antibiotic pairs that are not researched. One way of gaining a better mechanistic understanding is by studying whole genome sequences of bacterial strains to discover genetic differences between resistant and sensitive strains to find the genes that might be involved in collateral effects (Roemhild et al., 2020).

To explore more complex interactions, we explored three-way interactions between antibiotics, based on whether a group with resistance to two antibiotics had a lower resistance to a third antibiotic. Due to the discrete nature of the data, not all splits were based on both antibiotics. Interestingly, some combinations of three antibiotics showed collateral sensitivity, where the separate pair-wise combinations did not show such large CS effect or even a collateral resistance effect, such as gentamycin and imipenem (GEN & IMP) and ampicillin and imipenem (AMP & IMP) showed in K. aerogenes. This indicates why focusing on multiple drug interactions can uncover more complex CS responses that might be useful especially in chronic infections, where drug combinations and cycling regimens often include more than two antibiotics. Analyzing the multidimensional collateral effects, however, vastly increases the number of combinations of antibiotics that are tested. With the data that were available in this study, a three-way interaction was feasible, but when going to four- or five-dimensional data, the dimension becomes exponentially larger.

6.5 Conclusion

Our study showed that CS commonly occurs in clinically relevant bacterial pathogenic species, strains and antibiotics, with limited consistency between species and antibiotic classes. Our findings may guide prioritization of CS-based antibiotic treatment strategies that reduce the risk for AMR.

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Supplementary material

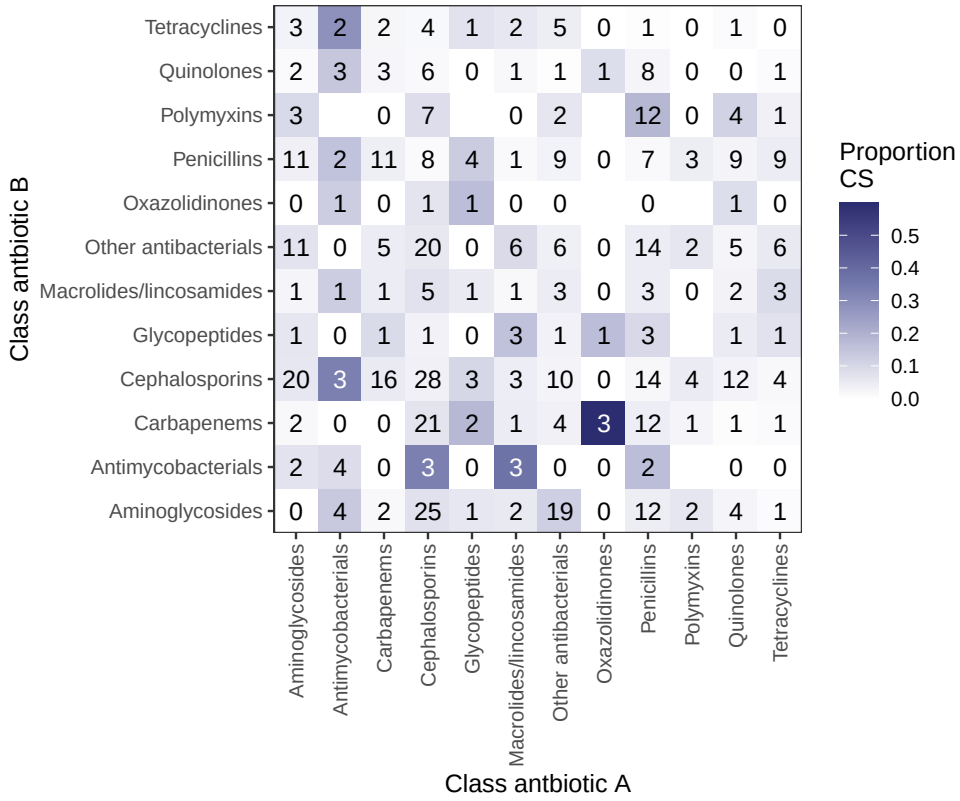

Figure S6.1: The number of CS responses found in at least one species for each combination of antibiotic classes. The color indicates the proportion of CS effects within all tested antibiotic pairs for each class and the numbers show the actual number of CS effects for that combination.

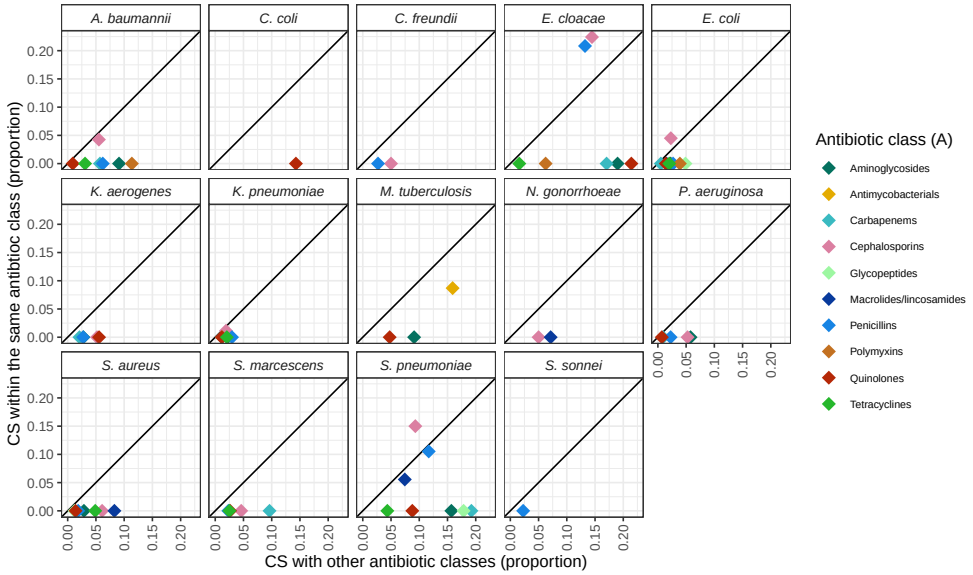

Figure S6.2: Collateral sensitivity (CS) between antibiotic classes. The proportion of CS effects detected within and between antibiotic classes for each species (C. jejuni was excluded, because there were no collateral effects estimated within the antibiotic class). On the x-axis the proportion of detected CS effects with other antibiotic classes. On the y-axis the proportion of detected CS effects within the same antibiotic class. The line denotes no difference between CS detected within and between antibiotic classes. All antibiotic classes in the lower right triangle more often show CS with other classes than within their own antibiotic class.

Table S6.1: All studied antibiotics, abbreviations, and antibiotic classes

Abbreviation	Antibiotic	Antibiotic class
AMA	4-aminosalicylic acid	Antimycobacterials
AMK	amikacin	Aminoglycosides
AMX	amoxicillin	Beta-lactams/penicillins
AMC	amoxicillin/clavulanic acid	Beta-lactams/penicillins
AMP	ampicillin	Beta-lactams/penicillins
SAM	ampicillin/sulbactam	Beta-lactams/penicillins
AZM	azithromycin	Macrolides/lincosamides
ATM	aztreonam	Beta-lactams/penicillins
CAP	capreomycin	Antimycobacterials
CEC	cefaclor	Cephalosporins (2nd gen.)
CFZ	cefazolin	Cephalosporins (1st gen.)
FEP	cefepime	Cephalosporins (4th gen.)
CFM	cefixime	Cephalosporins (3rd gen.)
CTX	cefotaxime	Cephalosporins (3rd gen.)
CTX-CLA	cefotaxime/clavulanic acid	Cephalosporins (3rd gen.)
CTT	cefotetan	Cephalosporins (2nd gen.)
CTF	cefotiam	Cephalosporins (2nd gen.)

FOX	cefoxitin	Cephalosporins (2nd gen.)
CPO	cefpirome	Cephalosporins (4th gen.)
CPD	cefpodoxime	Cephalosporins (3rd gen.)
CPX	cefpodoxime proxetil	Cephalosporins (3rd gen.)
CPT	ceftaroline	Cephalosporins (5th gen.)
CAZ	ceftazidime	Cephalosporins (3rd gen.)
CAZ-CLA	ceftazidime/clavulanic acid	Cephalosporins (3rd gen.)
CTF	ceftiofur	Cephalosporins (3rd gen.)
CEI	ceftolozane/enzyme inhibitor	Cephalosporins (5th gen.)
CRO	ceftriaxone	Cephalosporins (3rd gen.)
CXM	cefuroxime	Cephalosporins (2nd gen.)
CXA	cefuroxime axetil	Cephalosporins (2nd gen.)
LEX	cephalexin	Cephalosporins (1st gen.)
CEF	cephalothin	Cephalosporins (1st gen.)
CHL	chloramphenicol	Amphenicols
CIP	ciprofloxacin	Quinolones
CLR	clarithromycin	Macrolides/lincosamides
CLI	clindamycin	Macrolides/lincosamides
CST	colistin	Polymyxins
CYC	cycloserine	Oxazolidinones
DAP	daptomycin	Other antibacterials
DOR	doripenem	Carbapenems
DOX	doxycycline	Tetracyclines
ETP	ertapenem	Carbapenems
ERY	erythromycin	Macrolides/lincosamides
ETH	ethambutol	Antimycobacterials
ETI1	ethionamide	Antimycobacterials
FLR	florfenicol	Other antibacterials
FLC	flucloxacillin	Beta-lactams/penicillins
FOF	fosfomycin	Other antibacterials
FRM	framycetin	Aminoglycosides
FA	fusidic acid	Other antibacterials
GAT	gatifloxacin	Quinolones
GEN	gentamicin	Aminoglycosides
IPM	imipenem	Carbapenems
INH	isoniazid	Antimycobacterials
KAN	kanamycin	Aminoglycosides
LVX	levofloxacin	Quinolones
LZD	linezolid	Oxazolidinones
MEC	mecillinam (amdinocillin)	Beta-lactams/penicillins
MEM	meropenem	Carbapenems
MIN	minocycline	Tetracyclines
MXF	moxifloxacin	Quinolones
MUP	mupirocin	Other antibacterials
NAL	nalidixic acid	Quinolones
NET	netilmicin	Aminoglycosides
NIT	nitrofurantoin	Other antibacterials
NOR	norfloxacin	Quinolones
OFX	ofloxacin	Quinolones
OXA	oxacillin	Beta-lactams/penicillins
BPE	benzylpenicillin	Beta-lactams/penicillins
PNO	penicillin/novobiocin	Beta-lactams/penicillins

PIP	piperacillin	Beta-lactams/penicillins
TZP	piperacillin/tazobactam	Beta-lactams/penicillins
PMB	polymyxin b	Polymyxins
PRI	pristinamycin	Macrolides/lincosamides
PZA	pyrazinamide	Antimycobacterials
Q-D	quinupristin/dalfopristin	Macrolides/lincosamides
RFB	rifabutin	Antimycobacterials
RIF	rifampicin	Antimycobacterials
SPX	sparfloxacin	Quinolones
SPT	spectinomycin	Other antibacterials
STR	streptoduocin	Aminoglycosides
STR	streptomycin	Aminoglycosides
SMX	sulfamethoxazole	Trimethoprims
SXZ	sulfisoxazole	Other antibacterials
TEC	teicoplanin	Glycopeptides
TEL	telithromycin	Macrolides/lincosamides
TMC	temocillin	Beta-lactams/penicillins
TET	tetracycline	Tetracyclines
TIC	ticarcillin	Beta-lactams/penicillins
TIM	ticarcillin/clavulanic acid	Beta-lactams/penicillins
TGC	tigecycline	Tetracyclines
TOB	tobramycin	Aminoglycosides
TMP	trimethoprim	Trimethoprims
SXT	trimethoprim/sulfamethoxazole	Trimethoprims
VAN	vancomycin	Glycopeptides
AMA	para-aminosalicylic acid	Antimycobacterials
AMC	amoxicillin/clavulanate	Beta-lactams/penicillins
SAM	amipicillin/sulbactam	Beta-lactams/penicillins
CTX-CLA	cefotaxime/clavulanate	Cephalosporins (3rd gen.)
CAZ-CLA	ceftazidime/clavulanate	Cephalosporins (3rd gen.)
CRO	ceftriazone	Cephalosporins (3rd gen.)
CXM-S	cefuroxime/sodium	Cephalosporins (2nd gen.)
CEF	cefalotin	Cephalosporins (1st gen.)
CEF	cephalotin	Cephalosporins (1st gen.)
ETI1	ethiomide	Antimycobacterials
GEN	gentamycin	Aminoglycosides
PEN	penicillin	Beta-lactams/penicillins
PMB	polymyxin	Polymyxins
PMB	polymyxin B	Polymyxins
PZA	pyrazimide	Antimycobacterials
Q-D	synercid	Macrolides/lincosamides
RIF	rifampin	Antimycobacterials
SXT	sulfamethoxazole/trimethoprim	Trimethoprims
TIM	ticarcillin/clavulanate	Beta-lactams/penicillins

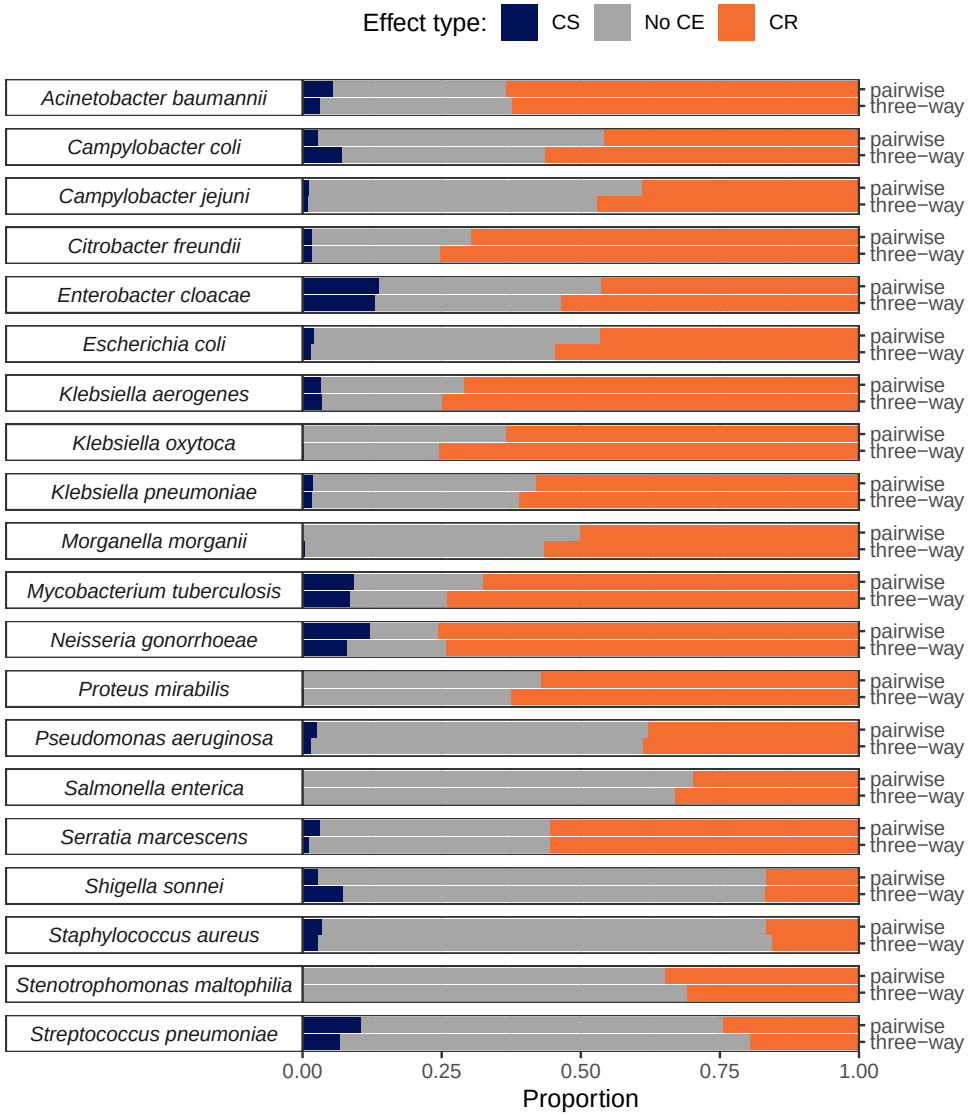

Figure S6.3: The proportion of collateral effects detected in the three-way analysis as compared to the pairwise analysis for each species. Abbreviations: CS; collateral sensitivity, no CE; no collateral effect, CR; collateral resistance