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Heatmap-Based and Regression Analysis of Antibiotic Co-Resistance Networks Among Gram-Positive Bacterial Isolates in a Tertiary Care Hospital

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Abstract

Background

Antimicrobial resistance (AMR) among Gram-positive bacteria is an escalating global health concern, increasingly complicated by the emergence of multidrug resistance (MDR). Traditional resistance surveillance often evaluates antibiotics individually, failing to capture co-resistance patterns that critically influence empirical therapy and clinical outcomes.

Methods

We conducted a retrospective observational study of Gram-positive bacterial isolates recovered from adult patients at a tertiary care hospital in Palestine between January 2021 and May 2025. Non-duplicate clinical isolates with complete antimicrobial susceptibility testing data were included. Resistance profiles were analyzed using pairwise phi-correlation matrices and visualized through hierarchical clustering heatmaps to identify antibiotic co-resistance patterns. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial classes. Univariable and multivariable logistic regression analyses were performed to identify clinical and demographic predictors of MDR.

Results

A total of 2,364 unique Gram-positive isolates were analyzed. Strong positive co-resistance was observed between fluoroquinolones, particularly ciprofloxacin and levofloxacin, as well as between β-lactam agents such as penicillin and amoxicillin/clavulanic acid. Distinct co-resistance patterns were identified across inpatient and outpatient settings, with overall consistency suggesting underlying biological mechanisms. Multivariable analysis demonstrated that inpatient status and specific specimen types were independently associated with increased odds of MDR, while outpatient sampling showed a protective effect.

Conclusion

Heatmap-based co-resistance analysis provides valuable insights beyond conventional single-drug resistance reporting, revealing clinically relevant resistance networks among Gram-positive pathogens. These findings underscore the importance of incorporating co-resistance patterns into antimicrobial stewardship strategies, particularly in resource-limited settings, to optimize empirical treatment and limit the spread of MDR organisms.

Introduction

Antimicrobial resistance (AMR) among Gram-positive bacteria is an escalating global health concern, with resistant pathogens such as *Staphylococcus aureus* and *Enterococcus faecium* listed among the World Health Organization's high-priority threats (1). In 2019 alone, AMR was linked to nearly 5 million deaths globally, with methicillin-resistant *S. aureus* (MRSA) among the leading causes. This burden falls disproportionately on low- and middle-income countries, where gaps in healthcare infrastructure and infection control amplify the impact of resistant infections (2). Once considered a greater problem in Gram-negative organisms, resistance in Gram-positive bacteria now demands equivalent attention and novel strategies for surveillance and intervention (1).

Conventional approaches to AMR surveillance typically assess resistance to individual antibiotics in isolation, summarizing the proportion of non-susceptible isolates per agent. However, this one-drug-at-a-time method overlooks how resistances often co-occur within a single organism (3). For instance, resistance to erythromycin often accompanies clindamycin resistance, and MRSA commonly shows resistance across several β-lactams. This fragmented view fails to capture the multidrug-resistant (MDR) phenotype and may mislead empirical treatment decisions. A more integrated analytical perspective is essential to understand resistance clustering and anticipate therapeutic failures (2).

Co-resistance the concurrent resistance to multiple antibiotic classes in one organism is frequently driven by the co-localization of resistance genes on plasmids or their co-selection under antibiotic pressure. This phenomenon is central to the emergence of MDR pathogens that compromise the effectiveness of routine treatments (4). By identifying which resistances tend to appear together, clinicians can more accurately predict treatment challenges. Recognizing co-resistance patterns also supports antibiotic stewardship by discouraging the use of drugs likely to fail concurrently (1, 4).

To uncover these relationships, statistical and visualization tools such as heatmaps and correlation matrices have proven valuable. These tools allow for the detection of clusters of co-resistant antibiotics by measuring the strength of association between resistance profiles. For example, a strong correlation between resistance to ciprofloxacin and levofloxacin may reflect shared resistance mechanisms or genetic linkages. Heatmaps provide a visual representation of such associations, highlighting co-resistance networks that might be missed in standard reporting. These insights can directly influence clinical decision-making and infection control measures (5).

Beyond microbial genetics, host-related factors also contribute significantly to MDR. Clinical and demographic variables such as prior antibiotic use, recent hospital admission particularly in intensive care units (ICUs) and underlying comorbidities are known to elevate the risk of acquiring MDR infections. For example, patients with prolonged hospital stays or indwelling devices are more likely to harbor or acquire resistant organisms. These contextual factors must be considered when analyzing resistance trends and designing interventions (6).

Despite increasing attention to co-resistance, most available data stem from high-income countries with robust laboratory infrastructure. There remains a critical shortage of co-resistance data from low-resource settings, where the burden of AMR is highest. Limited diagnostic capacity and fragmented

reporting hinder the ability to detect and respond to emerging resistance patterns in these regions, making empirical treatment particularly challenging (7).

To address this knowledge gap, our study analyzed Gram-positive bacterial isolates from a tertiary care center in a resource-limited setting over a four-year period. Using heatmap and correlation analysis, we investigated patterns of co-resistance across a broad panel of antibiotics. We also assessed the association of clinical and demographic factors such as ICU admission, prior antibiotic exposure, and comorbidities with MDR. Our objective was to characterize the local resistance landscape and provide evidence to guide empirical therapy and stewardship efforts in similar resource-constrained environments.

Methodology

Study Design and Setting

This study was conducted as a retrospective observational study at An-Najah National University Hospital (NNUH) in Nablus, Palestine, a tertiary care teaching hospital and regional referral center. Routinely collected electronic data were retrospectively extracted from the hospital's Microbiology Laboratory database over a four-year, from January 2021 to May 2025. The analysis focused on Gram-positive bacterial isolates obtained from clinical specimens to assess antimicrobial resistance and co-resistance patterns.

Study Population and Inclusion and Exclusion Criteria

The study population included all Gram-positive bacterial isolates obtained from all clinical specimens of adult participants (≥ 18 years) processed at the Microbiology Laboratory of NNUH during the study period. Eligible isolates were obtained from various clinical sources, including blood, urine, respiratory specimens, wound swabs, tissue specimens, and other body fluid specimens. Only laboratory records with available antimicrobial susceptibility testing (AST) results for at least one antibiotic and complete demographic and relevant clinical information were considered for inclusion.

Gram-positive organisms eligible for analysis included Staphylococcus aureus (including methicillin-resistant strains), Staphylococcus epidermidis, Enterococcus faecalis, Enterococcus faecium (including vancomycin-resistant strains), and other coagulase-negative staphylococci.

Records were excluded if they involved Gram-negative organisms, Gram-positive species outside the predefined scope of the study, young participants less than 18 years, specimens collected outside the designated study period, records lacking sufficient AST data, polymicrobial cultures, or follow-up cultures obtained from the same infectious episode. To ensure data completeness and analytical validity, isolates with substantial missing data exceeding 10% of required variables were excluded. Furthermore, isolates from patients with documented systemic antibiotic use occurring more than three months prior to

specimen collection were not considered eligible. Duplicate isolates recovered from the same patient were excluded to maintain analytical independence, with only one non-duplicate index isolate retained per participant.

Data Collection and Management

AST was performed routinely at NNUH in accordance with laboratory protocols based on Clinical and Laboratory Standards Institute (CLSI) guidelines. Relevant data were retrospectively retrieved from the hospital's electronic laboratory information system. Extracted variables included patient age and sex, specimen type, ward location at the time of specimen collection, bacterial species identification, and antimicrobial susceptibility results including antibiotics to which the organism was reported as susceptible or resistant. Available clinical information was reviewed to document comorbid conditions, prior antibiotic exposure, exposure to invasive procedures, and immunosuppressant use when accessible through the electronic system. To reduce potential clustering effects and ensure analytical independence, only one non-duplicate isolate per participant was included in the analysis. When multiple eligible specimens were available for the same participant, the isolate with the most complete microbiological and susceptibility data was selected. To preserve confidentiality, all personal identifiers were removed at the time of data extraction, and anonymized datasets were used for analysis. Prior to statistical analysis, the dataset was cleaned and standardized using Microsoft Excel. Antimicrobial susceptibility results were converted into binary variables, with resistant and susceptible isolates coded as 1 and 0, respectively. Antibiotics reported as "not tested" were excluded from analyses involving the corresponding agent. Additional derived variables were generated to identify multidrug resistance, prior antibiotic exposure, and major clinical comorbidities. The finalized anonymized dataset was then prepared for statistical analysis.

Study Variables

Study variables were classified into demographic and clinical variables, microbiological variables, and antibiotic susceptibility variables.

Demographic variables included patient age and sex. Clinical variables encompassed source of infection (urinary, respiratory, bloodstream, or other), infection origin, Comorbid conditions, prior antibiotic exposure, as well as ward location at the time of specimen collection, categorized as outpatient, intensive care unit (ICU) or general ward. Ward location was recorded to account for differences in disease severity, antimicrobial exposure, and drug resistance (8). Clinical characteristics also included specimen type, encompassing all specimen sources submitted to the microbiology laboratory. These included, but were not limited to, blood, urine, sputum, wound swabs, cerebrospinal fluid (CSF), tissue samples, and tracheal aspirates (TRAP). Infection origin was categorized as hospital-acquired or community-acquired. Hospital-acquired infections were defined as infections not present or incubating at the time of admission and diagnosed 48 hours or more after hospitalization (9), whereas community-

acquired infections were defined as infections identified within 48 hours of admission of patients (9). Comorbid conditions were documented as part of the past medical history and included diabetes mellitus, hypertension, chronic kidney disease, and heart failure. Prior antibiotic exposure was defined as receipt of systemic antibiotic therapy for more than 24 hours within the 90 days preceding specimen collection, a period known to influence antimicrobial resistance patterns (10). Exposure to invasive procedures within the 30 days prior to specimen collection was recorded. An invasive procedure was defined as any intervention in which intentional access to the body for the purpose was achieved through an incision or percutaneous puncture, or instrumentation via a natural body orifice (11). The 30-day time frame was selected as healthcare-associated infections are recognized to occur within 30 days following exposure to healthcare interventions, reflecting the period during which prior healthcare contact may significantly influence infection acquisition and antimicrobial resistance patterns (12). Procedures considered included urinary catheterization, central venous catheter use, mechanical ventilation, and other documented invasive interventions. In addition, immunosuppressant exposure was included as a clinical variable and was defined as a class of medications that inhibit or decrease the intensity of the immune response (13). This included documented exposure to immunosuppressive therapies such as systemic corticosteroids, cytotoxic chemotherapy, and transplant-related immunosuppressive agents, as recorded in the electronic medical record.

Microbiological variables included identification of Gram-positive bacterial pathogens isolated from clinical specimens using routine laboratory diagnostic methods.

Antibiotic susceptibility variables were derived from AST results. Susceptibility profiles were recorded for quinupristin/dalfopristin, penicillin, vancomycin, linezolid, erythromycin, tetracycline, amoxicillin/clavulanic acid, gentamicin, ciprofloxacin, levofloxacin, tigecycline, and nitrofurantoin. These variables were used to assess resistance patterns and co-resistance across different antimicrobial classes. Multidrug resistance (MDR) was defined as non-susceptibility to at least one representative agent in three or more antimicrobial classes (14).

Sample Selection and Isolate Inclusion Process

All microbiological cultures processed during the study period were reviewed, from which 4,231 clinical specimens yielding Gram-positive cocci were identified. To ensure analytical independence, repeated isolates recovered from the same patient were excluded (n = 1,466). Further exclusions were applied to specimens with incomplete datasets (n = 238), defined as missing more than 10% of required analytical variables (15). Samples from patients with documented antibiotic exposure occurring more than three months before specimen collection were also excluded (n = 94). Prior exposure to systemic antibiotics has been shown to be associated, at the individual patient level, with the subsequent emergence of antimicrobial resistance in bacterial isolates from different anatomical sites (16). In addition, specimens obtained from young participants less than 18 years of age were excluded (n = 33), owing to well-established differences in pharmacokinetics, antimicrobial dosing, and interpretation of antimicrobial susceptibility breakpoints compared with adults, as most susceptibility standards are derived from adult

dosing assumptions (17). Polymicrobial cultures (n = 19) and follow-up cultures obtained during the same infectious episode (n = 17) were also excluded. Following the application of all inclusion and exclusion criteria, the final study cohort comprised 2,364 unique, non-duplicate index isolates included in the analysis. (**Figure. 1**)

Statistical Analysis

Statistical analysis was performed using R version 4.5.1 (2025-06-13). Quantitative variables were summarized as means with standard deviations, whereas qualitative variables were presented as frequencies and percentages. Descriptive statistics were stratified according to patient location at the time of specimen collection (inpatient versus outpatient), as well as by intensive care unit (ICU) status, specimen type, and prior antibiotic exposure to explore variations in resistance patterns across subgroups.

Antibiotic susceptibility results were transformed into binary variables, with resistant coded as 1 and susceptible as 0. Isolates reported as intermediate were considered resistant for analytical purposes, in line with commonly adopted practices in antimicrobial resistance studies.

Pairwise correlation matrices were constructed to assess co-resistance patterns between antibiotics and were visualized using heatmaps with hierarchical clustering to identify groups of antibiotics sharing similar resistance profiles.

Univariable and multivariable logistic regression models were performed to identify factors associated with MDR, with results expressed as odds ratios (ORs) and 95% confidence intervals (CIs). Variables included in the multivariable model were selected based on clinical relevance and statistical significance.

Data completeness was assessed prior to analysis, and a complete-case approach was applied using listwise deletion. Only records with less than 10% missing data across key variables were retained in the final analysis. All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant.

Sample Size

The study sample size was defined by the total number of unique clinical isolates that met the predefined eligibility criteria after exclusion of duplicates. Owing to the retrospective, database-driven nature of the study, no a priori sample size calculation was performed. Instead, all eligible isolates identified during the study period were included through a complete enumeration (census) approach. This method was adopted to utilize the full available dataset, thereby enhancing the precision of statistical estimates and reducing the potential for selection bias inherent in sampling-based designs.

Results

Demographics and Clinical Characteristics of Patients

Table 1 shows patients demographics and clinical characteristics. The mean age of the cohort was 56 years (SD 19). Females accounted for 56% of the overall cohort. Urine was the most common sample type followed by wound and then blood samples. Prior antibiotic use in the cohort was common with 73% with similar distribution between inpatients and outpatient.

The most frequently identified organism was Enterococcus faecalis with 32% followed by Staphylococcus epidermidis with 17% and other coagulase-negative staphylococci with 16%. Vancomycin-resistant enterococci (VRE) accounted for 9.1% overall and were more frequent among inpatients (11%) than outpatients (5.3%).

Susceptibility Patterns by Pathogen

Table 2 summarizes the resistance profiles of different gram-positive organisms to different antibiotics. There was total resistance to levofloxacin, penicilin, amoxicillin/clavulanic acid, and erythromycin by VRE; however, it was totally susceptible to tigecycline. Marked between-organism differences were observed across tested antibiotics. VRE showed near-complete resistance to levofloxacin (131/216; 99%) and complete resistance to penicillin (215/216; 100%) and amoxicillin/clavulanic acid (216/216; 100%). In contrast, tigecycline susceptibility was preserved across organisms in the displayed results, including 100% susceptibility among VRE (208/208 reported susceptible) and similarly high susceptibility in other organism groups. Erythromycin resistance was high in several organism categories, including E. faecalis, E. faecium, Other CoNS, and Staphylococcus epidermidis. There was an increased resistance to fluoroquinolones (levofloxacin and cirpfloxacin) from E. faecium and Staphylococcus epidermidis.

Figure 2. shows the overall phi-correlation heatmap with clustering. Ciprofloxacin and levofloxacin showed prominent positive association (phi = 0.97) indication the co-occurrence of resistance with these two flouroquinolones. Another strong correlation was between penicillin and amoxicillin/clavulanic acid which both belong to beta-lactam family (phi ≈ 0.71). Strong Negative correlation was observed between penicillin and quinupristin/dalfopristin (phi ≈ − 0.70) and also between quinupristin/dalfopristin and amoxicillin/clavulanic acid (phi ≈ − 0.50).

Figures 3. and **4.** show the separate inpatient and outpatient heatmaps. Both heatmaps showed overall consistency in correlations. The correlations in flouroquinolones and beta lactams where roughly the same with overall heatmap indicating consistency. The penicillin–amoxicillin/clavulanic acid association also remained strong in both groups and in the overall heatmap. Negative associations involving quinupristin/dalfopristin were seen in both heatmaps, including a strong inverse relationship with penicillin in outpatients (phi ≈ − 0.71) and in inpatients (phi ≈ − 0.69). In the outpatient heatmap, penicillin

also showed negative correlations with tetracycline (phi ≈ − 0.45) and weaker negative associations with linezolid (phi ≈ − 0.15).

Figure 5. shows co-resistance heatmap after exclusion of Enterococcus faecalis and Enterococcus faecium. Again, the strongest positive association in the heatmap was between ciprofloxacin and levofloxacin resistance (phi ≈ 0.98). The previously negative associations seen in the overall heatmap between quinupristin/dalfopristin susceptibility and beta-lactams attenuated toward approximately zero (penicillin phi = 0.07; amoxicillin/clavulanic acid phi = 0.16), and moderate positive co-resistance among amoxicillin/clavulanic acid, erythromycin, gentamicin, and fluoroquinolones became more apparent (e.g., amoxicillin/clavulanic acid–ciprofloxacin phi = 0.55; amoxicillin/clavulanic acid–levofloxacin phi = 0.55)

Regression Model Results

In unadjusted analysis, male sex was associated with lower odds of MDR compared with female sex (OR 0.78; 95% CI 0.65–0.93; p = 0.007) with the association remaining statistically significant in the adjusted model (aOR 0.82; 95% CI 0.68–0.98; p = 0.032). Age did not demonstrate a statistically significant association in the unadjusted analysis (OR 1.00; 95% CI 1.00–1.01; p = 0.082) and hence it was not included in the adjusted model.

Sample type demonstrated heterogeneous associations relative to blood cultures. CSF samples were associated with lower odds of MDR in both unadjusted (OR 0.26; 95% CI 0.08–0.78; p = 0.019) and adjusted analyses (aOR 0.23; 95% CI 0.07–0.69; p = 0.010). TRAP samples showed higher odds of MDR compared with blood (unadjusted OR 2.35; 95% CI 1.45–3.95; p < 0.001; adjusted aOR 1.78; 95% CI 1.07–3.06; p = 0.031). Wound samples were associated with higher odds in unadjusted analysis (OR 1.43; 95% CI 1.07–1.90; p = 0.014) and showed a borderline association after adjustment (aOR 1.36; 95% CI 0.98–1.89; p = 0.066).

Time of sample was strongly associated with MDR: outpatients had lower odds of MDR than inpatients in unadjusted analysis (OR 0.59; 95% CI 0.49–0.71; p < 0.001) and this association remained after adjustment (aOR 0.58; 95% CI 0.48–0.71; p < 0.001). Several comorbidities (DM, HTN, CKD) did not show statistically significant associations in the presented unadjusted analyses. Prior antibiotic use within 90 days showed a borderline association in unadjusted analysis (OR 0.83; 95% CI 0.67–1.01; p = 0.068).

Recent invasive-procedure exposure was associated with reduced odds of MDR in unadjusted and adjusted analyses (unadjusted p < 0.001; adjusted p = 0.005), suggesting lower MDR among those with documented exposure in this dataset. Because this direction may be counterintuitive clinically, it is likely to reflect confounding by setting, sampling source, or case-mix and should be interpreted cautiously.

Comorbidities didn’t show any near strong association in the unadjusted model and thus were not included in the adjusted final model.

Discussion

While gram negative bacteria have historically posed significant challenges due to extensive resistance profiles compared to gram positive organisms, there is a continuous rise in antibiotic resistance among gram positive bacteria that made WHO release a list that includes most dangerous bacteria that are resistant to current treatments and requires the development of new antibiotics for combating the resistance (18, 19). The list includes *Methicillin-resistant Staphylococcus aureus (MRSA)*, *vancomycin-resistant Enterococcus faecium (VRE)*, and drug-resistant *Streptococcus pneumoniae* (18). Literature has described multiple mechanisms for gram positive organisms to develop resistance including changing affinity for penicillin binding protein (PBP) to β-lactams or acquiring point mutations that prevents fluoroquinolones (FQs) from binding to their targets resulting in resistance to the whole family of β-lactams or FQs (18, 20). Based on this biological background, this indicates that resistance to a single drug in family of antibiotics means resistance to all or most of antibiotics in the same family resulting in multidrug resistant (MDR) organisms.

In this study, we used heatmap analysis using phi correlation to visualize the different co-resistance patterns. In addition, we used logistic regression model to assess the main predictors of MDR. The main findings of heatmap analysis were the presence of a near perfect correlation (phi = 0.97) between ciprofloxacin and levofloxacin resistance, indicating that resistance to one highly predicts resistance to the other which limits the available antibiotic options for treatment. Different pathogens have demonstrated different mechanisms to resist FQs. For instance, *Staphylococcus aureus (S. aureus)*, especially *MRSA*, acquires point mutations in gyrB and grlA genes which encodes for DNA gyrase and topoisomerase IV enzymes (targeted enzymes by FQs). This results in decreased affinity of the mutated enzymes to the FQs which decreases their efficacy (18, 21). Moreover, FQ resistance is mediated by overexpression of endogenous efflux pumps (norA, noraB or noraC) that removes the drug outside of the cell (21). Similar mechanisms are seen in *Enterococcus faecium* and *Staphylococcus epidermidis* (18, 22). Important observation by Thuc Quyen Huynh et al was that seven *S. aureus* strains developed resistance to other antibiotics including β-lactams after exposure to FQs leading to a multi-drug resistance phenotype (22).

Another finding in the heatmap analysis was a strong positive correlation (phi ≈ 0.71) was observed between penicillin and amoxicillin/clavulanic acid which are both β-lactams. The main mechanism in *S. aureus* was the formation of β-lactamase enzyme that hydrolyses the β-lactam ring, thus making the organism resistant to penicillin but not to amoxicillin/clavulanic acid (20). However, *MRSA* has altered its PBP via acquiring new gene through horizontal gene transfer from another organism. The strong correlation between penicillin and amoxicillin/clavulanic acid is largely driven by the prevalence of the mecA gene in *MRSA*. mecA encodes PBP2a, a penicillin-binding protein with low affinity for beta-lactams, conferring resistance to the entire class regardless of beta-lactamase inhibitor presence making *MRSA* resistant to both penicillin and amoxicillin/clavulanic acid (20). In the case of *Enterococcus faecium*, the resistance to β-lactams was mainly due to PBP5 that has low affinity to β-lactams and bypass the inhibited PBP by shifting into L,D-transpeptidases (Ldts) to facilitate the crosslinking in the peptidoglycan to form the cell wall (23).

The resistance profile of *VRE* isolates (n = 216) predominantly followed the multidrug-resistant phenotype characteristic of *Enterococcus faecium*. In addition to glycopeptide resistance, these isolates exhibited high-level co-resistance to beta-lactams (penicillin/ampicillin) and FQs, reflecting the cumulative pressure of broad-spectrum antibiotic exposure. The main mechanism of VRE resistance to vancomycin is by changing the D-Ala-D-Ala terminus (binding site of vancomycin) into D-Ala-D-lactate terminus so that vancomycin binds with less affinity (24).

An interesting finding in the heatmap analysis was the strong negative correlations between penicillin and quinupristin/dalfopristin (Q-D) (phi ≈ − 0.70), a pattern that remained consistent across both inpatient and outpatient settings. However, this finding is mainly explained by the nature of intrinsic resistance for *Enterococcus faecalis* and *Enterococcus faecium* rather than a biological trade-off. *Enterococcus faecalis* isolates exhibited high intrinsic resistance to Q-D (99%) due to Isa gene which encodes for ABC transporter that acts as efflux pump for Q-D. Isa gene is found only in *Enterococcus faecalis* and absent in all other *enterococcus* species including *Enterococcus faecium* (25). Moreover, *Enterococcus faecalis* is highly susceptible to penicillin (93%) unlike *Enterococcus faecium* that has high intrinsic resistance to penicillin. These opposite profiles are the main cause of strong negative correlation between penicillin and Q-D. Thus, the negative correlation effect between Q-D and penicillin was weak (phi ≈ − 0.007) when both *Enterococcus faecalis* and *Enterococcus faecium* were excluded from the heatmap analysis.

The univariable model showed male sex as a risk factor for MDR. However, when adjusted with other factors in the multivariable model, male sex showed statistically significant protective effect compared to females. The association between female sex and higher MDR risk may reflect higher cumulative antibiotic exposure, potentially driven by the higher incidence of recurrent urinary tract infections in females, which necessitates frequent empiric therapy thus increasing resistance in their commensal flora (26).

Unexpectedly, invasive procedures appeared to be protective against MDR (aOR 0.73) in the multivariable regression model, contradicting reports that link ICU interventions to higher resistance (27). This is likely an artifact of sample distribution. The cohort without invasive procedures yielded a high volume of wound samples (n = 440), indicative of chronic infections with high rates of *MRSA* colonization (n = 134). In contrast, samples from the invasive procedure group were primarily blood (n = 344) and urine (n = 460), representing acute catheter-associated infections. These acute infections could be driven by *Staphylococcus epidermidis* and *Enterococcus faecalis*, pathogens that demonstrated a lower MDR risk profile than the polymicrobial, resistant flora found in chronic wounds. However, a subgroup analysis for the specific type of invasive procedure is required to confirm these assumptions.

Important applications can be drawn from this study for clinical practice and future research. The use of heatmap analysis provides an important method to visualize the patterns of co-resistance among different classes of antibiotics, which should be considered in empiric treatment to avoid adverse effects of unnecessary prescribed antibiotic. For instance, the strong positive correlation between ciprofloxacin

and levofloxacin prevents the usage of one antibiotic once resistance develops to one of them. This has been also observed by Alseqely et al. where resistance to ciprofloxacin and levofloxacin was 78% and 96% respectively (28). Furthermore, resistance to one β-lactam indicates the resistance to the other one except in case of *S. aureus* infection where amoxicillin/clavulanic acid showed to be effective even though resistance to penicillin by *S. aureus* was high. Another observation was the high efficacy of tigecycline even in MDR pathogens which should be saved as last option to avoid rise of tigecycline resistance.

In addition, our findings are consistent with other literature as outpatient cases have lower risk of developing MDR pathogen compared with inpatient cases (29). This can be explained by the fact that inpatients are exposed more to hospital acquired pathogens that are usually more resistant due to continuous exposure to antibiotics (30). However, the consistency of co-resistance patterns between outpatient and inpatient heatmap analysis indicates presence of biological factor that plays a key role in the resistance of these organisms. Even though regression analysis showed comorbidities risk was statistically insignificant, they should be considered as risk factors for antibiotic resistance especially in diabetes and chronic kidney disease participants due to their weaker immunity requiring recurrent antibiotic administration for prolonged periods in these conditions (31, 32).

The notable strengths of the present study are its large sample size (n = 2,364) combined with comprehensive antibiotic susceptibility coverage, and the use of advanced analytical methods such as heatmap analysis to visualize co-resistance patterns and logistic regression to adjust for confounders. It's worth mentioning that bias can be introduced into the results due to retrospective design and the collection of data from a single tertiary institution. Furthermore, potential residual confounding from unmeasured variables cannot be ruled out. Finally, the lack of minimum inhibitory concentration (MIC) data prevented the distinction between borderline and highly resistant isolates and the lack of subgroup analysis that clearly specifies the type of invasive procedure which should be addressed in future research.

Conclusion

This study provides a comprehensive evaluation of antimicrobial resistance and co-resistance patterns among Gram-positive bacterial isolates in a tertiary care hospital within a resource-limited setting. By applying heatmap-based correlation analysis, we identified clinically relevant co-resistance networks that are not captured by conventional single-antibiotic susceptibility reporting. In particular, strong associations among fluoroquinolones and between β-lactam agents highlight the limited therapeutic benefit of substituting antibiotics within the same class once resistance has emerged.

The consistency of co-resistance patterns across inpatient and outpatient populations suggests that shared biological resistance mechanisms play a substantial role alongside healthcare-associated factors. Inpatient status and specific specimen types were independently associated with increased

odds of multidrug resistance, underscoring the importance of contextual clinical factors when interpreting resistance data and guiding empirical therapy.

These findings emphasize the value of integrating co-resistance analysis into routine antimicrobial surveillance and stewardship programs. Heatmap-based visualization offers a practical and intuitive approach to identifying antibiotic combinations likely to fail together, thereby supporting more rational empirical prescribing and potentially reducing unnecessary antibiotic exposure.

In settings where diagnostic and therapeutic resources are limited, such analytical approaches may provide added value by maximizing the clinical utility of existing susceptibility data. Future studies incorporating multicenter data, molecular resistance profiling, and longitudinal surveillance are warranted to further elucidate the dynamics of co-resistance and to inform targeted infection control and antibiotic policy interventions.

Abbreviations

Abbreviation	Definition
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
CI	Confidence Interval
CoNS	Coagulase-Negative Staphylococci
CSF	Cerebrospinal Fluid
ICU	Intensive Care Unit
IQR	Interquartile Range
MDR	Multidrug Resistance
MRSA	Methicillin-Resistant Staphylococcus aureus
OR	Odds Ratio
SD	Standard Deviation
TRAP	Tracheal Aspirate
UTI	Urinary Tract Infection
VRE	Vancomycin-Resistant Enterococci
WHO	World Health Organization

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB) of the Faculty of Pharmacy, An-Najah National University, Nablus, Palestine (Protocol No. Pharm. Jan.2025/18).

The requirement for informed consent was waived by the approving ethics committee due to the retrospective design of the study and the use of anonymized data with no direct patient contact.

All methods were carried out in accordance with the principles of the Declaration of Helsinki and its subsequent amendments.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and analyzed during the current study are not publicly available due to institutional data protection policies but are available from the corresponding author upon reasonable request and subject to approval by the relevant authorities.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

OE and OAI contributed equally and shared first authorship. OE, OAI, NR, MNMM, and AH contributed to data collection and organization. AA conceived and designed the study. AF performed the statistical analysis and contributed to data interpretation. JM, MH, AS, and FA assisted in data verification and manuscript preparation. BMA, AS, MM, and AAT provided laboratory supervision and contributed to critical revision of the manuscript. All authors contributed to manuscript drafting and critical revision, read and approved the final version of the manuscript, and agree to be accountable for all aspects of the work.

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Tables

Tables 1 to 3 are available in the supplementary files section

Figures

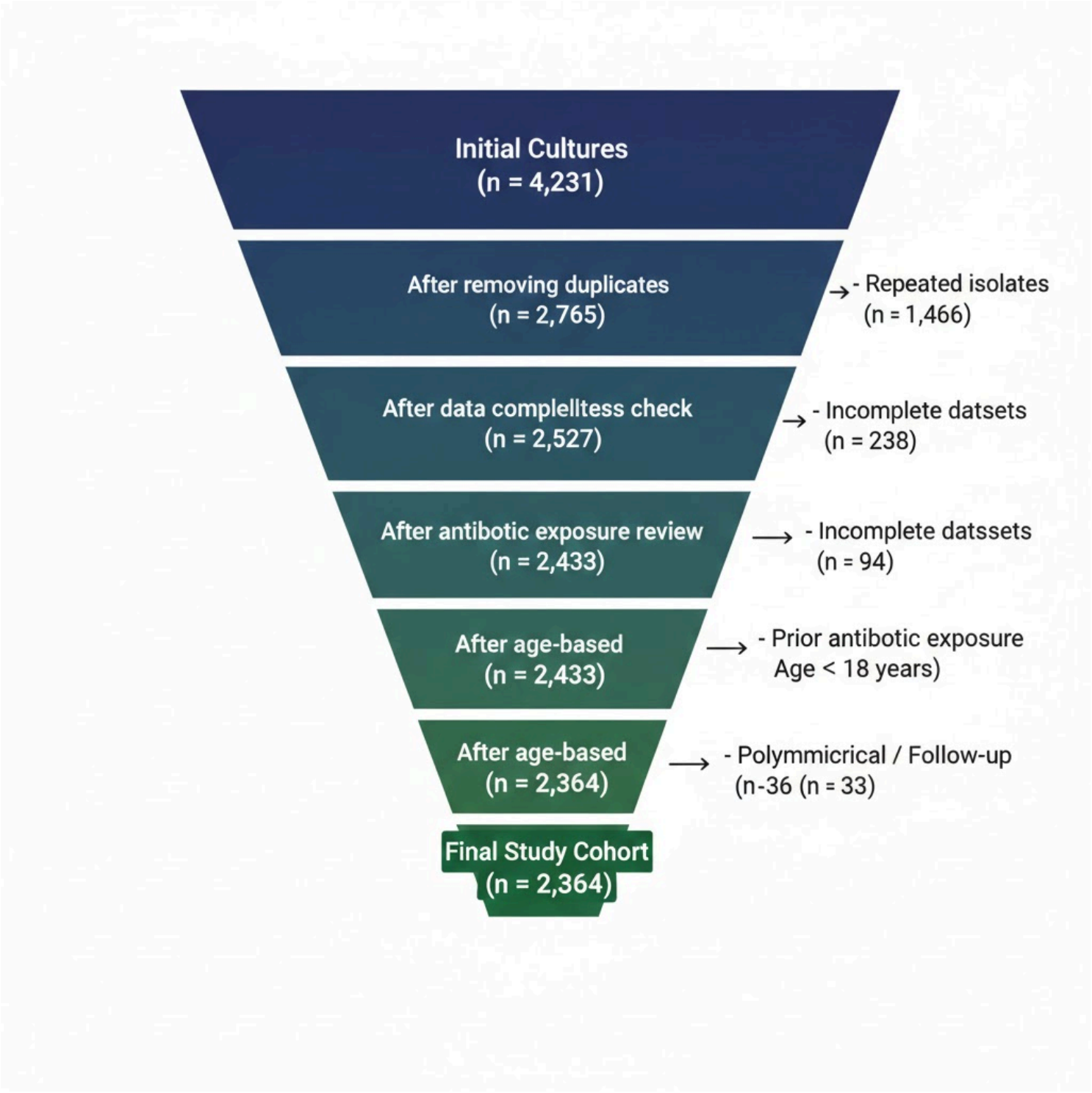


Figure 1

Flowchart of Sample Selection and Isolate Inclusion Process. The diagram illustrates the stepwise exclusion criteria applied to the initial 4,231 Gram-positive cocci cultures. Major reasons for exclusion included repeated patient isolates (n = 1,466), incomplete datasets (n = 238), and prior antibiotic exposure (n = 94). The final analysis was conducted on a cohort of 2,364 unique, non-duplicate clinical isolates.

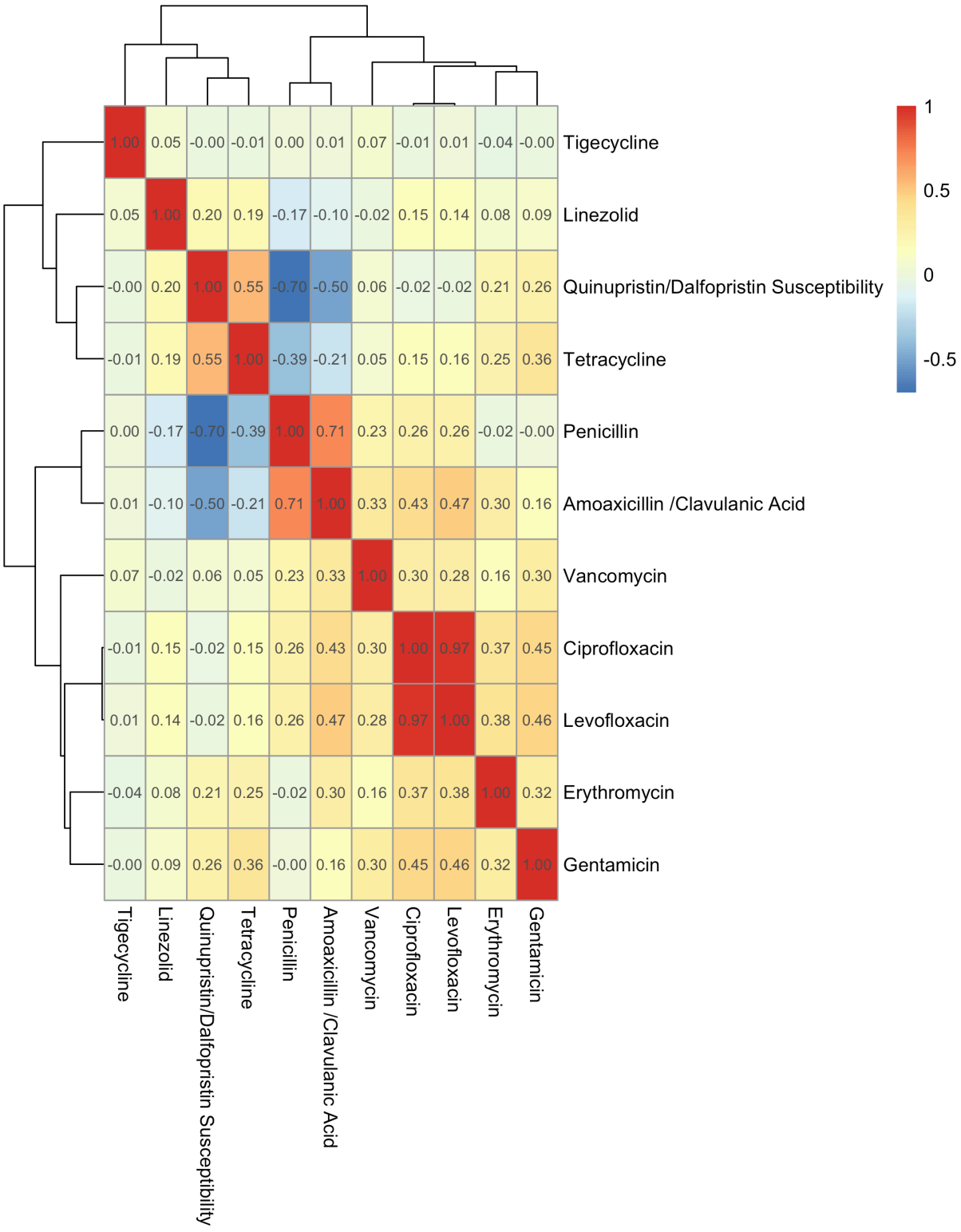


Figure 2

Overall Co-Resistance (Phi correlation) Heatmap for all Antibiotics

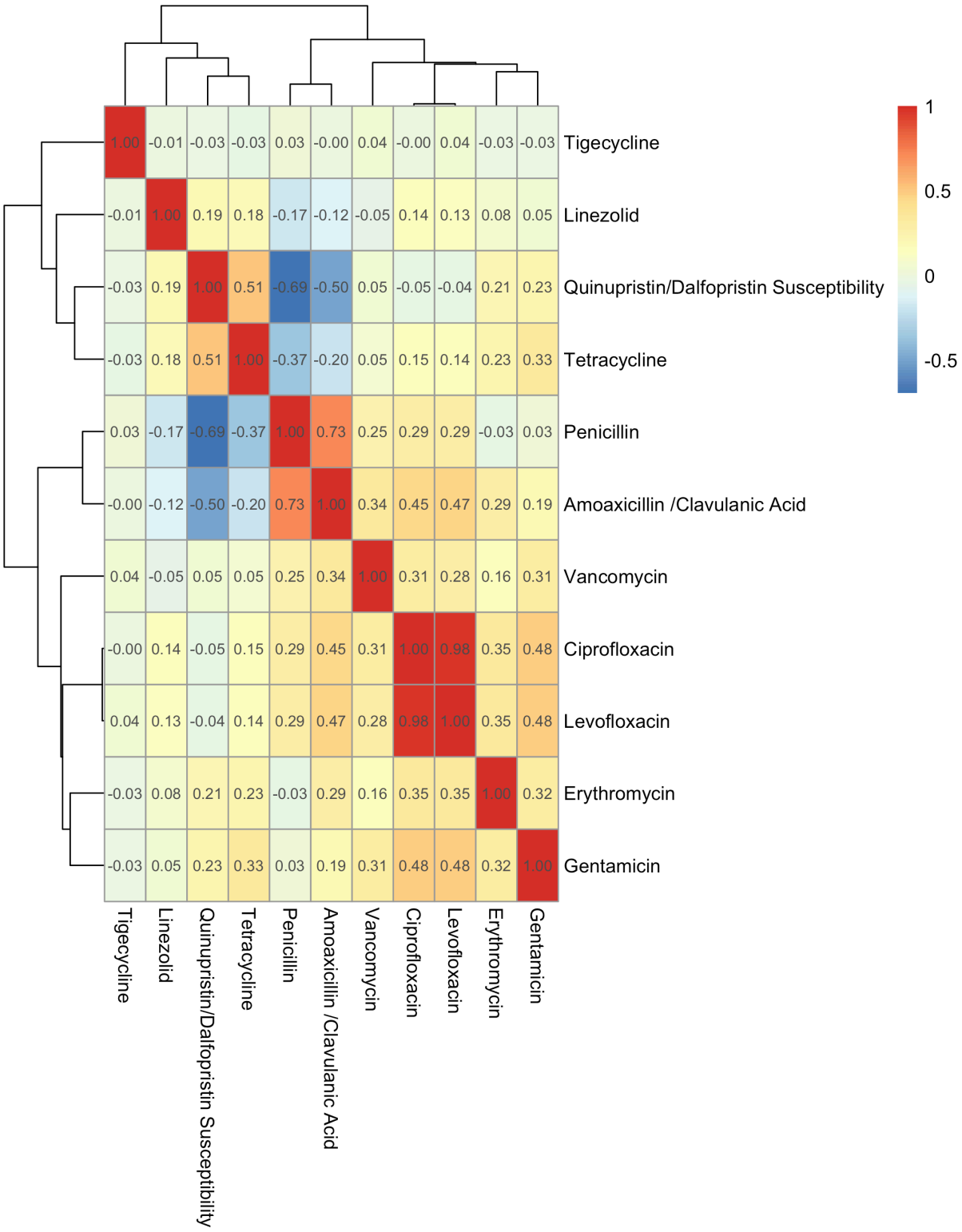


Figure 3

Inpatient Co-Resistance Heatmap

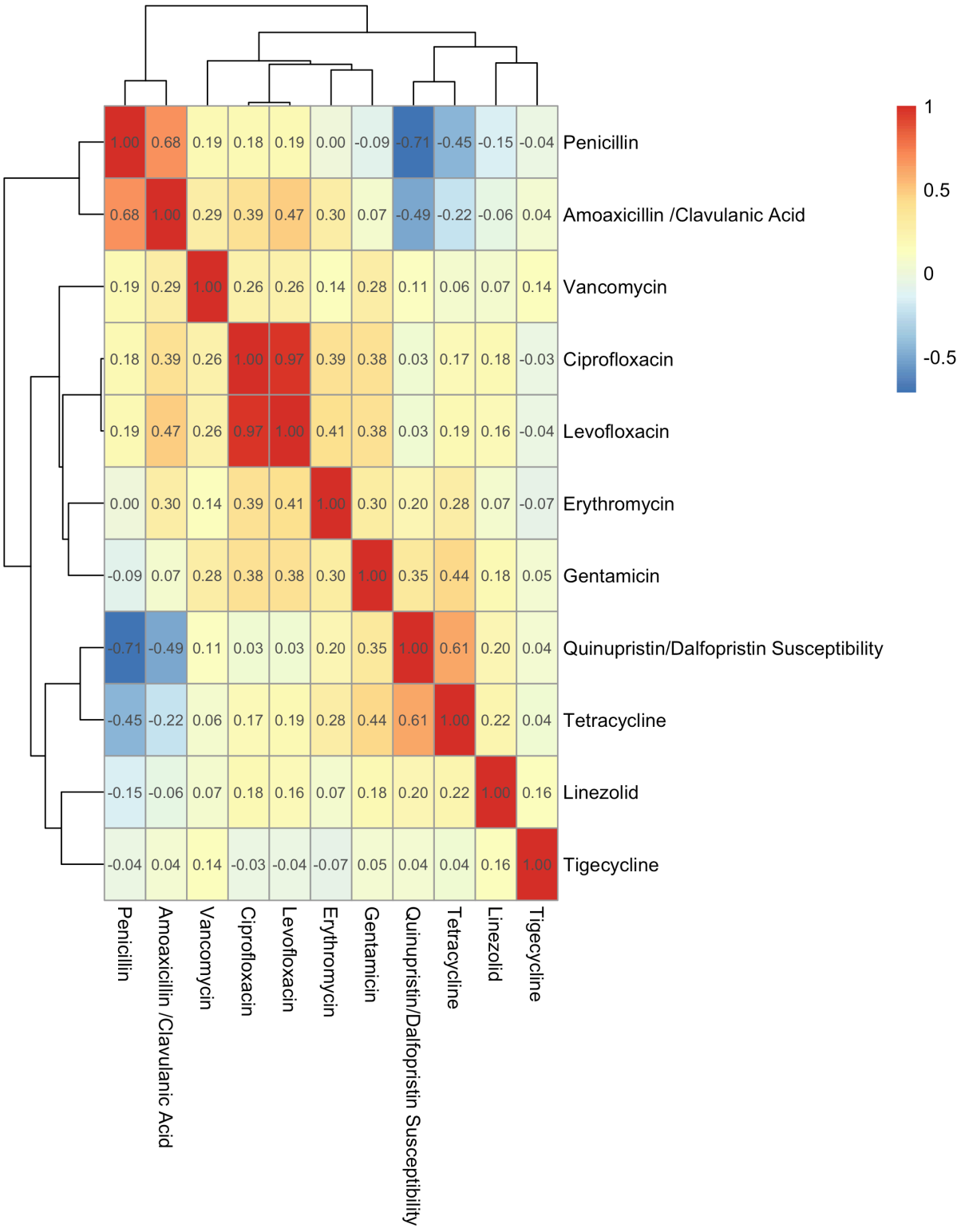


Figure 4

Outpatient Co-Resistance Heatmap

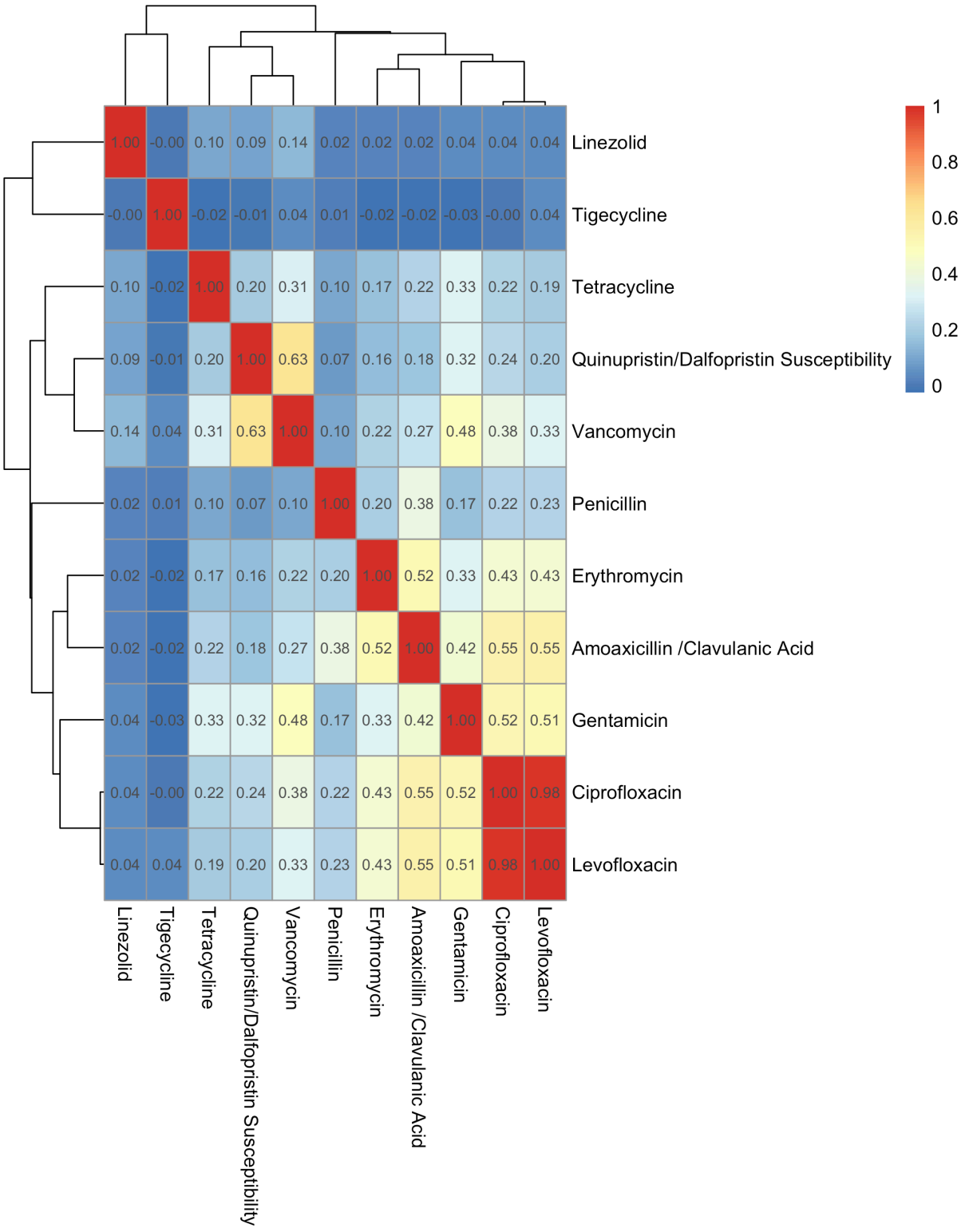


Figure 5

Co-Resisatance Heatmap with Exclusion of Enterococcus Faecalis and Enterococcus Faecium

Supplementary Files

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- Tables.docx