OPEN ACCESS

Original Article

Volume 26 | Number 1 | Jan-Mar 2026

Data Mining Antimicrobial Resistance: Hierarchical Clustering of Sensitivity Patterns across Thirty Antibiotics

Siddhartha Dhungana,[1] Puja Sharma[2]

[1]National Academy of Medical Sciences, Bir Hospital, Kathmandu, Nepal
[2]Tribhuvan University, Kathmandu,Nepal

Correspondence:
Siddhartha Dhungana
National Academy of Medical Sciences, Bir Hospital,
Kathmandu, Nepal
Email: siddharthadhungana@gmail.com
Phone: 9851201704

doi.org/10.56974/pmjn.956
www.pmjn.org.np

Submission Date:
2026, February, 09
Acceptance Date:
2025, March, 11
Published Date:
2025, March, 16

ABSTRACT

Introduction: Antimicrobial resistance (AMR) is a growing global public health threat, associated with increased morbidity, mortality, and healthcare costs. Conventional hospital antibiograms provide aggregate susceptibility rates but do not capture co-resistance relationships between antibiotics. This study applied hierarchical clustering to antimicrobial susceptibility data to identify clinically relevant co-resistance patterns and support evidence-based empirical therapy and strengthen antimicrobial stewardship efforts.

Methods: A retrospective observational study was conducted using microbiology laboratory data from a tertiary care hospital in Nepal from May 2023 to April 2025. Only the first isolate per patient per specimen type per patient encounter was included. Antimicrobial susceptibility results were dichotomized. Species-specific hierarchical clustering was performed using Jaccard distance and Ward's linkage method in R.

Results: Among 38,725 specimens processed, 12,134 (31.3%) yielded bacterial growth. Gram-negative organisms predominated (81.5%), with *Escherichia coli* (31.5%) and *Klebsiella* spp. (20.9%) being the most frequent isolates. Most isolates originated from urine (38.0%) and respiratory samples (17.5%). Hierarchical clustering revealed distinct species-specific co-resistance structures. In *E. coli*, four antibiotic clusters differentiated broad-spectrum β-lactams and oral agents from carbapenems and aminoglycosides. Klebsiella spp. demonstrated clustering patterns consistent with multidrug-resistant phenotypes, with reserve agents forming independent clusters. Comparable organism-specific patterns were observed for *Acinetobacter, Pseudomonas,* and *Staphylococcus* species.

Conclusion: Hierarchical clustering identified clinically meaningful co-resistance structures not evident in conventional antibiograms. Incorporating clustering-based analysis into routine AMR surveillance may enhance empirical therapy selection and strengthen antimicrobial stewardship in resource-limited settings.

Keywords: *Antimicrobial resistance, Co-resistance patterns, Empirical therapy, Hierarchical Clustering.*

INTRODUCTION

Antimicrobial resistance (AMR) is a critical global public health challenge that threatens the effective prevention and treatment of infectious diseases.[1,2] The World Health Organization estimates that AMR is responsible for more than 1.27 million deaths annually , disproportionately affecting low- and middle-income countries.[1,3] Inappropriate and excessive antibiotic use in both community and hospital settings has accelerated the emergence and spread of resistant pathogens, resulting in prolonged hospital stays, higher healthcare costs and increased mortality.[4]

In clinical practice, empirical antibiotic therapy is frequently initiated before antimicrobial susceptibility testing (AST) results become available. Cumulative hospital antibiograms are widely used to guide empirical therapy; however, they provide only aggregate susceptibility rates and do not capture co-resistance relationships between antimicrobial agents. As a result, conventional antibiograms may underestimate the complexity of multidrug-resistant (MDR) phenotypes and fail to inform rational sequential or combination therapy.[5] Data-driven analytical approaches, including hierarchical clustering, have been increasingly applied to large scale AST datasets to address these limitations.[6,7]

By identifying antibiotics that shared resistance profiles, clustering methods can reveal clinically meaningful co-resistance structures that may reflect common resistance mechanisms or common selective pressure.[8–10] Species-specific clustering enhances biological interpretability by minimizing confounding factors from intrinsic interspecific resistance differences.[8,11,12]

In this context, the present study applies hierarchical clustering to susceptibility data for thirty antibiotics from a tertiary care hospital in Nepal. The objective was to identify species-specific co-resistance clusters to support evidence-based empirical therapy, strengthen antimicrobial stewardship programs, and contribute to local AMR surveillance strategies in a resource-constrained setting.

METHODS

This was a retrospective, observational study used microbiology laboratory data from Bir Hospital, a tertiary-level referral center in Kathmandu, Nepal. Data spanned a two-year period (May 01, 2023, to April 30, 2025 AD). All clinical specimens processed for bacterial culture and antimicrobial susceptibility testing (AST) were considered. To prevent duplication, only the first isolate per patient per specimen type per hospital encounter was analyzed. The primary dependent variable was the antimicrobial susceptibility result for each antibiotic, initially recorded as sensitive, intermediate, or resistant. Independent variables included patient age, sex, inpatient/outpatient status and specimen type.

Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) 2025 guidelines. For hierarchical clustering analysis, results were binarized: isolates classified as resistant or intermediate were coded as 1, while susceptible isolates were coded as 0. This approach ensures that clustering reflects underlying co-resistance relationships, allowing the identification of antibiotics that frequently fail together.

As antibiotic testing was guided by clinical practice, the susceptibility matrix contained missing data coded as NA. For the primary analysis of antibiotic co-resistance, pairwise similarity was calculated using only the subset of isolates tested for both antibiotics in each pair. No imputation was performed, as 'not tested' reflects clinical protocols rather than random missingness.

Hierarchical Clustering of Antibiotics

Species-specific hierarchical agglomerative clustering was performed. Pairwise dissimilarity between antibiotics was calculated using the Jaccard distance. For two antibiotics A and B, the Jaccard similarity coefficient is:

$$J(A,B)=\frac{M11}{M11+M10+M01}$$

where M11 is the number of isolates resistant to both antibiotics, M10 is the number resistant to A but susceptible to B, and M01 is the number resistant to B but susceptible to A. The Jaccard distance was calculated as DJ=1−J (A, B). Clustering was performed on the resulting distance matrix using Ward's Linkage method (Ward.D2) to minimize within-cluster variances. The optimal number of clusters was determined by visual inspection of the dendrogram and assessment of cluster cohesion.

Data were extracted from the hospital's Laboratory Information System (LIS) and analyzed using R software (v4.5.2, R Foundation for Statistical Computing, Vienna, Austria). Data management utilized the **dplyr** and **tidyverse** packages, and descriptive statistics summarized resistance frequencies. Hierarchical clustering of the binary susceptibility matrix was performed using **hclust**, with dendrograms visualized via **ggplot2**. Antibiotics and isolates were ordered according to the clustering solution to reveal co-resistance patterns, and resulting clusters were compared with established antimicrobial classes to evaluate concordance and identify potential novel co-resistance groupings.

This study received approval from the Institutional Review Board of the National Academy of Medical Sciences (IRB-NAMS 456-2082/83). As this was a secondary analysis of de-identified laboratory data, informed consent was not required. Permission to access hospital records was formally granted by the administration of Bir Hospital.

RESULTS

During the study period, a total of 38,725 clinical specimens were processed, of which 12,134 (31.3%) yielded bacterial growth. Gram-negative organisms accounted for 81.5% of isolates. The most common pathogens were *Escherichia coli* (3,827; 31.5%) and *Klebsiella* spp. (2,535; 20.9%). Among Gram-positive organisms, *Staphylococcus* spp. (1,598; 13.2%) were the most common. The majority of isolates originated from urine (38.0%) and respiratory samples (17.5%), followed by pus (13.2%) and blood specimens (8.5%). The complete distribution of isolates by species and specimen type is summarized in Table 1. An additional 564 isolates (4.6%) were identified from other specimen types, including miscellaneous or less common sources not classified under the main categories.

Table 1. Distribution of bacterial isolates from clinical specimens by species and specimen type

Bacterial Species	Total Isolates	Blood	Urine	Sputum	Pus	Swab	Body Fluids	Other Specimens
Gram-negative								
Acinetobacter spp.	1189	197	199	402	67	116	170	38
Citrobacter spp.	633	58	172	181	85	32	28	77
Escherichia coli	3827	158	2629	294	379	159	66	142
Enterobacter spp.	69	3	30	18	8	-	3	7
Haemophilus spp.	209	2	-	204	-	-	-	3
Klebsiella spp.	2535	176	879	865	205	215	103	92
Morganella morganii	201	4	142	24	12	4	8	7
Proteus spp.	171	6	63	7	42	15	6	32
Pseudomonas spp.	965	29	256	308	175	76	76	45
Salmonella spp.	116	115	1	-	-	-	-	-
Gram-positive								
Staphylococcus spp.	1598	323	111	196	622	96	168	82
Streptococcus spp.	48	2	-	46	-	-	-	-
Enterococcus spp.	573	95	209	98	49	35	48	39
Total	12134	1168	4691	2643	1644	748	676	564

Descriptive Antimicrobial Resistance Patterns

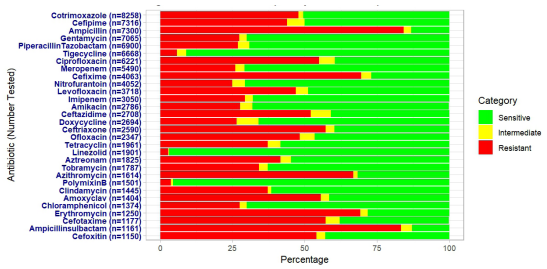

Figure 1. Antibiotics Susceptibility Pattern of Top 30 Antibiotics

As illustrated in Figure 1, high resistance frequencies were observed for several commonly used antibiotics. Resistance exceeded 70% for ampicillin (n=7300), amoxicillin/clavulanate (Amoxyclav, n=1404), and cefixime (n=4063), and was approximately 60% for ciprofloxacin (n=6221) and cotrimoxazole (n=8258). In contrast, susceptibility was comparatively preserved for later-generation agents; resistance to meropenem (n=5490) ranged from 8-15%, while amikacin (n=2786) and piperacillin/tazobactam (n=6900) demonstrated resistance rates of 10-20% and 15-25%, respectively. Among Gram-positive focused agents, linezolid (n=1901) and tigecycline (n=6668) retained near universal susceptibility (>98%). The conservative classification of intermediate results as resistant did not substantially alter these overall trends

Hierarchical clustering based on the Jaccard dissimilarity coefficient revealed distinct co-resistance patterns among the tested antibiotics in Figure 2A-2E. The dendrogram height represents the Jaccard distance (range: 0–1), where lower values indicate antibiotics that share a higher proportion of resistant isolates, and higher values indicate increasing dissimilarity in resistance profiles.

In *Escherichia coli*, four major antibiotic clusters were identified based on resistance profiles. One cluster comprised broad-spectrum -lactams, while carbapenems grouped closely with aminoglycosides, indicating shared resistance patterns. Fluoroquinolones formed a distinct cluster separate from older beta-lactam agents. Additionally, commonly used oral agents demonstrated independent clustering, suggesting divergent resistance behavior among isolate.

Similarly, in case of *Klebsiella* spp., carbapenems showed closely related resistance patterns and clustered together, while aminoglycosides formed a distinct group. Penicillin and cephalosporins were grouped separately. Polymyxin and tigecycline clustered together, indicating similar resistance behavior. Ciprofloxacin demonstrated a distinct clustering pattern.

In contrast, clustering in *Acinetobacter* spp., showed carbapenems clustering closely with piperacillin–tazobactam. Aminoglycosides and polymyxins formed

sub-clusters, while fluoroquinolones, cephalosporins, cotrimoxazole, and ampicillin/sulbactam grouped together. Doxycycline and tigecycline formed a separate cluster.

Among *Pseudomonas spp*., the dendrogram demonstrates two principal co-susceptibility clusters. Aminoglycosides (amikacin, tobramycin) clustered with carbapenems (imipenem, meropenem) and piperacillin–tazobactam, indicating that isolates susceptible to carbapenems were frequently susceptible to these agents. A second cluster included cefepime, ceftazidime, ceftazidime–avibactam, aztreonam, and levofloxacin, reflecting parallel susceptibility across β-lactams and fluoroquinolones. Polymyxin B formed a distinct branch, demonstrating an independent susceptibility pattern. Carbapenem susceptibility may act as a practical surrogate marker for broad β-lactam and aminoglycoside coverage in empirical therapy. Conversely, carbapenem resistance may signal limited activity within this cluster, prompting early consideration of polymyxins or alternative agents in suspected multidrug-resistant Pseudomonas infections.

Finally, *Staphylococcus* spp., revealed distinct groupings of β-lactam and macrolide–lincosamide antibiotics, with glycopeptides and advanced agents forming a separate cluster. Fluoroquinolones and tetracyclines grouped independently.

Table 2 presents the hierarchical clustering patterns of antimicrobial susceptibility among major isolates. Organism-specific clusters revealed distinct co-resistance patterns across major antimicrobial classes, highlighting clinically meaningful differences in susceptibility behavior.

Table 2. Antibiotic clusters and resistance patterns identified through hierarchical clustering in six bacterial species

Organism	Cluster Composition	Interpretation of Resistance Pattern
Escherichia coli	**C1:** Broad-spectrum β-lactams (penicillins + cephalosporins) **C2:** Carbapenems + Aminoglycosides **C3:** Fluoroquinolones **C4:** Oral agents (cotrimoxazole, tetracyclines)	Cluster 1 suggests ESBL-mediated resistance. Carbapenems retain activity. Oral agents show independent susceptibility behavior.
***Klebsiella* spp.**	**C1:** Carbapenems **C2:** Aminoglycosides **C3:** Penicillins + Cephalosporins **C4:** Polymyxin + Tigecycline	Carbapenems and aminoglycosides show related resistance patterns. Polymyxin and tigecycline cluster indicates similar salvage susceptibility.
***Citrobacter* spp.**	**C1:** Broad-spectrum β-lactams **C2:** Carbapenems + Aminoglycosides **C3:** Fluoroquinolones + Oral agents	Resistance resembles *E. coli* pattern, with divergence among oral agents.
***Acinetobacter* spp.**	**C1:** Carbapenems + Piperacillin–Tazobactam **C2:** Aminoglycosides + Polymyxins **C3:** Fluoroquinolones + Cephalosporins + Cotrimoxazole + Ampicillin–Sulbactam **C4:** Doxycycline + Tigecycline	Complex multidrug resistance pattern. Carbapenems cluster with piperacillin–tazobactam, separate from other β-lactams.
***Pseudomonas* spp.**	**C1:** Core anti-pseudomonal β-lactams **C2:** Aminoglycosides + Fluoroquinolones **C3:** Polymyxin B	Carbapenem susceptibility predicts broader β-lactam and aminoglycoside activity. Polymyxin B shows independent pattern.
***Staphylococcus* spp.**	**C1:** β-lactams **C2:** Macrolides + Lincosamides **C3:** Glycopeptides + Advanced agents **C4:** Fluoroquinolones + Tetracyclines	Clear separation of β-lactam and MLS groups. Glycopeptides remain active. Fluoroquinolones and tetracyclines show independent susceptibility.

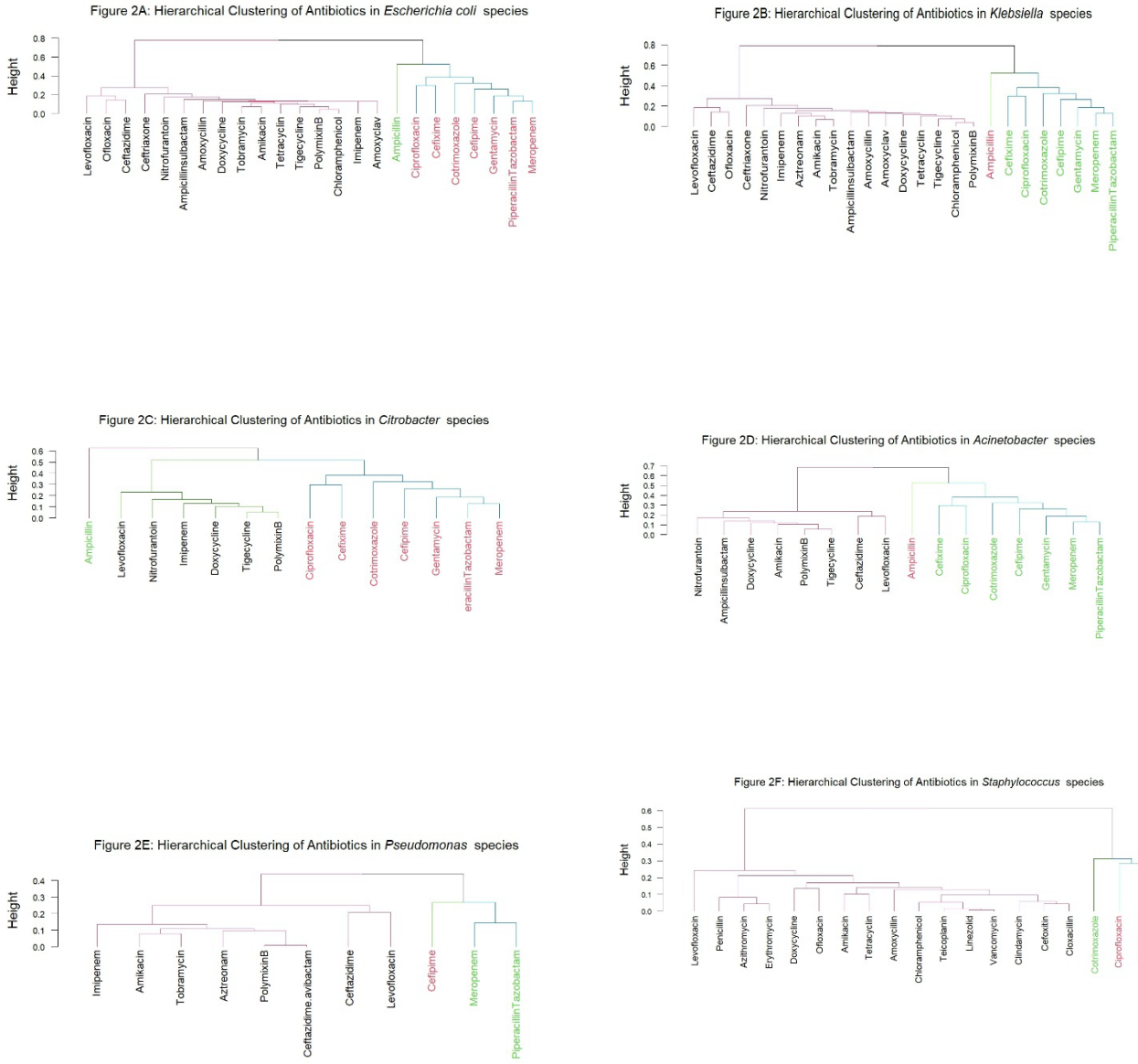

Figure 2. Heirarchial clustering of Antibiotics with different species

In summary, hierarchical clustering analysis proved effective in revealing species-specific patterns of antibiotic resistance among major bacterial pathogens. The analysis highlighted distinct co-resistance clusters for each organism ranging from four unique groupings in *E. coli* to the strong carbapenem–aminoglycoside associations in *Klebsiella*, as well as the varied clustering patterns observed in *Acinetobacter*, *Pseudomonas*, and *Staphylococcus* species. These results emphasize the complexity and organism-specific nature of resistance mechanisms, providing a useful framework for understanding cross-resistance, guiding empiric antibiotic therapy, and shaping targeted stewardship strategies.

DISCUSSION

Antimicrobial resistance in hospital settings remains a major challenge to effective clinical management. In this hospital-based study, we applied hierarchical clustering to large-scale antimicrobial susceptibility data to explore co-resistance patterns across thirty antibiotics in a tertiary care hospital. By moving beyond conventional antibiogram summaries, the analysis identified multidimensional resistance structures that have direct implications for empirical therapy and antimicrobial stewardship.

Over the two years, nearly one-third of processed cultures yielded bacterial growth, showing the increased burden of bacterial infections in a tertiary referral setting. The

predominance of Gram-negative organisms, particularly *Escherichia coli*, *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas* spp., is consistent with regional and global reports identifying these pathogens as major drivers of hospital-acquired infections and antimicrobial resistance.[13,14] The concentration of isolates from urine, bloodstream, respiratory, and wound specimens highlights clinical syndromes that contribute most to antibiotic exposure and resistance selection pressure in hospital environments in South Asia. This high burden of infections was accompanied by complex susceptibility profiles. Descriptive analysis of the susceptibility data revealed notable heterogeneity in resistance patterns across the thirty antibiotics and bacterial species studied**.** Commonly used therapeutic agents exhibited high resistance rates compared with reserve antibiotics.[15] In contrast, reserve antibiotics formed distinct clusters and demonstrated comparatively preserved susceptibility.[16] This pattern is consistent with differential antimicrobial exposure and associated selection pressure within the hospital setting. Furthermore, the decision to classify intermediate susceptibility as resistant provided a more conservative estimate of resistance, a clinically relevant approach in severe infections where borderline susceptibility may still result in treatment failure. Species-specific hierarchical clustering yielded clinically interpretable resistance groupings.[17,18]

In *Escherichia coli*, four distinct antibiotic clusters were identified, separating commonly used broad- spectrum and oral agents from carbapenems and aminoglycosides with closely aligned susceptibility profiles. This structure suggests shared resistance among widely used agents, while highlighting the therapeutic separation of reserved antibiotics. Such clustering provides insight into potential cross- resistance that is not evident from single-drug susceptibility percentages alone.[19] Non-clinical animal isolates from food-producing animals and food, and clinical animal isolates from food-producing and companion animals from national routine surveillance and monitoring for AR in Germany. Sixteen possible resistance combinations to four antibiotics ampicillin, cefotaxime, ciprofloxacin and gentamicin for these populations were used for hierarchical clustering (Euclidian and average distance.

Similarly in case of *Klebsiella* spp., clustering revealed a dominant multidrug-resistant pattern encompassing β-lactams, aminoglycosides, and fluoroquinolones, consistent with the global spread of ESBL- and carbapenems-producing strains.[20] The segregation of last-line agents such as polymyxins into distinct clusters underscores their retained but vulnerable role in managing drug-resistant infections extensively and reinforces the need for judicious use.[21] With *Staphylococcus* species, the clustering patterns reveal a functional map of co-resistance. Well-defined beta-lactam and MLSB clusters support the use of surrogate markers for rapid phenotypic detection. The identical profiles of vancomycin and teicoplanin, distinct from other classes, underscore their unique mechanism and confirm their role as last-line therapies. In contrast, the large heterogeneous cluster of oral and systemic alternatives suggests co-selection of diverse resistance genes, likely on mobile elements, indicating that empirical use of one may inadvertently drive multidrug resistance and complicate treatment, highlighting key targets for stewardship interventions.

Across species, the reproducibility and coherence of clusters support the biological validity of the approach. The use of Jaccard distance effectively accommodated selectively tested antibiotics, while Ward's linkage method produced compact and interpretable clusters, suggesting that observed patterns reflect true resistance relationships rather than analytical artifacts.[11] This approach has been successfully applied in previous studies to identify co-resistance patterns and multidrug-resistant phenotypes in clinically important pathogens.[15,12]

From a clinical perspective, antibiotics grouped within the same cluster are likely to fail concurrently, limiting their value as alternative or sequential therapies. Conversely, agents from distinct clusters may offer more rational empirical or step-down options. Integrating clustering-based analyses into routine AMR surveillance could therefore enhance empirical decision-making, strengthen antimicrobial stewardship programs, and provide a data-driven framework for local and national AMR control strategies, particularly in resource-limited hospital settings.

The strengths of this study include a large sample size, inclusion of multiple bacterial species, and the application of unsupervised machine learning techniques to routinely collected laboratory data. Stratification of clustering analyses by species enhanced biological interpretability, while the use of conservative resistance definitions improved clinical relevance. However, the retrospective design limited the ability to establish causal relationships, and the absence of molecular resistance data precluded direct correlation of phenotypic clustering with specific resistance genes. Additionally, selective antibiotic testing resulted in missing data; however, this was appropriately addressed using pairwise similarity metrics rather than data imputation.

CONCLUSIONS

Hierarchical clustering revealed distinct and clinically meaningful co-resistance patterns among major bacterial pathogens. The findings demonstrate substantial heterogeneity in antimicrobial susceptibility and highlight limitations of conventional antibiogram-based analyses, which may not fully capture inter-antibiotic resistance

relationships. Integrating hierarchical clustering into routine AMR surveillance frameworks could improve empirical treatment selection, strengthen antimicrobial stewardship efforts, and inform both hospital and national-level AMR control strategies.

Conflict of Interest: None

REFERENCES

1. World Health Organization. (2015). *Global action plan on antimicrobial resistance*. World Health Organization. https://www.who.int/publications/i/item/9789241509763

1. Centers for Disease Control and Prevention. (2019, November). *Antibiotic resistance threats in the United States, 2019* (Report No. 10.15620/cdc:82532). [DOI]
2. World Health Organization. (2022). *Global Antimicrobial Resistance and Use Surveillance System (GLASS) report: antibiotic use data for 2022* [Internet]. World Health Organization. https://www.who.int/publications/i/item/9789240108127
3. Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., Theuretzbacher, U., … WHO Pathogens Priority List Working Group (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet. Infectious diseases*, *18*(3), 318–327. [DOI | PubMed]
4. Klinker, K. P., Hidayat, L. K., DeRyke, C. A., DePestel, D. D., Motyl, M., & Bauer, K. A. (2021). Antimicrobial stewardship and antibiograms: importance of moving beyond traditional antibiograms. *Therapeutic advances in infectious disease*, *8*, 20499361211011373. [DOI | PubMed]
5. Xu, C., Zhao, L. Y., Ye, C. S., Xu, K. C., & Xu, K. Y. (2025). The application of machine learning in clinical microbiology and infectious diseases. *Frontiers in cellular and infection microbiology*, *15*, 1545646. [DOI | PubMed]
6. Sakagianni, A., Koufopoulou, C., Koufopoulos, P., Kalantzi, S., Theodorakis, N., Nikolaou, M., Paxinou, E., Kalles, D., Verykios, V. S., Myrianthefs, P., & Feretzakis, G. (2024). Data-Driven Approaches in Antimicrobial Resistance: Machine Learning Solutions. *Antibiotics (Basel, Switzerland)*, *13*(11), 1052. [DOI |PubMed]
7. Berrazeg, M., Drissi, M., Medjahed, L., & Rolain, J. M. (2013). Hierarchical clustering as a rapid tool for surveillance of emerging antibiotic-resistance phenotypes in Klebsiella pneumoniae strains. *Journal of medical microbiology*, *62*(Pt 6), 864–874. [DOI | PubMed]
8. Pattolath, A., Adhikari, P., & Pai, V. (2023). Clinical and Molecular Profile of Carbapenem Resistant *Klebsiella pneumoniae* Infections in a Tertiary Care Hospital -Mangalore. *Infection and drug resistance*, *16*, 4335–4348. [DOI | PubMed]
9. Hewapathirana, R., & Wijayarathna, G. (2010). Spatiotemporal antibiotic resistance pattern monitoring using geographical information system based hierarchical cluster analysis. *Studies in health technology and informatics*, *160*(Pt 1), 501–504. [PubMed]
10. Bardhan A, Qureshi QA, Abraham TJ. Cluster Analysis of Oxytetracycline and Chloramphenicol Susceptibility in *Aeromonas spp.* and *Escherichia coli* from an Aquaculture Environment. *Mediterranean Journal of Infection Microbes and Antimicrobials*. [DOI]
11. Hesp, A., Ter Braak, C., van der Goot, J., Veldman, K., van Schaik, G., & Mevius, D. (2021). Antimicrobial resistance clusters in commensal Escherichia coli from livestock. Zoonoses and public health, 68(3), 194–202. [DOI | PubMed]
12. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. *Antimicrob Resist Infect Control*. 2017;6:67. [DOI | PubMed]
13. Jean SS, Coombs G, Ling T, Balaji V, Rodrigues C, Mikamo H, et al. Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Asia-Pacific region: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2010-2013. *Int J Antimicrob Agents*. 2016;47:328–34[DOI |PubMed]
14. World Health Organization. (2025, September 5). *The selection and use of essential medicines, 2025: WHO AWaRe (Access, Watch, Reserve) classification of antibiotics for evaluation and monitoring of use* . World Health Organization. https://www.who.int/publications/i/item/B09489
15. Moja, L., Abbas, M., de Kraker, M. E., Zanichelli, V., Ombajo, L. A., Sharland, M., & Huttner, B. (2025). Reserve antibiotics: overcoming limitations of evidence generated from regulatory approval trials. Globalization and health, 21(1), 17. [DOI | PubMed]

16. Ali S, Fitzpatrick F. The lingering legacy of one letter in antimicrobial susceptibility testing. *J Med Microbiol*. 2025;74:002082. [DOI |PubMed]

17. Humphries, R. M. (2021, April 26). *Re-exploring the intermediate interpretive category* [Internet]. Clinical & Laboratory Standards Institute. https://clsi.org/resources/insights-blog/re-exploring-the-intermediate-interpretive-category/

18. Suwono B, Eckmanns T, Kaspar H, Merle R, Zacher B, Kollas C, et al. Cluster analysis of resistance combinations in *Escherichia coli* from different human and animal populations in Germany 2014-2017. *PLoS One*. 2021;16:e0244413.[DOI | PubMed]

19. Falagas, Matthew E et al. "Global Epidemiology and Antimicrobial Resistance of Klebsiella Pneumoniae Carbapenemase (KPC)-Producing Gram-Negative Clinical Isolates: A Review." *Microorganisms* vol. 13,7 1697. 19 Jul. 2025, [DOI | PubMed]

20. Xie, M., Zhang, Y., Chen, K. *et al.* Increasing polymyxin resistance in clinical carbapenem-resistant *Klebsiella pneumoniae* strains in China between 2000 and 2023. *Commun Med* 5, 73 (2025). [DOI]