Vol.3, No.6, Page 1291- 1298, 2025 https://doi.org/10.59628/jast.v3i6.2004 ISSN 2958-9568 JAST

SUN PUBLISHER

مجلة جامعة صنعاء للعلوم التطبيقية والتكنولوجيا

Sana'a University Journal of Applied Sciences and Technology

https://journals.su.edu.ye/index.php/jast/

Enzymatic Capabilities and Antibiotic Susceptibility of Bacteria Isolated from Medical Laboratory Surfaces in Sana'a, Yemen

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Abstract

Background: The surfaces of medical laboratories are potential reservoirs of multidrug-resistant (MDR) organisms, posing a significant biosafety risk. In Yemen, where national surveillance and laboratory biosafety data are limited, the extent of environmental contamination is unknown. This study aimed to provide the first characterization of bacterial flora, their antimicrobial resistance profiles, and virulence-associated enzyme production from isolates on laboratory surfaces in Sana'a.

Methods: In this descriptive microbiological study, 100 surface swabs were collected from ten medical laboratories. Bacterial identification was performed using standard biochemical methods. Antimicrobial susceptibility was determined by disk diffusion according to the EUCAST guidelines. The isolates were also screened for protease, lipase, and hemolysin production.

Results: Bacterial growth was detected in 94% (94/100) of the sampled surfaces, with the bioburden dominated by gram-positive bacteria (98.3%), primarily *Staphylococcus* and *Bacillus* spp.. The isolates exhibited high resistance rates, including near-universal resistance to ampicillin (100%) and high resistance to clindamycin (>86%) among staphylococci. Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 15.6% of *S. aureus* isolates. Many isolates, particularly *Bacillus* species, exhibit significant enzymatic activity.

Conclusion: Surfaces in medical laboratories in Sana'a harbor a significant bioburden of pathogenic and MDR bacteria, including MRSA, along with isolates with notable enzymatic capabilities. These baseline findings provide crucial data-driven evidence to inform local biosafety policies and highlight the urgent need to integrate environmental surveillance into Yemen's national AMR action plan.

Article Info

Keywords:
Bacterial Contamination , Medical Laboratories, Antibiotic Resistance, MRSA, Multidrug Resistance, Staphylococcus, Bacillus, Environmental Surveillance, Yemen

Article History:
Received: 28-July-2025,
Revised: 05-September-2025,
Accepted: 11-September-2025,
Available online: 28 December 2025.

1. INTRODUCTION

Antimicrobial resistance (AMR) is a pressing global health priority, particularly in resource-limited and conflict-affected settings such as Yemen, where the healthcare infrastructure is severely strained [1].

A critical yet often-overlooked component of AMR control is laboratory biosafety and environmental hygiene. This is a major concern in Yemen, where biosafety practices are reported to be poor, with one study finding that only 32% of laboratory staff consistently adhere to standard precautions [2].

This gap in practice is compounded by systemic weaknesses in national coordination, surveillance, and infection prevention and control (IPC), as highlighted by the World Health Organization's self-assessment survey for Yemen, which rated the country's national AMR surveillance capacity as "limited" [3].

Although prior local research has documented issues such as airborne bacterial pollution in Sana'a's clinical environments [4], a corresponding knowledge gap exists concerning the contamination of high-contact surfaces in the diagnostic laboratories themselves. These laboratories function as a central nexus where diverse pathogens

are concentrated; however, Yemen still lacks a structured national AMR surveillance network to monitor and respond to the threats emerging from such settings [5].

The absence of local, evidence-based data on environmental reservoirs means that current biosafety protocols are based on general guidelines rather than tailored risk assessments [6].

Gram-positive bacteria, particularly desiccation-resistant *Staphylococcus* species and endospore-forming *Bacillus* species, are common environmental contaminants [7].

Beyond their resistance profiles, their ability to persist on surfaces can be enhanced by functional traits such as the production of extracellular enzymes. These enzymes can play a role in virulence and, critically, environmental hygiene in the formation of biofilms, which are known to confer increased tolerance to both antimicrobial agents and disinfectants [8, 9].

To the best of our knowledge, this is the first study in Yemen that systematically investigated the antibiotic resistance profiles and functional traits of bacterial isolates from medical laboratory surfaces. Therefore, this study was designed as a descriptive baseline study with the following objectives: (1) to isolate and identify bacterial species on high-contact surfaces in medical laboratories in Sana'a, (2) to determine their antibiotic susceptibility profiles, with a focus on MDR phenotypes and Methicillin-Resistant *Staphylococcus aureus* (MRSA), and (3) to characterize the production of virulence-associated enzymes (protease, lipase, and hemolysin). These findings are intended to provide foundational data to inform laboratory administrators and biosafety officers about the urgent need to strengthen disinfection protocols and integrate environmental surveillance into Yemen's National Action Plan on AMR.

2. MATERIALS AND METHODS

2.1. Study Design and Sample Collection

This descriptive microbiological study was conducted between August 2024 and March 2025 in Sana'a, Yemen, Brazil. Ten medical laboratories (five public and five private laboratories) were included in the study. A total of 100 swabs were collected from ten pre-defined high-contact environmental surfaces. For flat surfaces (e.g., workbenches), a sterile 10 × 10 cm template was used to define the sampling area. For irregular surfaces (e.g., doorknobs and microscope eyepieces), the entire surface was thoroughly swabbed [10]. Sampling was performed during routine laboratory hours. Sterile cotton swabs (Bio-Rad, USA) moistened with sterile 0.9% saline solution were transported to the laboratory within one hour for immediate processing [11].

2.2. Bacterial Isolation and Identification

Samples were inoculated onto Nutrient Agar (Oxoid, UK) and incubated at 37 ℃ for 24–48 hours. Distinct colonies were sub-cultured on Blood Agar and Mannitol Salt Agar (MSA) (Oxoid, UK) to ensure purity [12]. Isolates were identified by macroscopic colony characterization [13] and Gram staining [10].

A panel of standard biochemical tests was used for further identification, including catalase, coagulase (slide method), and oxidase [14, 15].

Biochemical methods have limitations for definitive species-level discrimination within certain genera (e.g., *Bacillus*). This study focused exclusively on bacterial recovery; fungal contaminants were not targeted.

2.3. Screening for Virulence-Associated Enzyme Production

All confirmed isolates were screened for the production of key extracellular enzymes using established plate-assay methods. Protease activity was evaluated using Skim Milk Agar (15% w/v), with a clear zone of hydrolysis indicating casein degradation recorded as a positive result [16].

Additionally, lipase activity was assessed on basal medium supplemented with Tween 20 (1% v/v), where an opaque precipitate zone indicated a positive test [17]. Finally, hemolytic activity was observed on Blood Agar and the resulting hemolytic patterns (α, β, and γ) were recorded [10].

2.4. Antimicrobial Susceptibility Testing

AST was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) (Oxoid, UK) following EUCAST guidelines (v. 14.0) [18]. The antibiotic panel was selected based on its clinical relevance and included Ampicillin, Ciprofloxacin, Clindamycin, Gentamicin, Erythromycin, Cefoxitin, Tetracycline, and Vancomycin. The zone diameters were interpreted based on EUCAST breakpoints. MRSA was determined based on its resistance to cefoxitin. To ensure the accuracy and reliability of the AST results, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as quality control (QC) strains in full compliance with EUCAST recommendations for testing Gram-positive and Gram-negative bacteria, respectively. *S. aureus* ATCC 25923 was used to validate the biochemical identification protocols.

2.5. Statistical Analysis

Data were analyzed using SPSS Statistics, Version 26.0. Descriptive statistics are presented as frequencies and

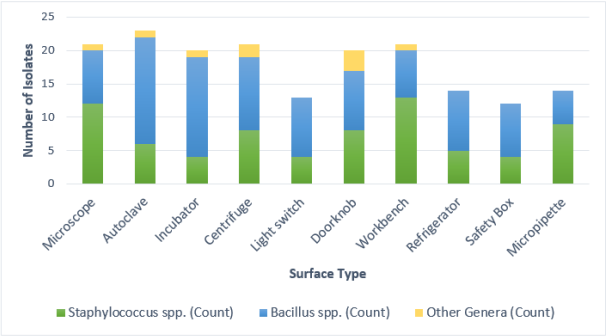

Figure 1. Distribution of major bacterial genera across different surface types.

percentages. The term "frequency of detection" was used to describe the proportion of positive samples. Chi-square or Fisher's exact test was used to compare categorical variables. The Shapiro-Wilk test was performed to assess normality. As the data were not normally distributed, the non-parametric Kruskal-Wallis H test was employed. The effect size for significance tests was calculated using eta-squared (η^2). Statistical significance was set at $P < 0.05$. Hierarchical Cluster Analysis (HCA) was performed using Ward's linkage method, and Principal Component Analysis (PCA) was used to visualize resistance patterns (N=179), with loading scores provided in the **Appendix**.

3. RESULTS

3.1. High Prevalence and Distribution of Bacterial Bioburden

Of the 100 sampled environmental surfaces, 94 were positive for bacterial growth, resulting in an overall **detection frequency** of 94%. Bacterial growth was detected on 100% of the surfaces, including the autoclaves, incubators, and workbenches. The detection rate in private laboratories (96.0%) was slightly higher than that in public laboratories (92.0%); however, this difference was not statistically significant (Fisher's Exact Test, P = 0.678). Of the 94 positive samples, 179 distinct bacterial isolates were identified. Gram-positive bacteria constituted the vast majority of isolates (98.3%). The microbial landscape was dominated by two genera, *Bacillus* (51.4%) and *Staphylococcus* (41.9%) (**Table1**. The distribution of these major genera varied by surface type, with *Staphylococcus spp.* being more frequently isolated from high-touch items such as micropipettes, whereas *Bacillus spp.* dominated surfaces such as incubators **(Figure 1)**.

3.2. Virulence-Associated Enzymatic Activity

The enzymatic capabilities of the isolates sharply diverged along the generic lines **(Table 2)**. Protease production is a hallmark of the *Bacillus* genus, with 100% of *B. licheniformis* and 94.7% of the *B. cereus* group showing activity. In contrast, all 73 Staphylococcus isolates tested negative for protease production. Kruskal-Wallis test confirmed a statistically significant difference in the strength of protease production among the bacterial groups (**Figure 2**; H=16.06, P=0.042).

Table 1. Frequency and distribution of bacterial isolates recovered from 94 positive surface samples (n = 179).

Bacterial Isolate	Frequency (n)	Percentage (%)
Staphylococcus aureus	45	25.1%
Bacillus sp.[*]	38	21.2%
Bacillus cereus group[*]	19	10.6%
Coagulase-Negative Staphylococci (CONS)	18	10.1%
Bacillus subtilis group[*]	14	7.8%
Bacillus licheniformis	13	7.3%
Other species[†]	32	17.9%
Total Isolates	**179**	**100.0**

**Note: Identification to the species or group level within the Bacillus genus was based on standard biochemical profiles. "Bacillus sp." refers to isolates that did not fit the precise profile of other identified species.*
†Other species include Staphylococcus epidermidis (n=9), Bacillus thuringiensis (n=12), and single isolates of other genera.

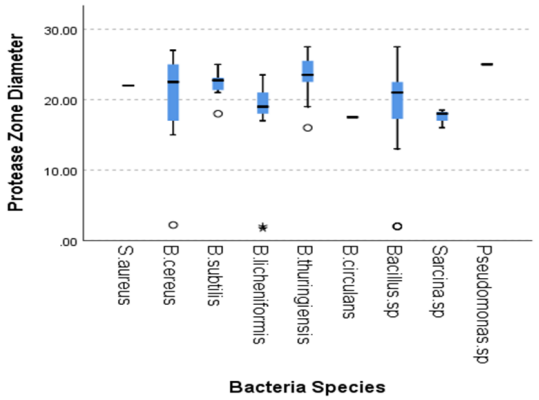

Figure 2. Distribution of protease zone diameters across bacterial species.

3.3. Antibiotic Resistance and MRSA Prevalence

The isolates exhibited high rates of resistance to the antibiotics tested **(Table 3)**. All the tested staphylococcal isolates (100%) were resistant to ampicillin. High rates of resistance to clindamycin were also observed, reaching 86.7% in *S. aureus*. Methicillin-resistant *Staphylococcus aureus* (MRSA), identified by its resistance to cefoxitin,

Table 2. Profile of virulence-associated enzyme production among bacterial isolates.

Bacterial Species	Total (n)	Protease Positive (%)	Lipase Positive (%)	Hemolysis Positive (%)
Staphylococcus aureus	45	0.0%	0.0%	0.0%
Bacillus sp.	38	60.5%	21.1%	15.8%
Bacillus cereus	19	94.7%	21.1%	94.7%
Coagulase-Negative Staphylococci	18	0.0%	0.0%	0.0%
Bacillus subtilis	14	85.7%	21.4%	35.7%
Bacillus licheniformis	13	100.0%	15.4%	76.9%
Bacillus thuringiensis	12	75.0%	50.0%	66.7%
Staphylococcus epidermidis	9	0.0	0.0	0.0
Sarcina sp.	3	100.0%	0.0%	0.0%
Micrococcus sp.	2	0.0%	0.0%	0.0%
Staphylococcus haemolyticus	1	0.0%	0.0%	100.0%
Bacillus circulans	1	100.0%	0.0%	0.0%
Enterococcus sp.	1	0.0%	0.0%	0.0%
Klebsiella pneumoniae	1	0.0%	0.0%	0.0%
Escherichia coli	1	0.0%	0.0%	0.0%
Pseudomonas sp.	1	100.0%	100.0%	100.0%

was detected in 15.6% of all *S. aureus* isolates.

3.4. Multidrug Resistance (MDR) and Risk Analysis

MDR phenotypes were frequently observed within staphylococcal isolates, with a rate of 77.8% among CONS and 51.1% among *S. aureus* **(Table 4)**. The mean Multiple Antibiotic Resistance (MAR) index for these groups (0.516 and 0.365, respectively) exceeded the 0.2 high-risk threshold. Kruskal-Wallis test showed that staphylococcal isolates had significantly higher MAR indices than *Bacillus* species, with a large effect size (H = 43.848, P < 0.001, (η^2) = 0.246).

3.5. Genus-Specific Resistance Profiles

Hierarchical Cluster analysis (HCA) and Principal Component Analysis (PCA) revealed that the isolates segregated into two distinct clusters that corresponded to their genus (*Staphylococcus* vs. *Bacillus*). The HCA dendrogram **(Figure 3)** and PCA scatter plot **(Figure 4)** visually represented this clear separation.

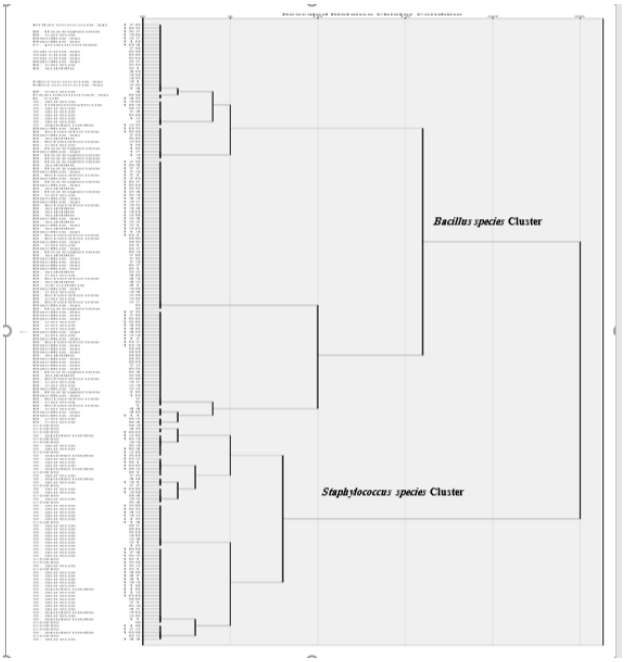

Figure 3. Dendrogram from Hierarchical Cluster Analysis (HCA).

4. DISCUSSION

The findings of this study provide the first baseline data on the microbial bioburden on medical laboratory sur-

Table 3. Antibiotic resistance profiles of predominant bacterial isolates (% Resistant).

Bacterial Species	Total (n)	AMP %R	CIP %R	CD %R	GEM %R	E %R	FOX %R	TE %R	VA %R
Staphylococcus aureus	45	100%	0%	86.7%	2.2%	37.8%	15.6%	13.3%	NT*
Bacillus sp.	38	NT	5.3%	81.6%	NT	44.7%	NT	NT	0%
Bacillus cereus	19	NT	15.8%	78.9%	NT	52.6%	NT	NT	0%
Coagulase-Negative Staphylococci	18	100%	27.8%	88.9%	44.4%	33.3%	27.8%	38.9%	NT*
Bacillus subtilis	14	NT	0%	85.7%	NT	21.4%	NT	NT	0%
Bacillus licheniformis	13	NT	0%	84.6%	NT	46.2%	NT	NT	0%
Bacillus thuringiensis	12	NT	0%	66.7%	NT	41.7%	NT	NT	0%
Staphylococcus epidermidis	9	100%	11.1%	88.9%	22.2%	33.3%	11.1%	33.3%	NT*
S. haemolyticus	1	100%	0%	0%	0%	0%	0%	0%	NT*
B. circulans	1	NT	0%	100%	NT	0%	NT	NT	0%
Enterococcus sp.	1	0%	0%	0%	0%	0%	NT	0%	0%
K. pneumoniae	1	0%	0%	NT	0%	NT	0%	0%	NT
E. coli	1	100%	0%	NT	0%	NT	0%	100%	NT
Pseudomonas sp.	1	NT	0%	NT	100%	NT	NT	100%	NT

Abbreviations: AMP, Ampicillin; CIP, Ciprofloxacin; CD, Clindamycin; GEM, Gentamicin; E, Erythromycin; FOX, Cefoxitin (MRSA marker); TE, Tetracycline; VA, Vancomycin. NT: Vancomycin disk diffusion is not a reliable method for staphylococci per EUCAST; MIC testing is required. NT: No established EUCAST clinical breakpoint for this organism-antibiotic combination.*

Table 4. Summary of Multidrug Resistance (MDR) and Multiple Antibiotic Resistance (MAR) Index analysis.

Bacterial Species	Total (n)	MDR Isolates ($n \geq 3$)	MDR (%)	MAR Index Range (Min–Max)	Mean MAR Index
S. aureus	45	23	51.1%	0.14–0.57	0.365
Bacillus sp.	38	0	0.0%	0.00–0.50	0.329
B. cereus	19	2	10.5	0.00–0.75	0.368
Coagulase-Negative Staphylococci	18	14	77.8%	0.29–0.86	0.516
B. subtilis	14	0	0.0%	0.00–0.50	0.268
S. epidermidis	9	5	55.6%	0.29–0.71	0.429

MDR: Resistant to ≥ 3 distinct classes of antibiotics. MAR Index calculated for each isolate as (number of antibiotics resisted / number of antibiotics tested).

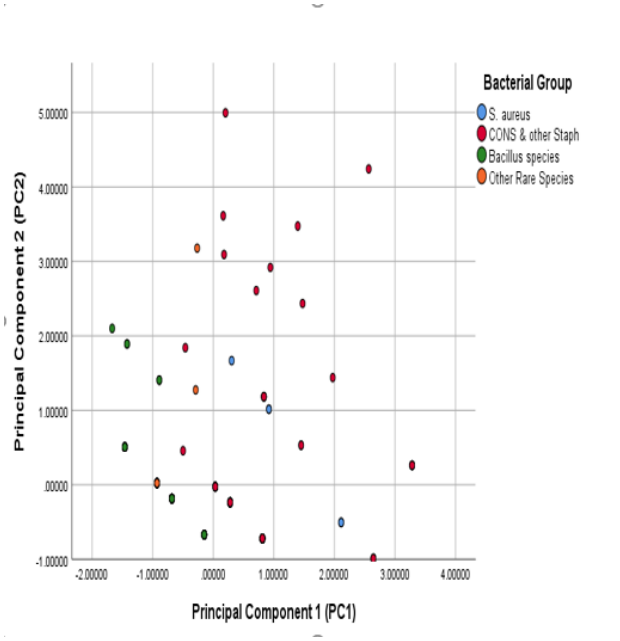

Figure 4. Principal Component Analysis (PCA) scatter plot.

faces in Yemen. The high frequency of bacterial detection (94%) is consistent with, and in some cases exceeds, rates reported in other resource-limited settings [19, 20], suggesting that current environmental hygiene protocols may be insufficient. The overwhelming dominance of gram-positive bacteria aligns with the literature on environmental microbiology, as these genera are well adapted to survive on dry, inanimate surfaces [7].

Our findings are also strikingly consistent with those of a recent local study by Alkhyat et al., who also found a predominance of gram-positive airborne bacteria in Sana'a's clinical environments [4].

These findings align with regional data from Khartoum, where Yagoub and El Agbash found similar pathogenic bacteria contaminating the air in hospital rooms [21].

This suggests a consistent environmental microbial signature in the region's healthcare facilities. The antibiotic resistance patterns found in these environmental isolates are of particular concern. The high resistance rates mirror trends observed in clinical settings and likely reflect the intense selective pressure from widespread antibiotic use in Yemen [1, 22].

Detection of MRSA in 15.6% of *S. aureus* isolates is a critical finding. This rate is similar to that reported for clinical isolates from a Sana'a hospital [23], underscoring the potential role of laboratory surfaces as environmental reservoirs. A key contribution of this study is the characterization of functional traits and resistance profiles. The high enzymatic activity observed in *Bacillus* isolates may contribute to their environmental persistence by facilitating biofilm formation [8, 9], a mechanism known to increase tolerance to disinfectants. This interplay between resistance and functional capabilities highlights the complexity of assessing biosafety risk. This study had several limitations. The reliance on phenotypic methods, descriptive design, and lack of microbial load quantification (CFU/cm²) prevents us from assessing transmission dynamics. However, this study provides crucial, previously unavailable baseline data that directly address the documented weaknesses of Yemen's national AMR surveillance and IPC practices [3, 5].

5. CONCLUSION

This study provides the first descriptive baseline data on the microbial bioburden, antibiotic resistance profiles, and functional traits of bacteria on medical laboratory surfaces in Sana'a, Yemen, Brazil. The high frequency of detection of pathogenic and multidrug-resistant bacteria, including MRSA, in an environment with documented weaknesses in biosafety practices confirms that these surfaces represent a significant, unmonitored risk. The characterization of both resistance patterns and enzymatic capabilities provides a comprehensive foundation for future targeted surveillance. These findings serve as a direct response to the national call for action to strengthen AMR surveillance, and underscore the urgent need to integrate environmental monitoring into Yemen's national infection control strategies.

6. RECOMMENDATIONS

Based on the findings of this study, a multifaceted approach is recommended to mitigate the identified risks. First, it is crucial to implement targeted data-driven disinfection protocols. Rather than a uniform strategy, protocols should utilize agents with proven sporicidal activity for surfaces such as incubators and autoclaves, where spore-forming *Bacillus* species predominate. Concurrently, for high-touch equipment, such as microscopes and workbenches, where *S. aureus* and MRSA are frequently detected, implementing enhanced daily cleaning and targeted weekly deep-disinfection protocols is advised. To support these measures, we recommend reinforcing biosafety training with a specific focus on proper disinfection of complex, high-touch equipment, as our data suggest that this is the primary route for staphylococcal colonization. Second, to ensure the long-term effectiveness of these interventions, it is imperative to establish a continuous environmental surveillance programme. The Multiple Antibiotic Resistance (MAR) index values calculated in this study can serve as an initial baseline, and regular monitoring of this index on specific surfaces can be used as a practical metric to evaluate cleaning effectiveness over time. Finally, to build upon this foundational work, future studies should prioritize the use of molecular techniques. This includes employing PCR for the detection of key resistance genes (e.g., *mecA* and *erm*) to better understand resistance mechanisms, and using molecular typing methods such as

Table 5. Rotated Component Matrix for PCA of Antibiotic Resistance Variables

Antibiotic Variable	Component 1	Component 2	Component 3
Ampicillin (AMP)	**.748**	.187	.071
Cefoxitin (FOX)	**.718**	.111	-.046
Tetracycline (TE)	.467	**.621**	-.149
Gentamicin (GEM)	.223	**.688**	-.052
Ciprofloxacin (CIP)	-.039	**.663**	.268
Clindamycin (CD)	.439	-.222	**.723**
Erythromycin (E)	-.263	.281	**.689**

MLST to definitively track potential transmission routes between the laboratory environment, staff, and clinical samples.

7. DECLARATIONS

- **Ethics Approval:** Not applicable.
- **Author Contributions:** G.A.A. conducted the study and prepared the initial draft. S.M.A. and H.J.A. supervised the study and revised the manuscript accordingly. All authors approved the final version of the manuscript.
- **Conflicts of Interest:** The authors declare no competing interests

8. APPENDIX

8.1. Appendix A: Principal Component Analysis of Antibiotic Resistance Profiles

Principal Component Analysis (PCA) was performed on the binary resistance data (resistant vs. non-resistant) for seven antibiotics across all 179 isolates. The analysis extracted three components with eigenvalues greater than 1.0, which explained **59.7%** of the variance in the resistance patterns. The table below presents the component-loading scores after varimax rotation. Loadings represent the correlation between the original antibiotic variable and the derived component. High absolute values (highlighted in bold) indicate that the variable strongly influences that component.

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