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IMPACT OF VARYING INCUBATION TEMPERATURES ON THE ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES

Ayesha Jabeen[*1], Usama Ali[2], Muhammad Waseem Irshad[3]

[*1]*Faculty of Allied Health Sciences, Superior University, Lahore Pakistan*
[2]*Superior University, Lahore Pakistan*
[3]*Centre for Applied Molecular Biology and Forensic Sciences, Punjab University, Lahore Pakistan*

[*1]ayesha.jabeen@superior.edu.pk, [2]su91-bmlsm-f22-184@superior.edu.pk, [3]waseem.res.camb@pu.edu.pk

Corresponding Author: *
Ayesha Jabeen
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ABSTRACT

Background: *Temperature influences bacterial physiology, affecting membrane fluidity, enzyme activity, gene regulation, and antibiotic susceptibility. This study compared antimicrobial resistance (AMR) among bacteria isolated from different thermal environments.*

Methods: *A comparative experimental study was conducted at the Sheikh Zayed Microbiology Laboratory over four months. A total of 150 bacterial isolates were collected from cold (4°C, n=50), moderate (25–37°C, n=50), and hot (50–60°C, n=50) environments. Bacteria were identified using Gram staining and biochemical tests. Antimicrobial susceptibility was assessed against 25 antibiotics from nine drug classes using the Kirby-Bauer disk diffusion method according to CLSI guidelines. Data were analyzed using Pearson's chi-square and Kruskal-Wallis tests.*

Results: *Multidrug resistance (MDR) was detected in 84% of isolates, with the highest prevalence in cold-environment bacteria (98%), followed by hot (80%) and moderate (74%) isolates (χ^2=11.607, p=0.003). Significant differences in resistance patterns were observed for 12 antibiotics and overall resistance (Kruskal-Wallis H=24.16, p<0.001). Cold-environment isolates showed greater resistance to aminoglycosides, fluoroquinolones, and vancomycin. Hot-environment isolates were more resistant to macrolides and linezolid, while moderate-temperature isolates displayed the widest variety of resistance traits, particularly to tetracycline and nitrofurantoin.*

Conclusion: *Environmental temperature significantly influences antimicrobial resistance patterns. Cold-adapted bacteria exhibited the highest MDR, whereas hot-adapted bacteria showed characteristic resistance to macrolides and oxazolidinones. These findings highlight the importance of considering thermal adaptation in AMR surveillance, antibiotic stewardship, and food safety.*

Keywords: *Antimicrobial resistance, temperature, multidrug resistance, Kirby-Bauer, thermal adaptation, antibiotic susceptibility.*

INTRODUCTION

Temperature is one of the most important physical factors regulating bacterial physiology. It influences enzymatic activity, membrane fluidity, DNA replication, and gene expression, all of which affect bacterial growth, survival, and antimicrobial susceptibility. Consequently, temperature plays a significant role in the development and expression of antimicrobial resistance (AMR). Based on their optimal growth

temperature, bacteria are classified as psychrophiles (<20°C), psychrotrophs (20–30°C), mesophiles (20–45°C), thermophiles (45–80°C), and hyperthermophiles (>80°C).[1] Each group possesses unique structural and physiological adaptations. Psychrophiles contain unsaturated and branched-chain fatty acids that maintain membrane fluidity at low temperatures, whereas thermophiles possess saturated membrane lipids and heat-stable enzymes. Mesophiles, including most clinically important pathogens, are adapted to approximately 37°C but can survive temperature fluctuations during fever or cold storage.[2]

Temperature stress induces physiological responses that influence antibiotic susceptibility. Heat shock activates the sigma factor σ32 (rpoH), leading to the production of molecular chaperones such as DnaK and GroEL/GroES that protect proteins from thermal damage. Conversely, cold shock stimulates the production of CspA-family proteins that stabilize RNA and maintain cellular functions.[3] These responses modify membrane permeability, efflux pump activity, and the expression of resistance genes. Several mechanisms explain the relationship between temperature and AMR. Temperature-dependent changes in membrane lipid composition alter antibiotic penetration into bacterial cells. Moriuchi et al. (2024) demonstrated that *Escherichia coli* rapidly adjusts membrane lipid composition through the temperature-regulated FabI and FabB pathway following thermal stress. Similarly, Hurton et al. (2025) reported reduced efflux pump activity in *Acinetobacter junii*, *Bacillus cereus*, and *Enterobacter cloacae* at both 7°C and 43°C compared with 36°C, suggesting that thermal stress can influence intracellular antibiotic accumulation. In addition, thermal stress activates global regulatory systems such as RpoS, which provide cross-protection against environmental stress and indirectly affect antimicrobial resistance.[4]

The clinical importance of temperature-associated resistance is increasingly recognized. Fever (38–41°C) can alter bacterial mutation rates and resistance development. Van Eldijk et al. (2024) reported that elevated temperatures increased the frequency of resistance mutations against ciprofloxacin, rifampicin, and ampicillin in *E. coli*.[5] Likewise, prolonged refrigeration has been shown to increase quinolone and aminoglycoside resistance in *Staphylococcus aureus* through overexpression of the *norA* efflux pump and mutations in the *grlA* gene. At the population level, MacFadden et al. (2018) analyzed 1.6 million bacterial isolates from 28 countries and found a positive association between increasing ambient temperature and antibiotic resistance in *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.[6]

Despite increasing evidence, most previous studies have investigated single bacterial species or laboratory-induced temperature changes, while direct comparisons among bacteria naturally adapted to different thermal environments remain limited. Therefore, this study compares the antimicrobial susceptibility profiles of bacteria isolated from cold (4°C), moderate (25–37°C), and hot (50–60°C) environments using the Kirby–Bauer disk diffusion method.[7]

Understanding the influence of temperature on antimicrobial susceptibility is important for clinical microbiology, food safety, and environmental health. Most available studies focus on individual bacterial species or specific resistance mechanisms, with limited comparative data on bacteria adapted to different thermal environments. Furthermore, local research on temperature-associated AMR is scarce.[8]

Bacteria isolated from refrigerated foods, febrile patients, or high-temperature industrial settings may exhibit different resistance phenotypes than those tested under standard laboratory conditions. Evaluating these differences will improve our understanding of temperature-associated AMR and support more accurate susceptibility testing, antibiotic stewardship, food safety practices, and AMR surveillance.

Literature Review:

In the existing literature, several studies have investigated the effect of temperature on antibiotic resistance from biophysical, biological, and epidemiological perspectives. The following review summarizes the theoretical basis of temperature-

dependent antimicrobial susceptibility and the available experimental evidence.

The CLSI/EUCAST guidelines recommend incubating clinically significant bacterial isolates at 35 ± 2°C for 16–20 hours, reflecting normal human body temperature.13 However, many environmental bacteria require lower incubation temperatures (18–28°C) for optimal growth. Smith and Kronvall (2014) analyzed 141 disc diffusion datasets and reported that the precision of disc diffusion results decreased as incubation temperature fell from 35°C to 22°C, whereas MIC results remained largely unaffected.[9]

A direct study evaluating *Escherichia coli* susceptibility at 25°C, 30°C, 37°C, and 44°C found significant differences in inhibition zone diameters for most antibiotics, with maximum variations of 7–8 mm between the lowest and highest temperatures. However, susceptibility results at 30°C and 37°C were not significantly different, indicating that small temperature changes within the physiological range have minimal impact on susceptibility interpretation.[10]

Al-Nabulsi et al. (2015) investigated the effect of environmental stress on the antibiotic susceptibility of *C. sakazakii* and found that prior exposure to cold stress altered the bacterium's response to different antibiotic classes, demonstrating that environmental conditions can influence antimicrobial susceptibility.[11]

The bacterial cell membrane plays a vital role in antimicrobial susceptibility as it regulates the entry of most antibiotics. Membrane fluidity is strongly influenced by temperature and is maintained through homeoviscous adaptation, which involves changes in fatty acid composition, acyl chain length, and branched-chain lipids. Low temperatures decrease membrane fluidity and permeability, whereas high temperatures increase membrane fluidity and may compromise membrane stability.[12]

Moriuchi et al. (2024) reported that *Escherichia coli* regulates the balance between saturated and unsaturated fatty acid synthesis through the temperature-dependent activity of the FabI and FabB enzymes.This rapid homeoviscous adaptation allows bacteria to adjust membrane composition within a single generation following temperature stress, thereby influencing membrane permeability and antibiotic uptake.[13]

The effectiveness of antibiotics is strongly influenced by temperature due to its effect on membrane fluidity. β-lactam antibiotics enter bacterial cells through porins and act by binding to penicillin-binding proteins; therefore, reduced membrane fluidity at low temperatures can slow antibiotic diffusion and increase minimum inhibitory concentrations. Koga also noted that temperature-driven lipid remodeling is a universal and evolutionarily conserved mechanism across bacteria and archaea.[14]

Efflux pumps are membrane-bound protein complexes that actively expel a wide range of antimicrobial compounds from bacterial cells before they reach inhibitory concentrations. They represent one of the most important mechanisms of both intrinsic and acquired antibiotic resistance in Gram-positive and Gram-negative bacteria.[15]

Efflux pump activity is energy-dependent, relying on the proton motive force, and is therefore strongly influenced by temperature. Hurton et al. (2025) examined efflux activity in *Acinetobacter junii*, *Bacillus cereus*, and *Enterobacter cloacae* using the agar–ethidium bromide cartwheel assay and found increased ethidium bromide accumulation at both 7°C and 43°C compared with 36°C, indicating reduced efflux activity at temperature extremes. This was associated with increased antibiotic sensitivity, including higher meropenem susceptibility at elevated temperature in *Bacillus cereus*.[16]

Cold stress also significantly affects efflux-mediated resistance. In *Staphylococcus aureus*, prolonged exposure to 4°C and −20°C increased MIC values for quinolones and aminoglycosides by at least four-fold compared to controls. This was linked to more than 50-fold overexpression of the *norA* efflux pump gene and mutations in the *grlA* target gene, reducing quinolone binding. These findings indicate that cold environments can induce both physiological and genetic changes that enhance resistance.[17]

Dawan and Ahn (2022) further emphasized that thermal stress influences multiple resistance pathways simultaneously by regulating global stress responses, including efflux pump expression, SOS

activation, and horizontal gene transfer. Temperature therefore acts as a multifactorial driver of antimicrobial resistance rather than a single isolated factor.[18] The heat shock response involves rapid transcriptional reprogramming when temperatures exceed the optimal range and is mainly controlled by the sigma factor σ32 (RpoH) in *E. coli*. This response induces molecular chaperones such as DnaK and the GroEL/GroES system, which maintain protein folding and cellular proteostasis under thermal stress.[19]

Meinen et al. (2023) reviewed antimicrobial resistance trends in Germany and Europe in the context of climate change and found that rising environmental temperatures are associated with increased prevalence and spread of antibiotic-resistant bacteria. This relationship is explained by temperature-dependent effects on bacterial growth rates, horizontal gene transfer, and shifts in microbial communities toward more intrinsically resistant organisms. The authors further emphasized that temperature acts as a continuous environmental selection pressure that shapes bacterial resistance profiles even before clinical exposure, meaning that environmental isolates reflect their long-term thermal adaptation.[20]

Rodríguez-Verdugo et al. (2020) similarly highlighted that temperature must be considered when predicting both individual bacterial responses and community-level dynamics of resistance genes. They identified growth rate, virulence, and especially horizontal gene transfer as key temperature-sensitive processes, with increased rates of conjugation and transformation observed at higher temperatures.[21] Supporting this, Li et al. (2022) analyzed CHINET surveillance data in China and reported a positive independent association between higher regional temperatures and increased carbapenem resistance in *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.[22]

Overall, the literature strongly supports temperature as a key determinant of antimicrobial susceptibility through multiple interconnected mechanisms, including membrane lipid remodeling, efflux pump regulation, stress-response activation, mutation rates, and horizontal gene transfer. However, further research is needed to understand how these mechanisms interact to produce distinct susceptibility phenotypes across different thermal environments.

Material and Methadology

This study employed a controlled comparative experimental design to investigate how thermal environment influences antimicrobial susceptibility. A total of 150 bacterial isolates were collected via sterile swabs from three distinct thermal groups: cold surfaces (4°C, n=50), moderate-temperature surfaces (25–37°C, n=50), and high-temperature surfaces (50–60°C, n=50) at Sheikh Zayed Medical University, Pakistan. Isolates were processed within two hours in BHI broth and cultured on nutrient and blood agar at their respective isolation temperatures. Pure cultures were identified using Gram staining and biochemical tests, while antimicrobial susceptibility was assessed via the Kirby-Bauer disk diffusion method on Mueller-Hinton agar following CLSI guidelines. Bacterial counts were determined through serial dilution and colony counting, with each sample plated in duplicate and averaged over three replicates. Biofilm formation was evaluated spectrophotometrically at OD570 after 24 and 48 hours of incubation in 96-well plates. The study adhered strictly to WHO and institutional biosafety protocols, excluding BSL-3/BSL-4 pathogens, contaminated samples, non-viable cultures, and specimens with unverified thermal history.

All experimental procedures were conducted in certified biosafety cabinets with appropriate personal protective equipment, and biological waste was autoclaved at 121°C and 15 psi for 30 minutes prior to disposal. Data were systematically recorded using a pre-coded proforma that captured sample identification, colony characteristics, biochemical profiles, CFU/mL values, antimicrobial susceptibility interpretations, and biofilm optical density readings. Ethical approval was obtained from the institutional review board, as the study exclusively utilized environmental bacteria from inanimate surfaces with minimal pathogenicity (BSL-1/BSL-2). Suspected high-risk isolates were immediately autoclaved and discarded. The four-month study

employed non-probability convenience sampling, ensuring rigorous standardization across all thermal groups to enable meaningful comparisons of resistance patterns, biofilm production, and growth characteristics across different temperature regimes.

RESULTS

A total of 150 bacterial isolates were used in this experiment. All 50 isolates for each of three thermally-defined ecological groups were obtained from various environmental sources including 4°C (Cold) and 25-37°C (Moderate), 50-60°C (Hot). All isolates were taken from 26 locations ranging from fridge surfaces and cold chain equipment to computer keyboards, lab bench tops, autoclave surfaces, and industrial hot water piping. All bacterial samples underwent Gram staining, biochemical testing, and antibiotic sensitivity testing to 25 antibiotics representing 9 drug classes via Kirby-Bauer method. Below are presented the results obtained in these experiments.

Frequency Analysis

All isolates were evenly distributed between Gram-positive (n=76; 50.7%) and Gram-negative (n=74; 49.3%) categories providing an equal ground for comparison among groups. Also, all three temperature categories included exactly 50 isolates each thus excluding possible effects associated with imbalanced sample size. MDR was a prevailing bacterial strain phenotype with MDR strains detected in 126 of 150 isolates (84%). The Cold group had a near-complete MDR saturation (98%; 49/50). However, the prevalence of MDR strains within the Moderate and Hot groups was relatively lower at 74% and 80% respectively. MDR strain prevalence between temperature categories varied significantly (χ^2=11.607, p=0.003). The detailed breakdown of all samples is provided in Table 5.1.

Table 5.1: Sample Composition, MDR Status, and Resistance Burden by Temperature Group (N=150)

Parameter	Cold	Moderate	Hot	Total
n	50	50	50	150
Gram-Positive, n (%)	21 (42%)	9 (18%)	46 (92%)	76 (50.7%)
Gram-Negative, n (%)	29 (58%)	41 (82%)	4 (8%)	74 (49.3%)
MDR, n (%)	49 (98%)	37 (74%)	40 (80%)	126 (84%)
Non-MDR, n (%)	1 (2%)	13 (26%)	10 (20%)	24 (16%)
Mean Drugs Resistant ± SD	6.52 ± 1.98	5.10 ± 2.60	4.58 ± 1.62	5.40 ± 2.15
Mean Drugs Susceptible ± SD	10.26 ± 1.90	11.30 ± 3.00	14.12 ± 1.87	11.89 ± 2.85
ESBL/Carbapenemase Suspected, n	17	28	4	49

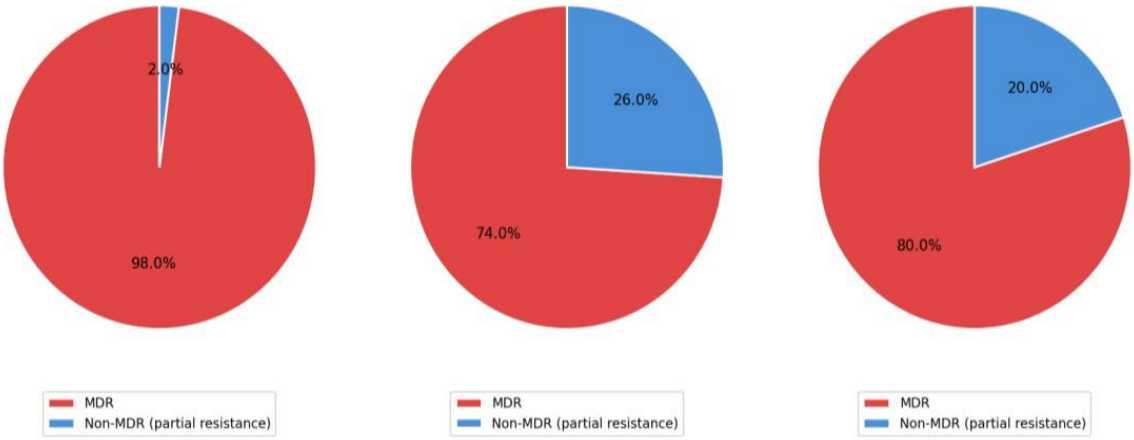

Figure 1: MDR Status Distribution by Temperature Group Cold 98%, Moderate 74%, Hot 80%

As seen above, there was a highly pronounced discrepancy in Gram stain results (χ^2=57.023, p<0.001). In particular, the Cold group consisted of mostly (58%) Gram-negative bacteria, the Moderate group – of even higher proportion (82%), while Gram-positive bacteria dominated in the Hot group (92%). This finding is expected in view of known bacteria ecology with respect to different temperatures.

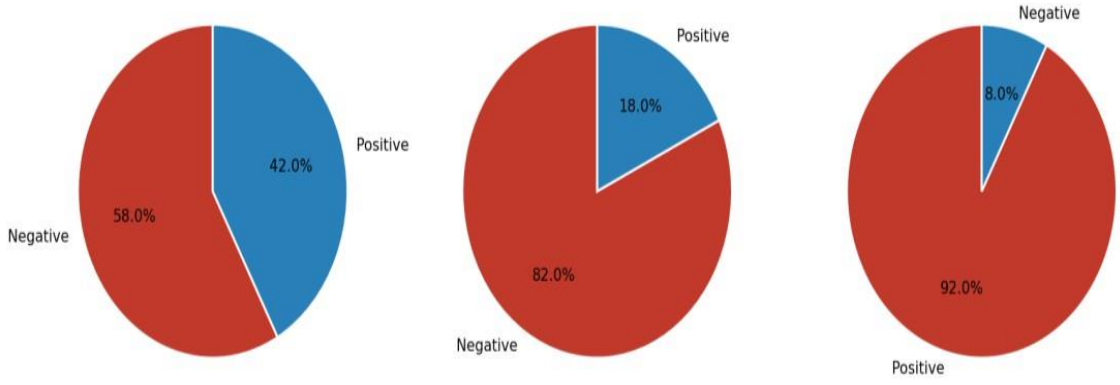

Figure 2: Gram Stain Distribution by Temperature Group (χ^2=57.02, p<0.001) Frequency Analysis with Respect to Each Variable

We identified thirty-four different kinds of bacteria. Bacteria that thrive in temperatures are mainly found in certain places, including Bacillus licheniformis, Brevibacillus brevis, Clostridium thermocellum and Geobacillus stearothermophilus. The Moderate group has bacteria that cause disease and are important in medicine, such as Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus mirabilis. You can see the types of bacteria in Figure 3.

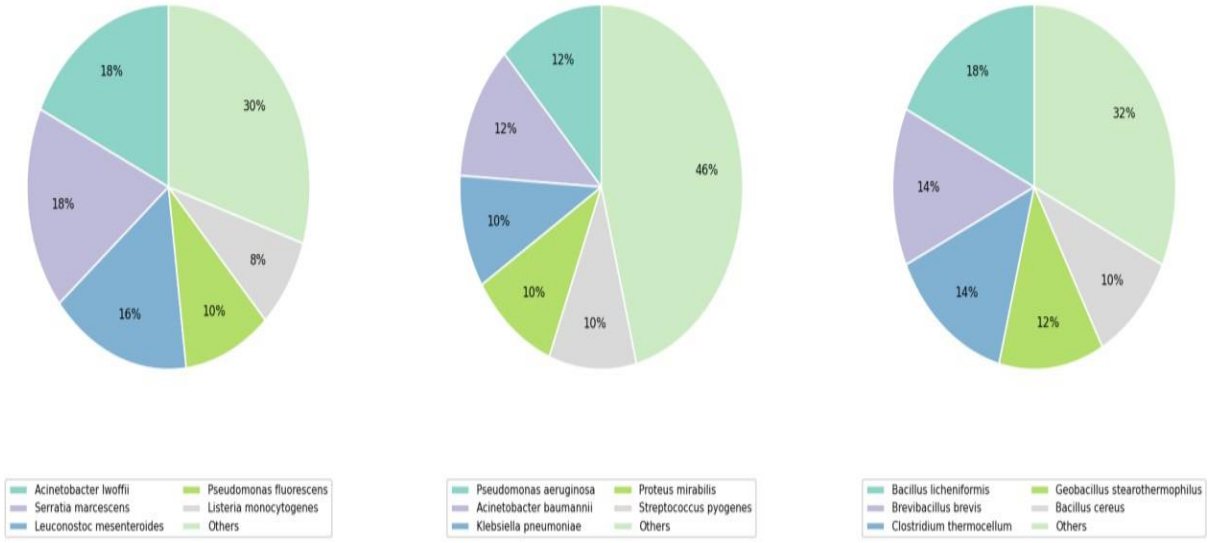

Figure 3: Bacterial Species Distribution Across Temperature Groups (Top 5 + Others per group)

Gram Stain and Cell Morphology

Most of the bacteria are rod shaped. In the groups Cold had 30 out of 50 that were rod shaped Moderate had 35 out of 50 and Hot had 47 out of 50. The Hot samples had the number of round bacteria only 3 out of 50. There were no bacteria that were shaped like both rods and spheres in the Hot samples. Some were found in the Cold samples 9 out of 50 and in the Moderate ones 8, out of 50. The near-total dominance of bacillary forms in the Hot group is explained by the fact that its composition is almost entirely made up of thermophilic Bacillaceae bacteria; the presence of different species types accounts for the morphological variety seen in the Cold and Moderate groups.

Biochemical Characterization

Biochemical testing revealed significant differences between the groups.

Oxidase positivity was most frequent in the Hot group (64%) and least frequent in the Cold isolates (16%) (χ^2=31.92, p<0.001). Catalase activity was present in most of the isolates of all the groups but it was the highest in the ones from the Moderate group (90%) and the Hot group (86%). Voges-Proskauer positive was mainly seen in the Hot isolates (72%), whereas the percentage of samples utilizing citrate changed from Cold (54%) to Hot (80%) gradually. Urease and indole tests also greatly varied among the groups.

Table 5.2: Biochemical Test Positivity by Temperature Group with Chi-Square Results

Biochemical Test	Cold (+/50)	Mod. (+/50)	Hot (+/50)	χ^2	p-value
Oxidase Test	8 (16%)	10 (20%)	32 (64%)	31.92	<0.001
Catalase Test	33 (66%)	45 (90%)	43 (86%)	10.60	0.005
Urease Test	15 (30%)	23 (46%)	9 (18%)	9.17	0.010
Indole Test	6 (12%)	6 (12%)	0 (0%)	6.52	0.038
Methyl Red (MR)	11 (22%)	21 (42%)	14 (28%)	4.95	0.084 (ns)
Voges-Proskauer (VP)	26 (52%)	12 (24%)	36 (72%)	23.26	<0.001
Citrate Utilization	27 (54%)	34 (68%)	40 (80%)	7.70	0.021

Growth Rate by Group

At cold temperatures, 60% of isolates grew slowly, while 66% of moderate temperature isolates were rapid growers. Conversely, the hot group was characterized by a more heterogeneous distribution of slow, moderate, and rapid growthers.

ESBL and Carbapenemase Screening

Based on their phenotype, 17.3% of all isolates were considered as potential carbapenemaseproducers, and 7.3% as potential ESBL producers. Surprisingly, the Cold group had the highest number of these resistant phenotypes, although the differences among groups were not found to be significant statistically (χ^2=3.795, p=0.434).

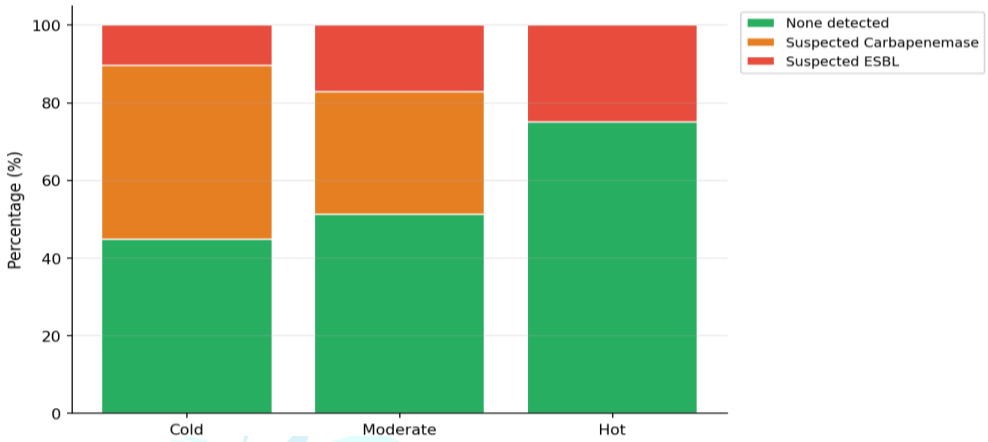

Figure 4: ESBL/Carbapenemase Screening Results by Temperature Group (%)

Analytical Calculations

Resistance phenotype data were analyzed using Pearson chi-square tests for categorical resistance classifications and Kruskal-Wallis test for the continuous drug-resistance count variable. Both analyses confirmed temperature group as a statistically significant determinant of antimicrobial susceptibility profile.

Impact of Temperature on Antibiotic Resistance

Significant differences in the amount of drugs were observed for the groups based on the results of Kruskal-Wallis test (H=24.161; p<0.001). Isolates obtained from cold conditions demonstrated the highest resistance rate, whereas Hot isolates appeared to be susceptible to the maximum number of tested antibiotics.

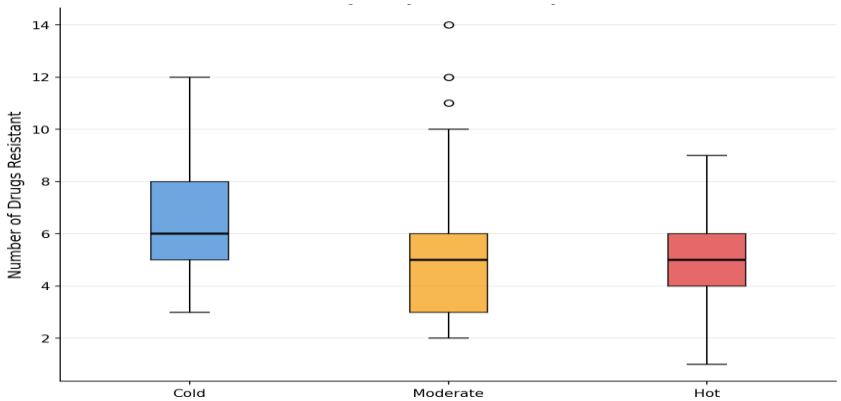

Figure 5: Drug Resistance Count Distribution by Temperature Group (Kruskal-Wallis H=24.16, p<0.001)

Table 5.3 presents resistance rates for all 25 antibiotics by group with chi-square statistics. Figures 3, 7, 8, and 9 provide visual summaries.

Table 5.3: Resistance Rates (%) and Chi-Square Statistics for All 25 Antibiotics by Temperature Group

Antibiotic	Cold R%	Moderate R%	Hot R%	χ^2 (p-value)
Ampicillin	44.0%	60.0%	18.0%	18.96 (p=0.001)
Amoxicillin-Clavulanate	38.0%	40.0%	10.0%	14.89 (p=0.005)
Piperacillin-Tazobactam	56.0%	48.0%	42.0%	2.52 (p=0.641)
Cefazolin	74.0%	52.0%	70.0%	15.49 (p=0.004)
Cefoxitin	22.0%	38.0%	44.0%	8.21 (p=0.084)
Ceftriaxone	42.0%	42.0%	32.0%	2.07 (p=0.722)
Cefepime	44.0%	28.0%	22.0%	7.75 (p=0.101)
Imipenem	20.0%	10.0%	2.0%	9.59 (p=0.048)

Resistance depended on the antibiotic group as follows:

1. Aminoglycoside resistance was mainly characteristic for the Cold group (resistance rates 68% for amikacin, 58% for tobramycin, 46% for gentamicin; all p<0.001).
2. Fluoroquinolones resistant isolates were mainly observed in the Cold group. The highest rate belonged to the ciprofloxacin resistance (28%). In addition, resistance to levofloxacin was found only in Cold isolates.
3. Resistance to macrolides and linezolid were characterized by the high rates in Hot group. They comprised 28% (erythromycin), 38% (azithromycin) and 60% (linezolid) correspondingly (all p<0.001).
4. Vancomycin resistance occurred in 30% of Cold isolates.

Higher carbapenem resistance rates were revealed for Cold isolates compared with the Hot ones. Isolates resistant to piperacillin-tazobactam and ceftriaxone were highly abundant among the studied isolates without any statistically significant differences, which indicates the presence of ecological resistance independent of the origin temperature.

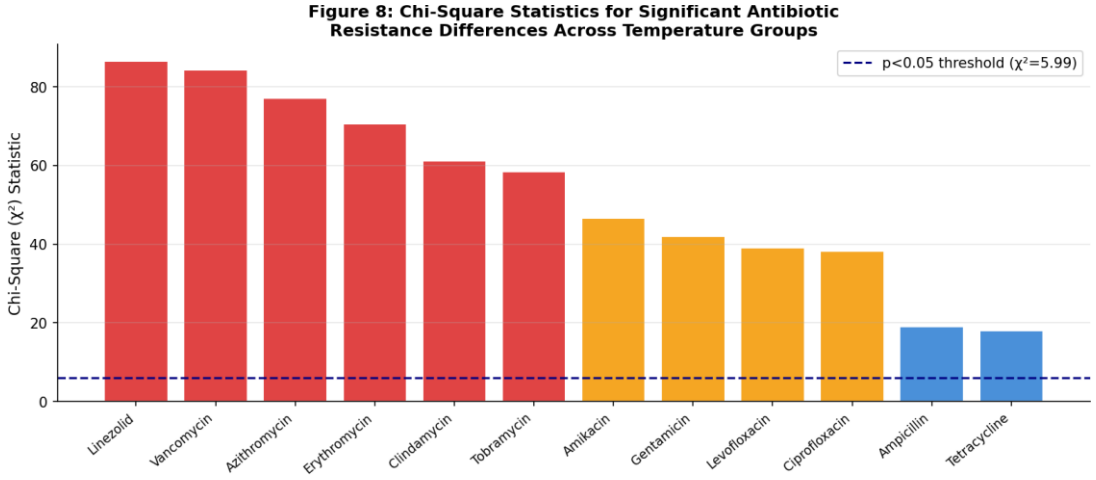

Figure 7: Chi-Square Statistics for Significant Resistance Differences (p<0.05 threshold shown)

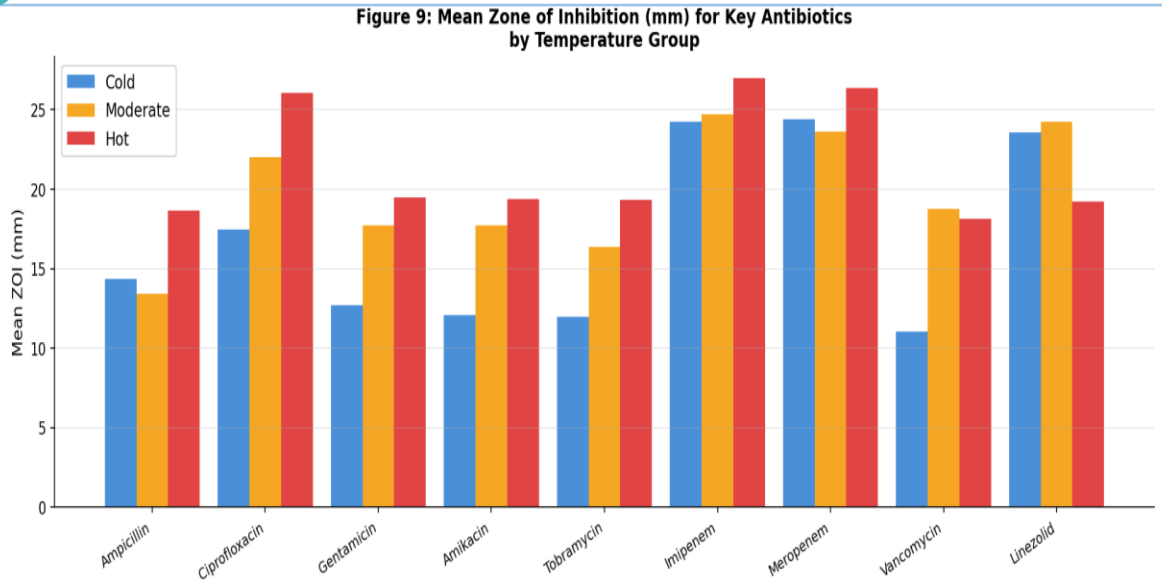

Figure 8: Mean Zone of Inhibition (mm) for Key Antibiotics by Temperature Group Evaluation of Susceptibility Perceptions Across Temperature Groups

Measuring the zone of inhibition which's the clear area around where an antibiotic was applied worked well. It helped check how resistant bacteria really were. The bacteria in the group usually had larger clear zones. This was especially true for fluoroquinolones and aminoglycosides. These bacteria were actually more susceptible to fluoroquinolones and aminoglycosides.

Table 5.4: Resistance Pattern Summary by Drug Class and Temperature Group

Drug Class	Cold	Moderate	Hot	Pattern	Significance
Aminoglycosides	57%	15%	5%	Cold dominant	p<0.001
Fluoroquinolones	27%	5%	0%	Cold dominant	p<0.001
Macrolides	0%	0%	33%	Hot dominant	p<0.001
Lincosamide / Oxazolidinone	2%	2%	38%	Hot dominant	p<0.001
Glycopeptides (Vancomycin)	30%	0%	12%	Cold dominant	p<0.001
1st Gen Cephalosporins	74%	52%	70%	Moderate lowest	p=0.004
Carbapenems	19%	17%	2%	Hot lowest	p=0.029–0.048
Beta-lactam combos	47%	44%	26%	Hot lowest	p=0.001–0.005
Tetracyclines	5%	22%	11%	Moderate dominant	p=0.001
Nitrofurantoin	16%	34%	8%	Moderate dominant	p=0.018
Colistin	8%	6%	0%	Cold/Mod dominant	p=0.001

Here are some specific resistance patterns we found:
Cold bacteria: They resisted aminoglycosides and fluoroquinolones.
Hot bacteria: They resisted macrolides, clindamycin and linezolid.
Moderate Bacteria: They showed a mix of resistance. They had trouble with tetracycline and nitrofurantoin.

DISCUSSION

The results indicate that isolation temperature significantly affects the antibiotic susceptibility of the isolates, which was demonstrated through chi-square tests and Kruskal-Wallis analysis ($H=24.16$, $p<0.001$). Resistance to aminoglycosides and fluoroquinolones was the most common among bacteria isolated from cold environments. This finding is supported by the results of other studies according to which cold temperature stress can result in increased pump activity and mutation rates, thereby increasing the risk of multidrug resistance development. Also, the organisms isolated from the Cold group, especially the Serratia and Acinetobacter genera, are characterized by the presence of natural aminoglycoside resistance genes. In case of the Hot group of bacteria, there was resistance to macrolides and linezolid. This is consistent with the MLSB mechanism of resistance typical of Gram-positive thermophilic bacteria such as Bacillus and Clostridium genera. It should be noted that bacteria from these groups do not have antibiotic resistance because of their natural ability to exhibit ribosomal resistance without hospital exposure to antibiotics.

The prevalence of Multidrug Resistant (MDR) isolates in the Cold group (98%) suggests simultaneous selection of multiple resistant pathways by cold temperature stress. This has important food safety concerns because it can cause colonization of refrigerators by MDR bacteria.While the Hot group also exhibited a high level of MDR, its resistance pattern was pharmacologically more limited and only primarily affected macrolides and oxazolidinones. On the other hand, the Moderate group exhibited the highest variation in resistance, which is consistent with the diversity of microorganisms found on room-temperature environmental surfaces.

Consistently high resistance to ceftriaxone and piperacillin, tazobactam in all the groups indicate that resistance to these widely used empirical therapies is ecologically very broad and cannot be accounted for by thermal origin alone.

There are two main weaknesses that must be kept in mind. Firstly, the variation in species between the groups may play a role in the resistance patterns seen in addition to thermal adaptation per se. Secondly, the study used phenotypic susceptibility testing without molecular verification of resistance mechanisms.

Still, the results are both statistically significant and biologically feasible. Hence, temperature must be regarded as a major ecological factor affecting antimicrobial resistance.

CONCLUSION

This work demonstrates that bacteria from different thermal environments have profoundly different resistance to antimicrobials. Isolates from cold environments had the highest resistance levels and MDR frequency, especially to aminoglycosides and fluoroquinolones. Bacteria from hot environments had selected resistance to macrolides and oxazolidinones, while Moderate isolates had the highest resistance diversity. Resistance to 12 out of 25 antibiotics significantly differed between temperature groups. Even total resistance burden varied significantly according to the Kruskal-Wallis test ($H=24.16$, $p<0.001$). These results mean a lot to the fields of microbiology, infection control, food safety, and antibiotic stewardship. Susceptibility testing and empirical antibiotic therapy should take into account the temperature where bacteria originated.

RECOMMENDATIONS

- Reporting of antimicrobial susceptibility should consider the thermal origin of isolates. Isolates from cold environments should be emphasized in aminoglycoside and fluoroquinolone resistance screening.
- Isolates from hot environments should be tested specifically for resistance to the macrolide and linezolid groups.
- Infection-control programs in hospitals should incorporate thermal-zone surveillance. Refrigerated environments should be acknowledged in food-safety regulations as possible reservoirs of MDR.
- Resistance mechanism elucidation should be the focus of future research using isogenic strains and whole-genome sequencing.
- National AMR surveillance programs need to document the temperature environment

of isolates separately.

STUDY LIMITATIONS

- Resistance could differ as a result of different proportions of species in the different groups.
- We performed only phenotypic resistance testing; no molecular confirmation was conducted.
- Since a convenience sample was used, generalizability is limited.
- The low sample size might restrict the power to find minor differences.
- The temperature conditions during laboratory incubation were different from those in natural environmental temperatures.
- There was no molecular confirmation of ESBL and carbapenemase screening.
- The study was a single-institution study, so the results cannot be generalized geographically.
- Not all antibiotics were suitable for the entire array of organisms; this minorly affected the comparisons.

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