



JOURNAL OF COMMUNITY MEDICINE AND PRIMARY HEALTH CARE

ORIGINAL ARTICLE

PREVALENCE OF MULTIDRUG-RESISTANT PHENOTYPES AND ANTIMICROBIAL RESISTANCE PATTERNS IN COMMUNITY-ACQUIRED RESPIRATORY PATHOGENS IN THE PERUVIAN HIGHLANDS DURING THE COLD SEASON

Gómez P, Huaranga M, Custodio M.

¹Faculty of Human Medicine, Universidad Nacional del Centro del Perú.

KEYWORDS

Altitudinal Stress;

Antimicrobial
Stewardship,

Drug Resistance
Bacterial,

Health Services
Accessibility,

Respiratory Tract
Infections,

Self-Medication.

ABSTRACT

Background: The Andean cold season (*friaje*) increases the incidence of acute respiratory infections (ARIs), thereby increasing the risk of antimicrobial resistance (AMR) due to inappropriate antibiotic use. This study aimed to determine the prevalence of multidrug-resistant phenotypes and characterize AMR patterns in community-acquired respiratory pathogens among residents of the Peruvian highlands during the 2025 cold season.

Methods: An observational cross-sectional study was conducted in Huancayo, Peru (3,250 masl). Through non-probabilistic convenience sampling, 100 oropharyngeal swabs were collected from symptomatic patients. Isolates were identified using selective media, and susceptibility was assessed via the Kirby-Bauer disk diffusion method per CLSI M100 guidelines. Data on self-medication and treatment adherence were gathered through structured questionnaires. Statistical analyses, including Chi-square and Fisher's exact tests ($p < 0.05$), were performed using IBM SPSS v.29.0. The protocol was approved by the UNCP Ethics Committee (Code: CEI-UNCP-N° 064-2025).

Results: Mixed microbiota was identified in 68% of samples. Primary pathogens included *Haemophilus influenzae* (11%), *Staphylococcus aureus* (10%), and *Streptococcus pneumoniae* (4%). *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated, posing a risk for multidrug resistance (MDR). Although high rates of self-medication (51.9%) and treatment non-adherence (48.1%) were observed among carriers of resistant isolates, no statistically significant association with AMR was found ($p > 0.05$).

Conclusion: The detection of key respiratory pathogens with high AMR potential during the Andean *friaje* represents a significant public health challenge. These findings underscore the necessity for localized microbiological surveillance and targeted educational interventions to optimize antibiotic stewardship in vulnerable high-altitude populations.

Article History:

Date received:31/03/2026

Date accepted:19/06/2026

Date Published:01/08/2026

Correspondence to:

Maria Custodio

mcustodio@uncp.edu.pe

INTRODUCTION

Acute respiratory infections (ARIs) remain a leading cause of global morbidity and mortality, disproportionately affecting vulnerable populations, including children, older adults, and individuals with chronic comorbidities.^{1, 2} In developing nations, ARI incidence exhibits marked seasonality, peaking during low-temperature periods; this trend is particularly pronounced during the Andean *friaje* phenomenon.^{3, 4} This seasonal surge is driven by a multifactorial interplay, including impaired upper respiratory immune responses, overcrowding in poorly ventilated spaces, and prolonged exposure to extreme environmental conditions. Within this epidemiological framework, there is heightened circulation of critical bacterial pathogens, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.^{5, 6} Recent data indicate alarming resistance patterns among these taxa toward frontline antibiotics, including penicillin, amoxicillin, ceftriaxone, erythromycin, and azithromycin.⁷ Antimicrobial Resistance (AMR) represents a preeminent threat to 21st-century public health, jeopardizing the efficacy of standard treatments and increasing the risk of severe complications.^{8, 9} In 2019, an estimated 4.95 million deaths globally were associated with resistant bacterial infections, with respiratory tract involvement serving as a primary driver.^{10, 11} In most Latin American settings, therapeutic decisions are predominantly empirical due to the scarcity of rapid susceptibility testing or significant diagnostic delays in public healthcare facilities. Furthermore, the unregulated availability and self-prescription of macrolides have likely accelerated the emergence of resistant phenotypes.¹² The World Health Organization (WHO) has designated AMR as one of the top ten global health threats. This phenomenon is no longer confined to nosocomial environments; it has permeated rural communities where microbiological diagnostic capacity is negligible and indiscriminate antibiotic use remains prevalent.¹³ International reports indicate that previously susceptible organisms, such as *H. influenzae*, now demonstrate multidrug-resistant

(MDR) profiles in up to 30% of isolates within similar community-based cohorts.¹⁴ In Peru, the cold season significantly affects regions above 3,000 meters above sea level (masl), leading to a seasonal spike in ARI cases, with medical consultations increasing by up to 40% during the peak winter months.¹⁵ This heightened healthcare demand unfolds in a high-risk environment characterized by intense selective pressure from inappropriate antimicrobial use. Persistent practices, such as self-medication, over-the-counter sales, and empirical prescribing without etiological confirmation, facilitate the dissemination of resistant strains, particularly in areas highly susceptible to the *friaje*.¹⁶ Despite this, the lack of decentralized microbiological surveillance in Peru remains a critical barrier to the early detection and containment of AMR. Although the province of Huancayo (Junin) is highly vulnerable to extreme climatic shifts, there is a conspicuous absence of studies investigating the link between extreme temperatures and AMR development in respiratory pathogens. This lack of localized evidence obscures whether the seasonal context actively promotes the emergence of resistant strains, thereby limiting the efficacy of local healthcare responses and the optimization of clinical regimens. This study addresses this knowledge gap by characterizing, for the first time, AMR patterns in ARI-causing pathogens within Andean rural communities during the *friaje*. The objective of this study was to determine the prevalence of multidrug-resistant phenotypes and characterize patterns of antimicrobial resistance among community-acquired respiratory pathogens identified in residents of the Peruvian highlands during the cold season.

METHODS

STUDY DESIGN: An observational, descriptive, cross-sectional study was conducted to characterize the prevalence and antimicrobial resistance (AMR) patterns of respiratory pathogens in the Peruvian Central Andes. The study was conducted during the cold season (*friaje*) of 2025 in the district of Huancayo, Junín, a high-altitude region at approximately 3,250 masl.

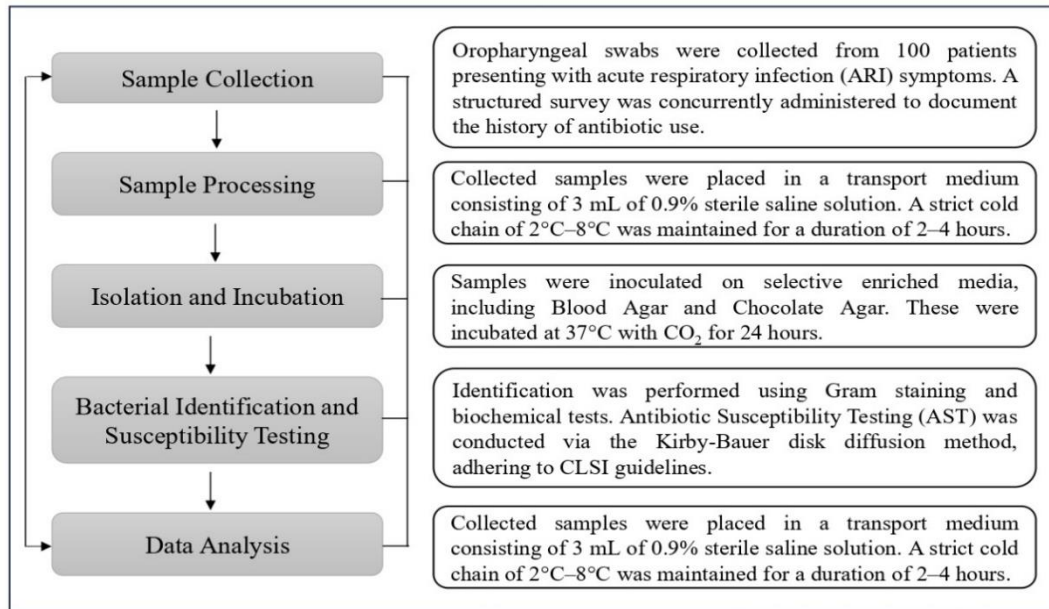


Figure 1: Methodological workflow for the collection, isolation, and antimicrobial susceptibility testing of bacterial samples from patients with acute respiratory infections.

This design facilitated a systematic mapping of the AMR landscape within a specific climatic and geographical context (Figure 1).

POPULATION AND ELIGIBILITY CRITERIA

The study population comprised residents of Huancayo presenting with acute respiratory infection (ARI) symptoms at primary healthcare centers. Given the operational complexities of the high-altitude study area, a non-probabilistic convenience sampling strategy was employed.

Inclusion criteria were: (i) permanent residents of Huancayo of any age or sex; (ii) clinical presentation of ARI symptoms (e.g., cough, fever, dyspnea); and (iii) provision of written informed consent by the participant or a legal guardian. Exclusion criteria included: (i) antibiotic therapy within the 14 days prior to sampling; (ii) current hospitalization; (iii) history of chronic respiratory diseases (e.g., active tuberculosis, cystic fibrosis); and (iv) refusal to participate.

SAMPLE COLLECTION AND CLINICAL DATA ACQUISITION

Through non-probability convenience sampling due to the limited flow of eligible patients during

the cold season, one hundred (100) oropharyngeal samples were collected. For each patient, a structured questionnaire documented clinical and epidemiological variables and antibiotic use history, including self-medication practices. Oropharyngeal swabs were obtained using standardized clinical techniques¹⁷ and immediately submerged in sterile saline solution as a transport medium.¹⁸ To ensure microbial viability, samples were transported to the laboratory within a 2 to 4-hour window under strict cold-chain conditions (2°C – 8°C) using thermal containers with refrigerant gels.

MICROBIOLOGICAL PROCESSING AND SUSCEPTIBILITY TESTING

Samples were manually homogenized to release adherent microorganisms. Inoculation was performed within a class II biological safety cabinet onto selective and enriched media, including defibrinated blood agar and chocolate agar, the latter to facilitate the growth of fastidious organisms.

Antimicrobial susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method.¹⁹ Bacterial inocula were adjusted to a 0.5

TABLE 1: Morphological, biochemical, and pathogenic characteristics of microorganisms isolated in ARIs from Andean population during the cold season.

Bacteria	Gram Stain	Morphology	Hemolysis	Identification Criteria	Clinical Relevance in ARI
<i>Haemophilus influenzae</i>	Gram-Negative	Bacillus Coccobacillus	Gamma (γ)	Non-hemolytic on blood agar. Opaque gray color on Chocolate agar.	Causative agent of pneumonia and chronic bronchitis.
<i>Staphylococcus aureus</i>	Gram-Positive	Cocci in clusters	Alpha (α) Beta (β)	Partial or total hemolysis on blood agar. Coagulase and Catalase positive.	Causative agents of pneumonia, abscesses, and bacteremia. Multidrug-resistant species are a nosocomial concern.
<i>Klebsiella pneumoniae</i>	Gram-Negative	Encapsulated bacillus	Gamma (γ)	Non-hemolytic on blood agar. Capsule production (mucoid colonies).	Causative agent of severe pneumonia. Multidrug-resistant species associated with hospital settings.
<i>Streptococcus pneumoniae</i>	Gram-Positive	Cocci in pairs / Diplococci	Alpha (α)	Partial hemolysis on blood agar. Greenish zone due to hemoglobin oxidation. Bile soluble	Causative agent of community-acquired pneumonia, otitis media, and meningitis.
<i>Pseudomonas aeruginosa</i>	Gram-Negative	Bacillus	Alpha (α) Beta (β) Gamma (γ)	Partial, total, or no hemolysis on blood agar. Green pigmentation Fruity odor	Opportunistic pathogen associated with nosocomial infections, pneumonia in cystic fibrosis patients, and bronchiectasis.
<i>Streptococcus viridans</i>	Gram-Positive	Cocci in chains	Alpha (α)	Partial hemolysis on blood agar. Bile insoluble	Oropharyngeal commensal agent.
<i>Streptococcus pyogenes</i>	Gram-Positive	Cocci in chains	Beta (β)	Complete hemolysis on blood agar. Bacitracin susceptible	Causative agents of pharyngitis and tonsillitis; less common in adult pneumonia.
<i>Fusobacterium nucleatum</i>	Gram-Negative	Fusiform bacillus	Gamma (γ)	Non-hemolytic on blood agar (Growth under anaerobic conditions)	Part of the normal oral flora. Associated with aspiration pneumonia and lung abscesses.

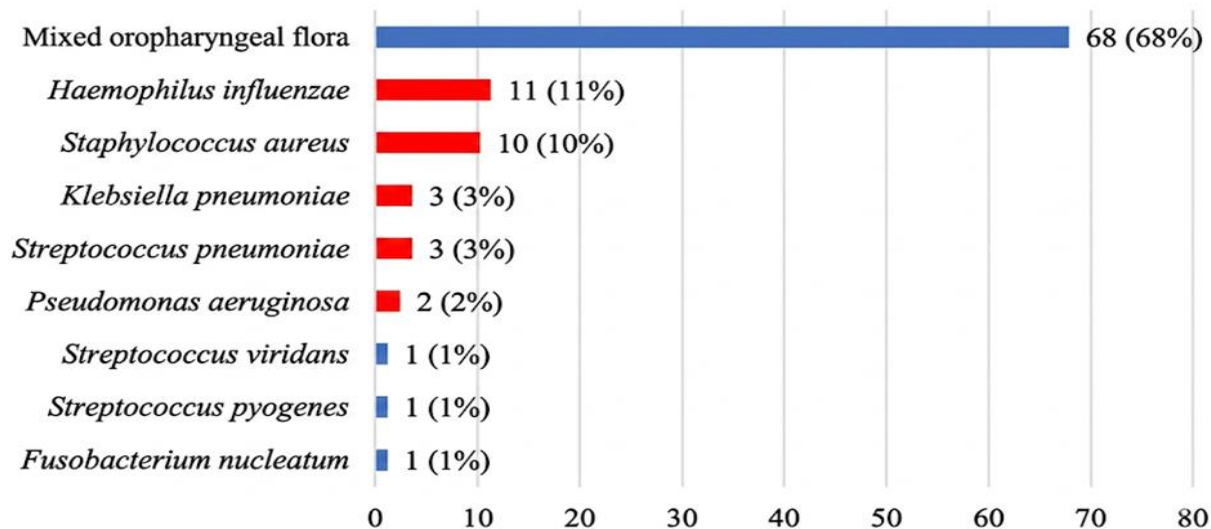


FIGURE 2: Distribution of bacterial isolates in acute respiratory infection samples during the cold season. Red bars indicate respiratory pathogenic bacteria. Blue bars represent non-pathogenic bacteria. X-axis values correspond to the percentage of isolates.

McFarland turbidity standard and inoculated onto Mueller-Hinton agar plates (supplemented where necessary for fastidious species). Following the application of specific antibiotic disks, plates were incubated at 37°C for 24 hours. Inhibition zone diameters were measured and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines to categorize isolates as Susceptible, Intermediate, or Resistant.²⁰ For *Staphylococcus aureus*, oxacillin and cefoxitin disks were used to identify methicillin-resistant phenotypes (MRSA), while third-generation cephalosporins were used to screen for potential extended-spectrum beta-lactamase (ESBL) production.

STATISTICAL ANALYSIS

Data were recorded in a dedicated database and underwent rigorous quality control to ensure integrity. Statistical analysis was performed using IBM SPSS version 29.0. Categorical variables – including sex, bacterial species, resistance profiles, and self-medication history – were expressed as absolute and relative frequencies. The association between clinical-epidemiological variables and microbiological resistance was assessed using the

Chi-square (X^2) test. Fisher's exact test was used for analyses in which expected cell frequencies were low ($n < 5$). Statistical significance was set at $p < 0.05$. Additionally, a multivariate binary logistic regression analysis was performed to control for potential confounders, including self-medication and incomplete antibiotic treatment, with antimicrobial resistance as the binary outcome. Adjusted odds ratios (aOR) and their 95% confidence intervals (95% CI) are reported.

ETHICAL CONSIDERATION

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the Universidad Nacional del Centro del Perú (Protocol code: CEI-UNCP-N° 064-2025). Written informed consent was obtained from participants prior to their participation in the study. Participation was voluntary, and participants could withdraw at any time if they felt uncomfortable with the interview and sampling process. The confidentiality of the collected data was guaranteed, ensuring that access to sensitive information was strictly limited to the research team.

TABLE 2: Antimicrobial susceptibility profiles of bacterial species isolated in ARI during cold season.

Bacterial species	Number of isolates	PS	AMP	CTX	CRO	CN	AMK	CIP	SXT	NOR	E	CAZ
<i>Haemophilus influenzae</i>	8	S	16.7	17	37.5	15	62.5	50	30	25	25	37.5
		I	16.7	0	25	25	12.5	12.5	25	12.5	0	12.5
		R	66.6	83	37.5	60	25	37.5	45	62.5	75	50
<i>Staphylococcus aureus</i>	6	S	0	33.3	50	66.6	66.7	66.7	50	15	15	16.7
		I	0	16.7	16.7	16.7	0	0	33.3	15	20	16.7
		R	100	50	33.3	16.7	33.3	33.3	16.7	70	65	66.6
<i>Streptococcus pneumoniae</i>	6	S	16.7	33.3	50	66.7	50	33.3	16.7	16.7	12.5	33.3
		I	16.7	16.7	16.7	0	0	16.7	16.7	16.7	29.5	33.3
		R	66.6	50	33.3	33.3	50	50	66.6	66.6	58	33.4
<i>Klebsiella pneumoniae</i>	5	S	0	0	15	20	40	40	0	0	0	0
		I	0	20	15	10	0	0	0	0	20	20
		R	100	80	70	70	60	60	100	100	80	80

PS: Antimicrobial Susceptibility Profiles; AMP: Ampicillin; CTX: Cefotaxime; CRO: Ceftriaxone; CN: Gentamicin; AMK: Amikacin; CIP: Ciprofloxacin; SXT: Sulfamethoxazole/Trimethoprim; NOR: Norfloxacin; E: Erythromycin; CAZ: Ceftazidime.

RESULTS

The distribution of bacterial isolates from acute respiratory infection (ARI) samples collected during the cold season exhibited significant heterogeneity (Figure 2). Mixed oropharyngeal flora was the predominant finding, accounting for 68% of the total isolates. This high prevalence suggests a substantial colonization rate within the study population or a primary viral etiology that may predispose individuals to bacterial co-infection. Among specific pathogens, *Haemophilus influenzae* was the most frequent isolate (11%), followed by *Staphylococcus aureus* (10%), *Klebsiella pneumoniae* (3%) and *Streptococcus pneumoniae* (3%). The identification of these primary etiologic agents, known for their potential to cause severe lower respiratory tract infections, underscores the critical need for continuous surveillance of local antimicrobial resistance (AMR) patterns. Other bacterial species, including *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *S. viridans*, and *Fusobacterium nucleatum*, were detected in marginal proportions (each representing < 3% of total isolates). This distribution profile

confirms the circulation of key bacterial pathogens for the clinical management of ARIs in rural Andean communities during periods of low temperatures.

FIGURE 2: Distribution of bacterial isolates in acute respiratory infection samples during the cold season. Red bars indicate respiratory pathogenic bacteria. Blue bars represent non-pathogenic bacteria. X-axis values correspond to the percentage of isolates. The identification of bacterial isolates from ARI samples was achieved by integrating Gram staining, colony morphology, and specific biochemical assays (Table 1). The taxonomic distribution was nearly balanced between Gram-positive cocci (51.5%) and Gram-negative bacilli (48.5%). Within the Gram-positive group, *Streptococcus* species were differentiated based on hemolysis patterns and biochemical susceptibility. Specifically, *S. pneumoniae* was identified by its characteristic diplococci morphology, alpha-hemolysis, and bile solubility, whereas *S. viridans* was confirmed by its alpha-hemolytic activity and bile insolubility.

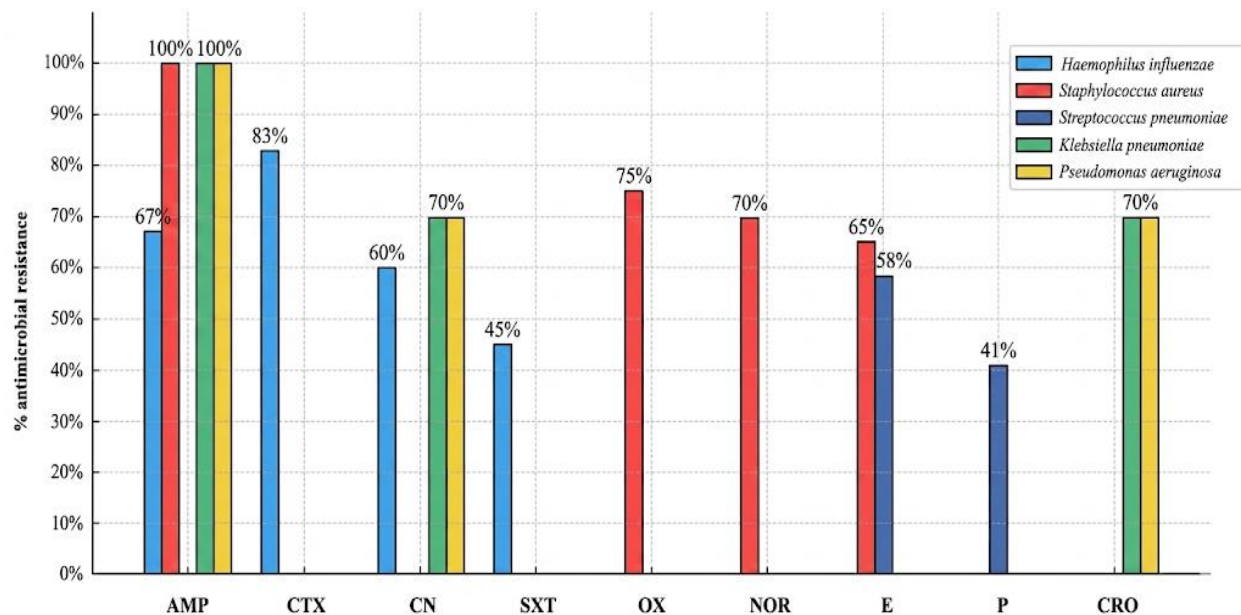


FIGURE 3: Antimicrobial resistance profile of isolated respiratory pathogens.

S. pyogenes was distinguished by beta-hemolysis and bacitracin susceptibility. Furthermore, *Staphylococcus aureus* was definitively characterized by its cocci-in-clusters arrangement and positive results for both coagulase and catalase tests. These Gram-positive pathogens carry significant clinical weight; *S. aureus* is associated with abscesses and the emergence of multidrug-resistant nosocomial strains, while *S. pneumoniae* remains a primary agent of community-acquired pneumonia. The Gram-negative group comprised *Haemophilus influenzae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. *H. influenzae*, a coccobacillus, was identified by its lack of hemolysis on blood agar and opaque gray colony growth on chocolate agar, highlighting its role as a causative agent of pneumonia and chronic bronchitis. *K. pneumoniae* was distinguished by its encapsulated, mucoid colony morphology, while *P. aeruginosa* was identified by its characteristic fruity odor and green pigmentation. Additionally, the anaerobic Gram-negative bacillus *Fusobacterium nucleatum* was isolated; although typically an oropharyngeal commensal, its presence is clinically relevant in cases of aspiration

pneumonia and lung abscesses. The detection of these pathogens, particularly those associated with severe pneumonia and multidrug-resistant profiles, such as *K. pneumoniae* and *S. aureus*, highlights a significant epidemiological risk in these rural Andean communities. (Table 1) The analysis of antimicrobial susceptibility revealed levels of non-susceptibility to several first-line and transition-group antibiotics among the prevalent pathogens (Table 2). Among the primary isolates, *Haemophilus influenzae* showed resistance rates of 66.6% to ampicillin and 75% to erythromycin, with a 62.5% susceptibility rate to amikacin. Non-susceptibility to ceftriaxone and ciprofloxacin was 62.5% and 50%, respectively. AMR patterns were documented in *Staphylococcus aureus* and *Klebsiella pneumoniae*. All *S. aureus* isolates (100%) were resistant to ampicillin, while resistance rates for norfloxacin and erythromycin were 70% and 65%, respectively. Additionally, 66.6% of *S. aureus* strains were resistant to ceftazidime. *Streptococcus pneumoniae* exhibited 66.6% resistance to both ampicillin and norfloxacin, and 58% resistance to erythromycin.

TABLE 3: Association between self-medication and antimicrobial resistance in patients with pathogenic bacteria.

Variable	Resistant (n = 27)	Non- Resistant (n = 5)	OR (95% CI)	p (Fisher's exact test)	OR (95% CI) [Multivariate]	p (Multivariate)
Self-Medication	14 (51.9 %)	3 (60.0 %)	0.72 (0.10 – 5.01)	1.00	0.76 (0.10 – 5.49)	0.782
Incomplete Treatment	13 (48.1 %)	1 (20.0 %)	3.71 (0.37 – 37.7)	0.33	3.67 (0.36 – 37.35)	0.272

Fisher's exact test applied in cells with expected frequencies < 5. OR: adjusted Odds Ratio from multivariate binary logistic regression, controlling for self-medication and incomplete antibiotic treatment simultaneously. Statistical significance set at $p < 0.05$.

K. pneumoniae showed 100% resistance to ampicillin, ciprofloxacin, sulfamethoxazole/trimethoprim, and norfloxacin. Resistance to third-generation cephalosporins was 70% for cefotaxime and ceftriaxone, and 80% for ceftazidime. *P. aeruginosa* also demonstrated 100% resistance to ceftriaxone.

Regarding aminoglycosides, resistance to gentamicin was 33.3% in *S. pneumoniae* and 60% in *H. influenzae*. (Table 2) The results presented in Figure 3 reveal significant therapeutic challenges, particularly with first-line drugs. Total resistance (100%) to ampicillin (AMP) was observed in *Staphylococcus aureus* and *Klebsiella pneumoniae*, while *Haemophilus influenzae* showed 67% resistance to this same agent. Among third-generation cephalosporins, a critical rate of resistance of 83% was observed in *H. influenzae* to cefotaxime (CTX). Meanwhile, both *K. pneumoniae* and *Pseudomonas aeruginosa* exhibited identical ceftriaxone (CRO) resistance levels (70%). The profile of *S. aureus* is particularly concerning due to the presence of 75% resistance to oxacillin (OX), which acts as a phenotypic marker for methicillin-resistant *S. aureus* (MRSA) strains in the studied sample; Additionally, this pathogen showed 70% resistance to norfloxacin (NOR) and 65% resistance to erythromycin (E). Finally, considerable levels of resistance were documented in *Streptococcus pneumoniae* against erythromycin (58%) and penicillin (P) (41%), underscoring the need for continuous epidemiological surveillance. (Table 2) An association analysis was conducted on the 32 pathogenic bacterial isolates to evaluate the

relationship between clinical history and the emergence of antimicrobial resistance (AMR), categorized as resistant (n = 27) or non-resistant (n = 5). Among patients with resistant isolates, 51.9% reported self-medication and 48.1% reported incomplete treatment. Fisher's exact test did not identify a statistically significant association between the variables (Table 3). Fisher's exact test identified no statistically significant association between resistance and self-medication (OR: 0.72; 95% CI: 0.10 – 5.01; $p = 1.00$) or incomplete treatment (OR: 3.71; 95% CI: 0.37 – 37.7; $p = 0.33$). To control potential confounders, a multivariate binary logistic regression analysis was subsequently performed including both variables simultaneously. After adjustment, neither self-medication (aOR: 0.76; 95% CI: 0.10 – 5.49; $p = 0.782$) nor incomplete antibiotic treatment (aOR: 3.67; 95% CI: 0.36 – 37.35; $p = 0.272$) was independently associated with antimicrobial resistance (Table 3). Although neither variable reached statistical significance, the results suggest that incomplete treatment increases the risk of resistance, with the null finding being attributable to low statistical power.

DISCUSSION

This study characterizes the regional baseline of respiratory pathogens acquired in high Andean communities of Huancayo and their phenotypic resistance patterns during the critical Andean cold season (*friaje*). Our principal findings reveal that among the 100 evaluated symptomatic outpatients, 32% yielded positive cultures for pathogenic bacteria, with *Haemophilus influenzae* (11%) and

Staphylococcus aureus (10%) identified as the most frequent isolates. Providing localized microbiological data where surveillance was previously unavailable, these community-level results highlight a critical public health challenge that aligns with the global health crisis of antimicrobial resistance (AMR), which disproportionately impacts developing regions due to systemic deficits in healthcare access and economic constraints.^{21,22} The resistance profile of *Klebsiella pneumoniae* was particularly alarming, characterized by an absolute lack of susceptibility (100% resistance) to ampicillin, ciprofloxacin, norfloxacin, and sulfamethoxazole/trimethoprim. These findings align with global reports of escalating multidrug resistance (MDR) in *K. pneumoniae*, a phenomenon frequently driven by the horizontal acquisition of mobile genetic elements, such as plasmids.^{23,24} Notably, the concurrent resistance to third-generation cephalosporins (ceftriaxone) suggests a phenotypic profile of Extended-Spectrum beta-lactamase (ESBL) production.^{25,26} This mechanism is clinically critical because it renders standard empirical therapies ineffective, often leaving carbapenems as the sole viable alternative.^{27,28} However, in resource-constrained Andean communities, carbapenems are frequently unavailable or restricted due to a lack of infrastructure and specialized personnel.²⁹ The presence of these MDR strains in a community setting implies a high risk of therapeutic failure. Seventy-five percent of *S. aureus* isolates were resistant to oxacillin, an indirect indicator of a high prevalence of methicillin-resistant *S. aureus* (MRSA) in this community. This high prevalence is consistent with contemporary meta-analytic data from community settings in developing regions, where the combined prevalence of community-associated MRSA (CA-MRSA) reached 27% (95% CI: 19–35%).³⁰ Our findings reflect the unique patterns of antimicrobial resistance observed in high-altitude ecosystems. Evidence from high-altitude regions, such as the Tibetan Plateau, demonstrates that hypoxic conditions alter bacterial susceptibility, with reported MRSA rates of 46–57.51%.³¹ This phenomenon is linked to

physiological adaptation, selective pressure, and alterations in the efflux pump systems of both bacteria and the host. In the case of *H. influenzae*, the 66.6% resistance rate to ampicillin is consistent with reports from pediatric populations in Brazil.³² The detection of such resistance, often mediated by beta-lactamases and the presence of the *blaTEM-1* gene, is highly predictive of treatment failure.³³ To investigate the behavioral drivers of this multi-resistance, we analyzed a cohort in which 63.5% reported antibiotic use without a prescription. Although statistical analysis using Fisher's exact test did not show a significant association ($p = 1.00$), this finding likely reflects a Type II error due to the limited sample size rather than a lack of biological correlation. This practice is recognized by the World Health Organization as a major global threat.^{34,35} To strengthen the association analysis and address potential confounding, a multivariate binary logistic regression was conducted. After simultaneous adjustment for self-medication and incomplete antibiotic treatment, neither variable reached statistical significance (self-medication: aOR 0.76, 95% CI 0.10 – 5.49, $p = 0.782$; incomplete treatment: aOR 3.67, 95% CI 0.36 – 37.35, $p = 0.272$). These results are consistent with the univariate findings and reinforce the interpretation that the absence of significance reflects insufficient statistical power rather than a true lack of biological effect, a limitation inherent to the small susceptibility-testing subgroup ($n = 32$). The adjusted OR for incomplete treatment (3.67) suggests a clinically meaningful, if statistically underpowered, association that warrants investigation in future, adequately powered studies. Despite the clinical significance of our findings, this pioneering characterization of AMR in Andean communities presents methodological limitations that warrant cautious interpretation. Primarily, the sample size ($n = 100$) and the small subgroup available for susceptibility testing ($n = 32$) likely limited the statistical power to detect a significant association between non-prescription antibiotic use and phenotypic resistance. Consequently, the lack of significance in Fisher's exact test ($p = 1.00$) should be viewed as a potential Type II error rather than an absence of

biological correlation, as unmeasured confounding variables may have obscured a relationship that is otherwise well documented in the international literature.

STUDY LIMITATIONS

This study provides the first systematic characterization of antimicrobial resistance in community-acquired respiratory pathogens in the Peruvian central Andes during the cold season. However, several limitations must be acknowledged when interpreting these findings. First, the resistance profile was determined using conventional phenotypic methods; although these capture functional resistance, they lack the resolution to differentiate overlapping enzymatic mechanisms. The absence of molecular techniques (PCR or WGS) precludes definitive genotypic characterization of specific determinants (*bla*KPC, *bla*CTX-M, or *mecA*). Second, due to logistical complexities associated with high-altitude fieldwork, sociodemographic data were omitted to streamline operations and prioritize direct clinical variables. Third, as a cross-sectional study conducted in a single Andean region, the results may not be generalizable to other high-altitude areas or different climatic periods. Finally, self-reported data regarding antibiotic consumption introduces potential recall bias.

CONCLUSION

This study provides the first characterization of antimicrobial resistance (AMR) in community-acquired respiratory pathogens within the central Peruvian Andes during the cold season. In the district of Huanayo, 32% of patients yielded positive cultures, with *Haemophilus influenzae* and *Staphylococcus aureus* identified as the primary isolates. The data reveal a high prevalence of multidrug-resistant (MDR) phenotypes, including methicillin-resistant *S. aureus* surrogates and

suspected ESBL-producing *K. pneumoniae*. These profiles restrict empirical therapeutic options for severe acute respiratory infections in this resource-constrained setting. While statistical analysis did not show a significant association between self-medication and resistance, the high frequency of antibiotic misuse observed remains a clinical concern. Consequently, these findings establish a baseline for the implementation of local antimicrobial stewardship programs and stricter regulations on over-the-counter antibiotic sales. Future efforts should prioritize community education on rational antibiotic use and improve access to microbiological diagnostics in primary care. Additionally, longitudinal research utilizing genomic sequencing is necessary to monitor resistance dynamics and transmission pathways in high-altitude Andean communities.

FUNDING

This work received no external funding.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

PG conceptualization, investigation, methodology, formal analysis, writing – original draft; MG conceptualization, investigation, methodology, writing – original draft; MC conceptualization, investigation, methodology, validation, writing – review & editing. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The authors wish to thank the technical staff of the Human Microbiology and Parasitology Laboratory of the Faculty of Human Medicine and the Water Research Laboratory-UNCP, microbiology area, for their support in the anaerobic bacterial culture.

REFERENCES

1. Ananth S, Adeoti AO, Ray A, Middleton PG, Ekkelenkamp M, Thee S, et al. Healthcare Worker Views on Antimicrobial Resistance in Chronic Respiratory Disease. *Antimicrobial Resistance and Infection Control*. 2025; 14(1): 1. doi:10.1186/s13756-025-01515-8
2. Sutton KF, Ashley LW. Antimicrobial Resistance in the United States: Origins and Future Directions. *Epidemiology and Infection*. 2024; 152: e33. doi:10.1017/S0950268824000244
3. Quino Sifuentes W, Alvarado Guerrero JJ. La Resistencia Antimicrobiana en Perú: Un Problema de Salud Pública. *Alpha Centauri*. 2021; 2(3): 15-22. doi:10.47422/ac.v2i3.38
4. Li Valverde VM, Aguirre Castañeda PC, Gonzales BE, Castillo-Tokumori F, Vidal JE, Ochoa TJ. Antibiotic Resistance and Factors Associated with Colonization Dynamics of *Staphylococcus aureus* and *Streptococcus pneumoniae* in Healthy Children in Lima, Peru. *Epidemiology and Infection*. 2025; 153: e106. doi:10.1017/S0950268825100277
5. Viteri-Dávila C, Morales-Jadán D, Creel A, Jop Vidal AG, Boldo XM, Rivera-Olivero IA, Bautista-Muñoz C, Alibayov B, Garcia-Bereguian MA, Vidal JE. The Crisis of Macrolide Resistance in *Pneumococci* in Latin America. *The American Journal of Tropical Medicine and Hygiene*. 2024; tpmd230913. doi:10.4269/ajtmh.23-0913
6. Gergova R, Boyanov V, Muhtarova A, Alexandrova A. A Review of the Impact of Streptococcal Infections and Antimicrobial Resistance on Human Health. *Antibiotics*. 2024; 13(4): 360. doi:10.3390/antibiotics13040360
7. Quiceno JM, Hernández-Pérez A, Thirión Romero I. Respiratory Conditions in the Latin American Context. *Handbook of Latin American Health Psychology*. 2025: 253-271. doi:10.1007/978-3-031-84433-1_12
8. Tekle MT, Sema FD, Worku MC, Jara AG, Ayenew WE. Dispensing of Antibiotics Without Prescription in Community Drug Retail Outlets of Bahir Dar City in Northwest Ethiopia: A Simulated Client Visit Before and After Educational Intervention. *Journal of Global Antimicrobial Resistance*. 2025; 45: 138-46. doi: 10.1016/j.jgar.2025.08.017
9. Ajulo S, Awosile B. Global Antimicrobial Resistance and Use Surveillance System (GLASS 2022): Investigating the Relationship Between Antimicrobial Resistance and Antimicrobial Consumption Data Across the Participating Countries. *PLoS ONE*. 2024; 19(2): e0297921. doi: 10.1371/journal.pone.0297921
10. Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, Gray AP, et al. Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *The Lancet*. 2022; 400(10369): 2221-2248. doi:10.1016/S0140-6736(22)02185-7
11. Rexe N, Kajee A, Singh R, Swe Swe-Han K. The Distribution of Methicillin-Resistant *Staphylococcus aureus* and Carbapenem-Resistant *Pseudomonas aeruginosa* in the Academic Complex Central Hospital in KwaZulu-Natal, South Africa: A 5-Year Retrospective Study From 2019 to 2023. *International Journal of Microbiology*. 2025; 2025(1): 6364811. doi:10.1155/ijm/636481
12. Birlutiu V, Birlutiu RM. An Overview of the Epidemiology of Multidrug Resistance and Bacterial Resistance Mechanisms: What Solutions Are Available? A Comprehensive Review. *Microorganisms*. 2025; 13(9): 2194. doi:10.3390/microorganisms13092194
13. Dharmayanti NLPI, Kusala MKJ, Nuradji H, Nurjanah D. Antimicrobial Resistance in Indonesia: A Comprehensive One Health Analysis and

- Strategic Roadmap for Mitigation. *International Journal of One Health*. 2025: 34-53. doi:10.14202/IJOH.2025.34-53
14. McMahon F, Ware RS, Grimwood K, Attack JM. *Haemophilus influenzae* and *Pneumococci*: Co-colonization, Interactions, Cooperation and Competition. *Pediatric Pulmonology*. 2025: 60(1): e27318. doi:10.1002/ppul.27318
 15. Polo-Pucho DA, Gonzales-Carrillo JJ, Arce-Huamani MA. Factors Associated with Acute Respiratory Infections in Children Under Five Years Old: Analysis of the Demographic and Family Health Survey. *Children*. 2025: 12(9): 1242. doi:10.3390/children12091242
 16. Paredes JL, Navarro R, Watanabe T, Morán F, Balmaceda MP, Reateguá A, et al. Knowledge, Attitudes and Practices of Parents Towards Antibiotic Use in Rural Communities in Peru: A Cross-sectional Multicentre Study. *BMC Public Health*. 2022: 22(1): 459. doi:10.1186/s12889-022-12855-0
 17. Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS. Epidemiology of Nasopharyngeal Carriage of Respiratory Bacterial Pathogens in Children and Adults: Cross-sectional Surveys in a Population with High Rates of Pneumococcal Disease. *BMC Infectious Diseases*. 2010: 10(1): 304. doi:10.1186/1471-2334-10-304
 18. O'Brien KL, Nohynek H. Report From a WHO Working Group: Standard Method for Detecting Upper Respiratory Carriage of *Streptococcus pneumoniae*. *The Pediatric Infectious Disease Journal*. 2003: 22(2): 133-140. doi:10.1097/01.inf.0000048676.93549.d1
 19. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. *Journal of Clinical Microbiology*. 2021: 59(12): e00213-21. doi:10.1128/JCM.00213-21
 20. Hanke CR, Grijalva CG, Chochua S, Pletz MW, Hornberg C, Edwards KM, et al. Bacterial Density, Serotype Distribution and Antibiotic Resistance of *Pneumococcal* Strains From the Nasopharynx of Peruvian Children Before and After *Pneumococcal Conjugate Vaccine 7*. *Pediatric Infectious Disease Journal*. 2016: 35(4): 432-439. doi:10.1097/INF.0000000000001030
 21. Aung MS, Osada M, Urushibara N, Kawaguchiya M, Ohashi N, Hirose M, et al. Molecular Characterization of Methicillin-susceptible/resistant *Staphylococcus aureus* From Bloodstream Infections in Northern Japan: The Dominance of CC1-MRSA-IV, the Emergence of Human-associated ST398 and Livestock-associated CC20 and CC97 MSSA. *Journal of Global Antimicrobial Resistance*. 2025: 41: 77-87. doi:10.1016/j.jgar.2024.12.010
 22. Hamza M, Traglia GM, Maccari L, Gomez S, Sanz MB, Akhtar U, et al. Emerging Resistance to Novel β -lactam β -lactamase Inhibitor Combinations in *Klebsiella pneumoniae* Bearing KPC Variants. *Journal of Global Antimicrobial Resistance*. 2025: 44: 297-305. doi:10.1016/j.jgar.2025.07.011
 23. Gao H, Liu Y, Wang R, Wang Q, Jin L, Wang H. The Transferability and Evolution of NDM-1 and KPC-2 Co-producing *Klebsiella pneumoniae* From Clinical Settings. *EBioMedicine*. 2020: 51: 102599. doi:10.1016/j.ebiom.2019.102599
 24. Zhou J, Meng X, Ni S, Yang Y, Zhang Q, Wu L, et al. IS26-mediated Cointegration Generates a Plasmid Co-harboring blaIMP-4 and blaKPC-2 in *Klebsiella pneumoniae*. *Journal of Global Antimicrobial Resistance*. 2025: 42: 61-65. doi:10.1016/j.jgar.2025.02.009
 25. Guitor AK, Wright GD. Antimicrobial Resistance and Respiratory Infections. *Chest*. 2018: 154(5): 1202-1212. doi:10.1016/j.chest.2018.06.019
 26. Saini J, Gautam S, Sharma D, Khanduri A, Venugopal D. Revisiting the Metallo- β -Lactamase-Mediated Antibiotic Resistance: Exploring Novel Mechanisms and Therapeutic Strategies. *International Journal of Microbiology*. 2025: 2025(1): 1574819. doi:10.1155/ijm/1574819

27. De Carvalho CX, Kipnis A, Thörn L, De Andrade JG, Pimenta F, Brandileone MCDC, Cobo ZR, Flannery B, Sgambatti S, Andrade AL. Carriage of *Haemophilus influenzae* Among Brazilian Children Attending Day Care Centers in the Era of Widespread Hib Vaccination. *Vaccine*. 2011; 29(7): 1438-1442. doi:10.1016/j.vaccine.2010.12.045
28. Belay WY, Getachew M, Tegegne BA, Teffera ZH, Dagne A, Zeleke TK, Abebe RB, Gedif AA, Fenta A, Yirdaw G, Tilahun A, Aschale Y. Mechanism of Antibacterial Resistance, Strategies and Next-generation Antimicrobials to Contain Antimicrobial Resistance: A Review. *Frontiers in Pharmacology*. 2024; 15: 1444781. doi:10.3389/fphar.2024.1444781
29. García-Betancur JC, Appel TM, Esparza G, Gales AC, Levy-Hara G, Cornistein W, Vega S, Nuñez D, Cuellar L, Bavestrello L, Castañeda-Méndez PF, Villalobos-Vindas JM, Villegas MV. Update on the Epidemiology of Carbapenemases in Latin America and the Caribbean. *Expert Review of Anti-infective Therapy*. 2021; 19(2): 197-213. doi:10.1080/14787210.2020.1813023
30. Patil S, Ramugesh K, Sukumaran S, Shivanand P. Prevalencia Global y Resistencia Antimicrobiana de *Staphylococcus aureus* Resistente a la Meticilina Asociado a la Comunidad (CA-MRSA): Una Revisión Sistemática y Metaanálisis. *Journal of Global Antimicrobial Resistance*. 2022. doi:10.1016/j.jgar.2022.04.015
31. Yang Z, Liu X, Wang Y, Zhang L. Perfiles de Resistencia Antimicrobiana y Características Genéticas de *Staphylococcus aureus* Aislado de Entornos Clínicos en Regiones de Gran Altitud. *Microbial Pathogenesis*. 2025. doi:10.1016/j.micpath.2024.106542
32. Zanella RC, Bokermann S, Galhardo M, Gava C, Almeida SCG, Pereira GA, et al. Trends in Serotype Distribution and Antimicrobial Susceptibility Pattern of Invasive *Haemophilus influenzae* Isolates From Brazil, 2009–2021. *International Microbiology*. 2024; 28(1): 157-163. doi:10.1007/s10123-024-00535-5
33. Andrzejczuk S, Kosikowska U, Chwiejczak E, Stępień-Pyśniak D, Malm A. Prevalence of Resistance to β -Lactam Antibiotics and bla Genes Among Commensal *Haemophilus parainfluenzae* Isolates From Respiratory Microbiota in Poland. *Microorganisms*. 2019; 7(10): 427. doi:10.3390/microorganisms7100427
34. Sunny T, Jacob R, K K, Varghese S. Self-medication: Is a Serious Challenge to Control Antibiotic Resistance? *National Journal of Physiology, Pharmacy and Pharmacology*. 2019; (0): 1. doi:10.5455/njppp.2019.9.0620508062019
35. Sachdev C, Anjankar A, Agrawal J. Self-Medication With Antibiotics: An Element Increasing Resistance. *Cureus*. 2022. doi:10.7759/cureus.3084