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# Antibiotic resistance patterns of co-isolated *Escherichia coli* and *Klebsiella* spp.: a cross-sectional study on their association with antibiotic usage intensity

Ayman M. Mustafa,[1] Taib A. Hama Soor[1,2]

[1]Medical Laboratory Department, Technical College of Health and Medical Technology, Sulaimani Polytechnic University, Sulaymaniyah; [2]Department of Medical Laboratory Analysis, College of Health Sciences, Cihan University - Sulaimaniyah, Sulaymaniyah, Iraq

## Abstract

Antimicrobial Resistance (AMR) in *Escherichia coli* (*E. coli*) and *Klebsiella* spp. presents a growing public health concern, particularly in low-resource developing countries. However, data on their concurrent resistance patterns within the same host remain limited. This study aims to address this gap by evaluating the resistance profiles of co-isolated strains. In the context of this cross-sectional study *E. coli* and *Klebsiella* spp. were isolated from stool samples of 138 individuals attending Smart Health Tower, in Iraq. Samples were processed using standard microbiological protocols and identified through Gram staining and biochemical tests. Antibiotic susceptibility was tested using the Kirby-Bauer disk diffusion method. Participants were categorized into low, moderate, and high antibiotic users based on their antibiotic usage patterns. Statistical analysis was performed using SPSS, with significance set at $p<0.05$. Among *E. coli* isolates, multidrug resistance was significantly more prevalent in high antibiotic users, with 38/46 (82.6%) exhibiting resistance, compared to 17/46 (37.0%) in moderate antibiotic users and 2/46 (4.3%) in low antibiotic users ($p < 0.001$). For *Klebsiella* spp. isolates, multidrug resistance was found in 36/46 (78.3%) of high antibiotic users. *Klebsiella* spp. showed higher resistance to Ampicillin-Sulbactam (59.9%) compared to *E. coli* (40.0%). Additionally, resistance to Gentamicin was more pronounced in *Klebsiella* spp. (80.0%) than in *E. coli* (20.0%). This study emphasizes the need for stricter antimicrobial stewardship in high antibiotic-use settings. The high multidrug resistance in *E. coli* and especially *Klebsiella* spp., necessitates targeted interventions to curb resistance and preserve antimicrobial efficacy.

Correspondence: Ayman M. Mustafa, Kani Spikah, Daban Street, House number 11, 46001 Sulaimani, Iraq.
Tel.: +9647711341037.
E-mail: Aymanmajid75@gmail.com

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## Introduction

Antibiotic resistance has emerged as a critical global health challenge, significantly complicating the treatment of bacterial infections. The World Health Organization (WHO) has identified antibiotic resistance as one of the top ten public health threats, highlighting its impact on the efficacy of commonly used antibiotics, particularly against pathogens like *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae*. Antimicrobial Resistance (AMR) contributes to increased morbidity, longer hospital stays, higher healthcare costs, and higher mortality rates.[1] In the United States, the Center for Disease Control and Prevention estimates that over two million people are affected by antibiotic-resistant infections annually, with at least 23,000 deaths directly attributed to these infections.[2] A study conducted in 2019 estimated that AMR was directly responsible for approximately 1.27 million deaths globally, with nearly 5 million deaths associated with drug-resistant infections in some capacity. Projections suggest that by 2050 AMR could cause up to 10 million deaths annually, surpassing deaths from cancer.[3]

The challenge of AMR is especially pronounced in developing

countries, where factors such as limited healthcare infrastructure, inadequate resources, poor hygiene, and irrational antibiotic use accelerate the spread of resistant pathogens.[4] In Iraq, the situation is particularly concerning due to the widespread, unregulated use of antibiotics, the absence of national AMR surveillance programs, and limited data on local resistance patterns. These conditions accelerate the emergence and dissemination of resistant strains, further complicating both treatment and public health efforts.[5] Enterobacteriaceae, a family of bacteria that includes *E. coli* and *Klebsiella pneumoniae*, is particularly notable for its resistance, primarily due to the production of Extended-Spectrum B-Lactamases (ESBLs), enzymes that confer resistance by breaking down third-generation cephalosporins and aztreonam. The emergence of novel ESBLs and carbapenemases has exacerbated AMR in Enterobacteriaceae over the past two decades, particularly in hospital environments where the intensive and often prolonged use of antibiotics creates strong selective pressure, promoting the survival and proliferation of resistant strains.[6]

AMR arises through various mechanisms, including enzymatic degradation of antibiotics, alterations in bacterial target sites, and changes in bacterial permeability or efflux pumps. These mechanisms are exacerbated by antibiotic overuse and misuse, and contributing factors such as poor hygiene, overcrowding, and international travel.[7] The relationship between antibiotic use and the development of resistance is well-established. Increased antibiotic consumption is directly correlated with higher rates of resistant infections, underscoring the need for effective antibiotic stewardship. *E. coli* and *Klebsiella pneumoniae* have exhibited concerning resistance trends, particularly against broad-spectrum antibiotics such as extended-spectrum cephalosporins and carbapenems, which complicate treatment regimens, increase healthcare costs, and prolong hospital stays.[8,9]

Despite extensive research on the bacteriological profiles and AMR of these bacteria, data on *E. coli* and *Klebsiella* spp. co-isolations within the same host remain scarce. This study aims to address this gap by analyzing their resistance patterns in the same host, offering insights to enhance clinical management and antimicrobial policies.

## Materials and Methods

### Study design and sample collection

This cross-sectional comparative study was designed to isolate *E. coli* and *Klebsiella* species from stool samples of 138 participants to investigate their antibiotic resistance profiles. The study was conducted at Smart Health Tower, located in Sulaymaniyah, Iraq. Participants were randomly selected based on the availability of stool samples during the designated study period. Stool samples containing both *E. coli* and *Klebsiella* spp. were collected from the same individuals, allowing for a comparative analysis of antibiotic resistance in both bacterial species.

The inclusion criteria were: individuals with stool samples containing both *E. coli* and *Klebsiella* spp., and individuals, or their parents/guardians if they were children, with the ability to provide informed consent for participation in the study. The exclusion criteria were: individuals who had stool samples containing only one of the two bacteria (*E. coli* or *Klebsiella* spp.), individuals were unable or unwilling to provide consent, and children's whose parents/guardians did not provide consent. Stool samples were collected using sterile bacteriological swabs, placed in Cary Blair transport medium, and immediately transported to the microbiological laboratory within 2–4 hours of collection to prevent bacterial overgrowth and contamination.

Ethical approval for this study was granted by the ethical committee of Sulaimani Polytechnic University, in accordance with their established ethical guidelines for research involving human subjects. The research was conducted in compliance with all relevant ethical standards, ensuring the protection of patient confidentiality and adherence to protocols for handling sensitive clinical and laboratory data. Informed consent was obtained from all participants, ensuring they understood the purpose of the study, the procedure of stool sample collection, and the confidentiality of their personal data.

### Microbiological isolation and identification

Samples were cultured following standard microbiological protocols. Upon arrival at the laboratory, stool samples were processed for bacterial culture. A suspension of each sample was prepared by aseptically emulsifying the swab in a small volume of sterile normal saline. The resulting suspension was thoroughly vortexed to ensure homogenous mixing and to disperse the bacterial content. A standardized inoculum was then obtained from the suspension and streaked onto selective media, namely MacConkey Agar and Eosin Methylene Blue Agar, to facilitate the isolation of *E. coli* and *Klebsiella* spp. These media were specifically chosen for their ability to differentiate lactose fermenters. On MacConkey Agar, *E. coli* and *Klebsiella* spp. typically appear as pink colonies due to lactose fermentation; however, *E. coli* often produces smaller, dry colonies, whereas *Klebsiella* spp. produces larger, mucoid ones. On Eosin Methylene Blue Agar, *E. coli* exhibits a characteristic metallic green sheen, whereas *Klebsiella* spp. forms large, mucoid pink to purple colonies without the sheen. This procedure was performed under sterile conditions to minimize the risk of contamination and to ensure the accuracy of subsequent microbial identification and antibiotic susceptibility testing.

### Bacterial identification and confirmation

Gram staining was performed to confirm the bacterial morphology, with both *E. coli* and *Klebsiella* spp. identified as gram-negative rods. *E. coli* typically exhibited small, straight rods, while *Klebsiella* spp. appeared larger, more encapsulated, and often demonstrated a characteristic mucoid appearance. To further differentiate the two species, a series of biochemical tests were conducted. The urease test revealed that *Klebsiella* spp. was urease-positive, with the reaction being read after 24 hours of incubation, while *E. coli* remained urease-negative. Similarly, the indole test demonstrated that *E. coli* was indole-positive, whereas *Klebsiella* spp. was indole-negative, providing additional support for their differential identification.

In cases where morphological and biochemical results were inconclusive, species confirmation was performed using the VITEK® 2 Compact system, an automated identification platform that applies Advanced Colorimetry™. This advanced technology analyzes subtle biochemical reactions to enhance species-level discrimination, ensuring high accuracy in distinguishing closely related organisms and minimizing the risk of misidentification or ambiguous results.

### Antibiotic sensitivity testing

Once the isolates were confirmed as *E. coli* or *Klebsiella* spp., antibiotic susceptibility testing was conducted using the Kirby-

Bauer disk diffusion method to determine the resistance patterns to seven commonly used antibiotics across different classes, Ampicillin/Sulbactam 10 µg (Beta-lactam/beta-lactamase inhibitor), Erythromycin 15 µg (Macrolide), Doxycycline 10 µg (Tetracycline), Trimethoprim-Sulfamethoxazole 25 µg (Folate pathway inhibitor), Gentamicin 10 µg (Aminoglycoside), Ceftriaxone 30 µg (Cephalosporin), Imipenem 10 µg (Carbapenem). The classification of these antibiotics was contextualized within the AWARE (Access, Watch, and Reserve) framework, as outlined by the World Health Organization (WHO). Four of the tested antibiotics (Ampicillin/Sulbactam, Doxycycline, Gentamicin, and Trimethoprim-Sulfamethoxazole) belong to the Access group, while Erythromycin and Ceftriaxone belong to the Watch group. Imipenem is categorized as a Reserve antibiotic. The Access group antibiotics represent first-line agents commonly used to treat bacterial infections and are intended to be widely available. Resistance to these agents was analyzed to assess baseline levels of resistance in the bacterial isolates. The Watch group antibiotics are prioritized for cautious use due to their higher potential for resistance development, and resistance to these agents was closely monitored to detect emerging trends. The Reserve group antibiotic, Imipenem, was included to evaluate the presence of Multidrug-Resistant (MDR) organisms, as it represents a last-resort treatment option for severe infections caused by resistant bacteria.[10]

In the Kirby-Bauer method, bacterial isolates were first standardized to specific turbidity (using a 0.5 McFarland standard) to ensure uniform inoculation. The inoculum was then spread evenly across the surface of Mueller-Hinton agar, using a sterile swab. Antibiotic-impregnated paper disks were placed on the inoculated agar surface at specific distances to ensure that each disk maintained an appropriate concentration gradient of the antibiotic. The plates were incubated at 37°C for 18 hours. After incubation, the growth inhibition zones around each disk were measured, and the results were interpreted based on established guidelines, such as those from the Clinical and Laboratory Standards Institute (CLSI).[11] The diameter of the inhibition zones was categorized as susceptible, intermediate, or resistant for each tested antibiotic (Figure 1). Quality control was ensured by using standard reference strains, including *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603, as positive controls. Sterile uninoculated Mueller-Hinton agar plates were included as negative controls to monitor for contamination. This method allowed for the assessment of AMR profiles for both *E. coli* and *Klebsiella* spp. isolates.

## Data collection on antibiotic usage habits

A comprehensive questionnaire was developed specifically for this study to gather detailed information regarding participants antibiotic usage history. The questionnaire included several key variables designed to assess various aspects of antibiotic usage, such as comorbidities, types of comorbidities, surgical history, reasons for recent antibiotic use, frequency of antibiotic usage, self-medication practices, completion of antibiotic courses, source of antibiotics (whether prescribed by a healthcare provider or obtained over-the-counter), hospitalization history, and the duration of antibiotic courses. Additionally, demographic information, including gender, age (years), residence, education level, and occupation, was recorded to explore potential correlations with antibiotic usage. The questionnaire was developed with input from ten experts in microbiology, pharmacology, and epidemiology to ensure its relevance, clarity, and comprehensiveness. Reliability testing of the questionnaire was performed using Cronbach's alpha, which yielded a value of 0.93, indicating excellent internal consistency and reliability. After these validations, the finalized questionnaire was used for data collection. In the absence of established guidelines for classifying individuals based on their antibiotic usage patterns, specific items from the questionnaire were selected to categorize participants into low, moderate, and high antibiotic use groups. The frequency of antibiotic usage and the source of antibiotics (whether prescribed or over-the-counter) were identified as key variables for classification. Participants were categorized into these three groups based on their responses, allowing for the comparative analysis of antibiotic resistance in *E. coli* and *Klebsiella* spp. isolates across varying levels of antibiotic consumption.

## Content validity index

The validation of the questionnaire incorporated expert input to ensure its clarity and relevance. Ten experts from the fields of microbiology, and pharmacology reviewed each item of the questionnaire. Their feedback was utilized to refine and enhance the questions. The Content Validity Index (CVI) was calculated by dividing the number of experts who agreed on each question by the total number of experts. The CVI scores ranged from 0 to 1. A CVI of 0.79 or higher indicated that the items were relevant, while a CVI between 0.70 and 0.78 suggested the need for revision. Items with a CVI below 0.70 were considered for potential elimination. In line with established guidelines, a CVI of 0.78 or higher is considered acceptable for studies involving more than five experts.[12] Among all items in the questionnaire, all except two demonstrated a CVI above 0.78. The two items with CVIs of 0.6 and 0.5 were excluded from the final version of the questionnaire.

## Statistical analysis

To categorize antimicrobial resistance profiles, bacterial isolates were categorized based on the number of antibiotic classes to which they exhibited resistance. The following classification sys-

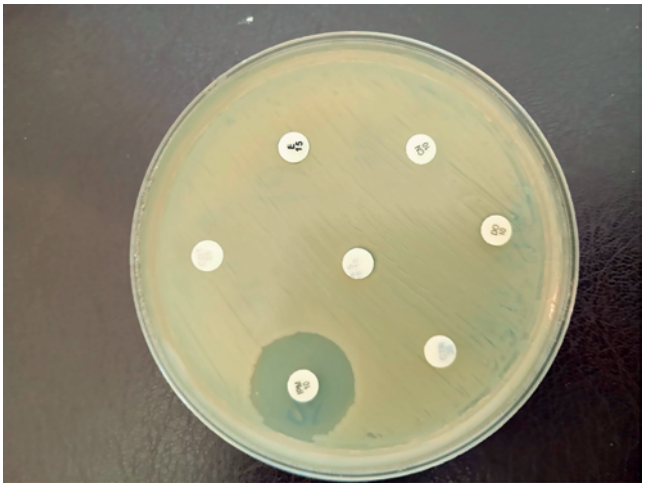

**Figure 1.** Antimicrobial susceptibility pattern of a multidrug-resistant isolate using the Kirby-Bauer disk diffusion method. The isolate demonstrates resistance to all tested antibiotics: Gentamicin (CN), Trimethoprim/Sulfamethoxazole (COT), Doxycycline (DO), Ceftriaxone (CTR), Erythromycin (E), and Ampicillin/Sulbactam (A/S), with susceptibility observed only to imipenem (IPM), as indicated by a distinct zone of inhibition.

tem was employed: R0 denoted isolates that were sensitive to all tested classes of antibiotics, R1 indicated resistance to one class, R2 to two classes, R3 to three classes, R4 to four classes, R5 to five classes, and R6 to six antibiotic classes. Multidrug Resistance (MDR) was defined as resistance to at least one antimicrobial agent from three or more distinct antimicrobial classes.[13] Statistical analyses were performed using IBM SPSS Statistics version 26.0. The normality of continuous data was assessed using the Shapiro-Wilk test. For data that were normally distributed, descriptive statistics, including means and standard deviations, were calculated, and intergroup comparisons were conducted using independent t-tests and one-way ANOVA. For data that were not normally distributed, medians and interquartile ranges were reported, and statistical comparisons were performed using the Mann-Whitney U test and Kruskal-Wallis test. Categorical variables were analyzed using chi-square test or Fisher's exact test, as appropriate. Statistical significance was set at a p-value of <0.05. Univariate analysis was first conducted to identify variables associated with MDR. Variables that demonstrated statistical significance in the univariate analysis were subsequently included in a multivariate logistic regression model to control for confounding factors and identify independent predictors of MDR. The multivariate logistic regression model included demographic factors, antibiotic usage history, and clinical variables as covariates to control for potential confounders. The dependent variable in the model was the presence of MDR, and the model aimed to determine which factors were independently associated with the development of resistance to multiple antibiotics.

## Results

In this study, 138 individuals were divided into three classes based on antibiotic usage: low (n=46), moderate (n=46), and high (n=46). Among them, 40 (29.0%) were children, while the remaining 98 (71.0%) were adults. Women comprised a slightly higher proportion 72 (52.2%) than men 66 (47.8%), with a median age of 31.5 years (Quartile Range (QR): 15.0–48.0). Regarding residence, 88 (63.8%) of individuals lived in urban areas, 37 (26.8%) in rural areas, and 13 (9.4%) in suburban regions. In terms of education level, 80 (58.0%) of individuals were illiterate, while 24 (17.4%) had higher education. Employment status showed that majority 76 (55.1%) were unemployed. Comorbidities were present in 77 (55.8%) individuals, with malnutrition reported in 50 (64.9%) and hypertension in 46 (59.7%) of applicable cases. Additionally, 76 (55.1%) of individuals reported completing their antibiotic course, while 41 (29.7%) engaged in self-medication. Hospitalization was recorded in 44 (31.9%) individuals, with a median hospital stay of 4 days (QR: 2.5–10 days) (Table 1).

Among participants, the median age varied significantly across antibiotic usage levels, with the low-usage group having a median age of 17 years (QR: 2–37), compared to 37 years (QR: 22–55) in the moderate group and 39.5 years (QR: 22–46) in the high-usage group (p <0.01). Additionally, education level was significantly associated with antibiotic use (p <0.001), with 16 (72.7%) of individuals with higher education in the low-usage group, compared to only 6 (27.3%) in the high-usage group. Maternal education also showed a significant association (p <0.01), as 12 (92.3%) of individuals with higher maternal education were in the low-usage group, while only 1 (7.7%) were in the high-usage group (Table 2).

Among *E. coli* isolates, resistance was significantly higher in high antibiotic users for Ampicillin-Sulbactam (53.5%, p <0.001, V=0.40, moderate effect), Gentamicin (77.8%, p =0.017, V=0.21, moderate effect), Ceftriaxone (63.9%, p <0.001, V=0.30, moderate effect), Trimethoprim-Sulfamethoxazole (66.7%, p <0.001, V=0.64, strong effect), Doxycycline (73.0%, p <0.001, V=0.38, moderate effect), and Erythromycin (42.0%, p <0.001, V=0.31, moderate effect). Sensitivity was highest in low antibiotic users. For *Klebsiella* spp. isolates, significant resistance was observed in high antibiotic users for Ampicillin-Sulbactam (35.8%, p=0.009, V=0.27, moderate effect), Gentamicin (50.0%, p=0.007, V=0.23, moderate effect), Trimethoprim-Sulfamethoxazole (40.0%, p=0.002, V=0.31, moderate effect), and Doxycycline (42.6%, p=0.003, V=0.22, moderate effect). Ceftriaxone and Erythromycin resistance showed no significant association (Table 3).

Resistance patterns varied significantly between *E. coli* and *Klebsiella* spp. isolates across multiple antibiotics. Resistance to Ampicillin-Sulbactam was higher in *Klebsiella* spp.: 106 isolates (59.9%) compared to *E. coli* 71 (40.0%), with a Cramér's V of 0.28. Gentamicin resistance was observed in 36 isolates (80.0%) of *Klebsiella* spp. and 9 (20.0%) of *E. coli* (Cramér's V=0.34). Doxycycline resistance was not significantly higher in *Klebsiella* spp.: 54 isolates (59.3%) than *E. coli* 37 (40.7%), with a Cramér's V of 0.13, indicating very low effect of bacterial isolate on resistance pattern. Erythromycin resistance was predominant in *Klebsiella* spp.: 117 isolates (57.1%) compared to *E. coli* 88 (42.9%) (Cramér's V=0.25) (Table 4).

Among *E. coli* isolates, 57/138 (41.3%) exhibited MDR, with a significantly higher prevalence among high antibiotic users 38/46 (82.6%) compared to moderate 17/46 (37.0%) and low antibiotic users 2/46 (4.3%) (p <0.001, Cramér's V=0.60). Resistance levels increased with antibiotic usage, as 20/464 (43.5%) of high antibiotic users exhibited resistance to four classes of antibiotics, while only 4/46 (8.7%) of moderate and none of the low antibiotic users did. Similarly, among *Klebsiella* spp. isolates, MDR was observed in 93/138 (67.4%), with significantly higher rates among moderate and high antibiotic users 36/46 (78.3%) compared to low antibiotic users 21/46 (45.7%) (p=0.002, Cramér's V=0.35).

For *E. coli*, 28 (20.3%) isolates exhibited no resistance (R0), while resistance increased across categories: 27 (19.6%) in R1, 26 (18.8%) in R2, 23 (16.7%) in R3, and 24 (17.4%) in R4. MDR was identified in 57 (41.3%) of *E. coli* isolates. For *Klebsiella* spp. isolates, only 7 (5.1%) isolates exhibited no resistance (R0), while 16 (11.6%) were in R1, 22 (15.9%) in R2, 22 (15.9%) in R3, and 38 (27.5%) in R4. MDR was prevalent in 93 (67.4%) of *Klebsiella* spp. isolates. The overall MDR rate was 54.3%. The association between bacterial species and resistance class was significant (p <0.001), with a moderate effect size (Cramer's V=0.34) (Table 5). Among the 1,932 antibiotic tests conducted on *E. coli* and *Klebsiella* spp. isolates from 138 individuals, 761 (39.4%) demonstrated resistance. Of these resistant cases, 445 (58.47%) belonged to the Access category, 313 (41.13%) were classified under the Watch category, and only 3 (0.4%) were in the Reserve category.

In the univariate regression analysis, high antibiotic users exhibited significantly higher odds of MDR compared to low antibiotic users, with an Odds Ratio (OR) of 11.455 (95% Confidence Interval (CI): 3.529–37.184, p<0.001). Moderate antibiotic users also had increased odds (OR: 8.945, 95% CI: 2.996–26.711, p<0.001). In the multivariate model, these associations remained significant, with ORs of 5.639 (95% CI: 1.748–18.189, p=0.004) and 5.163 (95% CI: 1.386–19.231, p=0.014), respectively. Regarding education level, illiterate individuals had a significantly higher risk of MDR compared to those with higher education (OR: 7.413, 95% CI: 2.661–20.651, p<0.001). Those with primary education also showed increased odds (OR: 5.909,

95% CI: 1.349–25.879, p=0.018). However, in the multivariate model, only higher education remained significant (OR: 4.035, 95% CI: 1.231–13.228, p=0.021). Self-medication with antibiotics was strongly associated with MDR (OR: 9.159, 95% CI: 2.077–40.384, p=0.003) in univariate analysis. The source of antibiotics also played a role, with individuals using leftover antibiotics from previous prescriptions having significantly higher MDR odds (OR: 12.682, 95% CI: 1.647–97.633, p=0.015) compared to those prescribed by healthcare providers. However, this effect was not significant in the multivariate analysis (OR: 4.792, 95% CI: 0.548–41.873, p=0.157) (Table 6).

## Discussion

AMR in *E. coli* and *Klebsiella* spp. is a major global health concern, with both pathogens showing significant resistance to commonly used antibiotics across clinical samples.[14] The World Health Organization (WHO) recognizes AMR as one of the foremost medical challenges, with misuse, overuse, and improper selection of antibiotics accelerating resistance. AMR has serious clinical implications, particularly in healthcare settings, leading to higher morbidity, mortality, and treatment costs. In some cases, bacteria are resist-

**Table 1.** Demographic, clinical, and antibiotic usage characteristics of the study participants.

| Variables | Frequency (%) |
|---|---|
| Gender | |
| Man | 66 (47.8) |
| Woman | 72 (52.2) |
| Age (Median, QR) | 31.5 (15.0-48.0) |
| Age group | |
| 0-10 | 30 (21.7) |
| 10-20 | 12 (8.7) |
| 20-30 | 26 (18.8) |
| 30-40 | 18 (13.0) |
| 40-50 | 24 (17.4) |
| 50-60 | 15 (10.9) |
| >60 | 13 (9.4) |
| Residence | |
| Urban | 88 (63.8) |
| Sub-urban | 13 (9.4) |
| Rural | 37 (26.8) |
| Education level | |
| Illiterate | 80 (58.0) |
| Primary education | 18 (13.0) |
| Secondary education | 16 (11.6) |
| Higher education | 24 (17.4) |
| Maternal education (No. of applicable individuals=33)* | |
| Illiterate | 12 (36.4) |
| Primary education | 4 (12.1) |
| Secondary education | 4 (12.1) |
| Higher education | 13 (39.4) |
| Occupation status | |
| Student | 23 (16.7) |
| Employed | 39 (28.3) |
| Unemployed | 76 (55.1) |
| Comorbidities | |
| Yes | 77 (55.8) |
| No | 61 (44.2) |
| Diabetes (No. of applicable individuals=77)** | |
| Yes | 40 (51.9) |
| No | 37 (48.1) |
| Hypertension (No. of applicable individuals=77)** | |
| Yes | 46 (59.7) |
| No | 31 (40.3) |
| Obesity (No. of applicable individuals=77)** | |
| Yes | 31 (40.3) |
| No | 46 (59.7) |
| Pregnancy (No. of applicable individuals=77)** | |
| Yes | 5 (6.5) |
| No | 72 (93.5) |
| Peptic ulcer (No. of applicable individuals=77)** | |
| Yes | 25 (32.5) |
| No | 52 (67.5) |
| COPD (No. of applicable individuals=77)** | |
| Yes | 11 (14.3) |
| No | 66 (85.7) |
| Renal insufficiency (No. of applicable individuals=77)** | |
| Yes | 21 (37.5) |
| No | 56 (72.7) |
| Gastrointestinal disease (No. of applicable individuals=77)** | |
| Yes | 45 (58.4) |
| No | 32 (41.6) |
| Malnutrition (No. of applicable individuals=77)** | |
| Yes | 50 (64.9) |
| No | 27 (35.1) |
| Surgical history | |
| Yes | 34 (24.6) |
| No | 104 (75.4) |
| Self-antibiotic medication | |
| Yes | 41 (29.7) |
| No | 97 (70.3) |
| Completion of antibiotic course | |
| Yes | 76 (55.1) |
| No | 62 (44.9) |
| Reason for recent antibiotic use | |
| Urinary tract infections | 15 (10.9) |
| Respiratory tract infections | 18 (13.0) |
| Gastrointestinal infections | 38 (27.5) |
| Soft tissue or skin infections | 3 (2.2) |
| Bloodstream infections | 3 (2.2) |
| Prophylaxis | 8 (5.8) |
| Bone and joint infections | 6 (4.3) |
| Broad spectrum use | 19 (13.8) |
| Viral infections | 28 (20.3) |
| Source of antibiotic | |
| Prescribed by a healthcare provider | 97 (70.3) |
| Purchased without prescription | 13 (9.4) |
| Leftover antibiotics from previous use | 28 (20.3) |
| Hospitalization | |
| Yes | 44 (31.9) |
| No | 94 (68.1) |
| Length of hospital stay (days) (Median, QR) | 4 (2.5-10) |

QR, Quartile range; *only those aged below 18 years were analyzed; **only those with comorbidity were included.

ant even to last-resort antibiotics.[15] The current study underscores the relationship between antibiotic usage patterns and MDR in *E. coli* and *Klebsiella* spp. co-isolations, highlighting the role of antibiotic consumption in shaping resistance profiles. The resistance trends observed align with global findings, further emphasizing the need for enhanced antimicrobial stewardship, improved prescription practices, and more robust resistance surveillance to address the growing AMR crisis.[16]

The demographic analysis revealed intriguing patterns that align with and at the same time diverge from existing literature. The significant age difference across antibiotic usage groups contradicts the findings by Schröder *et al.*, who reported higher antibiotic consumption among younger populations in European countries.[17] This divergence may reflect the cultural context of healthcare decision-making in Middle Eastern societies, where older family members often control medication acquisition, as described by Al-Mohamadi *et al*.[18] The demographic profile of antibiotic users in this study provides critical context for understanding the social determinants of AMR in developing countries. The strong association between education level and antibiotic usage patterns represents one of the most significant findings of the present study. The observation that 72.7% of individuals with higher education fell into the low-usage group aligns with comprehensive reviews by Gandra *et al*., who identified education as a critical determinant of appropriate antibiotic use behaviors.[19]

The graduated increase in resistance rates across low, moderate, and high antibiotic usage groups observed in the current study provides compelling evidence supporting antimicrobial stewardship programs. This relationship parallels findings from Holmes *et al.*, who demonstrated similar resistance gradients in hospital settings following implementation of structured antibiotic stewardship protocols.[20] These results reinforce recommendations by Pulcini *et al.* regarding targeted stewardship efforts focused on antibiotics with the highest resistance potential.[21]

The patterns of antibiotic resistance observed in this study reflect broader public health challenges faced by developing countries with limited regulatory frameworks for antibiotic dispensing. The relatively high rates of self-medication (29.7%) and incomplete antibiotic courses (44.9%) reported by the participants mirror findings from Ayukekbong *et al.*, who documented similar practices across resource-limited settings globally.[22] These behaviors create ideal conditions for resistance development, as described in the comprehensive review by Prestinaci *et al.* examining global drivers of AMR.[2] The socioeconomic context is particularly relevant, as demonstrated by Pouwels *et al.* who identified unemployment status as significantly associated with increased self-medication practices in similar populations.[23]

The study's findings on Ampicillin-Sulbactam resistance (53.5% in high antibiotic users) corroborate the results of Tacconelli *et al.*, who reported similar resistance rates (48–57%) in regions with high antimicrobial consumption.[24] A study identified prior exposure to penicillin antibiotics, including Ampicillin and Ampicillin-Sulbactam, as the only significant independent risk factors for Ampicillin-Sulbactam-resistant *E. coli*, confirming that prior antibiotic exposure is a critical determinant of resistance development.[25] The significant resistance to Trimethoprim-Sulfamethoxazole (66.7%) among high antibiotic users in our study exceeds the global average of 50.4% reported by the Global Antimicrobial Resistance Surveillance System.[26] This discrepancy likely reflects the unique challenges of unregulated antibiotic access in resource-limited settings, a phenomenon described by Klein *et al.* as "antibiotic accessibility without appropriate stewardship".[27] The particularly strong effect size (V=0.64) for Trimethoprim-Sulfamethoxazole resistance suggests a robust relationship with antibiotic usage patterns, supporting the assertion that certain antibiotics are especially vulnerable to resistance development under consistent selection pressure. Ceftriaxone resistance (63.9%) in the high-usage group similarly exceeds the global average of 45-55% documented by Cassini *et al.*, highlighting the severity of resistance development in settings with limited antimicrobial stewardship protocols.[28]

**Table 2.** Comparison of demographic and socioeconomic factors across different levels of antibiotic use.

| Variables | Frequency of antibiotic use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Low | Moderate | p value | Low | High | p value | Moderate | High | p value |
| Age (Median, QR) | 17 (2-37) | 37 (22-55) | 0.002 | 17 (2-37) | 39.5 (22-46) | 0.004 | 37 (22-55) | 39.5 (22-46) | 0.780 |
| Gender (N, %) | | | 0.404 | | | 0.532 | | | 0.834 |
| Man | 25 (55.6) | 20 (44.4) | | 25 (54.3) | 21 (45.7) | | 20 (48.8) | 21 (51.2) | |
| Woman | 21 (44.7) | 26 (55.3) | | 21 (45.7) | 25 (54.3) | | 26 (51.0) | 25 (49.0) | |
| Residence (N, %) | | | | | | | | | |
| Urban | 32 (56.1) | 25 (43.9) | 0.315 | 32 (50.8) | 31 (49.2) | 0.384 | 25 (44.6) | 31 (55.4) | 0.265 |
| Sub-urban | 5 (45.5) | 6 (54.5) | | 5 (71.4) | 2 (28.6) | | 6 (75.0) | 2 (25.0) | |
| Rural | 9 (37.5) | 15 (62.5) | | 9 (40.9) | 13 (59.1) | | 15 (53.6) | 13 (46.4) | |
| Education level | | | | | | | | | 0.164 |
| Illiterate | 19 (42.2) | 26 (57.8) | | 19 (35.2) | 35 (64.8) | | 26 (42.6) | 35 (57.4) | |
| Primary education | 5 (35.7) | 9 (64.3) | 0.074 | 5 (55.6) | 4 (44.4) | <0.001 | 9 (69.2) | 4 (30.8) | |
| Secondary education | 6 (54.5) | 5 (45.5) | | 6 (54.5) | 5 (45.5) | | 5 (50.0) | 5 (50.0) | |
| Higher education | 16 (72.7) | 6 (27.3) | | 16 (88.9) | 2 (11.1) | | 6 (75.0) | 2 (25.0) | |
| Maternal education | | | | | | | | | 0.467 |
| Illiterate | 1 (16.7) | 5 (83.3) | | 1 (14.3) | 6 (85.7) | | 5 (45.5) | 6 (54.5) | |
| Primary education | 2 (50.0) | 2 (50.0) | 0.002 | 2 (100.0) | 0 (0.0) | <0.001 | 2 (100.0) | 0 (0.0) | |
| Secondary education | 3 (100.0) | 0 (0.0) | | 3 (75.0) | 1 (25.0) | | 0 (0.0) | 1 (100.0) | |
| Higher education | 12 (92.3) | 1 (7.7) | | 12 (100.0) | 0 (0.0) | | 1 (100.0) | 0 (0.0) | |
| Occupation status | | | | | | | | | |
| Student | 10 (58.8) | 7 (41.2) | 0.619 | 10 (62.5) | 6 (37.5) | 0.467 | 7 (53.8) | 6 (46.2) | 0.453 |
| Employed | 12 (42.9) | 16 (57.1) | | 12 (52.2) | 11 (47.8) | | 16 (59.3) | 11 (40.7) | |
| Unemployed | 24 (51.1) | 23 (48.9) | | 24 (45.3) | 29 (54.7) | | 23 (44.2) | 29 (55.8) | |

**Table 3.** Antibiotic susceptibility patterns of *E. coli* and *Klebsiella* spp. isolates among different levels of antibiotic users.

| Bacterial isolates | Antibiotics | Total (N, %) | Low antibiotic user (N, %) | Moderate antibiotic user (N, %) | High antibiotic user (N, %) | p value | Cramer V |
|---|---|---|---|---|---|---|---|
| ***E. coli*** | | | | | | | |
| | Ampicillin-Sulbactam | | | | | | |
| | Sensitive | 61 (100.0) | 35 (57.4) | 19 (31.1) | 7 (11.5) | | |
| | Intermediate | 6 (100.0) | 4 (66.7) | 1 (16.7) | 1 (16.7) | <0.001 | 0.40 |
| | Resistance | 71 (100.0) | 7 (9.9) | 26 (36.6) | 38 (53.5) | | |
| | Gentamicin | | | | | | |
| | Sensitive | 124 (100.0) | 45 (36.3) | 42 (33.9) | 37 (29.8) | | |
| | Intermediate | 5 (100.0) | 0 (0.0) | 3 (60.0) | 2 (40.0) | 0.017 | 0.21 |
| | Resistance | 9 (100.0) | 1 (11.1) | 1 (11.1) | 7 (77.8) | | |
| | Ceftriaxone | | | | | | |
| | Sensitive | 100 (100.0) | 41 (41.0) | 36 (36.0) | 23 (23.0) | | |
| | Intermediate | 2 (100.0) | 0 (0.0) | 2 (100.0) | 0 (0.0) | <0.001 | 0.30 |
| | Resistance | 36 (100.0) | 5 (13.9) | 8 (22.2) | 23 (63.9) | | |
| | Trimethoprim-Sulfamethoxazole | | | | | | |
| | Sensitive | 81 (100.0) | 43 (53.1) | 30 (37.0) | 8 (9.9) | <0.001 | 0.64 |
| | Intermediate | 0 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | | |
| | Resistance | 57 (100.0) | 3 (5.3) | 16 (28.1) | 38 (66.7) | | |
| | Imipenem | | | | | | |
| | Sensitive | 138 (100.0) | 46 (33.3) | 46 (33.3) | 46 (33.3) | | |
| | Intermediate | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | * | * |
| | Resistance | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | | |
| | Doxycycline | | | | | | |
| | Sensitive | 99 (100.0) | 44 (44.4) | 36 (36.4) | 19 (19.2) | | |
| | Intermediate | 2 (100.0) | 1 (50.0) | 1 (50.0) | 0 (0.0) | <0.001 | 0.38 |
| | Resistance | 37 (100.0) | 1 (2.7) | 9 (24.3) | 27 (73.0) | | |
| | Erythromycin | | | | | | |
| | Sensitive | 47 (100.0) | 29 (61.7) | 10 (21.3) | 8 (17.0) | | |
| | Intermediate | 3 (100.0) | 1 (33.3) | 1 (33.3) | 1 (33.3) | <0.001 | 0.31 |
| | Resistance | 88 (100.0) | 16 (18.2) | 35 (39.8) | 37 (42.0) | | |
| ***Klebsiella* spp.** | | | | | | | |
| | Ampicillin-Sulbactam | | | | | | |
| | Sensitive | 32 (100.0) | 18 (56.3) | 6 (18.8) | 8 (25.0) | | |
| | Intermediate | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.009 | 0.27 |
| | Resistance | 106 (100.0) | 28 (26.4) | 40 (37.7) | 38 (35.8) | | |
| | Gentamicin | | | | | | |
| | Sensitive | 83 (100.0) | 35 (42.2) | 23 (27.7) | 25 (30.1) | | |
| | Intermediate | 19 (100.0) | 5 (26.3) | 11 (57.9) | 3 (15.8) | 0.007 | 0.23 |
| | Resistance | 36 (100.0) | 6 (16.7) | 12 (33.3) | 18 (50.0) | | |
| | Ceftriaxone | | | | | | |
| | Sensitive | 59 (100.0) | 24 (40.7) | 20 (33.9) | 15 (25.4) | | |
| | Intermediate | 7 (100.0) | 2 (28.6) | 4 (57.1) | 1 (14.3) | 0.173 | 0.15 |
| | Resistance | 72 (100.0) | 20 (27.8) | 22 (30.6) | 30 (41.7) | | |
| | Trimethoprim-Sulfamethoxazole | | | | | | |
| | Sensitive | 63 (100.0) | 31 (49.2) | 16 (25.4) | 16 (25.4) | | |
| | Intermediate | 0 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.002 | 0.31 |
| | Resistance | 75 (100.0) | 15 (20.0) | 30 (40.0) | 30 (40.0) | | |
| | Imipenem | | | | | | |
| | Sensitive | 133 (100.0) | 44 (33.1) | 45 (33.8) | 44 (33.1) | | |
| | Intermediate | 2 (100.0) | 2 (100.0) | 0 (0.0) | 0 (0.0) | 0.325 | 0.15 |
| | Resistance | 3 (100.0) | 0 (0.0) | 1 (33.3) | 2 (66.7) | | |
| | Doxycycline | | | | | | |
| | Sensitive | 82 (100.0) | 37 (45.1) | 23 (28.0) | 22 (26.8) | | |
| | Intermediate | 2 (100.0) | 0 (0.0) | 1 (50.0) | 1 (50.0) | 0.003 | 0.22 |
| | Resistance | 54 (100.0) | 9 (16.7) | 22 (40.7) | 23 (42.6) | | |
| | Erythromycin | | | | | | |
| | Sensitive | 21 (100.0) | 10 (47.6) | 6 (28.6) | 5 (23.8) | | |
| | Intermediate | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.416 | 0.13 |
| | Resistance | 119 (100.0) | 36 (30.8) | 40 (34.2) | 41 (35.0) | | |

**Table 4.** Antibiotic susceptibility and resistance in *E. coli* and *Klebsiella* spp. isolates.

| Antibiotics | Total (N, %) | *Escherichia coli* (N, %) | *Klebsiella* spp. (N, %) | p value | Cramer V |
|---|---|---|---|---|---|
| Ampicillin-Sulbactam | | | | | |
| Sensitive | 93 (100.0) | 61 (65.6) | 32 (34.4) | | |
| Intermediate | 6 (100.0) | 6 (100.0) | 0 (0.0) | <0.001 | 0.28 |
| Resistance | 177 (100.0) | 71 (40.1) | 106 (59.9) | | |
| Gentamicin | | | | | |
| Sensitive | 207 (100.0) | 124 (59.9) | 83 (40.1) | | |
| Intermediate | 24 (100.0) | 5 (20.8) | 19 (79.2) | <0.001 | 0.34 |
| Resistance | 45 (100.0) | 9 (20.0) | 36 (80.0) | | |
| Ceftriaxone | | | | | |
| Sensitive | 159 (100.0) | 100 (62.9) | 59 (37.1) | | |
| Intermediate | 9 (100.0) | 2 (22.2) | 7 (77.8) | <0.001 | 0.30 |
| Resistance | 108 (100.0) | 36 (33.3) | 72 (66.7) | | |
| Trimethoprim-Sulfamethoxazole | | | | | |
| Sensitive | 144 (100.0) | 81 (56.3) | 63 (43.8) | | |
| Intermediate | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.020 | 0.13 |
| Resistance | 132 (100.0) | 57 (43.2) | 75 (56.8) | | |
| Imipenem | | | | | |
| Sensitive | 271 (100.0) | 138 (50.9) | 133 (49.1) | | |
| Intermediate | 2 (100.0) | 0 (0.0) | 2 (100.0) | 0.06 | 0.14 |
| Resistance | 3 (100.0) | 0 (0.0) | 3 (100.0) | | |
| Doxycycline | | | | | |
| Sensitive | 181 (100.0) | 99 (54.7) | 82 (45.3) | | |
| Intermediate | 4 (100.0) | 2 (50.0) | 2 (50.0) | 0.079 | 0.13 |
| Resistance | 91 (100.0) | 37 (40.7) | 54 (59.3) | | |
| Erythromycin | | | | | |
| Sensitive | 68 (100.0) | 47 (69.1) | 21 (30.9) | | |
| Intermediate | 3 (100.0) | 3 (100.0) | 0 (0.0) | <0.001 | 0.25 |
| Resistance | 105 (100.0) | 88 (42.9) | 117 (57.1) | | |

**Table 5.** Antibiotic usage and resistance patterns in *E. coli* and *Klebsiella* spp.

| Bacteria | Resistance to classes | Total (N, %) | Low antibiotic users | Moderate antibiotic users | High antibiotic users | p value* | Cramer V* | p value** | Cramer V** |
|---|---|---|---|---|---|---|---|---|---|
| ***E. coli*** | | | | | | | | | |
| | R0 | 28 (20.3) | 23 (50.0) | 5 (10.9) | 0 (0.0) | | | <0.001 | 0.34 |
| | R1 | 27 (19.6) | 15 (32.6) | 10 (21.7) | 2 (4.3) | | | | |
| | R2 | 26 (18.8) | 6 (13.0) | 14 (30.4) | 6 (13.0) | | | | |
| | R3 | 23 (16.7) | 2 (4.3) | 12 (26.1) | 9 (19.6) | | | | |
| | R4 | 24 (17.4) | 0 (0.0) | 4 (8.7) | 20 (43.5) | | | | |
| | R5 | 5 (3.6) | 0 (0.0) | 0 (0.0) | 5 (10.9) | | | | |
| | R6 | 5 (3.6) | 0 (0.0) | 1 (2.2) | 4 (8.7) | <0.001 | 0.60 | | |
| | MDR | 57 (41.3) | 2 (4.3) | 17 (37.0) | 38 (82.6) | | | | |
| ***Klebsiella* spp.** | | | | | | | | | |
| | R0 | 7 (5.1) | 5 (10.9) | 1 (2.2) | 1 (2.2) | | | | |
| | R1 | 16 (11.6) | 10 (21.7) | 3 (6.5) | 3 (6.5) | | | | |
| | R2 | 22 (15.9) | 10 (21.7) | 6 (13.0) | 6 (13.0) | | | | |
| | R3 | 22 (15.9) | 8 (17.4) | 8 (17.4) | 6 (13.0) | | | | |
| | R4 | 38 (27.5) | 7 (15.2) | 20 (43.5) | 11 (23.9) | | | | |
| | R5 | 19 (13.8) | 6 (13.0) | 2 (4.3) | 11 (23.90 | | | | |
| | R6 | 14 (10.1) | 0 (0.0) | 6 (13.0) | 8 (17.4) | | | | |
| | MDR | 93 (67.4) | 21 (45.7) | 36 (78.3) | 36 (78.3) | 0.002 | 0.35 | | |

R0, Sensitive to all classes of antibiotics; R1, resistance to one class of antibiotic; R2, resistance to two classes of antibiotics; R3, resistance to three classes of antibiotics; R4, resistance to four classes of antibiotics; R5, resistance to five classes of antibiotics; R6, resistance to six classes of antibiotics; MDR, multidrug resistance. *p value, Cramer V for association between resistance rate with antibiotic usage intensity; **p value, Cramer V for comparing resistance rate between bacterial isolates.

The increased resistance to Gentamicin, Doxycycline, and Erythromycin in *E. coli* isolates among high antibiotic users reflects broader AMR trends. While specific *E. coli*-related data were limited, a Centers for Disease Control and Prevention (CDC) study on doxycycline resistance observed that Erythromycin-resistant strains frequently exhibited cross-resistance to Clindamycin. Notably, significantly higher minimum inhibitory concentrations for Minocycline, Erythromycin, and Clindamycin were reported in patients receiving prolonged antibiotic therapy compared to antibiotic-naïve individuals, supporting the association between chronic antibiotic exposure and resistance development.[29]

Significant resistance to Ampicillin-Sulbactam and Trimethoprim-Sulfamethoxazole in *Klebsiella* spp. isolates from high antibiotic users is corroborated by recent research. A retrospective study conducted in Turkey over a four-year period analyzed urine culture isolates to determine the antibiotic resistance patterns of *K. pneumoniae*, revealing notably high resistance rates (80%) to Amoxicillin-Clavulanic acid and 74.8% to Trimethoprim-Sulfamethoxazole. These findings were particularly evident among hospitalized patients undergoing prolonged antibiotic therapy.[30] These results reinforce the association between antibiotic exposure and resistance development in *Klebsiella* spp.

The significant association between high antibiotic usage and Gentamicin resistance in *Klebsiella* spp. has historical precedent. A study documented the emergence of Gentamicin-resistant *Klebsiella* spp. in a general hospital setting in Canada following seven years of Gentamicin use. In this study nine different Gentamicin-resistant serobiotypes of *Klebsiella* spp. were isolated from 35 patients. Additionally, R-factor-mediated resistance was identified, with co-transfer of resistance to aminoglycosides such as Kanamycin, Neomycin, and Tobramycin. However, susceptibility to Amikacin and Netilmicin was retained, highlighting the importance of antibiotic stewardship and strategic antibiotic selection.[31] Although Ceftriaxone resistance was notably high in *Klebsiella* spp., no significant association with antibiotic usage was observed in the present study, a finding that contrasts with trends reported in recent literature. A retrospective, laboratory-based cross-sectional study conducted at the Pathology Center for Diagnosis and Research, Faculty of Medicine, University of Gezira, Sudan, based on clinical specimens collected from patients in Wad Medani between January 2020 and October 2023, revealed that *Klebsiella* spp. exhibited complete (100%) resistance to Ceftriaxone. This exceptionally high resistance rate may reflect geographical variability, differing levels of antibiotic exposure, or other contextual factors. Moreover, the study docu-

**Table 6.** Univariate and multivariate regression analysis of factors associated with multidrug-resistant isolates.

| Variables | Univariate regression | | | Multivariate regression | | |
|---|---|---|---|---|---|---|
| | B (S.E.) | OR (95% CI) | p value | B (S.E.) | OR (95% CI) | p value |
| **Frequency of antibiotic use** | | | | | | |
| Low antibiotic user* | | 1 | | | 1 | |
| Moderate antibiotic user | 2.191 (0.558) | 8.945 (2.996-26.711) | <0.001 | 1.730 (0.598) | 5.639 (1.748-18.189) | 0.004 |
| High antibiotic user | 2.438 (0.601) | 11.455 (3.529-37.184) | <0.001 | 1.641 (0.671) | 5.163 (1.386-19.231) | 0.014 |
| **Education level** | | | | | | |
| Higher education* | | 1 | | | | |
| Illiterate | 2.003 (0.523) | 7.413 (2.661-20.651) | <0.001 | 1.395 (0.606) | 4.035 (1.231-13.228) | 0.021 |
| Primary education | 1.776 (0.754) | 5.909 (1.349-25.879) | 0.018 | 1.232 (0.847) | 3.427 (0.651-18.037) | 0.146 |
| Secondary education | 0.678 (0.659) | 1.970 (0.541-7.169) | 0.304 | 0.243 (0.768) | 1.276 (0.283-5.744) | 0.751 |
| **Comorbidities** | | | | | | |
| Yes* | | 1 | | | | |
| No | -0.548 (0.402) | 0.578 (0.263-1271) | 0.173 | ** | ** | ** |
| **Surgical history** | | | | | | |
| Yes* | | 1 | | | | |
| No | -.028 (0.465) | 0.972 (0.391-2.418) | 0.952 | ** | ** | ** |
| **Self-antibiotic medication** | | | | | | |
| Yes | 2.215 (0.757) | 9.159 (2.077-40.384) | 0.003 | 1.567 (1.106) | 4.792 (0.548-41.873) | 0.157 |
| No* | | 1 | | | 1 | |
| **Completion of antibiotic course** | | | | | | |
| Yes* | | 1 | | | | |
| No | 0.297 (0.406) | 1.346 (0.607-2.986) | 0.465 | ** | ** | ** |
| **Source of antibiotic** | | | | | | |
| Prescribed by healthcare provider* | | 1 | | | 1 | |
| Purchased without prescription | 1.729 (1.063) | 5.636 (0.701-45.304) | 0.104 | 1.078 (1.110) | 2.938 (0.334-25.875) | 0.331 |
| Leftover antibiotics from previous use | 2.540 (1.041) | 12.682 (1.647-97.633) | 0.015 | 1.567 (1.106) | 4.792 (0.548-41.873) | 0.157 |

*Reference category; **variables that were not significant in univariate were excluded from the multivariate analysis. B, beta; S.E., standard error; OR, odds ratio; CI, confidence interval.

mented an overall Ceftriaxone resistance rate of 70.7% across all bacterial isolates, with variations influenced by both the bacterial species and the type of clinical specimen.[32] The inherently elevated baseline resistance to Ceftriaxone among *Klebsiella* spp. may account for the absence of a statistically significant increase in resistance among individuals with high levels of antibiotic consumption. The association between high antibiotic usage and Doxycycline resistance in *Klebsiella* spp. is indirectly supported by antibiotic resistance studies. While specific data on Doxycycline resistance related to *Klebsiella* spp. was limited, according to a study in the systematic review of CDC, which was on the use of Doxycycline and antimicrobial resistance, in which 150 patients of acne vulgaris were investigated to determine the resistance rate using minimum inhibitory concentration culture method, a statistically significant increase in resistance was observed among patients undergoing prolonged antibiotic therapy compared to those who have not received antibiotics. Specifically, patients who never used antibiotics showed no resistant isolates. In contrast, among those who had received short-term antibiotic treatment, 6.25% of isolates exhibited resistance, while 21.6% of isolates from patients on long-term antibiotic therapy displayed resistance. The differences in the rates of resistance between patients with no prior antibiotic use and those receiving prolonged therapy were statistically significant ($p=0.015$), as were the differences between those receiving short-term versus long-term antibiotic therapy ($p=0.036$).[29] In the current retrospective cross-sectional study, which focuses on co-isolations within the same host in addition to antibiotic usage, 54 (39.1%) of the 138 *Klebsiella* spp. isolates exhibited resistance, with a notably higher resistance rate observed among individuals with high antibiotic usage. This pattern reinforces the notion that increased antibiotic exposure plays a significant role in promoting doxycycline resistance, particularly in *Klebsiella* spp. isolates.

A distinctive contribution of this study is the examination of antibiotic resistance patterns in *E. coli* and *Klebsiella* spp. co-isolation within the same host. This approach differentiates the present work from most previous studies, which typically focuses on either organism in isolation. This co-isolation perspective may offer unique insights into potential transmission of resistance genes between bacterial species within the gut microbiome, a phenomenon theoretically described in the literature but rarely investigated in clinical samples.[33]

Variations in antibiotic resistance patterns between *E. coli* and *Klebsiella* spp. have been widely reported in the literature, highlighting the differential capacity of these pathogens to develop resistance. A study analyzing isolates from hospital wastewater in Dodoma, Tanzania, found that *K. pneumoniae* exhibited higher resistance rates to multiple antibiotics including Gentamicin, Ceftriaxone, Trimethoprim-Sulfamethoxazole compared to *E. coli*, suggesting that *Klebsiella* species may have a greater propensity for acquiring resistance, particularly in environments with high antibiotic exposure.[34] Similarly, a study by Joseph *et al*. conducted to examine the correlation between antimicrobial consumption and resistance patterns demonstrated that *K. pneumoniae* exhibited higher resistance rates to commonly used antibiotics, indicating a direct relationship between antibiotic usage and resistance development.[35] Additionally, a five-year longitudinal analysis conducted in a multi-profile hospital observed a significant increase in antibiotic resistance among *K. pneumoniae* isolates, whereas *E. coli* exhibited a comparatively slower rise in resistance rates.[15] These findings collectively emphasize the differential resistance patterns between these two species, reinforcing the need for targeted surveillance and intervention strategies. The current study, which isolated both bacteria from the same host, detected significant variations in resistance patterns. Resistance to Ampicillin-Sulbactam, Gentamicin, and Erythromycin was notably higher in *Klebsiella* spp., while resistance to Doxycycline showed minimal variation between the two species. The effect size analysis indicates that the bacterial isolate had a moderate to strong influence on resistance patterns for certain antibiotics, reinforcing the observation that *Klebsiella* spp. exhibits greater resistance compared to *E. coli* under similar biological conditions.

Recent studies have underscored the escalating threat of MDR infections, particularly concerning *E. coli* and *Klebsiella pneumoniae*. In a comprehensive surveillance study spanning Kenya, Uganda, and Jordan, high rates of MDR strains were identified in both *E. coli* and *K. pneumoniae* isolates, highlighting the global nature of this resistance.[39] Additionally, a study from Iran identified antibiotic use and prolonged hospital stays as risk factors for MDR *Klebsiella* infections.[37] Furthermore, a five-year study in a multi-profile hospital demonstrated a growing trend of antibiotic resistance among *E. coli* and *K. pneumoniae* clinical isolates, emphasizing the dynamic nature of bacterial resistance patterns.[15] These findings align with the current study, which observed higher MDR rates in *Klebsiella* spp. (67.4%) compared to *E. coli* (41.3%) among all antibiotic users. Furthermore, this study confirmed that antibiotic use was a significant risk factor for MDR, reinforcing the urgent need for enhanced antimicrobial stewardship and the development of novel therapeutic strategies to combat the rising threat of drug-resistant bacterial infections.

The current methodological approach offers several innovations that enhance this study's contribution to the field. The classification of participants into low, moderate, and high antibiotic usage groups provides a more nuanced assessment than the binary classifications used in other studies. Additionally, the present study alignment with the WHO's AWARE framework represents a forward-thinking approach that contextualizes findings within global antimicrobial stewardship priorities, an approach endorsed by Sharland *et al.* for standardizing AMR research.[38] Despite its strengths, this study has several limitations that warrant consideration. The cross-sectional design limits its ability to establish causal relationships between antibiotic usage and resistance development. The reliance on a questionnaire to classify antibiotic users is due to the absence of standardized guidelines for such categorization. The geographical limitation to a single region in Iraq may restrict generalizability to other settings with different antibiotic accessibility patterns, a concern raised by Founou *et al.* regarding regional variation in resistance patterns.[39] While this study provides valuable insights into the phenotypic resistance patterns of *E. coli* and *Klebsiella* spp. co-isolations, future research should incorporate molecular and genomic analyses to further elucidate the underlying mechanisms of resistance. Whole-genome sequencing, plasmid profiling, and detection of specific resistance genes can provide deeper insights into resistance transmission pathways, clonal relationships, and the genetic basis of multidrug resistance.

The clinical implications of the current study findings are substantial for practitioners managing infections caused by *E. coli* and *Klebsiella* spp., particularly in settings with high rates of inappropriate antibiotic use. The high resistance rates observed for front-line antibiotics suggest that empirical treatment guidelines may need revision in certain populations. For patients with extensive antibiotic exposure history, clinicians should consider this history as a risk factor for resistant infections, potentially favoring broader-spectrum agents while awaiting culture results.

## Conclusions

This study highlights the urgent need for stricter antimicrobial stewardship, particularly in high antibiotic-use settings. The strong link between antibiotic consumption and MDR in *E. coli* and *Klebsiella* spp. underscores the necessity for targeted interventions, including stricter prescription policies and rapid diagnostic testing. Variations in resistance patterns suggest intrinsic bacterial differences, warranting further molecular investigation. Additionally, the reliance on questionnaire-based classification emphasizes the need for standardized antibiotic usage guidelines. Furthermore, the findings offer practical implications for healthcare providers, emphasizing the importance of judicious antibiotic use in preventing the emergence of MDR infections, which can inform treatment protocols and infection control strategies. Future research should focus on longitudinal surveillance and resistance mechanisms to combat rising AMR.

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