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Original Article
Prevalence of bacterial infections, antimicrobial sensitivity, and resistance patterns in respiratory samples

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Abstract: Objective: Respiratory tract infections (RTIs) are a significant global health concern, particularly with the rise of antimicrobial resistance (AMR). This study aimed to investigate the prevalence of bacterial pathogens, resistance patterns, and gender-specific differences among patients with RTIs admitted to a tertiary-level hospital in South Punjab, Pakistan. Methods: The retrospective study, which lasted from September 2023 to February 2024, included 194 patients with bacterial RTIs. Demographic data, clinical characteristics, and bacterial isolates were analyzed. The antibiotic susceptibility of 194 bacterial isolates was assessed using the disc diffusion method. Bacteria were classified as extensively drug-resistant (XDR), multidrug-resistant (MDR), or pan-drug-resistant (PDR) based on standard criteria. The impact of bacterial resistance on mortality and ICU admissions was examined using multivariate Cox regression analysis. Results: The study cohort had a mean age of 66.5 ± 10.8 years, with 76.4% being male. ICU admissions were higher among males (25%) than females (9%). *Pseudomonas aeruginosa* (12.89% in males; 15.46% in females) and *Klebsiella pneumoniae* (3.61% in males; 9.79% in females) were the most prevalent Gram-negative bacteria, whereas *Streptococcus* spp. and *Moraxella catarrhalis* were the most common Gram-positive bacteria. A higher mortality rate was observed among MDR-infected patients (12.22%) compared to those with non-resistant strains (4.89%). Resistance to beta-lactams, fluoroquinolones, and macrolides was particularly pronounced in ICU patients. Gender-specific differences in bacterial prevalence and resistance patterns were noted, with females exhibiting higher rates of *P. aeruginosa* and MRSA infections. Conclusion: The study underscores the growing burden of antimicrobial resistance in RTIs, with significant gender-based disparities. The high prevalence of MDR bacteria highlights the urgent need for targeted antibiotic stewardship programs and infection control measures to mitigate the impact of drug-resistant respiratory infections.

Keywords: Respiratory disease, bacterial infections/microbiology, drug resistance, drug sensitivity

Introduction

Infections of the respiratory tract are the most common cause of disease globally [1]. In developing countries, upper and lower respiratory tract infections are the most common causes of disease and death. As far as public health is concerned, respiratory infections are the leading cause [2]. The most common cause of illness in Pakistan are respiratory illnesses [3, 4]. Respiratory diseases have a significant economic impact due to lost production and the high cost of drugs doctors prescribe, even when bacteria are not the leading cause. The most prevalent respiratory bacterial pathogens are *Klebsiella* spp., *Pseudomonas, Streptococcus pneumonia, Staphylococcus aureus. M. catarrhalis, S. aureus*, and *S. pneumonia*e which are common causes of upper respiratory tract infections (URI). *Acinetobacter spp.,* *K. pneumoniae,* and other members of the *P. aeruginosa* family are some Gram-negative bacteria that most often cause lower respiratory tract infections (LRTIs). Gram-positive bacteria, on the other hand, cause LRTIs less often. *S. pneumoniae* and *S. aureus* are more common among Gram-positive bacteria [5]. One of the biggest concerns facing the global healthcare industry is antibiotic resistance. It has led to the emergence of mutated bacterial strains. *P. aeruginosa* and *S. pneumoniae* are two respiratory bacteria that are not very sensitive to antibiotics [6]. One of the main issues facing low- and middle-income countries (LMICs), such as Pakistan, is the rise in illness, death, and medical costs brought on by antimicrobial resistance (AMR) [7-15]. A big reason is that doctors do not always prescribe and give out antibiotics correctly, especially for illnesses that get better on their own. Another reason is that there are not enough comprehensive monitoring systems to track antibiotic use and trends in bacterial resistance. There are problems with infrastructure and sanitation, doctors who do not know much about AMR and antibiotics, and the wrong use of antibiotics in farming and agriculture in many LMICs, all of which make AMR worse [16]. It is hard for healthcare officials in different countries, especially LMICs that do not have a lot of money, people, or infrastructure, to deal with these issues and regularly monitor AMR patterns in all care areas [17]. This cross-sectional experimental study will evaluate the sensitivity of the bacterial strains to antibiotics, investigate their gender-specific resistance patterns, and determine the prevalence of bacterial infections in respiratory samples in the oldest city in Asia, Multan, Pakistan [18]. This research aims to thoroughly understand bacterial infections of the respiratory system, including the types of bacteria involved, how susceptible they are to antibiotics, and whether there are any changes in infection rates or resistance patterns between genders. Sputum, tracheal secretions, bronchial washes, and pleural fluid were among the 270 RP samples analyzed. We examined every sample's antimicrobial sensitivity, gram stain, and culture [19]. We applied Bartlett's grading system while grading sputum samples. Following incubation at 37°C, we checked the cultures for growth. Seventy-six RP exhibiting normal throat flora (NTF) or no bacterial growth were cautiously excluded. Of the 194 patients discharged with bacteria, 51% were men, and 49% were women. Males made up 25% of the ICU and females 10%. A total of 90% of females and 75% of males were from the outpatient department (OPD). There were differences in survival rates: OPD was 95% for males and 94.8% for females, whereas in the ICU, it was 72% for males and 77% for females. Unfortunately, the mortality rates in the ICU were greater than outside of it for both genders; 28% of males and 22% of females died there, compared to just 5% outside of the ICU [20].

Materials and methods

Study population

The retrospective study collected data from patients with respiratory tract disorders admitted to a South Punjab tertiary-level hospital in Multan, Pakistan, between September 2023 and February 2024. The hospital information system (HIS) was used to look at all the people who took part in the study who had a respiratory disease. They were asked about their age and gender, any other illnesses they had, how long they were in the hospital, whether they were admitted to the ICU or not. Data on bacterial pathogen species, antibiotic sensitivity, and resistance trends were gathered. The study enrolled participants with bacterial illnesses. Patients who did not have bacterial flare-ups were not considered.

Study definitions

Samples were taken from patients who needed treatment for a respiratory disease that affect the lungs and other parts of the respiratory system and have symptoms like coughing, phlegm, shortness of breath, or sputum. The study followed the rules set by Magiorakos et al. [21] to find pathogenic microorganisms that were resistant to many drugs (XDR), multiple drugs (MDR), and all drugs (PDR). According to our research, drug-resistant bacteria do not respond to at least one type of antibacterial drug. Single-drug-resistant (SDR) strains cannot be killed by more than one antibacterial agent from the same class. It was said that at least one antibiotic agent from three or more classes did not kill MDR strains. PDR strains, on the other hand, were resistant to all the antimicrobial agents used.

Microbiological study

Respiratory samples obtained for routine diagnostics at the microbiology laboratory were used in the inquiry. The respiratory system produces sputum, tracheal secretions, bronchial washings, and pleural fluids. The department's standard operating procedures were followed when handling the samples. In summary, Gram staining was used to evaluate the sputum sample's quality, and samples with Bartlet scores of 0-1 or 2 were disqualified from additional examination. To separate microorganisms, sputum samples with Bartlet scores of +1 and +2 were added to blood, chocolate, and MacConkey agar medium. The media were then incubated for a whole night at 37 degrees Celsius. We used biochemical tests such as the triple sugar iron test (TSI), citrate, sulfide indole motility (SIM), oxidase, catalase, and coagulase to identify the growth further. We used the disc diffusion method to assess the antibiotic susceptibility of the isolates [22, 23]. We screened all study participants' clinical and demographic data using HIS. We categorized the bacteria as potentially pathogenic pathogens (PPMs) or non-PPMs based on the isolated organism, following the guidelines outlined by Cabello et al. Microorganisms that were found to cause respiratory diseases were called PPMs [24]. It was true whether they were linked to the oropharyngeal or gastrointestinal flora. PPMs included *H. influenzae, Proteus, E. coli, S. aureus, Citrobacter, Enterobacter, P. aeruginosa, Acinetobacter spp.,* and *K. pneumoniae.* We classified non-PPM microorganisms from the gastrointestinal or oropharyngeal flora as typically not associated with respiratory illnesses in patients without immune deficiencies.

Antibiotic susceptibility

From September 2023 to February 2024, a total of 194 bacterial isolates were subjected to antibiotic susceptibility testing. This testing included carbapenems (ertapenem, doripenem, imipenem, and meropenem), aminoglycosides (gentamicin and amikacin), fluoroquinolones (norfloxacin, ciprofloxacin, gatifloxacin, and levofloxacin) other cephalosporins (ceftazidime, cefepime, cefoperazone/sulbactam, ceftriaxone, cefotaxime, cefoxitin, and cefuroxime), for gram-negative bacteria, and macrolides (erythromycin), tetracyclines (minocycline), glycopeptides (teicoplanin and vancomycin), penicillins (penicillin-G, oxacillin, amoxicillin-clavulanic acid, ampicillin, piperacillin/tazobactam, and ticarcillin/clavulanic acid), glycylcyclines (tigecycline), sulfonamides (trimethoprim/sulfamethoxazole), lipopeptide (colistin), phenicols (chloramphenicol), nitrofurantoin, and oxazolidinones (linezolid) for gram-positive bacteria.

Inclusion/exclusion criteria

The ICD-10 codes J44.1 and J44.9 were used to identify the eligible subjects. The study included both male and female participants, aged 40 and older, who had received a diagnosis of RTI and tested positive. The study excluded participants without bacterial growth, other respiratory samples, and pertinent missing data.

Statistical analysis

The descriptive data were presented as the mean (standard deviation) of continuous variables and as frequencies of discrete variables. The student t-test for continuous variables and the chi-squared test for the categorical variables were compared through a univariate analysis to assess the statistical significance. Statistical analyses were performed with SPSS software v.22 (IBM, Corp., Armonk, NY, USA) and the Stata 16 version (StataCorp., College Station, TX, USA). All statistical tests were two-tailed, and the factors were considered statistically significant at $P < 0.05$ and were included in the generalized linear model (GLM) and used a generalized estimating equation (GEE) if they were found to be statistically significant on a univariate analysis.

Results

As illustrated in **Figure 1**, the study comprised 194 patients (71.8%) admitted with a bacterial respiratory infection, whereas the study excluded patients with no bacterial growth (28.7%) and no relevant data. **Table 1** provides a summary of the patient's initial characteristics. Our study population had a mean age of 66.5 years ± 10.8, and 76.4% were men. We split ICU admissions and non-ICU admissions among the patients. Male deaths were almost twice as high as female deaths, and the research population's comorbidities were prevalent (**Figure 1**).

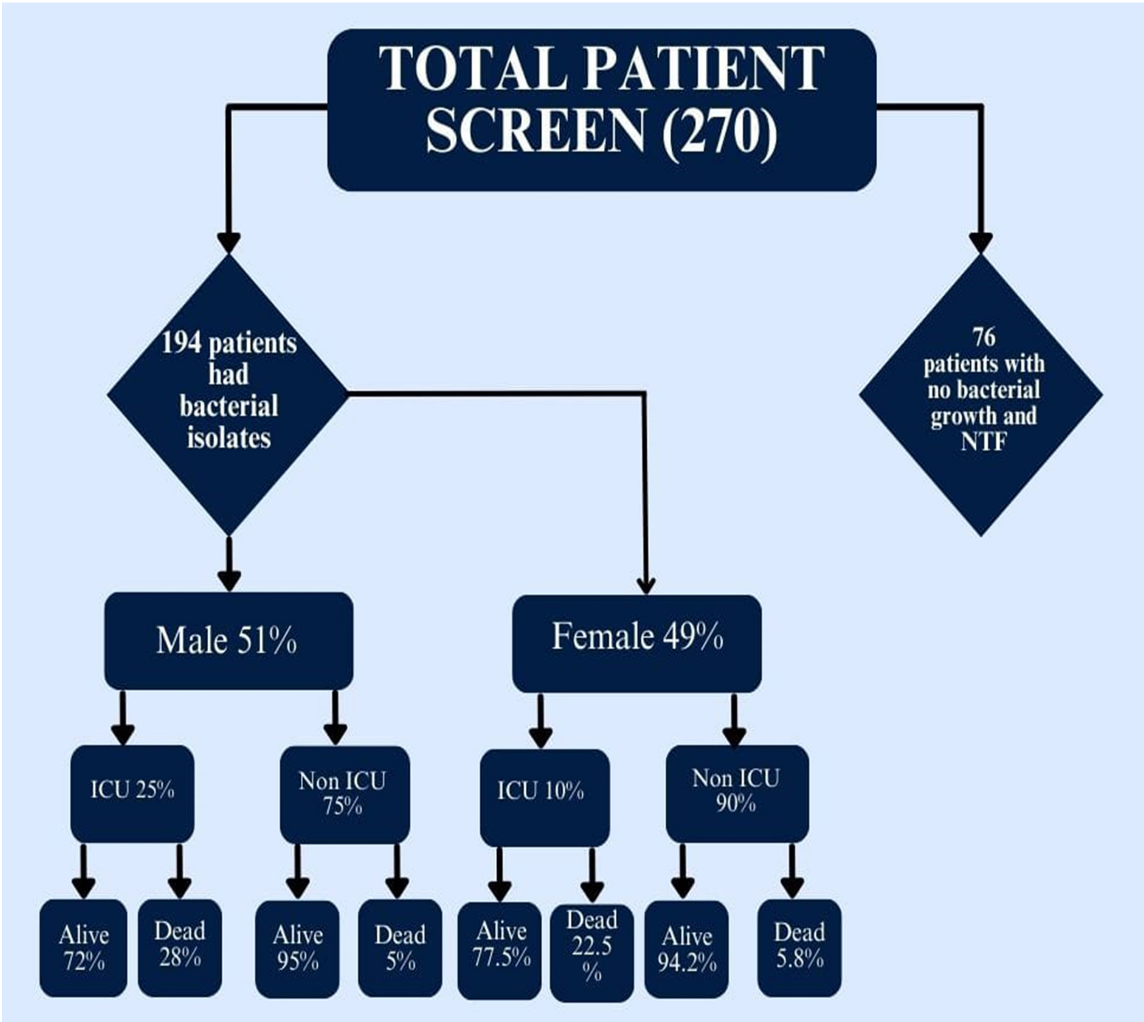

Figure 1. Study flowchart showing rates of in-hospital, mortality, gender, distribution, total number of participants screened, number of subjects in whom germs were identified, and place of admission (ICU or non-ICU).

Table 2 describes the Minimum Inhibitory Concentrations (MICs) of major bacterial isolates against several antibiotics, indicating resistance patterns. Pseudomonas aeruginosa showed resistance to amoxicillin/clavulanic acid, cefaclor, and clarithromycin but intermediate resistance to aztreonam, piperacillin/tazobactam, and ceftriaxone, and was sensitive to levofloxacin. Proteus mirabilis showed sensitivity to amikacin but intermediate resistance to aztreonam and piperacillin/tazobactam, while Proteus vulgaris was sensitive to cefixime. Klebsiella spp. demonstrated intermediate resistance to ceftriaxone and amikacin but was susceptible to levofloxacin. Escherichia coli exhibited resistance to cefaclor and amoxicillin/clavulanic acid. **Table 3** shows the average recovery time and ICU stay for various infections in bacteria, with Acinetobacter spp. and MRSA infections having the longest ICU stays (14 and 15 days, respectively) and the longest recovery times (25 and 28 days, respectively). Streptococcus spp., on the other hand. infections had the shortest ICU stay (6 days) and recovery time (10 days). These results highlight the effect of antimicrobial resistance on patient outcomes, with highly resistant infections having longer hospitalization and slower recovery.

Bacterial prevalence in different genders during study span

During the six-month study, the prevalence of various bacterial species in male and female samples was analyzed out of 194 samples.

Table 1. Participants' clinical, demographic, microbiologic, and survival data as well as the test of significance comparing the male and female bacterially infected subjects

Characteristics	Total (n=194)	Male (n=100)	Female (n=94)
Age (Mean ± SD)	66 (60 to 75)	66.8 ± 10.7	65.6 ± 11.3
Non-ICU	160	75	85
ICU	34	25	9
Alive	175	88	87
Dead	19	12	7
Potentially pathogenic organism			
Yes	194	100	94
No	76	48	28
Co-morbidities			
Asthma	36	20	16
COPD	15	07	08
Bronchitis	24	17	10
Pneumonia	17	10	07
Tuberculosis	13	07	06
Covid-19	07	05	02
Obesity	26	17	09
Sepsis	18	11	07
Hypertension	120	56	64
Pulmonary hypertension	64	28	36

Table 2. Minimum inhibitory concentrations (MICs) for Key bacterial isolates

Bacterial Species	Antibiotic	MIC Range (μg/mL)	Resistance Category
P. aeruginosa	Amikacin	4-16	Intermediate
	Aztreonam	8-32	Intermediate
	Piperacillin/tazobactam	2-64	Intermediate
	Amoxicillin/clavulanic acid	> 32	Resistant
	Cefaclor	> 31	Resistant
	Clarithromycin	100%	Resistant
	Levofloxacin	1-8	Sensitive
	Ceftriaxone	1-64	Intermediate
P. mirabilis	Amikacin	2-8	Sensitive
	Aztreonam	4-16	Intermediate
	Piperacillin/tazobactam	1-64	Intermediate
P. vulgaris	Cefixime	0.5-8	Sensitive
Klebsiella spp.	Amikacin	2-16	Intermediate
	Levofloxacin	0.5-8	Sensitive
	Ceftriaxone	1-64	Intermediate
E. coli	Amoxicillin/clavulanic acid	4-32	Resistant
	Cefaclor	2-16	Resistant

Among the gram-positive bacteria, *Streptococcus species* accounted for approximately 3.09% of male and 2.06% of female samples. We found *M. catarrhalis,* another gram-positive bacterium, in 7.22% of male samples and 8.76% of female samples. For gram-negative bacteria, *K. pneumoniae* was prevalent in 3.61% of male samples and notably higher in females at 9.79%. Of the gram-negative bacteria found in 12.89% of the male samples and

Table 3. Length of ICU stay and recovery time for different bacterial infections

Bacterial Species affecting patients	Average ICU Stay of patients (days)	Average Recovery Time of patients (days)
P. aeruginosa	10	18
P. mirabilis	7	14
P. vulgaris	5	12
Klebsiella spp.	12	20
E. coli	8	16
Streptococcus spp.	6	10
Acinetobacter spp.	14	25
MRSA	15	28

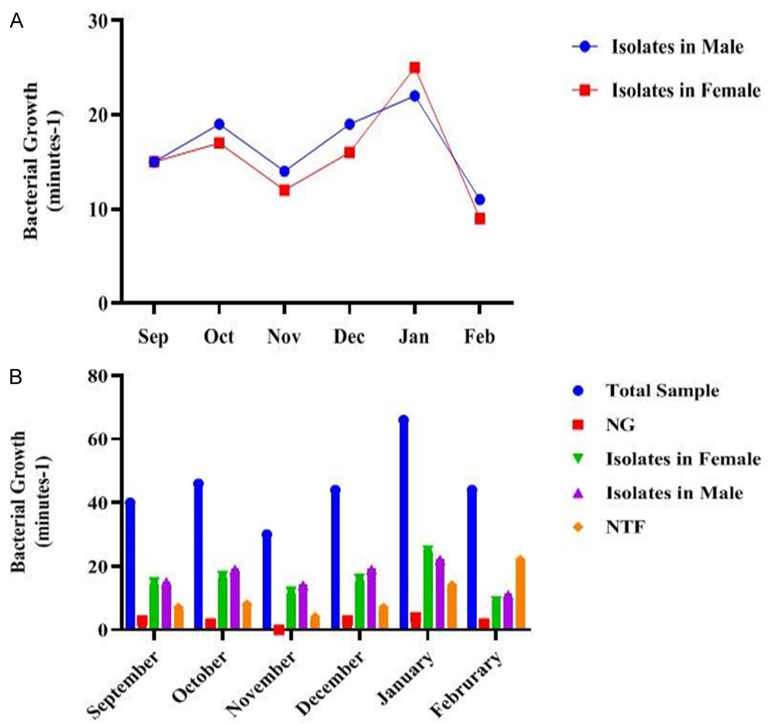

Figure 2. Bacterial prevalence was observed from September 2023 to February 2024 in males and females (A), while isolates from different genders observed in total samples detected from study span were shown in (B).

15.46% of the female samples, *P. aeruginosa* was the most common. *Acinetobacter spp.* accounted for 8.76% of male samples and 6.19% of female samples. *K. oxytoca* and *P. mirabilis* showed similar prevalence in both genders, each accounting for approximately 1.03% and 5.67% in male samples and 1.03% and 0.52% in female samples, respectively. *E. coli* and MRSA were also more prevalent in males, with *E. coli* accounting for 4.64% in males and 1.55% in females, while MRSA accounted for 4.64% in males and 3.09% in females. These results show that the prevalence of different types of bacteria is different in male and female samples. Some pathogens have different prevalence rates for men and women (**Figure 2**).

Bacterial distribution in different genders during study span

The comparison of bacterial isolates from male and female samples reveals unique trends in microbial composition. *P. aeruginosa, K. pneumoniae,* and *Acinetobacter spp.* are common in males, but *M. catarrhalis* is particularly abundant. Females have a higher frequency of *P. aeruginosa* and *K. pneumoniae*. *Acinetobacter spp.* and MRSA are less prevalent in female samples. The presence of Streptococcus species, *K. oxytoca*, *E. coli*, and *P. mirabilis* varies with gender. These connections show that microbes might colonize differently depending on gender, which can affect health and treatment efforts (**Figure 3**).

Trends in proportion of drug sensitivity

All bacteria isolated were subjected to antibiotic sensitivity testing, which includes commonly used drugs from different antibiotic classes for the treatment of bacterial infections such lincomycin and gentamicin (aminoglycosides), Penicillin-G, Ampicillin, Amoxicillin-clavulanic acid, Piperacillin/tazobactam (peni-

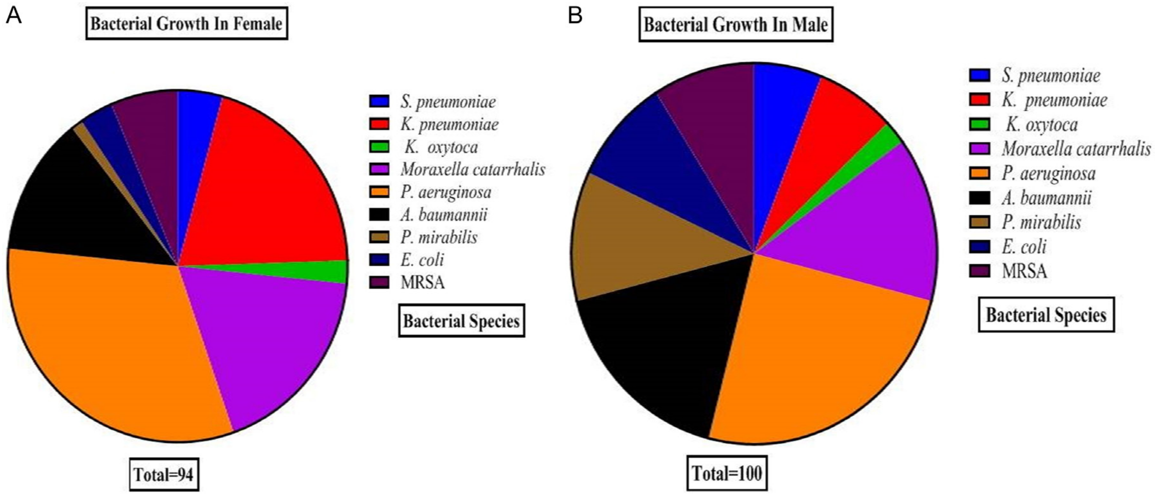

Figure 3. Distribution of the bacterial species in female patients. The pie chart displays the relative abundance of each species that cause bacterial growth among female patients and a total number of 94 isolates. It demonstrates the relative abundance of each of these species, ranging from *Streptococcus pneumoniae* to *Escherichia coli* and also Methicillin-resistant *Staphylococcus aureus* (MRSA). (A) Distribution of male patient bacterial species. The pie chart illustrates the distribution of the bacterial species responsible for bacterial growth among male patients, totaling 100 isolates. This demonstrates the relative incidence of each species, such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Escherichia coli*, and Methicillin-resistant *Staphylococcus aureus* (MRSA) (B).

cillin); Ceftazidime, Cefotaxime, Cefepime, Cefoperazone/sulbactam, Cefoxitin, Ceftriaxone, Cefuroxime (Cephalosporins); Meropenem, Imipenem (Carbapenems); Levofloxacin, Moxifloxacin, Ciprofloxacin (Fluoroquinolones), azithromycin and roxithromycin (macrolides), minocycline, glycylcyclinetigecycline (tetracycline), colistin (lipopeptide), and linezolid (oxazolidinones) for gram-positive bacteria (**Figure 4**).

In examining the sensitivities of various microbial agents to different antibiotics, notable differences emerge among the bacterial strains. Of the 55 samples of *P. aeruginosa,* 20% were moderately sensitive to amikacin, 20% to aztreonam, and 20% to piperacillin/tazobactam. However, it exhibited high resistance to amoxicillin/clavulanic acid (28%) and cefaclor (31%), while complete resistance was observed with clarithromycin. *P. aeruginosa* showed intermediate sensitivity to levofloxacin (13%) and ceftriaxone (14%). Thirteen samples of P. mirabilis showed slightly lower sensitivity to most antibiotics than P. aeruginosa. Amikacin and aztreonam had an 8% sensitivity, and piperacillin/tazobactam had a 9% sensitivity. *P. vulgaris,* with samples, exhibited similar patterns to *P. mirabilis,* although it showed slightly higher sensitivity to cefixime (4% compared to 2%). With only 30 samples, *Klebsiella* spp. was only slightly sensitive to amikacin (2%) and levofloxacin (3%), but they were very resistant to clarithromycin (13% of samples) and ceftriaxone (7% of samples). Finally, 9 samples of *E. coli* showed different susceptibility levels, similar *to P. aeruginosa.* Notably, 23% of the samples were resistant to amoxicillin/clavulanic acid, and 14% were resistant to cefaclor. Overall, some antibiotics worked the same way against different microbes, while others were more or less sensitive to different types of bacteria. This shows how important it is to choose an antibiotic based on how well it works against that particular strain of bacteria.

Discussion

RP experience elevated mortality and morbidity due to exacerbations caused by bacteria that have developed resistance to antimicrobial drugs. The rising prevalence of antibiotic-resistant bacteria in RTIs poses a significant challenge for clinical management and patient outcomes. In this study, we investigated bacterial prevalence, resistance patterns, and gender-specific differences among 270 patients with RD from September 2023 to February 2024. The study found that 71.85% of

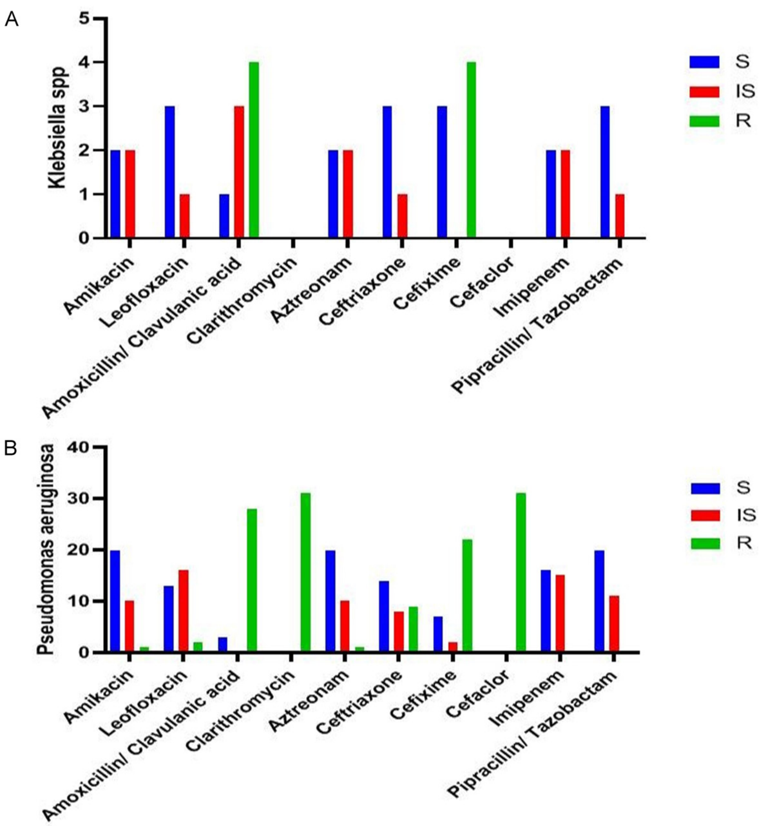

Figure 4. Antibiotic resistance profile of *Klebsiella* spp. and *Pseudomonas aeruginosa*. Antibiotic resistance profile for *Klebsiella* spp. isolates, representing the distribution of isolates categorized as sensitive (S), intermediate sensitive (IS), and resistant (R) to different antibiotics. The bars represent the relative proportion of isolates for every antibiotic, including Amikacin, Amoxicillin-Clavulanic acid, Ceftriaxone, Ceftazidime, and others. The comparison represents the variation in resistance among different antibiotics (A). Antibiotic susceptibility pattern of Pseudomonas aeruginosa, classifying isolates as sensitive (S), intermediate sensitive (IS), or resistant (R). The graph features antibiotics like Amikacin, Piperacillin-Tazobactam, Cefepime, and more, demonstrating the resistance pattern of Pseudomonas aeruginosa compared to Klebsiella spp. The trends depict the degree of antimicrobial resistance in the pathogens and assist in the comprehension of the efficacy of treatment regimens (B).

patients had bacterial isolates, indicating a high bacterial colonization or infection burden in this population. RP experience elevated mortality and morbidity due to exacerbations caused by bacteria that have developed resistance to antimicrobial drugs. The rising prevalence of antibiotic-resistant bacteria in RTIs poses a significant challenge for clinical management and patient outcomes. Specifically, our analysis revealed a mortality rate of 12.22% among patients infected with MDR bacteria, compared to a significantly lower mortality rate of 4.89% among patients with non-resistant strains.

Furthermore, the presence of extensive XDR bacteria, particularly in ICU settings, was associated with higher mortality rates, reflecting the serious clinical threat posed by these pathogens. This aligns with global trends, emphasizing the urgency of addressing antimicrobial resistance to improve patient outcomes. Our findings reveal several critical insights into the bacterial landscape in RTIs and the evolving antimicrobial resistance patterns.

Our results indicate that certain bacterial species display gender-specific differences in prevalence. According to the data, females were more likely to have *P. aeruginosa* (15.46%) than males (12.89%). Similarly, females were more likely to have *K. pneumoniae* (9.79%) than males (3.61%). These findings align with previous studies that suggest a possible role of gender in susceptibility to certain bacterial infections. For instance, Fink et al. [30] suggested that hormonal differences between genders might affect immune response, potentially influencing bacterial colonization. Other studies, such as those by Munoz-Price et al. [21], reported similar gender-specific discrepancies in bacterial prevalence, especially in ICU settings. Because *K. pneumoniae* and *P. aeruginosa* are more common in women, it is more important to focus on treating them specifically. It is due to their frequent association with severe RTIs and their high drug resistance [22].

On the other hand, Acinetobacter spp. was more common in men (8.76%) than in women (6.19%). Research indicates that the pathogen is associated with ICU populations predominantly composed of men [25]. Also, *Streptococcus* species were more common in males

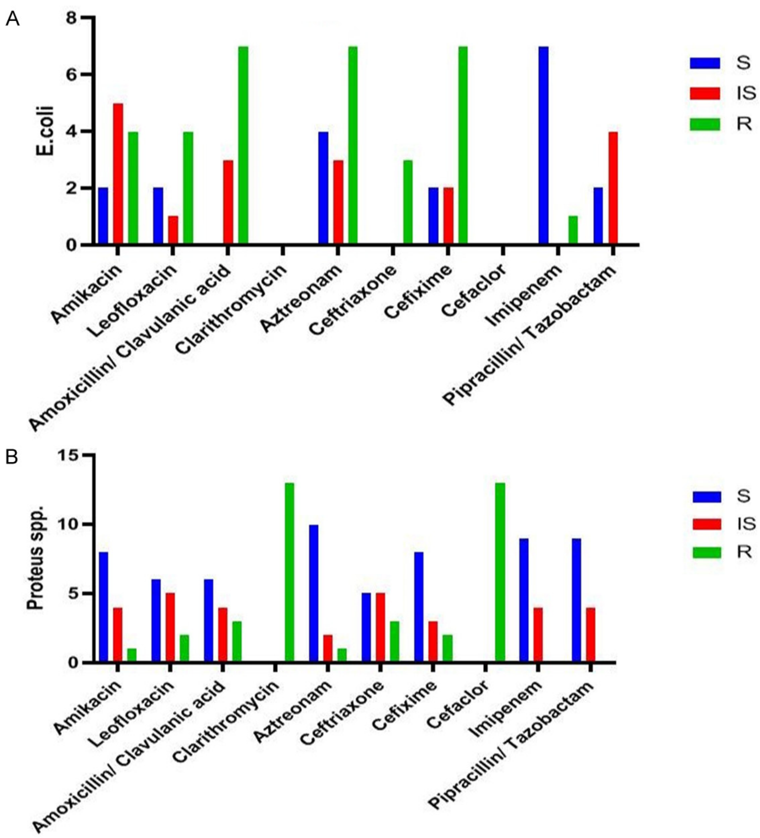

Figure 5. Susceptibility pattern of *Escherichia coli* and *Proteus* spp. isolates to antibiotics. Resistance patterns of *Escherichia coli* isolates to different antibiotics, divided into sensitive (S), intermediate sensitive (IS), and resistant (R) groups. The bars signify the reaction of isolates towards different antibiotics such as Amikacin, Levofloxacin, Amoxicillin-Clavulanic acid, Ceftriaxone, Ceftazidime, and Piperacillin-Tazobactam. A high percentage of resistance is seen towards some antibiotics, indicating antimicrobial resistance patterns among *E. coli* (A). Antibiotic susceptibility pattern of Proteus spp., again in the same order (S, IS, R). The prevalence of resistance against various antibiotics is indicative of the efficacy of the available treatment strategies for Proteus spp. infection. Comparison of E. coli and Proteus spp. is indicative of differences in patterns of resistance, and this information will be helpful for antimicrobial stewardship and selective treatment (B).

(3.09%) than females (2.06%), *and M. catarrhalis* were more common in females (8.76%) than males (7.22%), which shows that respiratory pathogens are different for men and women. These results are similar to those of Podschun and Ullmann [16], who also found differences between men and women in bacterial respiratory infections, mainly with *Streptococcus and M. catarrhalis.* Understanding these gender-based differences is essential for tailoring therapeutic strategies, particularly in light of growing antimicrobial resistance [26-29].

Our analysis revealed alarming trends in AMR, particularly among ICU patients [31]. Using regression analysis and generalized estimating equations (GEE), we accounted for factors such as age, gender, and comorbidities, finding that the prevalence of antibiotic resistance was significantly high across ICU and non-ICU patients. *P. aeruginosa*, with 55 samples, demonstrated intermediate sensitivity to amikacin, aztreonam, and piperacillin/tazobactam (20% each) yet showed high resistance to amoxicillin/clavulanic acid (28%) and cefaclor (31%), with complete resistance to clarithromycin (**Figures 5**, **6**). In comparison, *P. mirabilis*, represented by 13 samples, exhibited slightly lower sensitivities, with amikacin and aztreonam at 8% sensitivity and piperacillin/tazobactam at 9% (**Figure 4**). *P. vulgaris* followed a similar trend but displayed marginally higher sensitivity to cefixime (4% versus 2% for *P. mirabilis*), shown in **Figure 4**. Thirty samples of *Klebsiella* species had weak reactions to amikacin (2%) and levofloxacin (3%) but strong reactions to clarithromycin (13% of samples) and ceftriaxone (7% of samples). Finally, 9 samples of *E. coli* had sensitivity patterns that were similar to *P. aeruginosa.* They were very resistant to amoxicillin/clavulanic acid (23%) and cefaclor (14%). These results align with what the World Health Organization (18) and the Centers for Disease Control and Prevention (15) have said about global trends. They show how hard it is to fight antibiotic resistance and how important it is to choose antibiotics based on which strains of bacteria are most likely to become resistant to them. This will help treatments work better and stop the growing threat of multidrug-resistant infections.

The persistence of MDR bacteria in RTIs is a cause for concern, as it limits treatment options and increases the risk of poor clinical out-

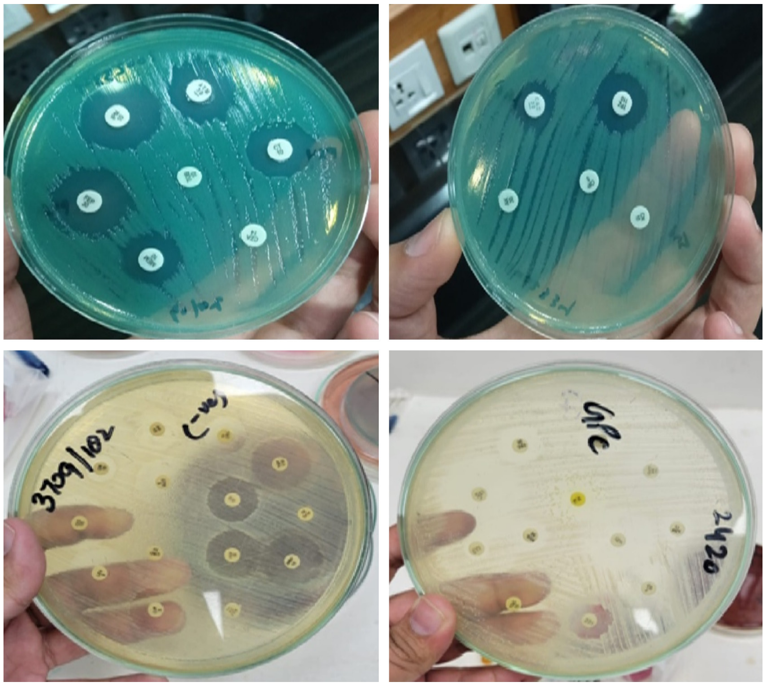

Figure 6. Culture plates showing culture sensitivity test against various pathogenic bacteria from respiratory tract samples.

Although our study provides valuable insights, several limitations should be acknowledged. First, the relatively short study duration (six months) may not fully capture the seasonal variability in bacterial prevalence and resistance. Additionally, while we included gender differences in bacterial prevalence, we did not investigate the underlying biological mechanisms contributing to these differences. Future research should explore the role of hormonal and genetic factors in shaping gender-specific susceptibility to respiratory pathogens. Moreover, our sample size, though sufficient for statistical analysis, may not reflect broader population-level trends. A larger, multicenter study would help validate our findings and provide a more comprehensive understanding of AMR in respiratory infections.

comes. Our study confirmed what Pouwels et al. [21] found: resistance patterns were more potent in females against some pathogens, like MRSA and *P. aeruginosa* (**Figure 7**). They reported higher rates of AMR in females due to increased antibiotic exposure, particularly in outpatient settings. It highlights the need for gender-specific antimicrobial stewardship programs to prevent the development and spread of resistance.

The significant differences in how well different types of bacteria in our group responded to antibiotics, especially *P. aeruginosa, P. mirabilis, Klebsiella spp.,* and *E. coli,* show how important it is to change how we treat infections immediately. Li et al. [19] say that empirical antibiotic regimens, which usually include drugs like amikacin and piperacillin/tazobactam, might not work anymore, especially in places where resistance is high. Although some antibiotics retained activity against specific strains, their effectiveness varies significantly. More research is needed to see how well these antibiotics work in situations where men and women are treated because the way men and women process drugs could affect the results of treatment (**Figure 8**).

In conclusion, our study shows how common bacterial respiratory infections are and how dangerous antimicrobial resistance is becoming, especially in intensive care units (ICUs). Gender-specific differences in bacterial prevalence and resistance patterns were evident, emphasizing the need for tailored therapeutic approaches. The high prevalence of MDR and XDR pathogens underscores the importance of implementing robust infection control measures and antimicrobial stewardship programs. As resistance trends change, more research should be done to find new ways to treat infections and learn more about the gender-related factors that affect bacterial colonization and resistance.

Strengths and limitations

As far as we know, this was the first study to look into patterns of medication resistance in Multan, Punjab, patients by gender, ICU admission type, and non-ICU admission type. We employed an innovative statistical methodology. This study is among the limited number of

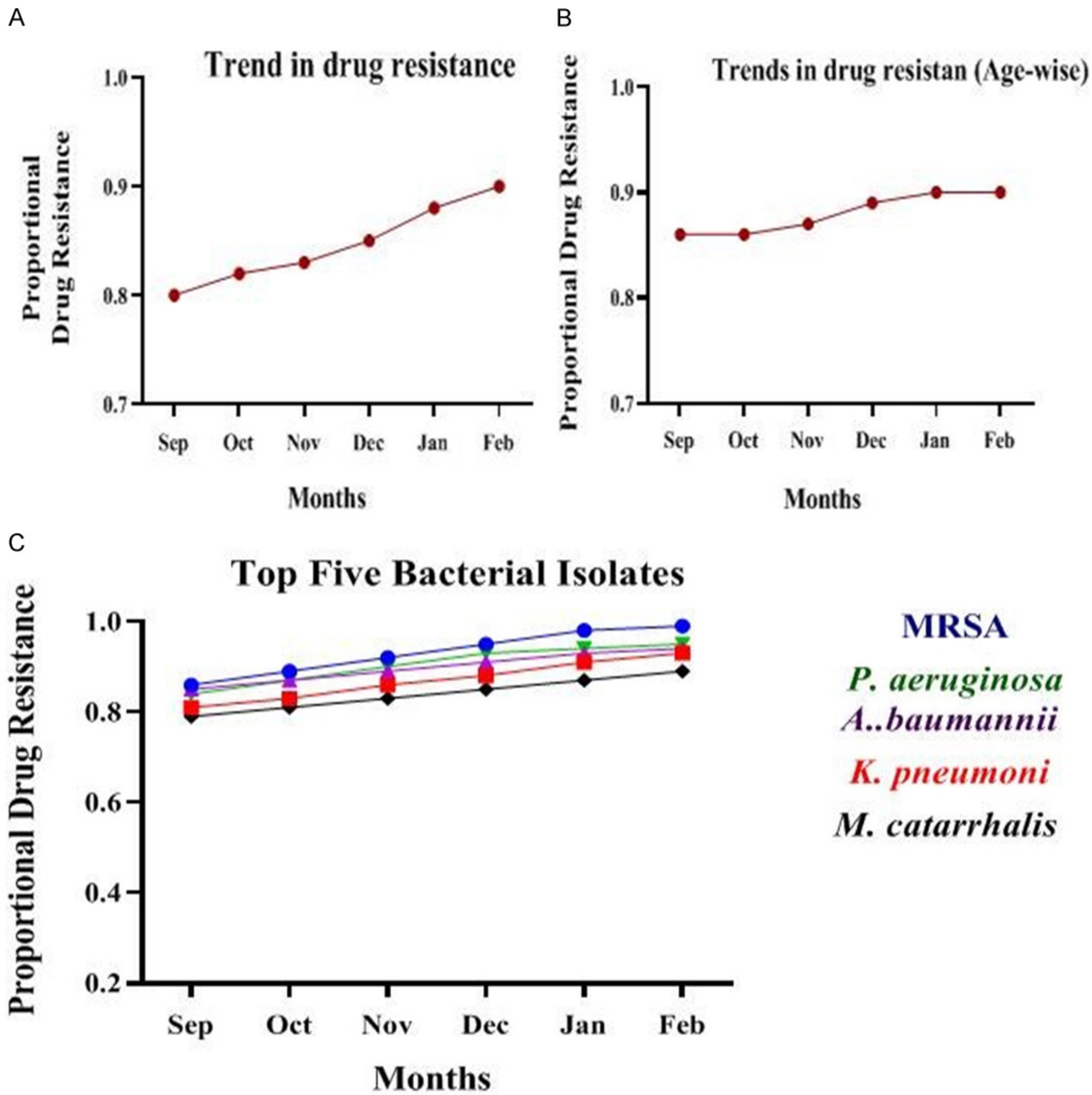

Figure 7. Temporal and age-wise pattern of drug resistance. Monthly pattern of proportional drug resistance between September and February. The graph reflects the increment in drug resistance over time, suggesting an increasing trend of antimicrobial resistance in bacterial isolates (A). Age-specific proportional drug resistance over the same period. The trend indicates fluctuations in the levels of resistance between age groups with a marginal upward trend. The results indicate the need for ongoing monitoring and intervention measures to regulate antibiotic resistance (B). Proportional drug resistance trends in the top five bacterial isolates. The figure demonstrates the monthly pattern of drug resistance between September and February for the top five most common bacterial isolates: MRSA, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Moraxella catarrhalis*. A sustained increase in resistance is seen among all isolates, with MRSA exhibiting the greatest resistance rates. These data highlight the emerging issue of antimicrobial resistance and the importance of specific therapeutic measures (C).

extensive investigations carried out in LMICs that have assessed the influence of gender on respiratory tract illness and death. The study's inherent constraint stems from the need for greater generalizability, given that data gathering occurred at a single centre. Detailed data on the frequency and specific antibiotics taken in recent months was not accessible for several instances and hence could not be included in the analysis. Since this is a cross-sectional study, we did not collect data on patient readmissions or prior admissions.

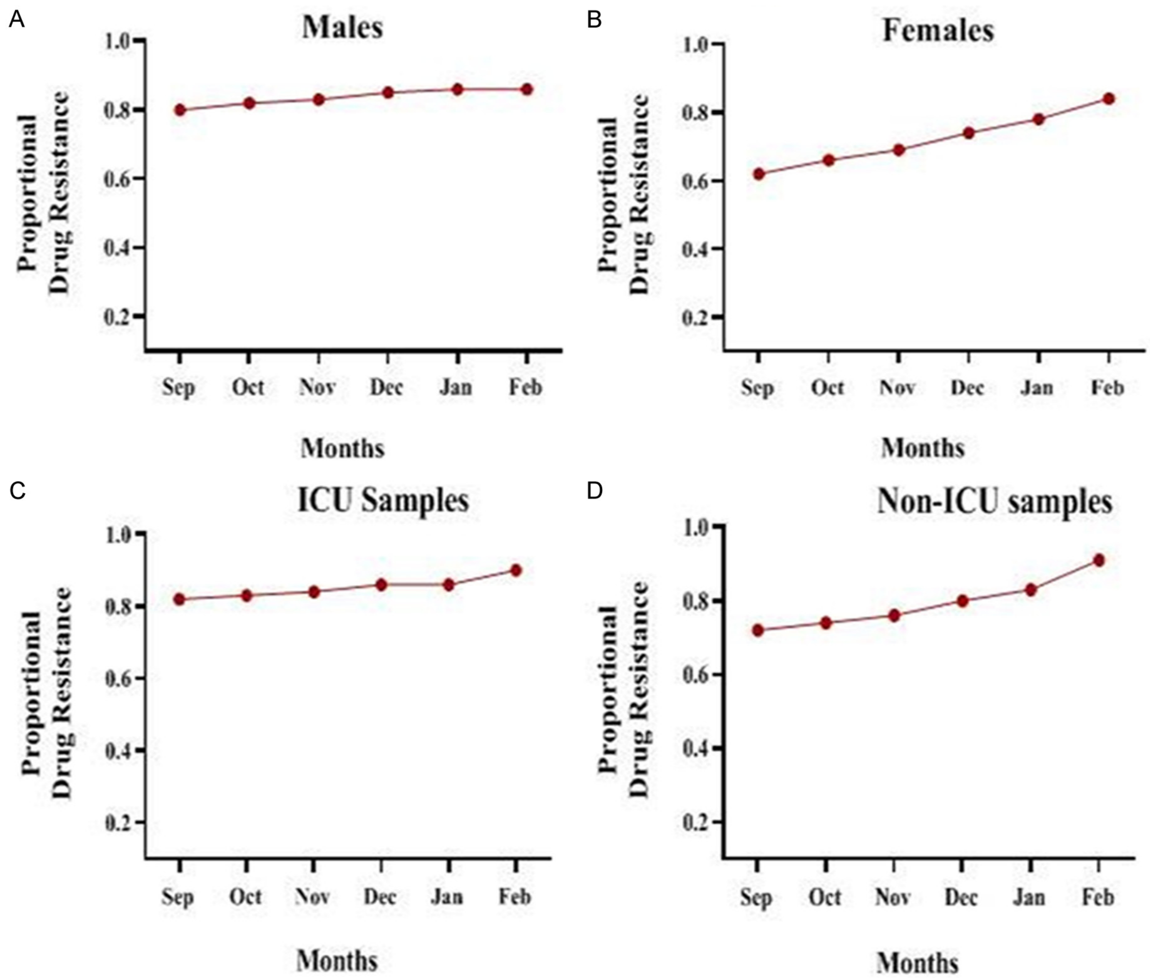

Figure 8. Trends in six-month (September to February) proportional drug resistance across various patient subgroups. In Males: The trend in the graph shows a consistent rise in proportional drug resistance in male patients throughout the period of the study, indicating a worsening antibiotic efficacy (A). Female patients indicate a more severe increase in drug resistance than their male counterparts, which may point to gender differences in bacterial resistance patterns (B). Patients in the ICU show persistently elevated levels of drug resistance with an upward drift, indicating the vital problem of antimicrobial resistance among intensive care unit patients (C). Although non-ICU patients also exhibit increasing drug resistance, the increase in the trend seems more rapid compared to ICU cases, indicating that community-acquired infections could be playing a role in the observed trend (D).

Conclusions

ICU and non-ICU environments identified a significant percentage of bacterial exacerbations in respiratory tract infections as drug-resistant. *S. pneumoniae* and *K. pneumoniae* were the predominant bacteria responsible for bacterial RTI, succeeded by *P. aeruginosa* and *Acinetobacter.* Hospital admissions for respiratory tract patients showed a rising trend in medication resistance over six months. Female sex and MRSA were independent risk factors for mortality and antimicrobial resistance. It is essential to use caution when using antimicrobial drugs so that resistant, multidrug-resistant, extensively drug-resistant, and pan-drug-resistant bacteria do not grow in people whose respiratory tract infections are getting worse quickly.

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Disclosure of conflict of interest

None.

Abbreviations

RP, Respiratory patients; RD, respiratory disease; MRSA, methicillin-resistant *Staphylococcus aureus*; GEE, generalized estimating equations; ICU, intensive care unit; URI, upper respiratory tract infection; LRTIs, lower respiratory tract infections; AMR, antimicrobial resistance; IPC, infection prevention and control; NTF, normal throat flora; OPD, outpatient department; HIS, hospital information system; LOS, length of hospital stay; XDR, widely drug-resistant; MDR, multidrug-resistant; PDR, pandrug-resistant; SDR, single-drug-resistant; TSI, triple sugar iron test; PPMs, potentially pathogenic pathogens; ICD-10, international classification of diseases, 10th revision; RTIs, respiratory tract infections; GCP, good clinical practice.

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