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Original Article

Microbiology Section

Changing Trends in Bacteriological Profile of Respiratory Isolates during Pre-COVID-19, COVID-19 and Post-COVID-19 Era: A Cross-sectional Study

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ABSTRACT

Introduction: The Coronavirus Disease-2019 (COVID-19) pandemic has exacerbated the global challenge of antibiotic resistance, particularly among respiratory pathogens. Findings from previous research highlight the urgent need to address antibiotic resistance in respiratory isolates during the COVID-19 era.

Aim: To investigate the changing trends in bacterial isolates, their prevalence, and antibiotic resistance patterns in respiratory samples from ward and Intensive Care Unit (ICU) settings during the pre-COVID-19 (2019), COVID-19 (2020), and post-COVID-19 (2022) periods.

Materials and Methods: A cross-sectional study was conducted in the Department of Microbiology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India. The study covered a three-year period from January 2019 to December 2022, excluding the year 2021. Respiratory samples such as Endotracheal Aspirates (ETA), Bronchoalveolar Lavage (BAL), and sputum that were culture positive were included for bacterial isolation and analysis of antibiotic resistance patterns. Data were collected from both ward and ICU patients, and statistical comparisons across the three study periods were performed using the Chi-square test to calculate p-values.

Results: A total of 830, 520, and 695 culture-positive respiratory isolates were analysed in 2019, 2020, and 2022, respectively. In the ICU, *Acinetobacter* spp. showed a significant increase from 26.49% in 2019 to 51.16% in 2020, followed by a marginal decline to 48.14% in 2022 (p-value <0.001). *Klebsiella* spp. exhibited a gradual rise in prevalence, while *Pseudomonas aeruginosa* initially decreased during the COVID-19 period but rebounded post-COVID-19. Antibiotic resistance patterns revealed a significant increase in resistance to colistin, imipenem, piperacillin-tazobactam, and tigecycline, particularly among *Klebsiella* spp. and *Acinetobacter* spp. in ICU settings.

Conclusion: The study highlights a concerning rise in antibiotic resistance among respiratory pathogens during the COVID-19 and post-COVID-19 periods, particularly in ICU settings. These findings emphasise the need for stringent antimicrobial stewardship, strengthened infection control measures, and continuous surveillance of resistance patterns to mitigate the spread of Multidrug-Resistant (MDR) bacteria.

Keywords: Antibiotic resistance, Bacterial infections, Respiratory tract infection

INTRODUCTION

Antimicrobial Resistance (AMR), accelerated by the misuse of antibiotics, caused an estimated 1.27 million deaths in 2019 and is projected to exceed 10 million annually by 2050 [1]. During the COVID-19 pandemic, global attention shifted away from AMR [2], while increased hospitalisations and the widespread use of broad-spectrum antibiotics led to a rise in MDR hospital-acquired infections [3,4]. Without urgent intervention, AMR could evolve into a slow-moving pandemic. The COVID-19 pandemic not only disrupted healthcare systems worldwide but also intensified the growing problem of antibiotic resistance. The overuse and misuse of antibiotics during this period contributed to the emergence and spread of antibiotic-resistant bacteria, posing a significant threat to public health. A study conducted at a tertiary care hospital in Italy reported a substantial increase in antibiotic resistance among respiratory pathogens, including Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Extended-Spectrum Beta-Lactamase (ESBL)-producing bacteria, in COVID-19 patients [5]. Similarly, research from Spain highlighted the emergence of antibiotic-resistant strains of *Streptococcus pneumoniae*, a common respiratory pathogen, in patients with COVID-19 [6].

A systematic review documented a rise in antibiotic resistance rates among respiratory isolates, particularly in Gram-negative bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [7]. Another study from India identified carbapenem-resistant *Acinetobacter baumannii*, a pathogen of major concern due to its resistance to multiple antibiotics, in COVID-19 patients [8]. These findings underscore the urgent need to address antibiotic resistance in respiratory isolates during the COVID-19 era. Inadequate diagnostic practices, poor adherence to treatment guidelines, and the use of broad-spectrum antibiotics without susceptibility testing have all contributed to the emergence and dissemination of resistant strains [9-13].

This study is novel in its temporal and setting-based comparative analysis of respiratory pathogens across three distinct periods—pre-COVID-19, during COVID-19, and post-COVID-19. While existing literature has documented rising resistance trends during the pandemic, this study provides one of the few comprehensive, institution-based longitudinal datasets differentiating between ICU and ward settings. By focusing specifically on high-priority Gram-negative pathogens (*P. aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp.), the research offers critical insights into the evolution of resistance and its correlation with pandemic-driven antimicrobial practices. These findings are crucial for informing future infection control strategies and antibiotic stewardship policies.

This study aimed to investigate the changing trends in the bacteriological profile, isolation rates, and antibiotic resistance patterns of key respiratory pathogens- *Pseudomonas aeruginosa*,

Klebsiella spp., and *Acinetobacter* spp.—in patients admitted to ward and ICU settings during the pre-COVID-19 (2019), COVID-19 (2020), and post-COVID-19 (2022) periods.

The primary objective was to compare the isolation rates and antibiotic resistance patterns of these key pathogens across the three time periods. The secondary objective was to evaluate temporal and setting-based differences in multidrug resistance trends, including carbapenem resistance, and to explore possible associations with changes in antibiotic usage during the COVID-19 pandemic.

MATERIALS AND METHODS

This study adopted a cross-sectional design, utilising data collected from both the wards and ICU during three distinct periods: the pre-COVID-19 era (2019), the COVID-19 era (2020), and the post-COVID-19 era (2022). The study was conducted over three years, from January 2019 to December 2022. Data from the year 2021 were excluded, as it did not represent a distinct COVID-19 or post-COVID-19 period. The inclusion of one representative year for each era—pre-COVID-19, COVID-19, and post-COVID-19—was intended to minimise statistical bias. Since the first half of 2021 had ongoing COVID-19 cases and the latter half showed a sharp decline, this year was omitted from analysis.

The study was carried out in the Bacteriology Section and the Hospital Infection Control Committee, Department of Microbiology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.

This study obtained ethical approval from the Institutional Research and Ethics Committee (IEC No.: ECR/483/Inst/UK/2013/RR-16). Patient data were anonymised and handled in accordance with data protection regulations. Informed consent was obtained from all participants, and confidentiality was maintained throughout the study.

Sample size: This was a time-bound study employing a census sampling strategy, including all available respiratory isolates collected from ICU and ward patients during the pre-COVID-19, COVID-19, and post-COVID-19 periods [13]. This approach ensured comprehensive coverage of bacterial prevalence and antibiotic resistance patterns across the three distinct time frames, providing valuable insights into the long-term impact of the COVID-19 pandemic on AMR trends.

Inclusion criteria: Respiratory samples such as ETA, BAL and sputum that were culture positive were included. Only samples showing significant bacterial growth, as per Clinical and Laboratory Standards Institute (CLSI) guidelines, were considered [14]. Patients admitted to various wards and ICUs with respiratory infections during 2019, 2020, and 2022 were included in the study.

Exclusion criteria: Duplicate samples from the same patient within 14 days were excluded to avoid redundancy. Respiratory samples showing commensal flora or mixed growth of more than two organisms were excluded. Additionally, patients with missing clinical details, incomplete antibiograms, or unconfirmed microbiological reports were not included. Data from 2021 were excluded for the reasons mentioned above.

Study Procedure

Respiratory isolates obtained from patients admitted to the wards and ICUs were included in the study. Samples were inoculated onto Blood agar and MacConkey agar plates and incubated at 35-37°C for 18-24 hours. The following day, colonies were examined, and distinct isolates were identified based on colony morphology. Identification and Antimicrobial Susceptibility Testing (AST) were performed using the VITEK® 2 automated system according to the manufacturer's guidelines.

Data collection: The bacteriological profile, including the prevalence and distribution of bacterial pathogens, was recorded. Antibiotic resistance data for each bacterial isolate were collected, focusing on commonly used antibiotics.

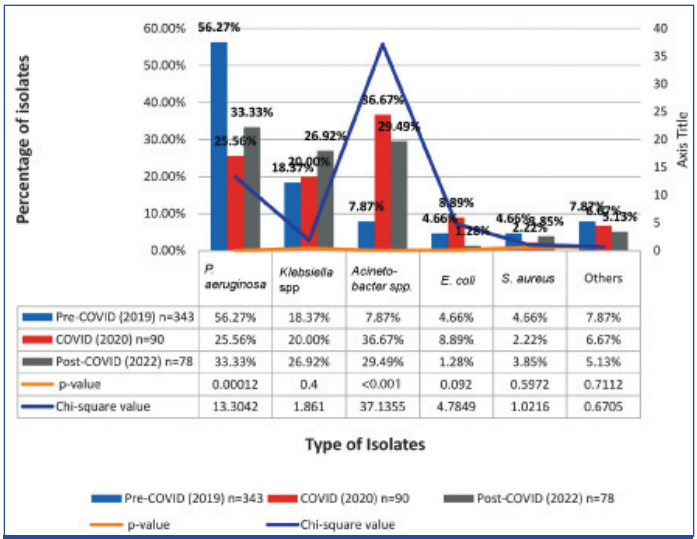
STATISTICAL ANALYSIS

Descriptive statistical analysis was performed to calculate the isolation percentages of different bacterial pathogens during the pre-COVID-19, COVID-19, and post-COVID-19 periods. Changes in isolation percentages across the three periods were assessed using the Chi-square test for p-value calculation. The antibiotic resistance patterns of *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp. were analysed and compared among the three study periods.

RESULTS

A total of 830 culture-positive respiratory isolates were analysed in 2019, of which 343 (41.3%) were from various wards and 487 (58.7%) from the ICU. In 2020, 520 culture-positive respiratory isolates were analysed, comprising 90 (17.3%) from wards and 430 (82.7%) from the ICU. In 2022, 695 respiratory isolates were analysed, with 78 (11.2%) from the wards and 617 (88.8%) from the ICU, indicating a sustained increase in ICU-associated respiratory infections post-pandemic.

The distribution of bacterial isolates in ward settings showed significant variations across the pre-COVID-19 (2019), COVID-19 (2020), and post-COVID-19 (2022) periods. *Pseudomonas aeruginosa*, the most frequently isolated organism in 2019 [193 (56.27%)], decreased significantly to 23 (25.56%) in 2020 but partially rebounded to 26 (33.33%) in 2022 (p-value=0.00012). This statistically significant trend may reflect changes in infection control practices or antibiotic pressures during and after the pandemic. *Acinetobacter* spp. showed a marked increase from 27 (7.87%) in 2019 to 33 (36.67%) in 2020, followed by a slight reduction to 23 (29.49%) in 2022 (p-value <0.001). This sustained rise was clinically significant, given the MDR nature of *Acinetobacter*, posing serious treatment challenges in ward settings. *Klebsiella* spp., *Escherichia coli*, and *Staphylococcus aureus* demonstrated minor fluctuations over the three-year period, without statistically significant changes. While these variations were not clinically impactful, ongoing surveillance remains important due to their potential role in hospital-acquired infections and AMR [Table/Fig-1].

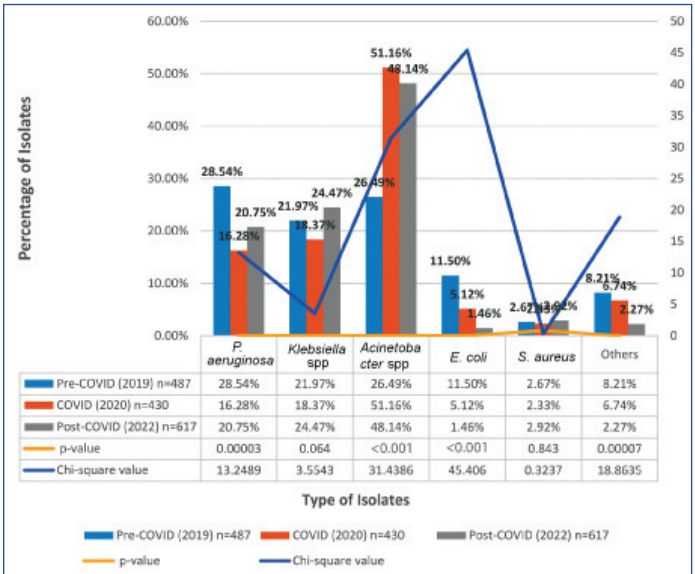


	P aeruginosa	*Klebsiella* spp	*Acineto-bacter* spp.	*E. coli*	*S. aureus*	Others
Pre-COVID (2019) n=343	56.27%	18.37%	7.87%	4.66%	4.66%	7.87%
COVID (2020) n=90	25.56%	20.00%	36.67%	8.89%	2.22%	6.67%
Post-COVID (2022) n=78	33.33%	26.92%	29.49%	1.28%	3.85%	5.13%
p-value	0.00012	0.4	<0.001	0.092	0.5972	0.7112
Chi-square value	13.3042	1.861	37.1355	4.7849	1.0216	0.6705

[Table/Fig-1]: Bacteriological profile of respiratory isolates from ward during pre-COVID-19 (2019), COVID-19 (2020), and post-COVID-19 (2022).
n (%): Chi-square test

The ICU microbiological profile revealed important shifts across the three study periods. *Pseudomonas aeruginosa* prevalence dropped from 28.54% in 2019 to 16.28% in 2020, with a partial resurgence to 20.75% by 2022 (p-value=0.00003), indicating a statistically significant trend. *Acinetobacter* spp. showed a marked surge during

the pandemic, increasing from 26.49% to 51.16%, and remained high at 48.14% post-pandemic (p-value <0.001), suggesting both clinical and statistical significance. *Klebsiella* spp. initially declined during COVID-19 (21.97% to 18.37%) but increased to 24.47% in 2022; however, this change was not statistically significant (p-value =0.064). *E. coli* displayed a consistent and significant downward trend, decreasing from 11.50% in 2019 to 1.46% in 2022 (p-value <0.001). Rates of *S. aureus* remained low and stable throughout the periods, with no significant difference observed (p-value=0.843). Additionally, other organisms decreased significantly over time, from 8.21% in 2019 to 2.27% in 2022 (p-value=0.00007), highlighting a shift in the broader microbial spectrum within the ICU environment [Table/Fig-2].



[Table/Fig-2]: Bacteriological profile of respiratory isolates from ICU during pre-COVID-19 (2019), COVID-19 era (2020), and post-COVID-19 (2022).
n (%): Chi-square test

The resistance patterns of *P. aeruginosa*, *Klebsiella* spp., and *A. baumannii* in ward settings exhibited dynamic changes across the study periods. *P. aeruginosa* maintained relatively stable resistance rates to most antibiotics (p-value >0.05); however, a notable increase in colistin resistance was observed in the post-COVID-19 era (p-value=0.05), raising potential clinical concerns. *Klebsiella* spp. demonstrated statistically significant resistance shifts, particularly to tetracycline (p-value=0.0068) and colistin (p-value=0.0003), suggesting emerging therapeutic challenges. *A. baumannii* showed the most pronounced changes, with significant increases in resistance to ceftazidime (p-value=0.028), piperacillin-tazobactam (p-value=0.006), imipenem (p-value=0.0029), tetracycline (p-value=0.0063), and cotrimoxazole (p-value=0.0063). Notably, ampicillin-sulbactam resistance initially decreased during the COVID-19 period but rebounded sharply post-COVID-19 (p-value <0.001), reflecting a concerning trend toward regained resistance. These shifts highlight the evolving AMR landscape during and after the pandemic, likely influenced by changes in antibiotic stewardship, infection control measures, and patient care dynamics during the COVID-19 crisis [Table/Fig-3].

In the ICU setting, resistance patterns evolved significantly across the study periods. *P. aeruginosa* resistance remained largely stable for most antibiotics (p-value >0.05); however, a significant increase in colistin resistance was observed post-COVID-19 (p-value=0.00005), raising important clinical concerns regarding last-resort treatment options. *Klebsiella* spp. demonstrated significant resistance changes to several critical antibiotics, including ceftazidime (p-value=0.047), piperacillin-tazobactam (p-value=0.0001), imipenem (p-value=0.00015), tetracycline, and tigecycline (both p-value <0.001), suggesting a worrying trend toward multidrug resistance. *A. baumannii* exhibited pronounced resistance shifts, notably against ampicillin-sulbactam, cotrimoxazole, and tigecycline (all p-value <0.001), further emphasising its role as a highly resilient ICU pathogen. These findings indicate a progressive escalation in AMR within the ICU during and after the COVID-19 pandemic, likely reflecting the combined effects of antimicrobial pressure, prolonged hospital stays, and evolving infection control challenges [Table/Fig-4].

DISCUSSION

The COVID-19 pandemic has significantly strained healthcare systems globally, thereby intensifying the challenge of AMR. The present study provides important insights into respiratory pathogen dynamics and resistance patterns across the pre-COVID-19, COVID-19, and post-COVID-19 eras-findings that broadly align with global observations [15,16].

A decline in the isolation rate of *Pseudomonas aeruginosa* during the COVID-19 era, followed by a partial rebound post-pandemic, was noted in this study. Similar trends were reported by Italian researchers, who observed a decrease in respiratory isolates during peak pandemic periods, potentially due to shifts in patient

Drug	*P. aeruginosa*				*Klebsiella* spp.				*A. baumannii*			
	Pre-COVID-19 (n=193)	COVID-19 (n=23)	Post COVID-19 (n=26)	χ^2, p-value	Pre-COVID-19 (n=63)	COVID-19 (n=18)	Post COVID-19 (n=21)	χ^2, p-value	Pre-COVID-19 (n=27)	COVID-19 (n=33)	Post COVID-19 (n=23)	χ^2, p-value
Ceftazidime	48 (25%)	8 (34.7%)	9 (34.6%)	0.761, p=0.383	50 (80%)	13 (72.2%)	19 (90.5%)	0.392, p=0.531	27 (100%)	27 (81.8%)	20 (87%)	4.828, p=0.028
Ampicillin sulbactam	NA	NA	NA	NA	NA	NA	NA	NA	20 (74%)	7 (21.2%)	17 (73.9%)	NA, p=<0.001
Piperacillin tazobactam	48 (25%)	6 (26.1%)	8 (30.8%)	0.058, p=0.810	50 (80%)	10 (55.6%)	15 (71.4%)	2.341, p=0.126	27 (100%)	25 (75.8%)	21 (91.3%)	7.55, p=0.006
Aztreonam	75 (39%)	9 (39.3%)	10 (38.5%)	0.0, p=0.999	NT	NT	NT	NA	IR	IR	IR	NA
Imipenem	39 (20.3%)	7 (30%)	9 (34.6%)	1.901, p=0.168	46 (73.0%)	8 (44.4%)	13 (61.9%)	3.214, p=0.073	27 (100%)	24 (72.7%)	20 (87%)	8.869, p=0.0029
Tigecycline	IR	IR	IR	NA	25 (40%)	4 (22.2%)	13 (61.9%)	1.242, p=0.265	6 (22.2%)	16 (48.6%)	11 (47.8%)	3.127, p=0.077
Tetracycline	IR	IR	IR	NA	50 (80%)	8 (44.4%)	19 (90.5%)	7.325, p=0.0068	27 (100%)	25 (75.8%)	22 (95.7%)	7.462, p=0.0063
Cotrimoxazole	IR	IR	IR	NA	46 (73.1%)	9 (50%)	15 (71.4%)	1.883, p=0.170	27 (100%)	25 (75.8%)	18 (78.3%)	7.462, p=0.0063
Colistin	28 (14.6%)	0	12 (46.2%)	3.841, p=0.05	0	5 (27.8%)	5 (23.8%)	13.07, p=0.0003	0	3 (9.1%)	1 (4.3%)	1.352, p=0.245

[Table/Fig-3]: Resistance pattern (R) of most (n-90) and Post-COVID-19 (n-78) era commonly isolated bacterial respiratory pathogens from ward during Pre-COVID-19 (n-343), COVID-19.
NA: Not applicable, NT: Not tested, IR: Intrinsic resistance; n (%): Chi-square test (χ^2)

Drug	*P. aeruginosa*				*Klebsiella spp.*				*A. baumannii*			
	Pre-COVID-19 (n=139)	COVID-19 (n=70)	Post COVID-19 (n=128)	χ^2, P-value	Pre-COVID-19 (n=107)	COVID-19 (n=79)	Post COVID-19 (n=151)	χ^2, P-value	Pre-COVID-19 (n=129)	COVID-19 (n=220)	Post COVID-19 (n=207)	χ^2, P-value
Ceftazidime	86 (61.8%)	40 (57.1%)	71 (55.5%)	1.188, p=0.552	94 (88%)	73 (92.4%)	145 (96%)	6.115, p=0.047	127 (98.5%)	214 (97.3%)	294 (99%)	0.671, p=0.715
Ampicillin sulbactam	IR	IR	IR	NA	NT	NT	NT	NA	75 (58.1%)	57 (26%)	281 (94.6)	18.421, p=0.0001
Piperacillin tazobactam	74 (53.2%)	46 (65.7%)	73 (57%)	2.966, p=0.227	74 (69.1%)	65 (82.4%)	136 (90%)	18.421, p=0.0001	122 (94.6%)	209 (95%)	294 (99%)	0.0, p=1.0
Aztreonam	82 (58.8%)	44 (62.9%)	80 (62.5%)	0.456, p=0.796	NT	NT	NT	NA	IR	IR	IR	NA
Imipenem	84 (60.5%)	47 (67.1%)	77 (60.2%)	1.1, p=0.577	61 (57.1%)	49 (62.3%)	121 (80.1%)	17.61, p=0.00015	127 (98.5%)	214 (97.3%)	295 (99.3%)	0.671, p=0.715
Tigecycline	IR	IR	IR	NA	18 (16.7%)	13 (16.5%)	89 (58.9%)	13.816, p=0.001	17 (13.1%)	37 (16.7%)	165 (55.6%)	13.816, p=0.001
Tetracycline	IR	IR	IR	NA	57 (53.3%)	52 (65.8%)	138 (91.4%)	13.816, p=0.001	119 (92.1%)	197 (89.6%)	297 (100%)	1.579, p=0.454
Cotrimoxazole	IR	IR	IR	NA	73 (68.2%)	63 (79.7%)	101 (66.9%)	4.451, p=0.108	107 (82.9%)	214 (97.3%)	236 (79.5%)	13.816, p=0.001
Colistin	19 (13.8%)	10 (14.3%)	44 (34.4%)	19.807, p=0.00005	17 (15.9%)	13 (16.5%)	32 (21.2%)	1.435, p=0.488	5 (3.9%)	12 (5.3%)	14 (4.7%)	0.451, p=0.798

[Table/Fig-4]: Resistance pattern (R) of most commonly isolated bacterial respiratory pathogens from ICU during Pre-COVID-19 (n-487), COVID-19 (n-430) and Post-COVID-19 (n-617) era.
NA: Not applicable; NT: Not tested; IR: Intrinsic resistance; n (%): Chi-square test (χ^2)

populations and the implementation of stringent infection control measures [17]. It has been proposed that altered healthcare utilisation patterns, such as reduced elective admissions and the prioritisation of critically ill COVID-19 patients, may have influenced these trends.

An increased prevalence of *Acinetobacter* spp. was detected during the COVID-19 era, consistent with findings from other studies that documented the emergence of MDR *Acinetobacter baumannii* in critically ill cohorts [18-20]. This rise may be attributed to prolonged mechanical ventilation, extended ICU stays, and the widespread empirical use of broad-spectrum antibiotics during the pandemic—all of which created a favourable environment for the proliferation of resistant strains.

Klebsiella spp. demonstrated a gradual increase across the study periods, mirroring global reports of *Klebsiella pneumoniae's* growing role in nosocomial infections [21,22]. This trend may reflect disruptions in antimicrobial stewardship programs during the pandemic, along with the overburdening of infection control practices, which allowed resistant organisms to spread more readily within healthcare settings.

Resistance pattern analyses further revealed a concerning rise in resistance to key antibiotics such as colistin and imipenem [23]. These findings are particularly alarming, given that these agents are often reserved as last-line therapies for MDR infections. The increased empirical use of broad-spectrum antibiotics, frequently without prior susceptibility testing during the pandemic, likely accelerated the selection of resistant strains [24,25]. These observations emphasise the urgent need for strengthened antimicrobial stewardship initiatives and the consistent application of infection prevention protocols [26].

Moreover, the dynamic resistance shifts observed in *A. baumannii* and *Klebsiella* spp. highlight the evolving nature of AMR. It has been suggested that pandemic-related changes in ICU demographics, including increased admissions of critically ill patients and extended hospitalisations, may have contributed to these patterns [27,28]. Variations in local antibiotic prescribing policies and laboratory diagnostic practices during different phases of the pandemic may also have introduced additional variability.

The rising antibiotic resistance among respiratory pathogens, particularly in ICU settings, poses a significant challenge for clinicians in managing infections. Increased resistance to last-resort antibiotics such as colistin and imipenem limits treatment options, leading to higher morbidity and mortality. These findings highlight the need for stringent antimicrobial stewardship programs to guide appropriate antibiotic use.

Regular surveillance of antibiotic resistance patterns is necessary to track emerging resistant strains and to modify treatment guidelines accordingly. Hospitals should enforce strict stewardship policies, ensuring adherence to evidence-based prescribing practices. Additionally, research into novel antimicrobial agents and alternative therapies, such as phage therapy and antimicrobial peptides, should be prioritised.

Limitation(s)

This study was conducted at a single tertiary care centre, which limits the generalisability of the findings to other healthcare settings. Its retrospective design may introduce selection bias and issues related to missing data. No molecular or genotypic analysis was performed to determine the genetic relatedness of the isolates. Furthermore, the study did not assess patient outcomes, such as mortality, treatment success, or length of hospital stay, in relation to bacterial resistance patterns.

CONCLUSION(S)

This study highlights the alarming rise in antibiotic resistance among respiratory pathogens during the COVID-19 pandemic, particularly in ICU settings. The findings emphasise the urgent need for enhanced antimicrobial stewardship and infection control measures to combat the escalating threat of antibiotic-resistant infections. The sustained increase in ICU-associated respiratory infections and the dynamic resistance patterns observed in key pathogens such as *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp. underscore the importance of continuous monitoring and research to inform clinical practice and public health policies. Addressing these challenges is crucial to preserving the efficacy of antibiotics for future generations.

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