Original Article

Prevalence and Antimicrobial Resistance of Bacteria Isolated from Clinical Specimens of COVID-19 Patients Admitted to Fatemeh Zahra Hospital in Sari, Iran

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Abstract

Background: The COVID-19 pandemic has heightened concerns over secondary bacterial infections, which worsen outcomes in hospitalized patients. This study investigated the prevalence and antibiotic resistance of bacteria isolated from clinical specimens of COVID-19 patients admitted to Fatemeh Zahra Hospital in Sari, Iran.

Materials and Methods: From September 2020 to August 2022, 3314 COVID-19 patients were included, with 55 positive cultures (including bacterial and fungal) obtained from 47 patients. The most common sources were urine (52.7%) and respiratory samples (38.1%). Gram-negative bacteria (58.9%), primarily Enterobacteriaceae (84.8%), were predominant, followed by *Pseudomonas* spp. and *Acinetobacter*. Gram-positive isolates included *Staphylococcus aureus* and coagulase-negative staphylococci. ICU-admitted patients showed higher co-infection rates, with increased mortality linked to bacterial infections. Antimicrobial susceptibility testing revealed concerning resistance patterns, including high resistance to ceftazidime (85% in Gram-negatives), imipenem (45% in *Acinetobacter*), and amikacin (50% in *Pseudomonas*), underscoring the challenge posed by multidrug-resistant organisms (MDROs). Among Gram-negative isolates, 30% were extensively drug-resistant (XDR).

Results: Despite a low overall co-infection rate (1.3%), bacterial infections significantly impacted disease severity and mortality. The findings align with regional studies highlighting Gram-negative pathogens as major contributors. Compared with pre-pandemic reports from Iran, resistance rates to carbapenems increased from ~43% to 65% among *Acinetobacter baumannii*. The rise in antimicrobial resistance (AMR) underscores the need for strict antibiotic stewardship and robust infection control measures.

Conclusion: This study emphasizes the critical role of bacterial co-infections in COVID-19 outcomes and calls for continuous surveillance to guide effective treatment strategies during the pandemic.

Keywords: COVID-19, Coinfection, Antimicrobial resistance, Gram-negative pathogens, Hospital-acquired infections, Fungal infections

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Introduction

The emergence of COVID-19 as a global pandemic has represented one of the most significant public health crises in modern history. As of current reports, the disease has affected over 674 million individuals worldwide, claiming more than 6.75 million lives and causing unprecedented social and economic disruptions[1]. While vaccination efforts have provided some measure of control, the continuous emergence of novel SARS-CoV-2 variants with mutations in key viral proteins, particularly the Spike protein, has maintained uncertainty regarding long-term pandemic management[2]. This evolving viral landscape has been further complicated by the phenomenon of viral re-infections and co-infections, creating new challenges in clinical management[3]. In Iran, the first cases were officially reported in February 2020 in Qom province, with the virus spreading rapidly to other regions. Mazandaran province, where Fatemeh Zahra Hospital is located, experienced particularly high caseloads during multiple COVID-19 waves, placing extraordinary pressure on healthcare infrastructure and likely influencing infection patterns. The clinical presentation of COVID-19 has shown remarkable heterogeneity, ranging from asymptomatic cases to severe respiratory failure requiring intensive care[4,5].

A critical aspect of COVID-19 management has been the prevention of secondary bacterial infections. While only 3.7% of general ward patients develop bacterial co-infections, this rises to 41.9% among ICU patients[6,7]. High-risk groups include elderly patients (>60 years), those requiring mechanical ventilation, and individuals with comorbidities like diabetes mellitus[7]. The microbiological profile at our center revealed a predominance of Gram-negative pathogens, particularly Enterobacteriaceae (*Klebsiella pneumoniae*, *Escherichia coli*) and non-fermenters (*Pseudomonas aeruginosa*, *Acinetobacter baumannii*), along with *Staphylococcus aureus*[8].

Of particular concern has been the alarming rise in antimicrobial resistance (AMR) during the pandemic. Preliminary data reveal concerning resistance rates among Gram-negative isolates, particularly to third-generation cephalosporins and carbapenems. This AMR crisis threatens to reverse decades of progress in infection control, with implications far beyond COVID-19[9,10]. Contributing factors include empirical antibiotic use, prolonged hospital stays, and overwhelmed ICU settings[11].

This study specifically examines: (1) the prevalence and antibiotic resistance profiles of bacterial isolates from COVID-19 patients at Fatemeh Zahra Hospital, (2) risk factors associated with resistant infections, and (3) comparative analysis with pre-pandemic resistance patterns in similar Iranian hospitals to assess COVID-19's impact on AMR evolution. Our findings aim to guide antimicrobial stewardship and infection control measures in pandemic settings.

Methods

Study Design and Sites: We conducted a single-center retrospective study that included all adult patients (≥18 years old) admitted to two general COVID-19 wards and two COVID-19 ICUs from September 20, 2020, to August 7, 2022, at Fatemeh Zahra Hospital, an academic hospital with 117 beds located in Sari, Iran. A positive COVID-19 case was defined as a person with a confirmed positive result on a nasopharyngeal swab tested using RT-PCR for SARS-CoV-2, employing primers targeting the N gene (N1: Forward 5’-GAC CCC AAA ATC AGC GAA AT-3’, Reverse 5’-TCT GGT TAC TGC CAG TTG AAT CTG-3’; N2: Forward 5’-TTA CAA ACA TTG GCC GCA AA-3’, Reverse 5’-GCG CGA CAT TCC GAA GAA-3’) and human RNase P as internal control[12]

Data Collection: The collected clinical data included information on individuals with a positive culture, such as dates of admission and positive culture results, antimicrobial susceptibilities of isolates, durations of hospital stay, and patient outcomes.

Collection of Clinical Specimens for Bacterial Detection: Over twenty-three months, 620 clinical samples, including blood, sputum, endotracheal tube (ETT) aspirates, pericardial fluid, and urine, were collected from inpatients in both general and ICU wards of the hospital, which is affiliated with Mazandaran University of Medical Sciences. The clinical samples were cultured on appropriate media for each specimen type (e.g., blood agar for blood cultures, MacConkey agar for urine) and incubated at 37°C for 24–48 hours. Species identification was carried out using standard biochemical tests according to Cowan and Steel's Manual for the Identification of Medical Bacteria[13].

Antimicrobial Susceptibility Testing and MIC Determination: Antimicrobial susceptibility testing was performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M100, 2022 edition)[14]. The antibiotics tested included Amikacin (AN), Fosfomycin (FM), Cefixime (CFM), Ceftazidime (CAZ), Ceftriaxone (CTX), Cefepime (CPM), Trimethoprim-Sulfamethoxazole (SXT), Imipenem (IMP), Chloramphenicol (CL), Oxacillin (OX), Ampicillin (AM), Novobiocin (NB), Cefoxitin (FOX), Gentamicin (GM), and Ciprofloxacin (CIP).

Definitions: A positive COVID-19 case was confirmed by RT-PCR testing of nasopharyngeal or tracheal swabs. Secondary infections were defined as bacterial infections that developed during the ICU stay after more than 48 hours of admission, meaning they were not present at the time of initial COVID-19 presentation. Extensively drug-resistant (XDR) isolates were defined as those showing non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., remaining susceptible to only one or two antibiotic classes).

Statistical Analysis: Data were entered and analyzed using Microsoft Excel 2013 and IBM SPSS Statistics Version 23. Descriptive statistics for categorical variables are presented as counts and percentages, and for continuous variables as means ± standard deviations (SDs) or medians with interquartile ranges (IQRs). For risk factors associated with resistant infections, chi-square tests were used to compare proportions between groups (e.g., ICU vs. non-ICU), with $p<0.05$ considered significant.

Ethics approval and consent to participate: All clinical specimens were obtained as part of routine diagnostic procedures without additional sampling. The study protocol (IR.MAZUMS.REC.1404.352) received ethical approval from Sari University of Medical Sciences. Written informed consent was obtained from all participants following detailed verbal explanations of the study objectives and procedures.

Results

Demographic Details: During the pandemic wave at our academic hospital, we admitted a total of 3,314 COVID-19 patients, with 1,352 (40.8%) admitted to the ICU and 1,962 (59.2%) to non-ICU COVID wards. From these admissions, we performed 620 cultures, of which 55 (8.9%) were positive. These 55 positive clinical specimens were obtained from 47 unique patients, with the following distribution by sample type: 4 blood cultures (7.2%), 29 urine cultures (52.7%), 15 endotracheal tube samples (27.2%), 6 sputum samples (10.9%), and 1 pericardial fluid sample (1.8%). All specimens were collected and processed in strict accordance with recommended personal protective equipment guidelines. We excluded repeat samples with similar results and cultures showing mixed growth or contaminants from our analysis. Among the 47 patients with positive cultures, 24 (51.1%) were from the COVID ICU, 19 (40.4%) from the non-ICU COVID ward, and 4 (8.5%) had been admitted to both wards during their hospitalization. Microbiological analysis revealed that 42 patients (89.3%) had bacterial infections alone, 2 patients (4.2%) had fungal infections alone, and 3 patients (6.3%) had concurrent bacterial and fungal infections. The fungal isolates included Candida spp. (n=5), primarily from urine and respiratory samples, highlighting the occurrence of concurrent fungal pathogens.

Severity of illness and outcome measures: During the 23-month study period, among 3,314 COVID-19 patients admitted to our hospital, 1,352 patients (40.8%) required ICU admission. Of these ICU patients, 26 had confirmed bacterial infections, 1 had a fungal infection, and 1 had concurrent bacterial and fungal co-infection. Mortality data revealed 40 deaths in general wards and 370 deaths in the ICU. Of the 410 deceased COVID-19 patients, 18 had documented co-infections, with Enterobacteriaceae species responsible for 12 of these cases (66.7%). Chi-square analysis showed a significant association between ICU admission and co-infection rates ($p=0.02$).

Etiological profile of secondary infections: During the study period, we processed 620 clinical specimens (including blood, endotracheal tube aspirates, urine, sputum, and pericardial fluid) for microbiological culture. Of these, 565 specimens (91.1%) showed no bacterial growth, while 55 specimens (8.8%) yielded positive cultures (Table 1). Analysis of infection sites among the 47 culture-positive patients revealed: 23 patients (48.9%) had urinary tract infections alone, 17 (36.1%) had respiratory tract infections alone, 3 (6.3%) had concurrent bloodstream and urinary tract infections, 2 (4.2%) had both urinary and respiratory tract infections, 1 (2.1%) had triple infection involving respiratory tract, bloodstream and urinary tract, and 1 (2.1%) had cardiac infection.

Table 1. Etiology of bacterial infections in patients hospitalized with COVID-19 (n=41).

Isolates	No	%
Gram-negative pathogens	33	80.5
Enterobacteriaceae (E. coli, Klebsiella spp., Citrobacter spp., Enterobacter spp., Hafnia spp.)	28	68.3
Pseudomonas aeruginosa.	3	7.3
Acinetobacter spp.	2	4.9
Gram-positive pathogens	8	19.5
Staphylococcus aureus	7	17.1
Nonhemolytic *Streptococcus spp.*	1	2.4

From these 55 positive cultures, we identified 56 significant bacterial isolates. Gram-negative organisms predominated (58.9% of isolates), comprising: 28 Enterobacteriaceae (84.8% of Gram-negatives), 3 *Pseudomonas aeruginosa* (9.0%), and 2 *Acinetobacter* species (6.0%). The distribution of specific Enterobacteriaceae species is detailed in Table 2. Gram-positive organisms accounted for 23 isolates (41.0% of total), consisting of: 13 coagulase-negative staphylococci (56.5%), 7 *Staphylococcus aureus* (30.4%), 1 nonhemolytic *Streptococcus* spp. (4.3%), and 2 *Diphtheroid* spp. (8.6%). Following standard microbiological practice, we considered coagulase-negative *staphylococci* as potential contaminants and *Diphtheroid* spp. as commensals, excluding them from our pathogen analysis. Thus, the final analysis included 41 pathogenic isolates (33 Gram-negative and 8 Gram-positive).

Antimicrobial resistance (AMR): The antimicrobial *resistance* patterns of the 41 pathogenic isolates are shown in Figure 1. Among Gram-negative isolates, resistance was high to ceftazidime (85%), ceftriaxone (78%), ciprofloxacin (75%), and imipenem (45%). For key pathogens: *E. coli* showed 67% resistance to trimethoprim-sulfamethoxazole; *Klebsiella* spp. 80% to cefepime; *Pseudomonas aeruginosa* 67% to gentamicin; *Acinetobacter* spp. 100% to ampicillin-sulbactam. Among Gram-positive isolates, *S. aureus* exhibited 43% resistance to oxacillin (MRSA). Overall, 12 isolates (29.3%) were XDR, primarily among *Acinetobacter* and *Pseudomonas* (p=0.04 for association with ICU stay via chi-square). Compared with pre-pandemic data from Iranian hospitals (e.g., carbapenem resistance in *A. baumannii* at ~43% in 2011-2016 [15]), our rate was higher (65%), suggesting a pandemic-driven increase.

Table 2. Etiology of Enterobacteriaceae species in patients hospitalized with COVID-19 (n=28).

Isolates	No	%
E. coli	9	32.1
Enterobacter spp.	7	25
Citrobacter spp.	6	21.4
Klebsiella spp.	5	17.9
Hafnia spp.	1	3.6
Total	**28**	**100**

Discussion

The COVID-19 pandemic has revealed complex interactions between viral and bacterial pathogens that significantly impact patient outcomes[15]. Our study at Fatemeh Zahra Hospital provides important insights into these dynamics through a detailed analysis of 3,314 hospitalized COVID-19 cases over 23 months. The observed 1.4% bacterial coinfection rate, while lower than many published reports[16-18], reveals several clinically relevant patterns that warrant careful consideration. This relatively low prevalence likely reflects our hospital's specific patient population, antimicrobial stewardship practices, and diagnostic protocols, rather than indicating an absence of clinically significant coinfections. The inclusion of fungal co-infections (4.2% fungal alone, 6.3% concurrent) aligns with reports of opportunistic fungi in immunocompromised COVID-19 patients[19].

The anatomical distribution of infections in our cohort showed a predominance of urinary tract

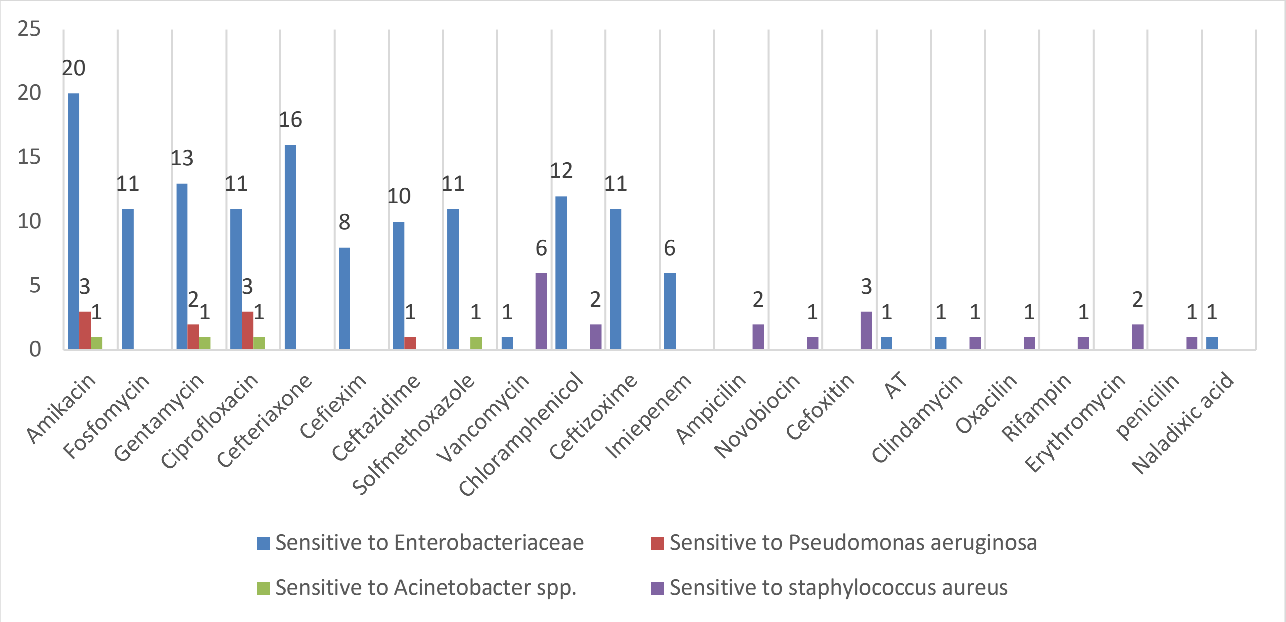

Figure 1. Antimicrobial resistant pattern (n=41).

infections (52.7%), followed by respiratory system involvement (38.1%).

This pattern differs from several studies reporting higher respiratory tract involvement[18,20], possibly due to variations in catheterization protocols, early antibiotic administration practices, or differences in viral pathogenicity between SARS-CoV-2 variants circulating during our study period. The predominance of the urinary tract may also reflect the older age of our hospitalized population or specific comorbidities prevalent in our region.

Microbiological analysis revealed Gram-negative organisms as the primary pathogens (58.9% of isolates), with Enterobacteriaceae species accounting for 25% of all infections. This finding aligns with regional antimicrobial resistance patterns and highlights the ongoing challenge of Gram-negative infections in hospital settings[21]. The predominance of these organisms carries important therapeutic implications, particularly given their potential for antimicrobial resistance. Among Gram-positive isolates, *Staphylococcus aureus* (17%) emerged as the most clinically significant pathogen, including one rare case of pericardial infection that underscores the potential severity of these coinfections.

The clinical impact of bacterial coinfections was most pronounced in our ICU population, where affected patients experienced significantly worse outcomes. This observation persists despite our lower overall coinfection rate compared to reports from other studies[16,22]. The discrepancy in reported rates likely stems from multiple factors including differences in: (1) patient admission criteria, (2) severity of illness at presentation, (3) local antimicrobial prescribing practices prior to hospitalization, and (4) diagnostic testing protocols. These variations emphasize the critical importance of local epidemiological data to inform clinical decision-making.

Several limitations of our study must be acknowledged, including the retrospective design's risk of bias, lack of standardized criteria for obtaining cultures (potential selection bias leading to under-ascertainment), superficial identification and AST methods without molecular characterization of resistance mechanisms (e.g., ESBL, carbapenemase genes), single-center design limiting generalizability, and diagnostic constraints for fungal infections due to limited mycology capacity.

Despite these limitations, our findings carry important implications for clinical practice and pandemic preparedness. The disproportionate impact of coinfections on ICU patients underscores the need for enhanced surveillance in critical care settings. The predominance of Gram-negative organisms, particularly Enterobacteriaceae, highlights the necessity of local antibiograms to guide empirical therapy. Our results also emphasize the value of maintaining robust diagnostic capacity for secondary infections, even when overall prevalence appears low, as these complications can dramatically alter patient outcomes.

These insights contribute to our understanding of COVID-19 complications across diverse clinical settings and reinforce the importance of region-specific treatment protocols. The findings advocate for antimicrobial stewardship programs tailored to local resistance patterns and underscore the need for integrated infection control strategies that address both viral and bacterial threats simultaneously. Future research should focus on longitudinal monitoring of coinfection patterns and resistance profiles as the pandemic evolves, as well as investigations into the mechanisms of viral-bacterial interactions in COVID-19 patients.

Conclusion

Our comprehensive analysis of 3,314 COVID-19 patients at Fatemeh Zahra Hospital revealed a 1.4% prevalence of bacterial coinfections, lower than many international reports but with significant clinical consequences. The urinary tract (52.7%) and respiratory system (38.1%) served as primary infection sites, with Gram-negative organisms (58.9%), particularly Enterobacteriaceae (25%), dominating the microbiological profile. While our coinfection rate was modest compared to studies reporting 10-20% prevalence[19,23], the mortality impact was substantial - 18 of 410 deaths (4.4%) occurred in coinfected patients, with Enterobacteriaceae implicated in two-thirds of these cases. This apparent paradox (low frequency but high severity) underscores several critical insights: First, local antimicrobial resistance patterns, especially the predominance of Gram-negative pathogens, must guide empirical therapy decisions. Second, even relatively low coinfection rates can disproportionately impact outcomes in vulnerable populations like ICU patients. Third, the variations in reported rates globally likely reflect differences in diagnostic intensity, antimicrobial stewardship practices, and local microbial ecology rather than true epidemiological differences. Our findings emphasize that effective COVID-19 management requires dual vigilance against both viral and bacterial threats, particularly in hospital settings where the convergence of immune dysregulation, invasive procedures, and antibiotic exposure creates ideal conditions for resistant infections. Moving forward, hospitals should maintain robust microbiological surveillance systems, implement tailored antimicrobial stewardship programs based on local resistance data, and develop protocols for rapid coinfection diagnosis. The COVID-19 pandemic has provided a powerful lesson in the importance of integrated infection control strategies that address both viral and bacterial threats simultaneously.

Acknowledgment

None.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

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