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Original Article

Prevalence, Etiology, and Antimicrobial Resistance Pattern of Bacterial Isolates From Bloodstream Infections in Ganjavian Hospital, Dezful, Iran

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Abstract

Background: Sepsis is a life-threatening condition with high mortality rates. The emergence of antimicrobial resistance (AMR) among pathogens that cause sepsis poses a significant challenge to effective treatment. This study was conducted to determine the AMR patterns of bacterial isolates from septic patients' blood cultures with emphasis on extended-spectrum beta-lactamases (ESBLs), carbapenemase, and methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence.

Methods: This cross-sectional laboratory study examined blood culture samples of 1248 patients. The blood cultures were subcultured on MacConkey and chocolate agar media. Bacterial identification was based on gram-staining and biochemical tests. Initial and confirmatory antibiotic susceptibility testing was then performed according to CLSI 2022 for MRSA, vancomycin-resistant *Enterococcus* (VRE), and ESBLs. The statistical analysis of the study findings was conducted using SPSS. The accuracy level of the evaluations was determined with a 95% confidence interval.

Results: Among the 174 cases of bloodstream infection (BSI) (13.9% positivity), Enterobacterales (59.8%) and gram-positive cocci (32.7%) were dominant isolates. Interestingly, the prevalence of MRSA was 67.5%, with a significantly higher prevalence in ICUs (81.3%; $P=0.037$). In gram-negative isolates, resistance to third-generation cephalosporins and carbapenems was 48.7% and 22.2%, respectively. Significant resistance to carbapenems (80%) was observed in *Acinetobacter* isolates, but all strains remained susceptible to colistin. ESBL producers included *Escherichia coli* (36.3%) and *Klebsiella pneumoniae* (16%). Notably, 52.4% of multidrug-resistant Enterobacteriaceae cases were isolated from patients in emergency departments and intensive care units.

Conclusion: This study highlights the alarming prevalence of MDR bacteria among sepsis isolates. Precautions should be taken against this growing threat by focusing on three priorities: enhancing infection control, maintaining continuous surveillance, and exploring novel therapeutic approaches.

Keywords: Blood culture, Antibiotic resistance, ESBL, MRSA, VRE

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Introduction

Sepsis, a life-threatening systemic response to infection, claims millions of lives annually. In 2020, there were approximately 48.9 million global sepsis cases, resulting in 11 million deaths (nearly 1 in 5 global fatalities). Even more alarming, the incidence of sepsis has risen steadily worldwide over the past two decades (1,2). Key factors include: aging populations, immunocompromising conditions, increased prevalence of chronic comorbidities, and suboptimal antibiotic use (e.g., inappropriate treatment protocols or incomplete courses). These prescribing errors inadvertently train bacteria to become drug-resistant superbugs (3).

For bloodstream infections (BSIs), every minute counts; in other words, delaying appropriate antimicrobial therapy significantly increases the risk of mortality. Initial

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empirical therapies (based on informed guidelines) may take several days to show their effects (4). Surprisingly, sepsis can arise from pneumonia, urinary tract infections, or even skin wounds. In recent decades, evolving pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, especially extended-spectrum and metallo-beta-lactamase-producing strains, as well as methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA) and vancomycin-resistant *Enterococcus* (VRE), have become a growing crisis for healthcare systems due to their rising resistance to last-line drugs (5).

Effective antimicrobial therapy for infectious diseases depends on precise pathogen identification and antimicrobial susceptibility testing to optimize treatment, minimize unnecessary antibiotic use, and protect the commensal microbiota. Although empirical therapy remains critical for severe infections, the rising threat of antimicrobial resistance (AMR) is diminishing its efficacy. Consequently, laboratory diagnostics are crucial for detecting resistant pathogens, informing evidence-based treatment decisions, and preventing the spread of resistance. Nevertheless, diagnostic challenges often delay optimal therapy, exacerbating both clinical outcomes and public health risks (6).

AMR is a significant global health threat, particularly in BSIs, where delays in effective treatment lead to high mortality rates. Accurate epidemiological data and regional antibiotic resistance patterns are critical for appropriate antibiotic prescribing. AMR leads to multiple negative consequences, including increased complications, prolonged hospital stays, and higher rates of therapeutic failures. This crisis is exacerbated in developing countries due to inadequate surveillance, poor infection control practices, overuse of antibiotics, and limited access to newer therapies (7). Therefore, active monitoring of resistance trends is essential for guiding responsible antibiotic use and stewardship programs (8). Understanding the prevalence and resistance patterns of BSIs in hospitalized patients is crucial to ensure timely and effective antibiotic treatment. This study aimed to evaluate the prevalence, causative pathogens, and AMR profiles of bacterial BSIs in Ganjavian Hospital to inform therapeutic decisions and enhance infection control measures.

Materials and Methods

This descriptive cross-sectional study aimed to examine the antibiotic resistance profiles of bacteria isolated from blood culture samples collected from patients admitted to Ganjavian Hospital in 2022. Sequential sampling was used to include all patients suspected of sepsis.

In the current study, blood culture bottles were subcultured on days 1, 3, and 6 onto chocolate agar and MacConkey agar media (Condalab, Spain) at 35 °C for 18 hours to optimize microbial recovery of both early and late-growing pathogens. Following incubation, bacterial identification was carried out through a series of tests, including gram staining, catalase, oxidase, DNase, coagulase, Mannitol Salt Agar, Bile Esculin Agar, CAMP (Christie, Atkinson, Munch, Peterson) test, glucose Oxidizer-Fermenter reactions, and IMViC (Condalab) (9).

According to the distinguishing criteria for true BSIs from possible contamination, including common organisms associated with pseudobacteremia (coagulase-negative staphylococci, *Corynebacterium* species, and *Bacillus* species), we performed repeat blood cultures. According to the current diagnostic standards, only isolates that showed persistent growth in serial blood cultures were considered pathogenic. In our study, blood culture samples presenting this challenge were obtained from the pediatric department, where repeat cultures of initially positive samples for these organisms yielded negative results, confirming the contamination. Consequently, these cases were excluded from the final analysis of BSI prevalence (10,11).

For antimicrobial susceptibility testing, a bacterial suspension was prepared to match the turbidity of the 0.5 McFarland standard and inoculated on Mueller-Hinton agar (MHA) (Condalab, Madrid, Spain). The test was performed using the Kirby-Bauer disk diffusion method for various antibiotics, including amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), piperacillin-tazobactam (100/10 µg), ampicillin (10 µg), penicillin (10 units), erythromycin (15 µg), tetracycline (30 µg), clindamycin (2 µg), linezolid (30 µg), rifampin (5 µg), and vancomycin (30 µg) (MAST Company). Antibiotic susceptibility was determined by measuring the growth inhibition zones and interpreting according to the Clinical and Laboratory Standards Institute (CLSI) 2022 M100 (32nd edition) guidelines (12).

Cefoxitin (30 µg) was used to detect methicillin-resistant *S. aureus* (MRSA). Vancomycin resistance in Enterococcus and Staphylococcus was evaluated using the Vancomycin Agar test, and the minimal inhibitory concentration (MIC) (0.016 to 256 µg/mL) was determined by Etest assay (Liofilchem). To detect extended-spectrum beta-lactamase (ESBL) and carbapenemase production in Enterobacteriaceae and Pseudomonas, ceftazidime (CAZ) combined with clavulanic acid and modified carbapenem inactivation method (mCIM) was used, respectively. Additionally, the EDTA-modified carbapenem inactivation method (eCIM) and mCIM tests were simultaneously used to differentiate between serine carbapenemase and metallo-beta-lactamase resistance. Colistin resistance was determined based on CLSI guidelines, with an MIC value of ≥ 4 µg/mL indicating resistance (12).

The statistical analysis of study findings was conducted using IBM SPSS version 21.0. The chi-square and Fisher's exact test were used for comparisons. Categorical variables were analyzed with a predetermined significance level of $P < 0.05$ and 95% confidence intervals.

Results

Demographics

Bacterial growth was detected in 174 (13.9%) of 1248 blood cultures. Among these isolates, 89 (51.1%) belonged to male patients and 85 (48.9%) belonged to female patients. The age of the patients ranged from 1 day to 89 years. Most cultures were collected in the emergency department (n = 494, 39.6%), followed by intensive care units (ICUs), internal medicine, and surgical departments, which accounted for 31.6% (n = 394), 25.3% (n = 316), and 3.5% (n = 44), respectively.

Distribution of Bacteria

Microbiological analysis revealed that the frequency of gram-positive cocci was 32.7% (57/174) and the frequency of Enterobacterales was 59.8% (104/174) of the isolates. Additionally, non-fermenting bacteria, including *P. aeruginosa* and *A. baumannii*, were isolated in 7.5% (13/174). The most frequently isolated pathogens were *E. coli* (25.3%), *S. aureus* (23%), and *K. pneumoniae* (14.4%).

Resistance Profiles

Staphylococcus aureus isolates exhibited the highest resistance rates to ampicillin and tetracycline, while vancomycin and linezolid showed the greatest efficacy. Clindamycin resistance was observed in 22.5% (9/40) of strains, with an MRSA prevalence of 67.5% (27/40). No vancomycin resistance was detected among *S. aureus* isolates using vancomycin agar and MIC testing (MIC range: 0.125-1.5 µg/mL). A significant association emerged between the prevalence of MRSA and hospital wards ($P = 0.037$), particularly in ICUs, where 81.3% of S. aureus isolates were MRSA. Fisher's exact test revealed no association between MRSA occurrence and patient gender ($P = 0.107$).

Enterococcus isolates (n = 11) comprised *E. faecalis* (27.3%, n = 3) and *E. faecium* (72.7%, n = 8). All isolates demonstrated ciprofloxacin resistance, while 36.4% (n = 4) of isolates were vancomycin-resistant *enterococci* (VRE) (MIC > 256 µg/mL). No significant associations were found between VRE prevalence and gender or hospital departments ($P > 0.05$).

Gram-negative bacteria (n = 117) showed 48.7% (n = 57) resistance to third-generation cephalosporins. Among the 104 isolates of *Enterobacterales*, 21 strains (20.1%) were multiple-drug resistant (MDR), predominantly from emergency (52.4%, n = 11) and ICU (23.8%, n = 5). ESBL production was identified in 20 strains (*E. coli*: 36.3%, n = 16; *K. pneumoniae*: 16%, n = 4).

Eight isolates of *A. baumannii* were isolated from ICU patients, which demonstrated the highest level of antibiotic resistance. The rate of multidrug-resistant *A. baumannii* (MDR-AB) was 87.5% (7/8), with all strains showing susceptibility to colistin as indicated by the Colistin E-Test. Following colistin, piperacillin-tazobactam was the next most effective antibiotic against *A. baumannii*, with 37% (3/8) susceptibility.

In our research, 5 strains of *P. aeruginosa* were isolated. Four strains exhibited sensitivity to ciprofloxacin, gentamicin, cotrimoxazole, and third-generation cephalosporins, and 3 isolates demonstrated resistance to meropenem and amikacin.

Figure 1 illustrates the patterns of antibiotic resistance among gram-negative isolates. Furthermore, carbapenem resistance rates in *Enterobacterales*, *P. aeruginosa*, and *A. baumannii* were 16.3% (17/104), 40% (2/5), and 87.5% (7/8), respectively. Carbapenemase production was detected in 14.4% (15/104) of Enterobacterales and 40% (2/5) of *P. aeruginosa* isolates using the modified carbapenem inactivation method (mCIM). The investigation examined the production of serine carbapenemases and metallo-beta-lactamases through the simultaneous use of mCIM and eCIM methods. Serine carbapenemase production among Enterobacterales isolates was distributed as follows: *E. coli* (2.3%), *K. pneumoniae* (32%), *K. oxytoca* (11.1%), and *K. aerogenes* (25%). Only 2 Klebsiella strains produced metallo-beta-lactamases. No significant associations were found between carbapenem resistance and gender/ward distribution ($P < 0.05$). Three isolates (1 *K. pneumoniae*, 1 *K. aerogenes*, and 1 *P. aeruginosa*) were

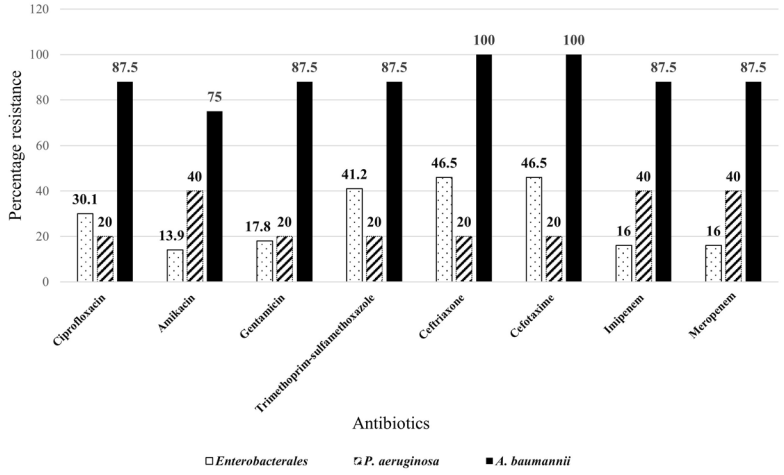

Figure 1. Antibiotic-Resistance Pattern of Gram-Negative Bacteria by Disk Diffusion Method

categorized as intermediate (MIC > 2 µg/mL) according to CLSI guidelines, while all others remained susceptible.

Discussion

The increasing prevalence of antibiotic resistance is a significant challenge for health systems worldwide. Region-specific data on antibiotic resistance patterns is crucial for effective treatment, especially in critical care, due to its increasing prevalence. In this study, bacterial infections accounted for 13.9% of positive blood cultures, with *E. coli* (25.3%), *S. aureus* (23%), and *K. pneumoniae* (14.4%) being the predominant isolates. While these results align with the results of the study by Oyekale et al (13) (*E. coli*: 29.4%), they contrast with the findings of the study conducted by Mohammadi et al (14), who reported *S. aureus* (31.5%) as most common, and Mahmoudi et al (15), who identified coagulase-negative staphylococci and *Proteus vulgaris* as prevalent pathogens. These variations may stem from differences in diagnostic methods (e.g., automated systems like BacTec/MALDI-TOF vs. conventional techniques) (16), as limited access to advanced tools persists in many settings (17).

Consistent with studies from Shiraz (17) (63.3%) and other places (18,19), gram-negative bacteria dominated (67.3%), though Abayneh et al (18) found no significant differences in the frequency of gram-positive or Enterobacteriaceae. These findings underscore the need for region-specific resistance monitoring and standardized diagnostics to optimize treatment strategies.

Moradi reported a higher prevalence of positive blood cultures among emergency department patients (60.4%) compared to our findings (52.4%), highlighting the notable frequency of such cases in emergency settings (20). The observed overrepresentation of positive blood cultures among emergency department patients can be attributed to the standardized practice of obtaining blood cultures upon admission before initiating antibiotic therapy (21). This routine procedure, prevalent in emergency settings, likely contributes to the higher isolation rates identified in our study compared to those reported in studies focusing on intensive care unit patients. The absence of a dedicated infectious diseases department may have further influenced these findings.

Our study revealed an ESBL prevalence of 21.5% among Enterobacterales, which aligns with the study by Bandy (22) but demonstrates notably lower rates than those reported in China (23). This geographical variation is particularly evident when comparing specific pathogens. In our study, *E. coli* (36.3%) and *K. pneumoniae* (16%) emerged as key ESBL producers, whereas in the study by Amanati et al (24), substantially higher rates were documented (66.7% and 60.7%, respectively). These differences highlight the importance of localized resistance monitoring for clinical practice.

The extreme resistance observed in *Acinetobacter* requires an urgent revision of our region's empirical treatment guidelines. Prolonged hospitalizations, the use of invasive devices, and overprescription of antibiotics exacerbate gram-negative resistance, creating selection pressure that fuels resistance cycles (25). The susceptibility of immunocompromised patients and the occurrence of healthcare-associated transmission underscore the urgent need for enhanced infection control and coordinated antimicrobial stewardship. Carbapenems and colistin remain among the most critical last-line antibiotics for treating multidrug-resistant infections. Our findings revealed alarming resistance patterns, particularly among *A. baumannii* isolates, which showed complete resistance to cephalosporins and 87.5% resistance to carbapenems, exceeding previously reported rates (26). Interestingly, while regional studies reported colistin resistance rates of 13.6% in Enterobacteriaceae (27) and 13.4% in *A. baumannii* (28), we detected no colistin-resistant strains. However, carbapenemase resistance (14.6%) and metallo-beta-lactamase resistance (2.2%) were present, as confirmed by mCIM/eCIM testing. The rate of carbapenem resistance in our study showed significant interspecies variation. The overall rate of resistance among gram-negative isolates (22.2%) was closely consistent with the findings reported by Balkhair et al (27.7%) (29) in Oman, indicating similar regional resistance patterns. This is particularly concerning given its association with a three-fold increase in 30-day mortality. Nasiri et al (30) reported carbapenem resistance of 24% in *K. pneumoniae* and 5% in *E. coli*. Although resistance patterns differ regionally, the broader trend remains clear. In other words, resistance to carbapenem and colistin is an escalating threat.

Our study found *E. coli* (25.3%) and *S. aureus* (23%) to be the most prevalent pathogens. These findings align with those of Maham et al (31) and Pourakbari et al (32), who reported similar challenges with MRSA. Specifically, we observed a high frequency of MRSA (67.5%) and VRE (36.4%), consistent with the study by Besharati et al (33). In contrast to the report by Mohammadi et al (14), all of our isolates remained susceptible to vancomycin. However, widespread resistance to older antibiotics, such as ampicillin (82%), erythromycin (75%), and tetracycline (68%), suggests that these agents may no longer be reliable for empirical therapy. These findings emphasize the importance of vancomycin as a critical therapeutic option, particularly for MRSA infections. Notably, we found no association between MRSA prevalence and patient gender ($P = 0.107$), contradicting studies that propose demographic risk factors. These discrepancies may reflect regional variations in antibiotic prescribing practices or infection control measures.

Our observation of 100% resistance to ciprofloxacin and erythromycin among enterococci aligns with trends noted by Shokoohizadeh et al (34). The high rates of resistance to penicillin and ampicillin among enterococci isolates (54%) observed in our region align with broader AMR trends, underscoring the urgent need for continuous local surveillance and the implementation of

targeted therapeutic protocols. Our findings, supported by An et al (35), reveal that the prevalence of MRSA varies significantly across geographic regions, highlighting the importance of having access to regional data for clinical decision-making.

This study has several limitations worth acknowledging. First, the reliance on phenotypic methods without complementary molecular testing (e.g., PCR or whole-genome sequencing) precluded the identification of specific genetic resistance determinants. Second, although our sample size (n = 1248) provided meaningful data, expanding to multiple centers may improve the detection of rare resistance patterns. Nevertheless, our findings provided important insights into regional AMR trends in BSIs, including: (1) high ESBL prevalence, (2) alarming MRSA rates in ICUs (81.3%), (3) emerging carbapenem resistance in *A. baumannii* (87.5%), and (4) preserved colistin susceptibility across all isolates. Future multicenter studies incorporating genotypic characterization are needed to validate these trends and guide regional infection control strategies.

In response to these challenges, it is crucial to support infection prevention and control practices in our hospital. This includes rigorous hand hygiene, appropriate use of invasive devices, continuous monitoring of disinfection processes, and immediate isolation of patients with multidrug-resistant organisms. An effective surveillance program will enable early detection of resistance patterns and facilitate necessary interventions, ultimately limiting the spread of resistant pathogens.

Conclusion

The high prevalence of multidrug-resistant organisms (ESBL-producing *Enterobacteriaceae*, MRSA, and VRE) in our hospital could have serious clinical and economic consequences. Based on AMR patterns detected in the current study, we propose the following evidence-based hospital-specific interventions. AMR surveillance systems should be strengthened by implementing active screening for resistant strains in high-risk wards. Targeted surveillance programs should be implemented to educate physicians to limit unnecessary antibiotic prescribing. Advanced infection control measures should include reducing contact precautions for carriers of MDR pathogens and monitoring hand hygiene. This triad, supported by lab-clinician collaboration, can effectively combat drug resistance while preserving the therapeutic options.

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Authors' Contribution

Conceptualization: Behnaz Deihim.
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Writing-original draft: Behnaz Deihim, Ahmad Ali Assarian, Mohammad Shoja.
Writing-review & editing: Behnaz Deihim, Ahmad Ali Assarian, Mohammad Shoja.

Competing Interests

The authors have no conflicts of interest to declare.

Ethical Aproval

Ethical committee approval received from the Ethics Committee of Dezful University of Medical Sciences (Approval no: IR.DUMS.REC.1401.002).

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