Antibiotic Susceptibility of Body Fluids and Urine Cultures from ICU Patients in a Tertiary Care Center in Iran

Abstract:
Background: Rising antibiotic resistance among prevalent pathogens poses a critical challenge, especially in ICU patients. This study aimed to evaluate antibiotic susceptibility and resistance patterns in body fluid and urine samples from ICU patients at a tertiary care center in Tehran, Iran.
Methods: A cross-sectional study analyzed cultures from body fluids and urine samples collected from ICU patients at Imam Khomeini Hospital between 2019 and 2022. Cultures were processed per standard protocols and interpreted based on growth inhibition zones to classify bacterial isolates as sensitive, intermediate, or resistant. Data analysis was performed using STATA V.17.0.
Results: A total of 4232 body fluid and 4062 urine samples were examined; 2400 body fluid and 2824 urine cultures were negative. The most common bacteria in urine were Klebsiella pneumoniae (16.7%), Escherichia coli (15.43%), and Pseudomonas aeruginosa (6.08%), while body fluids predominantly grew Klebsiella pneumoniae (37.19%), Acinetobacter baumannii (25.52%), and Pseudomonas aeruginosa (8%). Klebsiella pneumoniae showed highest sensitivity to Gentamicin (12.37% in body fluids, 17.93% in urine) and Imipenem (9.72% in body fluids, 14.67% in urine). Resistance was notably high against Ampicillin-Sulbactam (over 90%), Ciprofloxacin (85-89%), and Cotrimoxazole or Nitrofurantoin (84-88%) depending on the sample type.
Conclusion: There is significant antimicrobial resistance in key ICU pathogens including Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. These findings underscore the necessity for hospital-based active surveillance and effective antibiotic stewardship programs in ICU settings to monitor and mitigate resistance patterns, aiding in optimized infection control and patient management.

Keywords: antibiotic resistance, antibiotic susceptibility, antibiogram, intensive care unit, Iran

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Introduction:

Antibiotic resistance has become one of the most important determinants of outcomes in patients with severe infections. A recent estimate reports that more than ten million patients can die each year globally from antibiotic resistance. This rising number reflects a growing public health crisis that threatens to undermine the effectiveness of existing antibiotic treatments. The report emphasizes that global action is necessary to tackle this issue efficiently and sustainably. The impact of such a response is particularly relevant in settings with high rates of multidrug resistance such as hospitals and more importantly, intensive care units (ICUs). These critical care units are especially vulnerable due to factors like the frequent use of broad-spectrum antibiotics, prolonged patient stays, invasive procedures, and patients' compromised immune statuses. In these environments, antibiotic-resistant bacteria can spread rapidly, complicate treatment regimens, and increase mortality rates significantly. Without effective interventions including enhanced surveillance, antibiotic stewardship programs, infection prevention, and control measures, the burden of antibiotic resistance in ICUs will continue to rise, threatening patient outcomes and healthcare systems worldwide

(1-4). National action plans and surveillances are indeed necessary to mitigate this The problem of antibiotic resistance is an emerging and serious challenge worldwide. Unfortunately, Iran still lacks a well-organized national surveillance system to comprehensively collect and monitor data on antimicrobial resistance and antibiotic susceptibility. This deficiency makes it difficult to fully understand the scope and details of bacterial resistance in different regions and healthcare settings across the country. Therefore, conducting epidemiological studies in each geographic area is essential to gather accurate data, demonstrate local resistance patterns clearly, and develop strategies to control the spread of resistant bacteria effectively. The ultimate benefit of such surveillance programs lies in preventing the wider distribution of antimicrobial resistance within that specific region, protecting both public health and the effectiveness of current antibiotic treatments.

(3, 5-7).

The currently available data predominantly focuses on isolated outbreaks and particularly severe cases of resistant bacterial infections. Unfortunately, this limited scope does not provide sufficient information regarding the broader epidemiological trends, transmission dynamics, or established patterns of resistance within the population. Therefore, laboratory reports that systematically document the existing patterns of pathogens responsible for infections gain significant importance. These detailed microbiological surveillance reports play a crucial role in informing and guiding clinicians and healthcare providers. They enable healthcare teams to select the most effective and targeted treatments for managing drug-resistant infections. Additionally, such an evidence-based framework helps reduce the likelihood of further development and spread of antibiotic resistance by promoting rational and precise use of antimicrobial agents, ultimately contributing to better patient outcomes and public health preservation.

(8-11). Hence, this study aimed at evaluating the antibiotic susceptibility and antibiotic resistance pattern among the body fluids and urine cultures of ICU patients in a tertiary care center in Tehran, Iran.

Methods and Materials:

In this cross-sectional study, we used data from body fluids (including pleural and ascites fluid) and urine samples of ICU patients of Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran. This hospital is affiliated with the top-rank medical school in Iran and is a referral center. Hence, numerous patients from different socio-economic backgrounds are referred to this hospital. Thus, the data can be seen as a viable depiction of antibiotic susceptibility and resistance in the general population of Iran. The laboratory reports from 2019 to 2022 were gathered and examined in our study. Also, informed consent was taken from all the patients whose data has been used in our study.

The body fluids and urine cultures were taken according to the currently available standard protocols (3, 7, 12-14). After taking the sample, all types of microorganisms and susceptibility testing were recorded. These bacteria were isolated using the streak plate method on blood agar and MacConkey agar cultures. Culture plates were incubated at 37°C for 24 hours. A culture consisting of more than 10^5 colonies of a particular bacterium was considered a positive culture. Incubation of negative 24-hour cultures was extended for an additional 24 hours. Species were identified by biochemical tests (indole, citrate, oxidase and H2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase, mannitol fermentation, and susceptibility testing for novobiocin).

After identification, the antibiotic susceptibility and resistance were tested using the diffusion method on the Mueller-Hinton medium (Merck, Germany). After inoculating the bacteria on Mueller-Hinton agar and inserting antibiotic discs, the plates were incubated in an incubator for 24 hours. Results were then classified according to growth inhibition zone size and international number and reported into three

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groups: (1) susceptible and sensitive, (2) intermediate susceptibility or sensitivity, and (3) resistance. We used STATA V.17.0 to analyze and report the results.

Results:

Overall, 4232 body fluids samples and 4062 urine samples were gathered. Among the body fluids samples, 2400 cultures were negative and among the urine samples, 2824 cultures were negative. Among the body fluids samples, 97 (5.31%) cultures were positive for yeast (*Candida species*). Among the urine samples, 430 (36.94%) cultures were positive for yeast (*Candida species*). The most common bacterial species found in the urine cultures were *Klebsiella pneumonia* (184, 16.70%), followed by *Escherichia coli* (170, 15.43%) and *Pseudomonas aeruginosa* (67, 6.08%). The most common bacterial species found in the body fluids cultures were *Klebsiella pneumonia* (679, 37.19%), followed by *Acinetobacter baumannii* (466, 25.52%) and *Pseudomonas aeruginosa* (146, 8%). More details regarding the bacteria species found in the samples are summarized in Table 1.

Among the body fluids samples: the highest sensitivity of *Klebsiella pneumonia* was against Gentamicin (12.37%) and Imipenem (9.72%), respectively. Also, *Klebsiella pneumonia* was the most resistant against Ampicillin-sulbactam (91.61%), Ciprofloxacin (88.66%), and Cotrimoxazole (88.37%), respectively. The highest sensitivity of *Escherichia coli* was against Piperacillin-tazobactam (67.71%%) and Imipenem (58.82%) and the highest resistance was against Cotrimoxazole (76.47%) and Ciprofloxacin (72.55%), respectively. Further detail regarding the sensitivity and resistance of other species among the urine samples is summarized in Tables 2 and 3.

Among the urine samples: the highest sensitivity of *Klebsiella pneumonia* was against Gentamicin (17.93%), Imipenem (14.67%), and Piperacillin-tazobactam (12.50%), respectively. Also, *Klebsiella pneumonia* was the most resistant against Ampicillin-sulbactam (90.76%), Ciprofloxacin (84.78%), and Nitrofurantoin (84.24%), respectively. The highest sensitivity of *Acinetobacter baumannii* was against Imipenem (88.82%) and Nitrofurantoin (88.24%) and the highest resistance was against Cotrimoxazole (68.82%) and Ciprofloxacin (52.35%), respectively. Further detail regarding the sensitivity and resistance of other species in the urine is summarized in Tables 4 and 5. Also, details regarding the intermediate sensitivity of other species are available in Appendix 1.

Discussion:

The rise of bacterial co-infection or secondary infection among ICU-admitted patients has been alarming globally. Bacterial infection is one of the most severe complications of critically ill patients that have been admitted to ICU. Proper antibiotic therapy requires precise and timely isolation, identification, and performing sensitivity tests. Inappropriate or delayed antibiotic treatment may increase the chance of antibiotic resistance, prolonged infection, and increased mortality in ICU patients. Intrahospital surveillance programs are necessary to identify these agents and offer proper treatment. Such studies can enlighten us, regarding the changing pattern of antibiotic resistance and susceptibility (15-17).

In the present study, *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* were the most prevalent bacterial species found in the collected cultures. All of these aforementioned species showed diminished antibiotic susceptibility and increased antibiotic resistance, which should be alarming at a national and international level. Such a claim is supported by the number of studies during the last decade both in Iran and internationally. According to a recent study, *Klebsiella pneumonia, Staphylococcus aureus, and Acinetobacter baumannii* were the most prevalent bacterial species that were collected from critically ill patients with secondary infection. Such findings are following the results of our study (4, 18-21).

In a study that was designed to assess the antibiotic resistance pattern changes in Iran, Khoshbakht, et al. reported that the highest species with antibiotic resistance were *Klebsiella pneumonia, Pseudomonas aeruginosa, and Acinetobacter baumannii* (22). Such species were resistant to Ampicillin, Imipenem, and Ceftazidime. Similar results have been obtained in our study as *Acinetobacter baumannii* was resistant to Imipenem likewise. According to this study, *Klebsiella pneumonia* has shown a rising resistance, up to 30%, during one year, to a wide spectrum of antibiotics. Another similar study has reported the rising resistance of *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumonia* during the COVID-19 outbreak in Iran, up to 89% to multiple antibiotics. According to the results of our study, *Escherichia coli* was resistant to more than 80% Imipenem and Nitrofurantoin (23-30).

Another study reported the rising trend of antibiotic resistance of *Escherichia coli* against Beta-lactams, Aminoglycosides, Quinolones, Tetracycline, Chloramphenicol, and Cotrimoxazole (31). According to this study, although the pattern of resistance varies from region to region in Iran, however, the prevalence of *Escherichia coli* infection is significantly high in almost more than half of the provinces in Iran. There is a lack of evidence regarding the prevalence and resistance pattern of *Escherichia coli* in other provinces of

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Iran (31). A similar study which was conducted on patients in a hospital in Tehran has reported that Colistin and Tigecycline were the most effective antibiotic therapy choices against *Escherichia coli* (32-34).

Conclusion

The result of our current study suggests the high resistance of prevalent bacteria species such as *Klebsiella pneumonia, Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* against a variety of antimicrobial agents. Such a finding should be alarming for policymakers and necessitates the implementation of active surveillance programs in hospitals, and especially in ICU wards, to monitor the prevalence, susceptibility, and resistance of such infectious microorganisms.

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Author Contribution:

Author Contributions: Study concept and design: **A. A.** and M. T. B.; analysis and interpretation of data: **M. N.** and **P. A.;** drafting of the manuscript: **S. S., E. N. and A. N.;** critical revision of the manuscript for important intellectual content: **E. N.**, **S. S., A. N.**; statistical analysis: **S. N. and Z. P.**.

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