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Phenotypic Assessment of Antimicrobial Resistance in Nosocomial Bacterial Isolates from Hospitalized Patients: Disk Diffusion-Based Antimicrobial Susceptibility Testing and Epidemiological Patterns

Amin Pouresmail[1], Abbasali Dehpour Jouibari[1]*

[1] Department of Biology, QaS.C., Islamic Azad University, Qaemshahr Branch, Iran.

***Corresponding Author:** Abbasali Dehpour Jouibari. Department of Biology, QaS.C., Islamic Azad University, Qaemshahr Branch, Iran. Email: abbasalidehpour@gmail.com. Phone: 01142155025.

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Abstract

Introduction: Healthcare-associated infections represent a persistent global threat due to the increasing emergence of multidrug-resistant pathogens. This study aimed to phenotypically characterize antimicrobial resistance profiles among nosocomial bacterial isolates from hospitalized patients and establish epidemiological baseline data

Methods: This cross-sectional study analyzed 209 nosocomial bacterial isolates obtained from 13,850 hospitalized patients meeting Iranian Ministry of Health HAI criteria. Bacterial identification was performed using standard culture methods, and antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method according to CLSI 2023 guidelines. Sixteen antibiotics were evaluated using Mueller-Hinton agar and quality control strains. Resistance profiles were analyzed using WHONET 2024, while statistical comparisons were performed using SPSS v22.

Results: : Species-specific resistance associations were evaluated according to bacterial groups. Significant associations were observed for 11 of the 16 tested antibiotics ($p < 0.001$). Klebsiella spp. showed high resistance rates to ceftriaxone (93.2%), cefotaxime (94.6%), ceftazidime (93.2%), levofloxacin (87.8%), and imipenem (78.4%). Pseudomonas aeruginosa demonstrated high resistance to co-trimoxazole (87.5%) and imipenem (77.5%), while Acinetobacter spp. showed resistance to piperacillin-tazobactam (66.7%). Staphylococcus spp. remained susceptible to most tested agents except ampicillin-sulbactam (79.5%).

Conclusion: The phenotypic antimicrobial resistance patterns identified in this study provide epidemiological baseline data to support antimicrobial stewardship, optimize empirical therapy, and strengthen infection prevention and control strategies in healthcare settings.

Keywords: Nosocomial Infections; Antimicrobial Resistance; Disk Diffusion; Cross-Sectional Study; Iran

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Introduction

Healthcare-associated infections (HAIs) continue to pose a significant burden on healthcare systems worldwide. Beyond prolonging hospitalization and increasing medical costs, these infections are closely linked to poor clinical outcomes, greater use of broad-spectrum antimicrobials, and the emergence and spread of resistant pathogens within hospital settings [1]. HAIs highlight the need for continuous patient surveillance and comprehensive infection-control measures to minimize contamination risks in healthcare settings. The burden of healthcare-associated infections is especially pronounced in developing countries, where prevalence in some hospital settings has been reported to exceed 30%, reflecting the combined effects of limited infection-control infrastructure, high patient acuity, and constrained antimicrobial stewardship resources [2].

In recent years, the rise and spread of multidrug-resistant (MDR) bacteria, including Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli,

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have posed severe challenges to clinical therapy. These MDR strains, characterized by resistance to at least one agent across three or more antimicrobial categories, undermine conventional treatments, elevate therapeutic failure risks, prolong hospital stays, and amplify morbidity in vulnerable patient populations [3].

Recent worldwide data reveal a sharp escalation in antimicrobial resistance among clinical pathogens. For example, analysis of 475 bacterial strains from 910 patient samples showed critically high resistance rates in multiple species [4]. Similarly, research on hospital-acquired infections identified E. coli as the dominant isolate, with notable sensitivity to imipenem but extensive resistance to third-generation cephalosporins [5]. Considerable variability in resistance profiles was also observed across MDR bacteria such as E. coli, P. aeruginosa, K. pneumoniae, and A. baumannii, derived from diverse specimens including blood, wounds, sputum, and urine [6].

Antimicrobial resistance has become a major challenge in the management of nosocomial infections, particularly among hospitalized patients exposed to prolonged antimicrobial therapy and invasive procedures. Accurate phenotypic characterization of bacterial antimicrobial susceptibility profiles remains essential for guiding treatment decisions and understanding local resistance epidemiology. The Kirby–Bauer disk diffusion method is a standardized and widely used approach for antimicrobial susceptibility testing and provides reliable phenotypic assessment of bacterial resistance patterns [7].

This study aimed to phenotypically characterize antimicrobial resistance profiles among nosocomial bacterial isolates recovered from hospitalized patients and establish epidemiological baseline data using the standardized Kirby–Bauer disk diffusion method.

Materials and Methods

Ethical Considerations

The research protocol received approval from the Ethics Committee of Babol University (Approval Code: IR.IAU.BABOL.REC.1405.002). Written informed consent was secured from all participants or their authorized legal representatives prior to enrollment. Patient anonymity and data confidentiality were rigorously upheld throughout the entire study.

Study Design and Setting

This cross-sectional, descriptive-analytical study was conducted at Shahid Beheshti Hospital, Babol, Iran. The study aimed to investigate epidemiological aspects and antibiotic resistance spectrum using disk diffusion technique.

Reagents and Instruments

All reagents and materials used for bacteriological procedures were of analytical grade. Culture media including Blood Agar Base (Merck, Germany; Cat. No. 146876), Eosin Methylene Blue (EMB) Agar (HiMedia, India; Cat. No. M317), Mueller–Hinton Agar (Merck, Germany; Cat. No. 110333), Mannitol Salt Agar (HiMedia, Cat. No. M260), MacConkey Agar (Merck, Cat. No. 110347), and Tryptic Soy Broth (TSB) (HiMedia, Cat. No. M011) were used for primary culture and maintenance of isolates.

Biochemical identification reagents included TSI (Cat. No. M021), SIM (Cat. No. M108), citrate (Cat. No. M101), MR–VP (Cat. No. M127), urease (Cat. No. M195), DNase (Cat. No. M342), catalase and coagulase reagents (Merck, Germany; Cat. Nos. 1.12733 and 1.13397, respectively).

Antibiotic disks for susceptibility testing were purchased from Oxoid Ltd. (UK) and Liofilchem (Italy), including ampicillin–sulbactam (Cat. No. CT0023B), gentamicin (CT0047B), cotrimoxazole (CT0146B), nalidixic acid (CT0055B), nitrofurantoin (CT0059B), chloramphenicol (CT0015B), amikacin (CT0458B), cefazolin (CT0390B), tetracycline (CT0056B), penicillin (CT0043B), neomycin (CT0050B), cephalexin (CT0269B), streptomycin (CT0048B), erythromycin (CT0044B), oxacillin (CT0309B), and cloxacillin (CT0177B).

Instruments included an incubator (Memmert, Germany; Cat. No. IN55), biosafety cabinet Class II (ESCO, Singapore), autoclave (Sanyo, Japan), laminar flow hood (Nuaire, USA), Nano Drop 2000 spectrophotometer (Thermo Fisher), Eppendorf thermal cycler (Eppendorf AG, Germany; Cat. No. 6331000), gel electrophoresis apparatus (Bio-Rad, USA; Cat. No. 170–4486), UV transilluminator, and gel documentation system (Bio-Rad, Cat. No. 170–8195).

All consumables, including sterile pipette tips (Axygen, USA; Cat. No. T-200-Y), microtubes (Eppendorf; Cat. No. 0030123.344), and Petri dishes (Greiner Bio-One, Germany; Cat. No. 639160), were sterile and single-use grade to prevent contamination. Laboratory equipment and work surfaces were decontaminated with 70% ethanol and UV exposure before and after procedures to maintain aseptic conditions.

Study Population and Sampling

From 13,850 hospitalized patients, 209 cases that met the Iranian Ministry of Health criteria for HAIs were included. Entry criteria required onset of infection after at least 48–72 hours of hospital stay and absence of active

infection at admission. Patient demographic characteristics, infection type, microorganism category, previous invasive procedures, and underlying conditions were recorded in standardized data forms verified by both infection control nurses and infectious disease specialists[8].

Clinical Specimens Collection and Processing

Specimens (including blood, urine, sputum, wound discharge, cerebrospinal fluid (CSF), tracheal aspirates, and occasionally stool) were collected using aseptic techniques and promptly transferred to the bacteriology laboratory. Primary cultures were inoculated on Blood Agar and EMB Agar plates, incubated at 37°C for 24 hours. Gram staining and colony morphology were assessed microscopically. Gram-positive cocci were differentiated by catalase, DNase, mannitol salt agar, and coagulase tests. Gram-negative bacilli were identified using TSI, SIM, citrate, urease, oxidase, indole, and MR–VP assays. Pure isolates were sub-cultured and preserved in TSB with 20% glycerol at -20°C to -80°C to maintain isolate viability for further microbiological analysis [9].

Antimicrobial Susceptibility Testing by Disk Diffusion (Kirby–Bauer Method)

Antimicrobial susceptibility of the bacterial isolates was evaluated using the Kirby–Bauer disk diffusion method according to the current recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2023). The assay was performed on freshly prepared Mueller–Hinton Agar plates (150 mm Petri dishes, 4 mm depth), which were stored at 4°C and warmed to room temperature before use to ensure uniform agar thickness and moisture. Each bacterial isolate was subcultured from a pure colony onto a blood agar plate and incubated at 37°C for 18–24 hours to obtain a homogeneous growth. A representative colony was then suspended in sterile physiological saline to prepare a bacterial inoculum with an optical density equivalent to 0.5 McFarland standard, as measured by a McFarland turbidimeter. The turbidity of the suspension was adjusted visually when necessary to ensure reproducibility.

The standardized inoculum was then uniformly inoculated onto the surface of the Mueller–Hinton agar plate using a sterile swab, with the swab streaked in three directions (rotating the plate by 60° each time) to achieve a confluent, lawn-like growth. After allowing the inoculated agar surface to dry for 3–5 minutes under a laminar airflow cabinet, commercial antibiotic disks (Oxoid, UK; Liofilchem, Italy) were placed onto the agar using sterile forceps.

The selected antibiotics included ampicillin–sulbactam, gentamicin, cotrimoxazole, nalidixic acid, nitrofurantoin, chloramphenicol, amikacin, cefazolin, tetracycline, penicillin, neomycin, cephalexin, streptomycin, erythromycin, oxacillin, and cloxacillin, following local and international guidelines for testing common Gram-positive and Gram-negative pathogens.

Disks were evenly distributed across the plate, with at least 24–26 mm between centers, and a distance of at least 15 mm from the margin of the plate, to avoid overlapping inhibition zones. The inoculated plates were incubated upside down at 37°C for 18–24 hours in an aerobic atmosphere. After incubation, the plates were examined for the presence of inhibition zones around each disk. The diameter of the inhibition zone (including the disk diameter) was measured in millimeters using a calibrated ruler or digital caliper under transmitted light, with measurements taken to the nearest whole millimeter.

Zone sizes were interpreted according to the CLSI 2023 breakpoints as susceptible (S), intermediate (I), or resistant (R). Isolates resistant to at least thre e different classes of antimicrobial agents were classified as multidrug-resistant (MDR). All susceptibility tests were performed in parallel with appropriate quality-control strains, including Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853, to ensure methodological accuracy and reproducibility. Zone-diameter measurements for these control strains fell within the acceptable ranges specified by CLSI.

The susceptibility data for each isolate were recorded in standardized case report forms and subsequently entered into WHONET 2024 software (WHO Collaborating Centre, USA) for further analysis. WHONET was used to generate resistance profiles, cumulative susceptibility rates, and prevalence of MDR phenotypes across different specimen types and clinical wards, enabling both epidemiological surveillance and clinical decision-support for antimicrobial use in the hospital setting [10].

Statistical Analysis

Clinical and microbiological data were entered into SPSS version 22 (IBM Corp., Armonk, NY, USA; Serial No. 1022-0018). Descriptive statistics were calculated for bacterial distribution and resistance profiles. The Chi-square (χ^2) test was used to explore correlations among species, antimicrobial resistance profiles, specimen type, and clinical characteristics. A p-value < 0.05 was considered statistically significant.

Results

Inferential Statistics

According to Table 1, a binary logistic regression model was employed to assess the association between bacterial species and resistance profiles for the 16 tested antibiotics. Overall, 11 of the 16 antibiotics showed statistically significant associations between bacterial species and resistance profiles ($p < 0.001$), whereas resistance to five antibiotics (Erythromycin, Cefazolin, Cefoxitin, Oxacillin, and Vancomycin) was not significantly related to bacterial species ($p > 0.05$). Resistance patterns were interpreted according to the clinical relevance of each antimicrobial agent and the distribution of bacterial groups.

Table 1. Association between bacterial species and resistance to 22 antibiotics as determined by binary logistic regression and contingency-table analysis.

Antibiotic	Resistance (%)	Most resistant species (%)	p-value
Gentamicin	65.1	Klebsiella spp. (85.1)	<0.001
Ceftriaxone	71.3	Klebsiella spp. (93.2), E. coli (92.7)	<0.001
Cefepime	68.9	Klebsiella spp. (83.8), E. coli (82.9)	<0.001
Cefotaxime	81.8	Klebsiella spp. (94.6), E. coli (95.1)	<0.001
Trimethoprim-sulfamethoxazole	73.7	P. aeruginosa (87.5), Klebsiella spp. (85.1)	<0.001
Ceftazidime	71.8	Klebsiella spp. (93.2), E. coli (92.7)	<0.001
Piperacillin-tazobactam	51.2	Klebsiella spp. (84.1)	<0.001
Amoxicillin-clavulanic acid (or similar penicillin/β-lactamase inhibitor)	90.4	Klebsiella spp. (97.3), P. aeruginosa (95.0)	0.001
Nalidixic acid (or alternative quinolone)	66.0	Klebsiella spp. (83.8)	<0.001
Imipenem	57.4	Acinetobacter spp. (80.0), Klebsiella spp. (78.4)	<0.001
Levofloxacin	62.7	Klebsiella spp. (87.8), P. aeruginosa (77.5)	<0.001
Cefepime	58.9	Klebsiella spp. (81.1), P. aeruginosa (67.5), Acinetobacter spp. (66.7)	<0.001
Fosfomycin	21.5	P. aeruginosa (65.0)	0.001
Clindamycin	13.9	Staphylococcus spp. (69.2)	<0.001
Erythromycin	14.4	Staphylococcus spp. (76.9)	0.988
Cefoxitin	10.5	Staphylococcus spp. (53.8)	<0.001
Cefazolin	3.8	None (all species <5%)	0.990
Cefixime	8.6	None (all species ≤6.8%)	0.990
Oxacillin	11.0	None (all species ≤11.0%)	0.989
Vancomycin	8.1	None (all species ≤8.6%)	0.990

The most pronounced resistance was observed for 3rd- and 4th-generation cephalosporins and β-lactam-related agents, particularly among Klebsiella spp. and Pseudomonas aeruginosa. In contrast, Acinetobacter spp. and Staphylococcus spp. exhibited relatively lower resistance levels or predominant susceptibility for several agents.

Among the tested antibiotics, Klebsiella spp demonstrated the highest resistance rates for Gentamicin (85.1%), Ceftriaxone (93.2%), Ciprofloxacin (83.8%), Cefotaxime (94.6%), Ceftazidime (93.2%), Cefepime (81.1%), Nalidixic acid (83.8%), Imipenem (78.4%), and Levofloxacin (87.8%), whereas Pseudomonas aeruginosa displayed the highest resistance for Gentamicin (72.5%), Trimethoprim -sulfamethoxazole (co-trimoxazole) (87.5%), and Imipenem (77.5%).

In contrast, Acinetobacter spp. showed pronounced resistance to Piperacillin-tazobactam (66.7%) and IPM (80.0%), while Staphylococcus spp. exhibited very low or no resistance to Gentamicin, Ceftriaxone, Ciprofloxacin, Cefotaxime, Ceftazidime, Piperacillin-tazobactam, Cefepime, Imipenem, Levofloxacin, and Cefoxitin, but relatively higher resistance to Nalidixic acid (12.8%) and Ampicillin-sulbactam (79.5%).

The fluoroquinolone levofloxacin and aminoglycoside gentamicin showed high resistance prevalence among Gram-negative isolates, whereas erythromycin, cephalosporins, oxacillin, and vancomycin demonstrated predominantly susceptible phenotypes among the applicable bacterial groups, without significant association with species distribution ($p > 0.05$).

Disk Diffusion Assay

Antimicrobial susceptibility testing was performed on 209 consecutive nosocomial bacterial isolates recovered from hospitalized patients, using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with CLSI 2023 recommendations. The isolates originated primarily from urine (U/C), blood (B/CI/B/CII), (respiratory (SP/C), and wound (W/C) specimens, and were distributed across surgical (surgical ICU, general surgery, orthopedic, maxillofacial, neurosurgery), medical (internal medicine, emergency ward, CCU, cardiology, neurology/neurosurgery), and specialized (urology, transplant, dialysis, CPR) wards.

Cultures were processed on appropriate media (e.g., MacConkey, EMB, chocolate, Hektoen), and identification was based on conventional microbiological criteria; species-level identification and resistance pattern analysis were performed for the recovered bacterial isolates.

Figure 1 illustrates representative Kirby–Bauer disk diffusion test results for predominant nosocomial pathogens recovered from the study cohort. Panels A–F show conflu ent bacterial lawns on Mueller–Hinton agar inoculated with 0.5 McFarland standardized suspensions of Escherichia coli, Enterococcus faecalis, Klebsiella sp., Proteus sp., Pseudomonas aeruginosa, and Staphylococcus aureus, respectively, following placement of commercial antibiotic disks and 18–24 h incubation at 37°C.

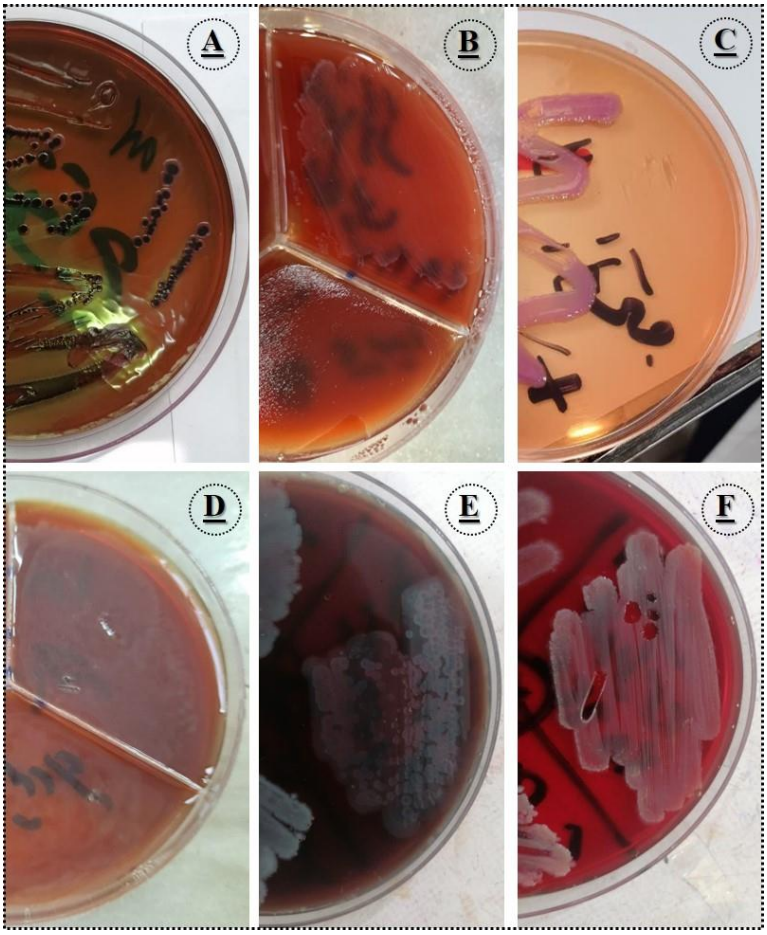


Figure 1. Kirby-Bauer disk diffusion results for key nosocomial isolates recovered from the study.
Plates demonstrate variable zone sizes corresponding to S (sensitive) and R (resistant) interpretations used to generate susceptibility profiles. **A)** Escherichia coli, **B)** Enterococcus faecalis, **C)** Klebsiella sp., **D)** Proteus sp., **E)** Pseudomonas aeruginosa, and **F)** Staphylococcus aureus.

Clear zones of inhibition surrounding individual disks demonstrate antimicrobial susceptibility (S), while absent or minimal zones indicate resistance (R), with diameters measured to the nearest millimeter using a calibrated ruler under transmitted light per CLSI 2023 criteria. These phenotypic results validate the S/R patterns reported in Table 1 and confirm methodological reproducibility across Gram-negative and Gram-positive clinical isolates.

Discussion

This study provides critical phenotypic insights into antimicrobial resistance patterns among nosocomial pathogens, addressing a major public health challenge by documenting resistance trends. Binary logistic regression analysis revealed strong species-specific associations with resistance for 11 of 16 tested antibiotics, with Klebsiella spp. and Pseudomonas aeruginosa exhibiting particularly alarming resistance profiles against third/fourth-generation cephalosporins (>90% for ceftriaxone/ceftazidime/cefotaxime), fluoroquinolones, and even carbapenems like imipenem (78–80%). In contrast, Acinetobacter spp. showed notable piperacillin-tazobactam resistance (66.7%), while Staphylococcus spp. maintained relatively preserved susceptibility to most agents except ampicillin-sulbactam (79.5%). Five antibiotics (including erythromycin, cefazolin, cefoxitin, oxacillin, and vancomycin) demonstrated no significant species-resistance correlation, within the applicable bacterial groups, suggesting broader susceptibility among the evaluated isolates. The Kirby-Bauer disk diffusion results from 209 clinical isolates across diverse specimens and wards validated these patterns through standardized Mueller-Hinton agar testing.

According to the findings, robust species-specific antimicrobial resistance patterns among 209 nosocomial isolates, with 11 of 16 antibiotics exhibiting highly significant associations, underscore the critical role of bacterial identification in interpreting resistance phenotypes. Klebsiella spp. displayed exceptionally high resistance rates exceeding 80% against third/fourth-generation cephalosporins (ceftriaxone 93.2%, cefotaxime 94.6%, ceftazidime 93.2%), fluoroquinolones (ciprofloxacin 83.8%, levofloxacin 87.8%), and even carbapenem imipenem (78.4%), consistent with global trends of ESBL-producing and carbapenem-resistant Klebsiella strains reported in hospital-acquired infections[11].

Similarly, Pseudomonas aeruginosa showed substantial resistance to gentamicin (72.5%), co-trimoxazole (87.5%), and imipenem (77.5%), reflecting the well-documented ability of this organism to develop multidrug-resistant phenotypes through multiple adaptive resistance mechanisms, including intrinsic and acquired resistance pathways [12]. Notably, resistance to erythromycin, cefazolin, cefoxitin, oxacillin, and vancomycin showed no species correlation, which may reflect the limited distribution of resistant phenotypes among the tested bacterial groups rather than universal activity across all isolates. Vancomycin, oxacillin, and cefoxitin interpretations were considered according to CLSI criteria and the clinical relevance of each bacterial group.

Acinetobacter spp. exhibited selective piperacillin-tazobactam resistance (66.7%), which is consistent with the complex resistance profiles commonly observed in this pathogen, while Staphylococcus spp. demonstrated broad susceptibility except ampicillin-sulbactam (79.5%). These patterns align with recent hospital surveillance reports showing high levels of Gram-negative resistance to cephalosporins and fluoroquinolones, whereas preserved activity of specific agents against susceptible Gram-positive isolates remains clinically relevant [13].

The Kirby-Bauer disk diffusion methodology employed in this study represents a widely accepted and standardized approach for phenotypic antimicrobial susceptibility testing, offering high reproducibility and epidemiological utility through Mueller-Hinton agar zone diameter measurements according to CLSI recommendations [7]. Testing 209 nosocomial isolates from diverse specimens (urine, blood, respiratory, wounds) across surgical, medical, and specialized wards confirmed the method's ability to detect species-specific resistance patterns, validating interpretable inhibition zones for E. coli, Enterococcus faecalis, Klebsiella sp., Proteus sp., P. aeruginosa, and S. aureus after standardized 18–24 h incubation at 37°C. Zones of inhibition around antimicrobial disks indicate susceptible phenotypes, whereas resistant isolates demonstrate reduced or absent inhibition zones according to CLSI interpretive criteria. These phenotypic resistance patterns may reflect the combined effects of intrinsic and acquired bacterial resistance mechanisms, including enzymatic activity, altered permeability, and other adaptive responses. These findings align with multicenter studies reporting high categorical agreement between disk diffusion and reference antimicrobial susceptibility methods for Enterobacterales and non-fermenting Gram-negative bacteria. However, the high level of Gram-negative cephalosporin resistance observed in our study (particularly among Klebsiella spp. with resistance rates exceeding 90% for several third-generation cephalosporins) highlights the regional burden of antimicrobial resistance and the need for continuous surveillance [14].

The preserved susceptibility to vancomycin, oxacillin, and cefoxitin among the clinically relevant

bacterial groups tested (p>0.05 species association) indicates maintained activity of these agents against susceptible isolates according to CLSI criteria. These findings should be interpreted considering the specific bacterial species and clinical indications of each antimicrobial agent, rather than as a universal susceptibility pattern across all bacterial isolates. Differences in resistance prevalence compared with other healthcare settings may reflect variations in antimicrobial exposure, infection-control practices, and local epidemiological characteristics [15].

Compared with molecular genotypic approaches, phenotypic disk diffusion testing provides a practical assessment of the overall antimicrobial response of clinical isolates, including the combined effects of multiple resistance mechanisms that influence antimicrobial susceptibility outcomes. Additionally, the standardized disk spacing (24–26 mm) and CLSI-based interpretation criteria ensured accurate measurement of inhibition zones and reproducible susceptibility classification.

Limitations

While this phenotypic survey provides valuable epidemiological insights into nosocomial antimicrobial resistance patterns, several limitations warrant consideration. Reliance on conventional culture-based identification may have missed fastidious organisms or polymicrobial infections, potentially underestimating the true diversity of nosocomial pathogens among the 209 isolates. The single-center design limits the generalizability of the findings beyond the hospital setting investigated, and the binary susceptibility classification may oversimplify subtle differences in antimicrobial susceptibility. Furthermore, the absence of molecular characterization precluded identification of the genetic mechanisms underlying the observed phenotypic resistance patterns. Finally, the lack of longitudinal patient outcome data prevented assessment of the relationship between antimicrobial susceptibility results and clinical treatment outcomes.

Future Research Directions

Future investigations should combine phenotypic antimicrobial susceptibility testing with molecular methods to further characterize the mechanisms underlying antimicrobial resistance in nosocomial pathogens. Prospective studies tracking temporal resistance trends over 2–3 years within the same hospital wards would help evaluate the impact of antimicrobial stewardship and infection-control interventions on the emergence of multidrug-resistant (MDR) organisms. In addition, optimization of rapid phenotypic antimicrobial susceptibility testing protocols could shorten the turnaround time for clinical decision-making and facilitate earlier optimization of antimicrobial therapy. Comparative multicenter surveillance across regional hospitals would determine whether the high resistance rates observed for cephalosporins and carbapenems represent broader regional trends or are specific to the study institution. Finally, predictive analytical models based on phenotypic antimicrobial susceptibility data may improve early identification of MDR pathogens and support evidence-based empirical antimicrobial therapy.

Conclusion

This comprehensive phenotypic analysis of 209 nosocomial isolates documented alarming species-specific antimicrobial resistance patterns, particularly in Klebsiella spp. (>90% resistance to third-generation cephalosporins) and Pseudomonas aeruginosa (77.5% resistance to imipenem), as determined by standardized Kirby–Bauer disk diffusion testing across diverse clinical specimens and hospital wards. The preserved susceptibility observed for selected antimicrobial agents within the appropriate bacterial groups provides valuable guidance for empirical therapy while emphasizing the urgent need for antimicrobial stewardship and strengthened infection-control strategies. These findings establish an important baseline for local antimicrobial resistance surveillance and underscore the indispensable role of routine phenotypic susceptibility testing in monitoring and controlling the growing burden of antimicrobial resistance.

Highlights

What Is Already Known?

Healthcare-associated infections (HAIs) and the rising prevalence of multidrug-resistant (MDR) bacterial pathogens represent a persistent and major global health threat. It is well-established that nosocomial pathogens (such as Klebsiella spp., Pseudomonas aeruginosa, Acinetobacter spp., and Staphylococcus spp.) exhibit high levels of resistance to standard empirical antibiotics, varying significantly by geographical region, hospital setting, and bacterial species. Standard diagnostic methods like the Kirby-Bauer disk diffusion method and guidelines (such as CLSI) are routinely used to monitor phenotypic resistance profiles.

What Does This Study Add?

Local and Regional Epidemiological Baseline: It provides robust, up-to-date local epidemiological baseline data on phenotypic antimicrobial resistance profiles among 209 confirmed nosocomial bacterial isolates from a large cohort of hospitalized patients (13,850 patients) based on strict Iranian Ministry of Health HAI criteria. Species-Specific Resistance Insights: It highlights specific, high-risk resistance patterns in the studied setting—notably extreme resistance rates in Klebsiella spp. (e.g., >93% resistance to third-generation cephalosporins and 78.4% to imipenem), high resistance of Pseudomonas aeruginosa to co-trimoxazole and imipenem, and resistance profiles for Acinetobacter and Staphylococcus species. Actionable Evidence for Stewardship: It supplies localized empirical evidence to directly support antimicrobial stewardship programs, optimize regional empirical therapy, and strengthen infection prevention and control (IPC) strategies in clinical settings.

Authors' Contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the findings, and drafting and revising the manuscript. All authors read and approved the final version.

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Conflicts of Interest Disclosures

The authors declare that there are no conflicts of interest.

Consent For Publication

Not applicable.

Ethics approval

This study was conducted in accordance with the ethical standards of the institutional research committee and was approved by the Ethics Committee of Islamic Azad University, Babol Branch (Ethical Approval Code: IR.IAU.BABOL.REC.1405.002). The study was performed as part of a doctoral research project at Islamic Azad University, Qaemshahr Branch. Clinical bacterial isolates were obtained from routine diagnostic specimens at Shahid Beheshti Hospital, Babol, and only anonymized microbiological data were analyzed. No patient-identifying information was collected or used in this study.

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The extent of AI use

Not applicable.

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