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Antimicrobial Resistance Trends of Gram-negative bacteria in ICU-acquired Healthcare-Associated Infections: A Surveillance over 6 years and Comparative Review with MENA Region

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Antimicrobial Resistance Trends of Gram-negative bacteria in ICU-acquired Healthcare-Associated Infections: A Surveillance over 6 years and Comparative Review with MENA Region

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Abstract

Introduction: Antimicrobial resistance (AMR) in healthcare-associated infections (HAIs) poses a significant global health challenge, particularly in intensive care units (ICUs). The COVID-19 pandemic has exacerbated AMR trends due to disproportionate antibiotic priscriptions. Updated data on post-pandemic AMR patterns of Gram-negative pathogens in ICUs of the Middle East and North Africa (MENA) region remain scarce.

Methods: This cohort was conducted retrospectively on 472 clinical isolates from 242 ICU patients. Bacterial identification and antibiotic susceptibility testing were determined by updated CLSI protocols. The distribution of bacterial species, AMR pattern (MDR, XDR, PDR), and antibiotic-specific resistance were assessed during the study period, which partially overlapped with the pandemic. Logistic regression was performed to evaluate AMR profiles.

Results: Klebsiella pneumoniae was the predominant isolate, followed by Pseudomonas aeruginosa and Escherichia coli. Respiratory tract specimens were more prevalent than urinary specimen. Overall prevalence of MDR, XDR, and PDR reached 76.9%, 64.8%, and 1.5%, respectively with no significant transformation in the temporal trends. XDR prevalence increased by 10-15% (particularly in elderlies) during the pandemic, and ~78% XDR and 90% MDR was observed in patients admitted with respiratory diseases. Acinetobacter baumannii exhibited the highest AMR (>90% XDR, 100% carbapenem resistance). AMR was highest to ceftriaxone, fluoroquinolones, and carbapenems, while colistin remained the most effective antibiotic.

Conclusion: This study highlights the alarming MDR and XDR burden in ICU settings following the COVID-19 pandemic. These findings underscore the urgent need for region-specific surveillance and antibiotic stewardship to guide rational empirical therapy and curb AMR in ICU settings.

Trial registration: Not applicable.

Keywords: Antimicrobial Resistance, Hospital-Acquired Infections, Intensive Care Units, Gram-Negative Bacterial Infections, COVID-19 Pandemic, Middle East and North Africa (MENA)

Introduction

Antimicrobial resistance (AMR) refers to genotypic or phenotypic changes in bacteria that reduce the efficacy of drugs intended to treat infections (1). The prevalence of AMR among healthcare-associated infections (HAIs) is rising dramatically. It has aggravated on a global scale as one of the foremost public health challenges of the 21st century. The burden of HAIs includes prolonged hospital length of stay, AMR, long-term disabilities, financial burdens on healthcare systems, patients, and families. This issue intensifies tremendously within intensive care units (ICUs), where critical patients are most vulnerable as a result of their compromised conditions. Some studies have proposed ICU-acquired HAIs during hospitalization as an independent factor linked to mortality, with a two-fold rise of the rate (2-4).

Gram-negative bacteria (GNB) are the proven culprits for most ICU-acquired HAIs (5). Remarkable GNB pathogens capable of developing resistance to multiple antibiotics consist of Klebsiella species, Pseudomonas aeruginosa (P.a), Acinetobacter baumannii (A.b), and Escherichia coli (E. coli) species. The aforementioned bacteria are frequently implicated in HAIs, in particular within ICU settings, such as ventilator-associated pneumonia, bloodstream infections, and urinary tract infections (UTIs) (6, 7). The rapid increase in resistance among bacteria to first- and second-line antimicrobial agents, coupled with the emergence of multidrug-resistant (MDR) organisms, extensively drug-resistant (XDR) organisms, and pan drug-resistant (PDR) organisms is particularly alarming. This situation has significantly narrowed the range of available therapeutic options for treating these infections. The limited number of new antimicrobial agents approved recently further exacerbates this situation (4).

The World Health Organization (WHO) estimated AMR was the main reason of 1.27 million deaths across the world and was accountable for 4.95 million deaths indirectly in 2019. Moreover, the burden of AMR is 2-3 times higher in medium- and lower-income countries with higher mortality (33.6% vs <20%) (8). The challenge posed by AMR at the Middle East and North Africa (MENA) region remains obscure (9). Although Iran is formally among the countries with established national and hospital-level Infection Prevention and Control (IPC) policies, implementation gaps still remain (10). According to a study conducted across 940 hospitals in Iran, HAI incidence rate was 4.2 per 1000 patient-days with 15.65% mortality rate. The mode of afflicted patients had respiratory tract infection (RTI), and after that in order to frequency, UTI, surgical site infections, and sepsis (11). Nonetheless, these figures predated the Coronavirus Disease-19 (COVID-19) pandemic.

The COVID-19 pandemic profoundly altered infection control practices and antibiotic indications worldwide. The Centers for Disease Control and Prevention (CDC) revealed a 20% increase of AMR in HAIs during the pandemic, peaking in 2021 and remaining above pre-pandemic levels in 2022 (12). Healthcare strategists must carry out regular evaluation of surveillance data to monitor the imperative trends and proactively avert outbreaks (13). Sharing epidemiological information

reporting the particularities of each region is a crucial measure to mitigate or degrade the advance of AMR according to the WHO Global Action Plan (14). Yet, such aspects remain insufficiently studied in Iran and across MENA region. The most updated comprehensive Iranian report on AMR and HAIs prior to this study dates back to before the COVID-19 pandemic (11). In the current study, the cornerstone of our investigations was to introduce an update on local epidemiology of HAIs and AMR trends of major bacterial pathogens in ICU settings over an approximately six-year period. Bank Melli Hospital in Tehran provides an ideal setting to study the incidence of AMR due to its high ICU patient turnover in this region.

Methods

Study Design and Subjects

The current observational cohort study was conducted retrospectively on 358 hospitalized patients in three separate ICU wards to evaluate AMR at Bank Melli Hospital, a tertiary center, Tehran, Iran over a 6-year period from March 2018 to December 2023, encompassing the COVID-19 pandemic. The official onset of the COVID-19 epidemic in Iran dates back to February 17, 2020. All of the demographic and clinical data including age, gender of the patients, medical records, clinical course during hospitalization, and microbiological laboratory results were reviewed from hospital databases for all patients. An infection was considered ICU-acquired HAIs if all elements of the CDC/NHSN site-specific infection criterion were present: new pathogen isolated >48 hours after admission with compatible clinical, radiological, and laboratory features (15). Positive specimen prior to 48 hours of admission, contaminated samples (i.e., polymicrobial cultures), and duplicated samples (i.e., repeated positive specimen similar in both bacterial species and site during the treatment course or a simultaneous positive blood culture to another prior infection) were also excluded. All of the previously listed criteria were put into place to avoid the possibility of duplication bias or miscalculating recurring situations. Missing data were handled with listwise deletion.

We included 472 non-duplicate clinical samples from 242 ICU patients with positive cultures of Klebsiella spp., A.b, P.a, and E. coli. The patients were treated in parallel to the results of clinical bacteriology identification procedures on the samples. The patients were also assessed for immunodeficiency. Immunodeficiency was acknowledged as primary or secondary (e.g., viral, malignancies and iatrogenic immunosuppression) (16).

Laboratory Evaluations

Clinical specimens were processed immediately in the hospital microbiology laboratory in accordance with the following established protocols guidelines to identify isolates (17). In detail, conventional biochemical tests including oxidase, catalase, motility, metabolic procedures such as citrate, indole production, methyl red, Voges-Proskauer, and presence of lysine decarboxylase and arginine dehydrogenase enzymes were performed. Blood samples were loaded in the BD BACTEC™ Automated Blood Culture System (BD Diagnostics, Sparks, MD, USA) at 36 °C for

up to 5 days and positive samples were subcultured. In addition, a molecular sepsis panel including PCR assays was performed in selected suspicious bacteremia cases, but we included only GNB isolates in our analysis. Bronchoalveolar lavage (BAL), sputum, pus swab and wound specimens, catheters and third-space fluids (pleural and peritoneal tap) samples were inoculated on MacConkey agar, sheep blood agar, and chocolate blood agar (Hi Media Laboratories LLC, India) and incubated in 37 °C overnight at both ambient air and 5% CO2. Urine samples were inoculated on cysteine-lactose electrolyte deficient agar, as well as aerobically.

Antimicrobial Susceptibility Testing

Isolates confirmed by biochemical and molecular tests underwent the disc diffusion susceptibility test to each tested drug using antibiotic disks provided by Rosco™, Switzerland and Padtan Teb™, Iran. In summary, a 0.5 McFarland suspension of each isolate was inoculated on a whole plate surface Mueller-Hinton agar plate by streaking the swab in back and forth motions. Then, they were incubated for 24 hours at 37°C. Pivotal points were interpreted according to the latest available clinical and laboratory standards institute (CLSI) guidelines. Interpretation of antibiotic susceptibility was performed as MDR: resistant to at least one drug in three or more antimicrobial classes, XDR: susceptible to only one or two classes, PDR: resistant to all drug classes (18-21). In addition, a gradient minimum inhibitory concentration (MIC) determining method according to manufacturer's instructions was implemented in certain cases. While converting to patient-level data, the highest AMR category was considered in patients with multiple non-duplicate smaples. Quality control was performed with E. coli ATCC 25922 for disk diffusion and MIC results according to the mentioned guidelines.

Statistical analysis

Patient-level data were used for demographic and clinical analyses, whereas isolate-level data were used for microbiological characteristics, antimicrobial susceptibility patterns, and AMR classification. Continuous variables were presented as means and standard deviations (SD), and categorical variables were summarized as frequencies and percentages. Logistic regression was implemented to evaluate variables and trend of AMR across the timeline (non-MDR VS MDR). The model was adjusted with sex, age, cause and length of hospitalization, immunodeficiency, diabetes, and smoking/substance abuse. Data were collected and analyzed using SPSS version 27 (IBM; NY, USA). A $P<0.05$ was considered statistically significant for all analyses.

The findings of the present study were compared descriptively and narratively with previously published reports on AMR patterns of GNB pathogens in ICU-acquired HAIs from the MENA region.

Results

Demographic characteristics of the study

A total of 472 non-duplicate clinical isolates from 242 hospitalized patients were recovered. Pneumonia was a major cause of hospitalization, particularly following the COVID-19 pandemic (Figure 1). The mean age of the patients was 75.6 ± 15.5 years and the majority were elderly (over 75 years old, 61.6%). Men comprised 59.9% of the cohort. The median duration of hospitalization was 18 days. Table 1 presents further demographic features of the study.

Distribution of Isolates

Klebsiella pneumoniae (K.p) was the most frequently isolated organism overall. Respiratory tract specimens (sputum and BAL) accounted for more than two-thirds of isolates (Table 2). Temporal observations revealed an increasing prevalence of Klebsiella spp. after the onset of the COVID-19 pandemic (peaking in 2022), while P.a and E. coli declined over time. Notably, A.b was not detected until 2021 but appeared thereafter only in respiratory tract and blood cultures (Figures 2,3).

AMR categories

The overall incidence of MDR, XDR and PDR pathogens was 76.9%, 64.8% and 1.5%, respectively. MDR strains were more frequent among females. The prevalence of XDR strains slightly increased with advancing age, and PDR isolates were not detected in patients under 60 years of age. AMR profile was similar between active or former smokers/substance abusers and non-smokers/-substance abusers. Immunocompromised patients were affected by relatively lower AMR. Interestingly, the patients who were primarily admitted for respiratory diseases suffered from insignificant higher AMR (~ 78% XDR, 90%MDR, P = 0.15) (Table 3). The lowest AMR rate was noted in E. coli. On the other hand, the highest XDR pattern was observed in A.b (Figure 4).

Although AMR was already remarkable at baseline and the prevalence of XDR pathogens increased after the pandemic (Figure 5), the overall increasing trend was not significant (P = 0.79, OR = 0.97). No significant transformation of AMR trend was noted in the timeline after stratification (P = 0.45) (Table 3).

Antibiotic-Specific Resistance

AMR varied considerably across antibiotic classes (Table 4). The highest overall AMR was observed against ceftriaxone (90.3%), followed by trimethoprim-sulfamethoxazole (83%), fluoroquinolones (FQs) (80.5%), carbapenems (79%), and ceftazidime (78.3%). Resistance to piperacillin-tazobactam was also high (70.4%). Aminoglycosides (AGs) (e.g., gentamicin and amikacin) showed relatively lower AMR (58.7%). Colistin retained the greatest activity, with resistance documented in only 2.8% of isolates. A.b consistently exhibited the highest AMR among species and across most antibiotic classes, while E. coli demonstrated the lowest, particularly against carbapenems and AGs.

Discussion

The increasing rates of AMR and MDR isolates of GNB represent excessive global healthcare concerns pointing to indiscriminate use of antibiotics in healthcare (e.g., prescriptions without indication, incorrect administration, and over-the-counter availability). This issue has amplified in Iran and it is more prevalent in ICUs than in other wards (5, 22, 23). This may be due to the frequent utilization of invasive medical devices among ICU patients and their heightened exposure to a greater diversity of antibiotic-resistant pathogens in addition to horizontal gene exchange of various resistant traits, namely plasmid-encoded betalactamases, AG modifying enzymes, quinolone resistance gene (24). WHO has defined the AWaRe (Access, Watch and Reserve) classification (Table 5) as a global action plan to counter the progression of AMR (25). Qatar introduced an effective stewardship policy managed to steadily diminish the MDR P.a prevalence from 9% to 5.46% over 3 years (26).

Source of Isolates and Empirical Therapy

The respiratory tract samples, sputum and BAL, were the most common type in terms of the type of HAIs, although most regional studies consistently found UTI as the leading cause of HAI (27-29). Frequent indication of ventilators during the COVID-19 pandemic may explain the mentioned discrepancies. A considerable prevalence (36.6%) of K.a and P.a was observed in the respiratory tract samples.

The incidence of E. coli as a cause of UTI is reportedly three-fold higher than Klebsiella spp. Effective empirical therapy includes nitrofurantoin in uncomplicated UTIs and AGs, ceftazidime, cefepime, or piperacillin-tazobactam in complex cases (30).

Some studies argue about the concern of less effectiveness of meropenem in vascular catheters and blood stream infections than other sites. These escalating reports of community-acquired carbapenem-resistant infections are attributable with higher mortality (31).

It is noteworthy to cover Gram-positive cocci in addition to GNB in skin/soft tissue, vascular catheter infections, and bacteremias because Staphylococcus aureus is generally the most common MDR isolate at these sites (32).

Isolates

Our data elucidated an overall preponderance of K.p (with the exception of E. coli emerging as the most common infectious agent in UTIs), comparable with the similar studies in the region (33-36). In the opposite view of us, all Klebsiella isolates depicted low AMR to levofloxacin per Molana et al. findings (37). Urinary tract and pus isolates of Klebsiella spp. often demonstrates remarkable resistance to carbapenem (38). Unlike Sekar et al. (38), the AMR rate in Klebsiella spp. was notably lower compared with E. coli; however, Klebsiella spp. had a higher carbapenem-resistance

in both studies. An Iranian study calculated the pooled prevalence of colistin-resistant K.p isolates (6.9%) in 2022 and measured a 3.4% increase compared to prepandemic studies (39).

E. coli had the least AMR in our study, though it is still concerning. It is a conditional pathogen with high uropathogenic propensity, which can also trigger RTIs (40). Studies have demonstrated higher AMR in blood samples of E. coli compared with urine samples (41). AMR of E. coli is reportedly lower in synthetic antibiotics such as sulfonamides than ampicillin (42). Among the penicillins, only betalactamase inhibitors, namely piperacillin-tazobactam remain considerably efficient against the growing challenge of ESBL strains (43). Our findings demonstrated growing resistance of E. coli to third-generation cephalosporins and FQs, in line with the current trend of studies reporting levofloxacin and ofloxacin as the most effective FQs (38, 44). However, extended spectrum betalactamases (ESBL)-producing strains are developing AMR to levofloxacin and AGs. This can be attributed to administration of high-grade and often unwarranted FQs or third-generation cephalosporins to provide empiric coverage in UTIs, which may restrict available treatment options to carbapenems (42, 45). Fortunately, the rate of carbapenem resistance was not as high as A.b and K.p isolates in our study.

P.a was identified as the second most prevalent strain in our study. MDR P.a is a major pathogen causing wound and burn HAIs in ICUs and its rate varies across the MENA region, where it ranges from 22.5% in Egypt to 61% in Saudi Arabia (9). A handful of prepandemic Iranian investigations (including burn centers) already reported considerable rates of ESBL, MDR (up to 94%), and XDR (up to 85%) genotypes of P.a with significant AMR to nearly all tested antibiotics: ciprofloxacin (62.7-93.7%), gentamycin (67.5%), amikacin (52-90%), cefepime and aztreonam, except colistin (2%). The data regarding AMR of ceftazidime (32-57.5%) and carbapenem (64-97.5%) was heterogeneous (46-51). In the current study, we also observed challenging XDR and PDR patterns of P.a; but the frequency of AG and carbapenem-resistant P.a was substantially higher in our study compared to pediatric referral centers in Tehran (36, 52). This higher overall AMR might be attributable to differences in patient populations (adult versus pediatric patients) and greater exposure to risk factors such as invasive devices in the COVID-19 pandemic.

A.b is an opportunistic pathogen with the capability to colonize in hospital settings, persist for an extended time, and acquire diverse virulence factors by dissemination of the plasmid-mediated AMR genes. Although A.b is less virulent than P.a, it represents the highest AMR to tested antibiotics. It has emerged as a crucial cause of ICU-acquired HAIs outbreaks, particularly in immunocompromised patients (53). The most prevalent HAI with A.b is ventilator-associated pneumonia through skin and airway defects penetration and attachment to bronchial epithelial cells (54) , which was also apparent in our study. In 2013, Jafari et al. (55) documented relatively lower AMR rates to carbapenems (41%-60), ciprofloxacin (78%), and retained susceptibility to colistin and ampicillin-sulbactam. The recent aggravation of ESBL and AMR challenge of A.b (up to 92-97%) in almost all tested agents (cephalosporins like ceftazidime, carbapenems, AGs and FQs) (54, 56, 57) was comparable with our observations. Carbapenems are considered as the mainstay treatment in practice for MDR A.b due to ongoing narrowed options; however, numerous reports of carabapenem-resistant isolates are noted locally and worldwide (53, 56). However, there were Iranian reports of sharp elevation of MDR and colistin-resistant A.b even

before the COVID-19 pandemic (56, 58, 59). In our observations, there was 100% carbapenem resistance and 0% colisitin resistance in A.b isolates.

Antibiotic agents and empirical therapy

The Infectious Diseases Society of America discourages administration of antimicrobials for empirical treatment if the regional AMR is greater than 10-20% (60). Considerable AMR to third and fourth generations of cephalosporins were previously noted in E. coli and Klebsiella spp. in developing countries (45). FQs were frequently favored in empirical therapy of UTI (45), yet we also found a relatively high AMR to FQs in E. coli (potential UTI pathogens). Therefore, cefepime combined with metronidazole or piperacillin-tazobactam are good alternatives for intra-abdominal infections, while meropenem must be reserved for sepsis (30).

The rising trends of AMR of broad-spectrum and antipseudomonal agents in most countries of our region is alarming: Bahrain, Qatar, Saudi Arabia, Iraq, Egypt, Syria, Libya, Tunisia, and Lebanon consistently showed elevated AMR levels for piperacillin-tazobactam, antipseudomonal cephalosporins (ceftazidime and cefepime), carbapenems, AGs, FQs, and aztreonam (9). Thus, rapid AMR transmission of carbapenem-resistant P.a (and other GNB) pose an international public health concern as it worryingly restricts available treatment options and accounts for high morbidity, mortality, and healthcare costs, especially in developing countries (38, 61). We also found a concerning AMR to carbapenems (80%). Carbapenem-resistant cases are managed by AGs, tigecycline, ceftazidime-avibactam, and colistin (59).

Colistin is the highly active last-resort against MDR, XDR, or carbapenem-resistant GNB with roughly 100% efficacy in most regions, although Qatar, Saudi Arabia, Egypt, and Syria report developing AMR to colistin (3.4 to 30%) (9, 62). A national survey calculated that Tehran had the highest resistance to colistin in Iran (16%), whereas Urmia had the lowest (0.3%). The overall rate of colistin resistance increased significantly over time (2013-2018, 2019-2021), which might be due to the increased use in the recent years (39). The increasing prevalence of carbapenem- and colistin-resistant GNB has exacerbated mortality of in bloodstream infections, along with alarming emergence of PDR organisms in ICUs (39, 63). Nevertheless, colistin resistance was very low in our study.

The novel betalactamase inhibitor combinations such as ceftazidime-avibactam and ceftolozane-tazobactam depicted good antimicrobial susceptibilities in the Middle East, despite of some high regional AMR (such as in Qatar 31.2 - 37.1%), even before their introduction into clinical practice. However, these specific last-line antibiotics plus linezolid, colistin, tigecycline and daptomycin were often not available (26, 64). Some futuristic studies are evaluating the promising results of synergism of bacteriophage therapy and antimicrobial agents in AMR outbreaks (65).

COVID-19

K.p became more prevalent concurrently with nosocomial RTIs after the pandemic in our observations. Moreover, we noted a high AMR development (~ 78% XDR, 90%MDR) among

patients who were initially admitted due to various respiratory diseases. This is possibly due to more ventilator utilization (especially during the COVID-19 pandemic) and it might necessitate more focus on ventilator-associated pneumonia in HAI empirical management guidelines. P.a, A.b, Mycoplasma pneumonia, and Haemophilus influenza were other frequently suspected bacterial superinfections in the COVID-19 pandemic. On the other hand, escalating trends of unjustified prescriptions of broad-spectrum antibiotics, especially the Reserve agents (like linezolid), occurred out of fear of bacterial coinfections or paraclinical overlap with bacterial RTIs. The phenomenon led to deterioration of AMR during and after the COVID-19 pandemic. For instance, the wide prescription of azithromycin due to its additional immunomodulatory and antiviral properties resulted in rapid transformation of AMR (66). Similarly, some reports claimed a 20% increase in carbapenem resistance in a 4-year period (67). We observed an overall >10-15% increase in the prevalence of XDR isolates at ICUs after the pandemic.

Risk factors

The optimal preventive healthcare protocols to combat dissemination of AMR should respond appropriately against risk factors. MDR GNB survive in the hospital environment and easily spread between patients through the hands of health care workers; therefore, enhancing routine hygiene practices and reducing invasive procedures are essential (68). We observed a slightly more frequency of MDR and XDR ICU-acquired HAI patients with prolonged hospitalization (>14 days), age over 60, and male gender, similar to the existing literature (2, 69). Importantly, prior hospitalization within the past year is a key determinant, and each additional day at ICU increased HAI risk by approximately 1% with a mean acquisition time of 11 days for MDR HAI and 24.5 days for XDR HAI. Furthermore, ICU patients with impaired consciousness, cirrhosis, respiratory or cardiovascular comorbidities are consistently prone to GNB HAI (70-72).

Underlying immunocompromising conditions are possible contributors to HAI and consequent morbidity in ICU patients; however, it did not affect the AMR rate in our study. Nonetheless, MDR GNB are a common cause of sepsis in patients undergoing transplantation or chemotherapy, which are typically managed by carbapenems (63). Some studies have documented invasive bloodstream infections caused by MDR and ESBL strains of Enterobacteriaceae in malignancies (e.g., neutropenic hematopoietic stem cell recipients) even with sufficient prophylaxis (73).

Limitations

Our retrospective observation had some limitations, as the genotypic and molecular data of the isolates were not documented. Furthermore, the disk diffusion method might not be sensitive enough as the broth microdilution method to identify AMR measurements. Moreover, most patients were already under antimicrobial treatment before referring to our center. Differentiating between pathogen and contamination were sometimes challenging, particularly in patients with urine catheterization. Moreover, the bias regarding retrospective and single-center design probably affects the generalizability of our findings.

Conclusion

This study demonstrates a substantial burden of AMR among GNB pathogens causing ICU-acquired HAIs, with 76.9% of isolates classified as MDR and 64.8% as XDR. AMR considerably inflicted patients who were hospitalized with respiratory diseases. We also noted a possible shift from UTIs to RTIs in terms of HAIs and a marked increase in XDR phenotypes (especially in elderlies) following the COVID-19 pandemic. Compromised/intact immune system, and smoking/substance abuse status were similar in terms of AMR profile. A.b exhibited the most critical AMR profile, including >90% XDR and 100% carbapenem-resistance. The AMR trend did not transform across the timeline. The high-risk GNB organisms dominated AMR in ICU-acquired HAIs with notable resistance to cephalosporins (ceftriaxone and ceftazidime), FQs, AGs, piperacillin-tazobactam and carbapenems, while colistin remained as an effective last-line option in MDR cases. We recommend futuristic replication of analytical studies in various hospitals and cities in this region for a more definitive comprehensive analysis to monitor AMR in ICU-acquired HAIs.

Abbreviations:

A.b: Acinetobacter baumannii

AG: Aminoglycosides

AMR: Antimicrobial Resistance

BAL: Bronchoalveolar Lavage

CDC: Centers for Disease Control and Prevention

COVID-19: Coronavirus Disease-19

E. coli: Escherichia coli

ESBL: Extended Spectrum Betalactamases

FQ: Fluoroquinolones

GNB: Gram-negative bacteria

HAI: Healthcare-Associated Infections

ICU: Intensive Care Units

K.p: Klebsiella pneumoniae

MDR: Multidrug Resistant

MENA: Middle East and North Africa

P.a: Pseudomonas aeruginosa

PDR: Pan Drug Resistant

RTI: Respiratory Tract Infection

UTI: Urinary Tract Infections

WHO: World Health Organization

XDR: Extensively Drug Resistant

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Authors' Contributions
R.B. and K.K. extracted the study data. M.R. and A.E. contributed to data analysis. A.E. and S.O. wrote the primary draft and together with K.K., R.B., and M.R. completed the final manuscript. S.S. supervised the current study and provided expert and critical opinions to resolve any discrepancies. All authors take full responsibility for their contribution to this study and also they all reviewed and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
The study protocol was registered and approved by the ethics committee of Farhikhtegan Hospital, Islamic Azad University, Tehran Medical Sciences (IAUTMU) (ethical code: IR.IAU.FARHIKHTEGANH.REC.1404.005||*2026.01.07*) (74) and consent for participation or anonymous publication was waived accordingly due to retrospective design and use of anonymized data. Hence, clinical trial registration was not applicable. Bank Melli Hospital administration granted access to medical and microbiological records in accordance with institutional regulations. All procedures were adhered to the tenets of the Declaration of Helsinki (1964) and its later amendments about biomedical studies involving human participants.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

Use of AI tools
The authors used AI-based tools only for language editing and grammar checking. No content was generated by AI, and the authors take full responsibility for the integrity and originality of the manuscript.

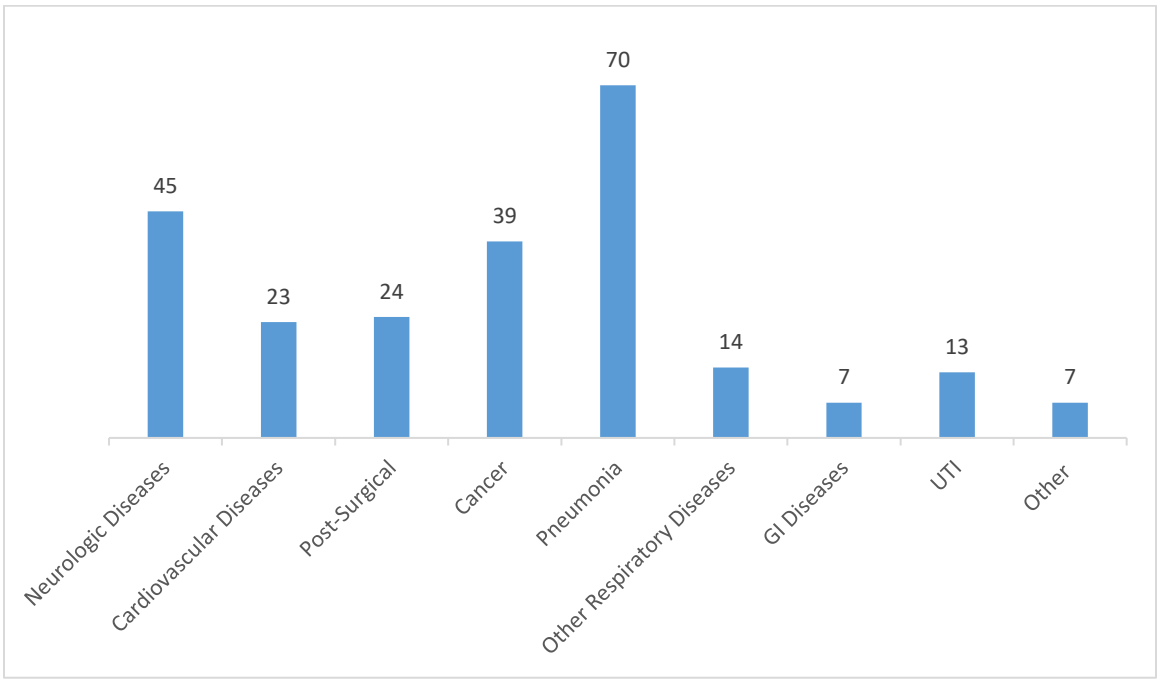


Figure 1. Cause of hospitalization

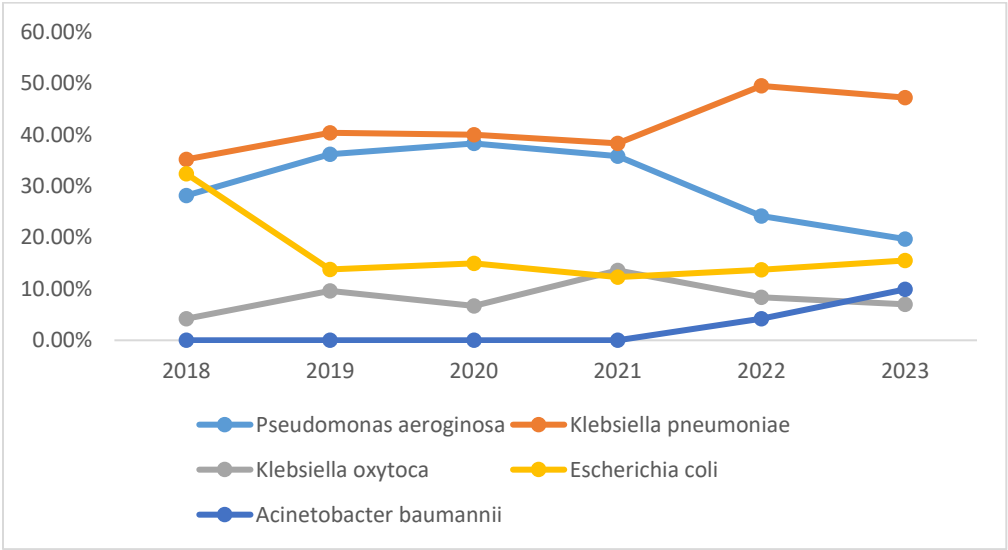


Figure 2: Prevalence of isolated strains during study period

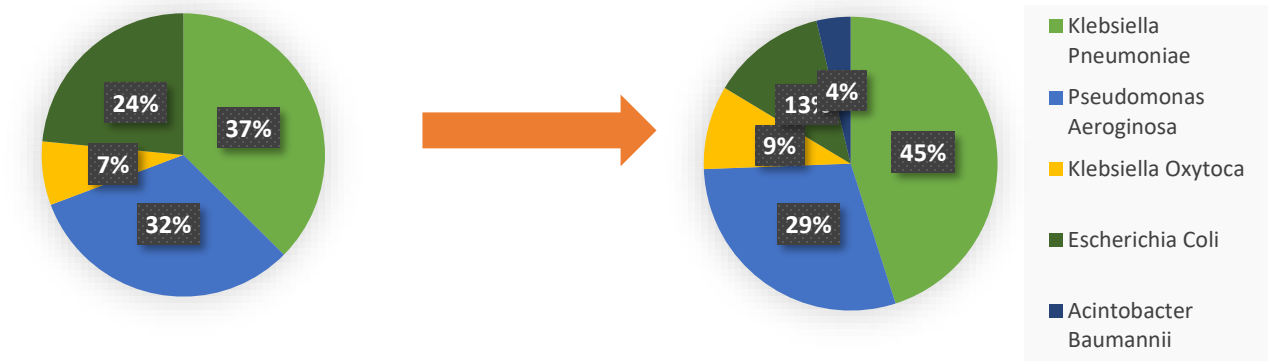


Figure 3: Prevalence of GNB before and after COVID-19

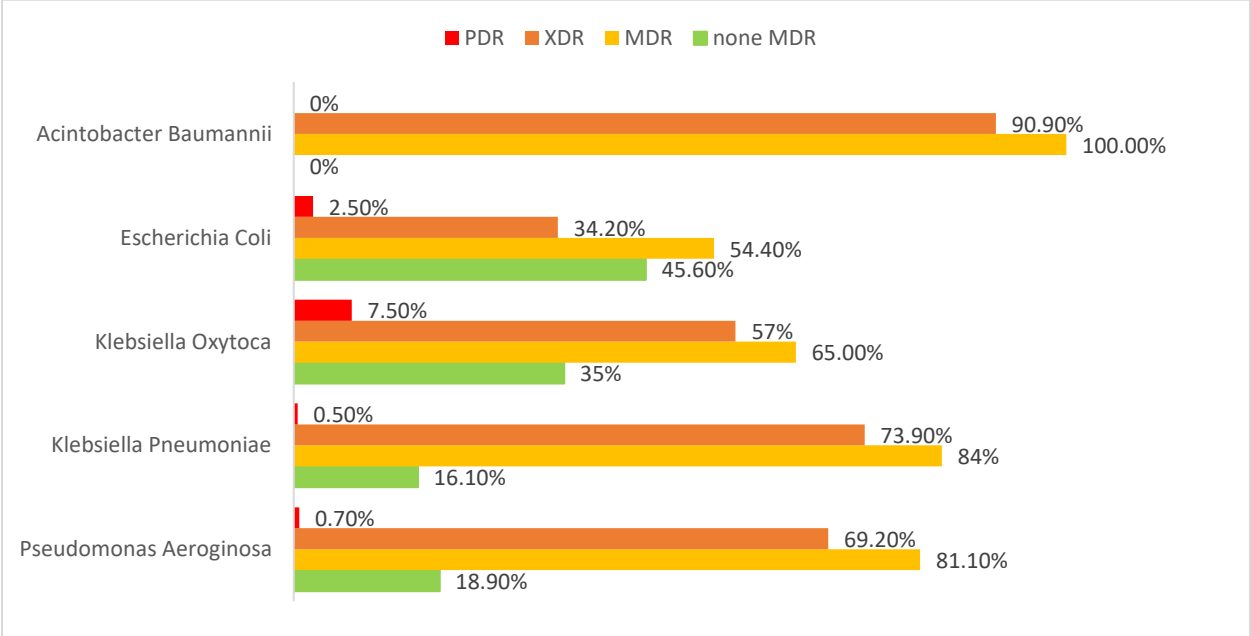


Figure 4: AMR prevalence of isolated strains

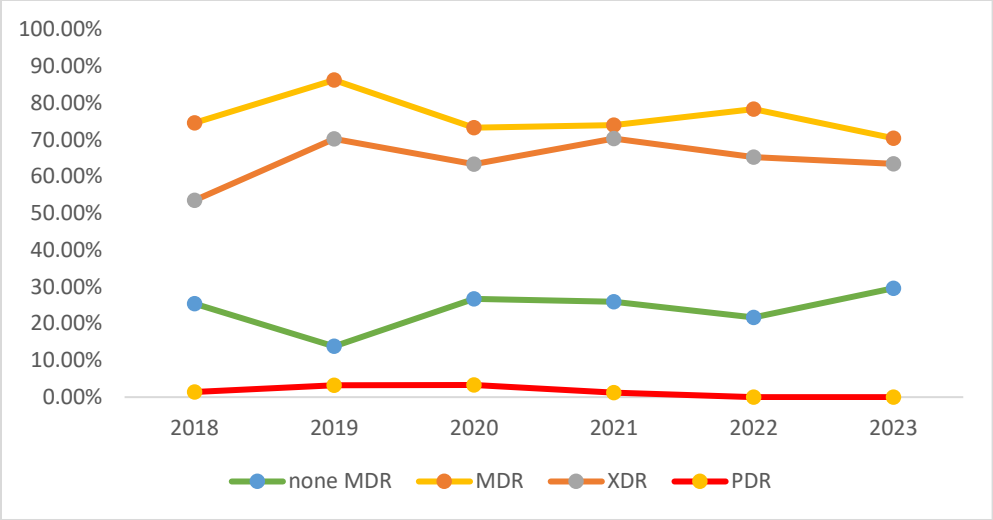


Figure 5: Overall AMR during study period

Table 1: Demographic and clinical characteristics of the participants (n = 242)

Variables		Frequency	Percent
Date	**2018**	39	16.1%
	2019	44	18.2%
	2020	27	11.1%
	2021	45	18.6%
	2022	43	17.8%
	2023	44	18.2%
Covid-19*	**Before**	88	36.4%
	After	154	63.6%
Length of hospitalization	**≤ 14 days**	103	42.6%
	> 14 days	139	57.4%
Age	**≤ 59 Y/O**	38	15.7%
	60-74 Y/O	55	22.7%
	≥ 75 Y/O	149	61.6%
Gender	**Male**	145	59.9%
	Female	97	40.1%
Smoker or substance abuse	**Yes**	42	17.4%
	No	200	82.6%
Diabetes Mellitus	**Yes**	61	25.2%
	No	181	74.8%
Immunodeficiency	**Yes**	58	24%
	No	184	76%

*Post-COVID-19 period: from February 2020 onward

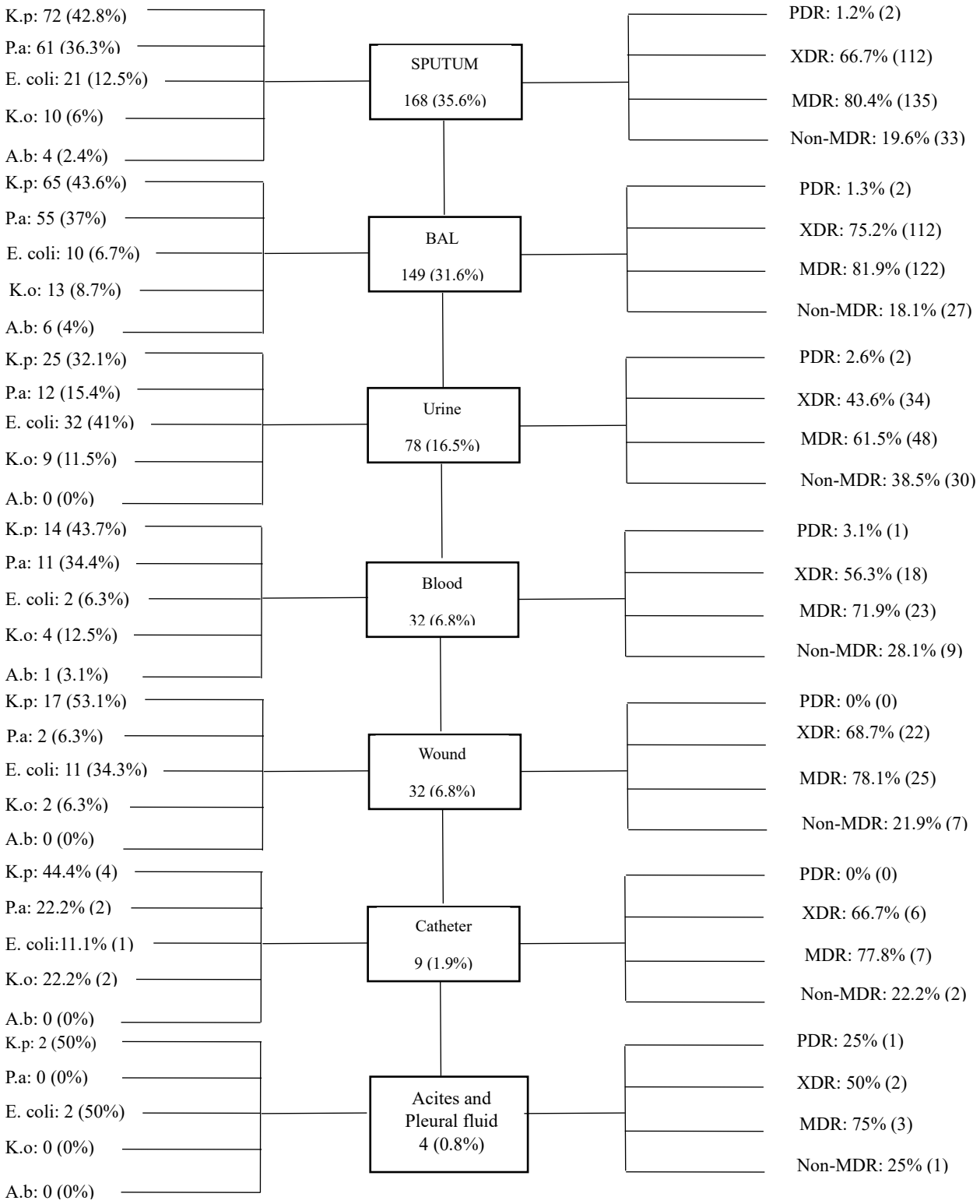


Table 2: Distribution of GNB strains and specimen sources along with AMR (n = 472)

Table 3: Patient-level AMR profile (n = 242)

Categories		Resistance (%)				P*	Adjusted OR*	95% Confidence Interval*
		PDR	XDR	MDR	Non-MDR			
Gender	Male	1.4	64.1	74.5	25.5	0.063	1.87	0.96 – 3.64
	Female	2.1	71.1	84.5	15.5			
Cigarettes and/or Substance abuse	Yes	2.4	71.4	78.6	21.4	0.551	1.33	0.52 – 3.43
	No	1.5	66	78.5	21.5			
Diabetes Mellitus	Yes	1.6	68.8	80.3	19.7	0.529	1.29	0.59 – 2.83
	No	1.7	66.3	77.9	22.1			
Immunodeficiency	Yes	0	62.1	75.9	24.1	0.932	1.05	0.37 – 2.97
	No	2.2	68.5	79.4	20.6			
Cause of hospitalization	Post-surgical	0	62.5	79.2	20.8	0.115	ref	ref
	Pneumonia	2.3	78.6	87.1	12.9	0.949	1.07	0.14 – 8.00
	Other respiratory dis.	7.1	78.6	92.9	7.1	0.480	0.43	0.04 – 4.50
	UTI	0	61.5	84.6	15.4	0.332	2.55	0.39 – 16.82
	Cancer	0	61.5	74.4	25.6	0.819	1.27	0.17 – 9.76
	Neurologic dis.	2.2	64.4	75.6	24.4	0.571	1.98	0.19 – 20.80
	Cardiovascular dis.	0	56.5	60.9	39.1	0.813	1.26	0.18 – 8.76
	GI dis.	0	57.1	71.4	28.6	0.252	4.96	0.32 – 76.58
	Others	0	42.9	57.1	42.9	0.400	0.43	0.06 – 3.12
Length of hospitalization		-	-	-	-	0.185	1.01	0.99 – 1.03
Age		-	-	-	-	0.658	1.01	0.98 – 1.03
Year	2018-2023	-	-	-	-	0.449	0.93	0.76 – 1.13

*calculated with logistic regression between MDR and non-MDR

Table 4: Overall AMR classification (n = 472)

ABs Class	Overall Resistance	Resistance	P.aeroginosa	K.pneumoniae	K.oxytoca	E.coli	A.baumannii
Ceftriaxone	385	R	114 (91.2%)	173 (92%)	28(84.8%)	59 (85.5%)	11 (100%)
	(90.3%)	S	11 (8.8%)	15 (8.0%)	5 (15.2%)	10 (14.5%)	0 (0%)
Ceftazidime	359	R	108 (77.1%)	163(84.9%)	28 (70%)	50 (65.8%)	10 (100%)
	(78.3%)	S	32 (22.9%)	29 (15.1%)	12 (30%)	26 (34.2%)	0 (0%)
Fluoroquinolones	368	R	110 (80.3%)	165(85.9%)	26(66.7%)	57 (73.1%)	10 (90.9%)
	(80.5%)	S	27 (19.7%)	27 (14.1%)	13(33.3%)	21 (26.9%)	1 (9.1%)
Aminoglycosides	275	R	85 (60.3%)	131(65.8%)	22 (55%)	27 (35.1%)	10 (90.9%)
	(58.7%)	S	56 (39.7%)	68 (34.2%)	18 (45%)	50 (64.9%)	1 (9.1%)
Trimethoprim-Sulfamethoxazole	274	R	90 (90%)	115(85.2%)	19(79.2%)	44 (74.6%)	6 (85.7%)
	(83%)	S	10 (10%)	20 (14.8%)	5 (20.8%)	15 (25.4%)	1 (14.3%)
Pipperacillin-Tazobactam	298	R	90 (72%)	144(80.9%)	23(65.7%)	30 (40.5%)	11 (100%)
	(70.4%)	S	35 (28%)	34(19.1%)	12(34.3%)	44 (59.5%)	0 (0%)
Carbapenems	328	R	114 (82%)	155(80.3%)	19 (50%)	30 (40%)	10 (100%)
	(79%)	S	25 (18%)	38 (19.7%)	19 (50%)	45 (60%)	0 (0%)
Colistin	13	R	2 (1.4%)	5 (2.5%)	3 (7.5%)	3 (3.8%)	0 (0%)
	(2.8%)	S	141 (98.6%)	194 (97.5%)	37 (92.5%)	76 (96.2%)	11 (100%)

Table 5. A brief list of the 2023 AWaRe classification

Access	Watch	Reserve
Amikacin	Azithromycin	Ceftazidime-avibactam
Amoxicillin	Cefepime	Ceftolozane-tazobactam
Amoxicillin-clavulanic acid	Cefixime	Colistin
Ampicillin	Cefotxime	Linezolid
Ampicillin-sulbactam	Ceftazidime	Tigecycline
Cefalexin	Cefteriaxone	
Cefazolin	Ceftizoxime	
Clindamycin	Cefuroxime	
Cloxacillin	Ciprofloxacin	
Doxycycline	Clarithromycin	
Gentamycin	Imipenem	
Metronidazole	Levofloxacin	
Nitrofurantoine	Meropenem	
Pennicillins	Moxifloxacin	
Sulfamethoxazole - trimethoprim	Piperacillin-tazobactam	
Tetracycline	Vancomycine	

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