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Infection control interventions against carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* in an Iranian referral university hospital: A quasi-experimental study

Mojtaba Akbari[1], Christian G. Giske[2], Malihe Alenaseri[3], Ahmad Zarei[3], Narjes Karimi[3] and Hamid Solgi[1,3*]

Abstract

Background In the past decades, the prevalence of carbapenem-resistant Gram-negative bacteria (CR-GNB) has increased on a global scale. Here, we outline the infection prevention and control (IPC) interventions for addressing the prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) and carbapenem-resistant *Klebsiella pneumoniae* (CRKP).

Methods A quasi-experimental study design was performed during the seven periods of 6-months from September 2018 to September 2021 in a large ICU in an Iranian hospital. IPC interventions were implemented from period 2 onward, with the exception of period 4 (March-September 2020) due to COVID-19 pandemic-related service disruptions. CRKP and CRAB prevalence and antibiotic resistance of GNB were compared across the seven periods.

Results In total, 1,862 GNB isolates were identified across seven periods, with *K. pneumoniae* (41%) being the most prevalent pathogen, followed by *Escherichia coli* (24.6%) and *A. baumannii* (14%). The highest antibiotic resistance rates, including 65.5% for meropenem, were observed during the fourth period. From September 2019 to March 2022, 178 CRKP and 97 CRAB isolates were identified, with infection rates of 78.1% and 62.9%, respectively. Following IPC interventions, a significant reduction in CRKP and CRAB prevalence was noted in the second and third periods, although an increase occurred during the fourth period. By the seventh period, the lowest prevalence of CRKP (26 isolates) and CRAB (5 isolates) was observed. Finally, a moderate inverse correlation (-0.571) was found between hand hygiene compliance and mortality incidence.

Conclusion The implementation of targeted IPC interventions significantly reduced the prevalence of CRKP and CRAB infections in the ICU, demonstrating their effectiveness in controlling antibiotic-resistant pathogens. However, the resurgence of these infections during the COVID-19 pandemic highlights the need for continuous monitoring and adaptation of IPC strategies. Ongoing training and adherence to hygiene protocols are essential to sustain improvements and prevent future outbreaks. Our findings underscore the importance of proactive infection control measures in managing antibiotic resistance in critical care settings.

*Correspondence:
Hamid Solgi
hamid.solgi@gmail.com
Full list of author information is available at the end of the article

Introduction

In recent years, carbapenem-resistant Gram-negative bacteria (CR-GNB) infections, particularly in intensive care units (ICUs), have become a major global health concern [1–4]. CR-GNB, namely, carbapenem-resistant Enterobacteriaceae (CRE), carbapenem-resistant and *Acinetobacter baumannii* (CRAB) have been highlighted as critical pathogens in the World Health Organization (WHO) prioritization of pathogens to guide research and development of new antibiotics. These pathogens were listed as "number one priority" for implementation of prevention and control measures in healthcare settings [5]. Infections caused by these pathogens are increasingly associated with higher morbidity, mortality and healthcare costs, mainly due to high levels of antimicrobial resistance, limited treatment options, additional infection-prevention and control (IPC) interventions, and prolonged length of hospital stay [1]. In the Europe and USA, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) are the main pathogens associated with healthcare-associated infections (HAIs) within ICUs [6–8]. In contrast, in Iran, the prevalence of CR-GNB greatly exceeds both MRSA and VRE, and the frequent expansion of CR-GNB poses considerable challenges to control HAIs, especially those occurring in ICUs [9–11]. The implementation of IPC measures for reducing in-hospital transmission of CR-GNB and of antimicrobial stewardship (AMS) programs can effectively reduce the incidence of HAIs caused by multidrug-resistant (MDR) pathogens [1, 12, 13].

Dissemination of the CR-GNB is facilitated by inadequate infection prevention and control practice in healthcare settings and uncontrolled or poorly controlled antimicrobial use. Patient-to-patient transmission and hand carriage by healthcare workers are the major modes of dissemination of these pathogens. Effective IPC measures—including contact precautions, hand hygiene, active surveillance, patient isolation, cohorting of patients, and environmental cleaning—are essential for reducing in-hospital transmission of CR-GNB. Similarly, AMS programs play a crucial role in controlling the spread of CR-GNB outbreaks [13, 14].

In our 370-bed teaching hospital, since 2018, by recruiting a bacteriology specialist, our hospital implemented IPC programs in a more active and targeted manner, with particular attention to the ICUs setting and AMS program.

The aims of this study were to evaluate the pattern of antibiotic resistance among CR-GNB in the hospital, the frequency of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and CRAB isolates in a large ICU during seven 6-month periods (September 2018–September 2021), assess the effect of collaborative IPC interventions to prevent the spread of these pathogens and examine clinical outcomes including the correlation between hand hygiene compliance (HHC) and mortality rates in a teaching hospital in Isfahan, Iran.

Methods

Settings

The quasi-experimental before – after study design was performed in Amin medical center,

a 370-bed teaching hospital in Isfahan, Iran. It is one of the largest health care centers in the north of Isfahan city. During the study period, the ICU comprised of 22 beds including four private rooms and 18 beds in a row with a distance of almost one meter (Fig. 1), with approximately 1100 adult surgical and medical admissions per year. During the study period, ICU and single-bed room types remained unchanged, while nursing and nursing assistant personnel changed and rotated throughout all periods, particularly during the fourth period with the start of the COVID-19 pandemic. The ICU cares for a mixed population of post-surgical patients and patients suffering from

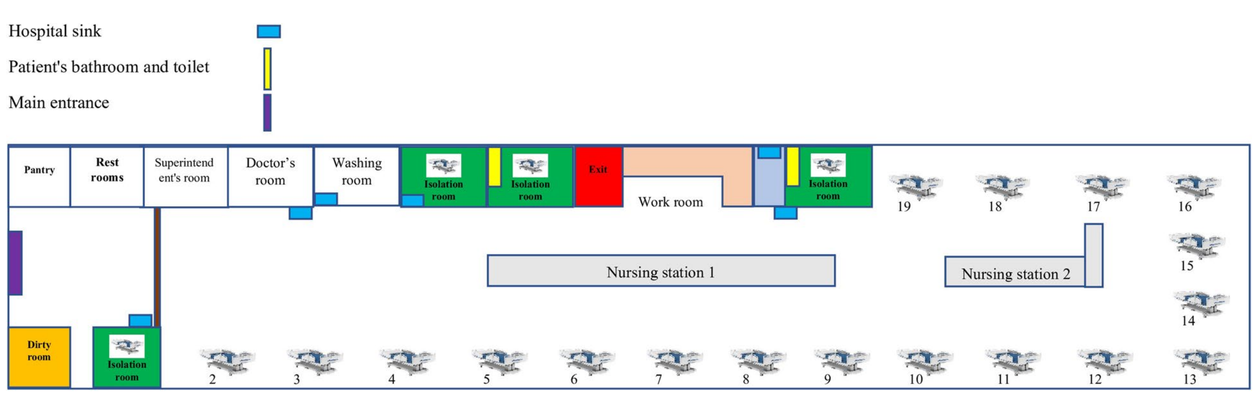

Fig. 1 Intensive care unit internal layout plan

life – threatening respiratory critical conditions as well as trauma and septic shock.

In this study, the frequency of CRKP and CRAB isolates in patients hospitalized in the ICU was investigated in seven periods of six months from 23 September 2018 to 20 March 2022, while the pattern of antibiotic resistance only for GNB was investigated for all the patients admitted to the hospital at the same time.

The IPC and AMS team, during period 1 (from 23rd of September till 20 th of March and prior to this period, consisted of an infectious disease specialist, an IPC nurse, a microbiologist with a master's degree, and the hospital head. Starting from the second 6-month period, a medical bacteriologist joined the team. Before period 2 (form 21rd of March 2019 till 22 th of September 2019), the IPC activity at our hospital was led by one IPC nurse subordinated to the head nurse office.

Bacterial isolation and antimicrobial susceptibility testing

Routine antibiotic susceptibility tests were performed by the disk-diffusion assay to identify carbapenem resistance; susceptibility breakpoints were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. The minimal inhibitory concentration (MIC) of colistin was determined by broth microdilution method using colistin sulfate (Sigma-Aldrich), and CLSI breakpoints were used for interpretation [16]. *E. coli* ATCC 25922 was used as quality control strain.

Active surveillance cultures (ASCs) from various samples (sputum, endotracheal aspirate, urinary tract, wound, and other possible infection sites) had been collected to monitor the frequency of CRKP and CRAB colonization/infection in the ICU. It is worth mentioning that the surveillance cultures were not done in the first and second periods. From the second 6-month period onwards, two infectious diseases and a medical bacteriologist specialist helped collect patients' clinical and microbiological data on colonization and/or CRKP infection. The microbiology laboratory information system was used to identify antibiotic resistance patterns in the hospital and to evaluate the frequency of CRKP and CRAB isolates among patients hospitalized in the ICU.

Infection control management

The IPC interventions implemented before and after the presence of a medical bacteriologist in our center and their timing are shown in Table 1. The study included all patients admitted to the ICU across all seven periods (September 2018-September 2021). During the first period, routine IPC programs were implemented with microbial cultures obtained only for clinically suspected CRKP infections. From the second period onward (except the fourth COVID-19 period), the enhanced IPC interventions shown in Table 1 were implemented, including routine surveillance cultures.

Hand Hygiene Compliance

Hand hygiene compliance (HHC) of ICU HCWs, including nurses and nurse's assistants, was assessed by direct observation using the WHO hand hygiene observation tool. We documented hand hygiene opportunities and actions according to the following five WHO indications: (1) before touching a patient; (2) before a clean/aseptic procedure; (3) after body fluid exposure risk; (4) after touching a patient, and (5) after touching patient's surroundings. For each infection control opportunity, the types of hand hygiene products available (alcohol dispenser, soap and water, or both) and hand hygiene behavior of the observed nurses and nurse's assistants (hand rubbing or hand washing) were recorded in a checklist. Overall, HHC was determined by dividing the number of observed hand hygiene practices performed by the total number of opportunities.

Definitions

CRKP and CRAB were defined as meropenem-or imipenem-nonsusceptible isolates according to the CLSI breakpoints. We classified the presence of CRKP and CRAB as infection or colonization, according to the clinical situation in which CR-GNB-positivity was identified. An infection was defined as follows: (i) CRKP and CRAB isolates were identified in a microbiologic specimen taken for diagnostic purposes, and (ii) the patient showed clinical signs and symptoms of infection, based on a combination of clinical and laboratory criteria. Colonization was defined as follows: (i) in the absence of signs and symptoms of infection (e.g., fever, leukocytosis, purulent secretions), and (ii) where no anti-CR-GNB antibiotics were used. The patient's medical team determined the categorization of infection or colonization. The prevalence of CRKP and CRAB infection/colonization was detected in ICU cultures.

Ethics

The study protocol received approval from the Ethical Committee of the Isfahan University of Medical Sciences (approval number IR.ARI.MUI.REC.1402.159). This study was conducted in accordance with the tenets of the Declaration of Helsinki.

Statistical analysis

The statistical analyses were done by SPSS version 25 statistical software package (SPSS Inc., Chicago, IL, USA). Data are reported as numbers (percentages), and descriptive statistics are presented in tables and figures.

Table 1 Implementation timeline and intensity of infection prevention and control (IPC) interventions across study periods

	Period-1 (23 September 2018 to 20 March 2019)	Period-2 (21 March 2019 to 22 September 2019)	Period-3 (23 September 2019 to 20 March 2020)	†Period-4 (21 March 2020 to 21 September 2020)	Period-5 (22 September 2020 to 19 March 2021)	Period- 6 (21 March 2021 to 22 September 2020)	Period- 7 (23 September 2021 to 20 March 2022)
Contact precautions (i.e. at least use of disposable gowns and gloves) education/monitoring	√	√	√	-	√	√	√√
Active surveillance using urine, wound and respiratory samples of high-risk patients on admission in ICU (colonization/infection)	-/√√	√√/√√	√√/√√	-/√	√√/√√	√√√/√√√	√√√/√√√
Patient isolation	√	√√	√√√	-	√√	√√√	√√√
Patient and staff cohorting	√	√√	√√√	-	√√√	√√√	√√√
Daily prevalence reporting to management	-	√√√	√√√	-	√√√	√√√	√√√
Immediate laboratory notification of cases	-	√√√	√√√	-	√√√	√√√	√√√
Healthcare worker education and adherence monitoring including a focus on hand hygiene	√√	√√	√√√	√	√√√	√√√	√√√
Healthcare worker education and adherence monitoring (on MDR organisms)	-	√	√√√	-	√√√	√√√	√√√
Distribution of guidelines for active CRO surveillance	-	√√√	√√√	-	√√√	√√√	√√√
Environmental cleaning education to nursing assistants		√	√√√		√√√	√√√	√√√
Daily Chlorhexidine gluconate mouth (three times)	-	√√√	√√√	-	√√√	√√√	√√√
Enhanced environmental cleaning/decontamination	-	-	-	-	√√√	√√√	√√√
Bedside cleaning with hypochlorite three time a day	√	√√	√√√	√	√√√	√√√	√√√

Table 1 (continued)

	Period-1 (23 September 2018 to 20 March 2019)	Period-2 (21 March 2019 to 22 September 2019)	Period-3 (23 September 2019 to 20 March 2020)	†Period-4 (21 March 2020 to 21 September 2020)	Period-5 (22 September 2020 to 19 March 2021)	Period- 6 (21 March 2021 to 22 September 2020)	Period- 7 (23 September 2021 to 20 March 2022)
Environmental surveillance cultures in the event of an outbreak	-	-	√√√	-	√√√	√√√	√√√
Enhanced antimicrobial stewardship and restriction of carbapenem use	-	√√	√√√	-	√√√	√√√	√√√
Audit and feedback (on hand hygiene, contact precautions, environmental cleaning)	√	√	√√	-	√√√	√√√	√√√
Encouraging personnel in the field of infection control by the management team	-	-	√√√	-	√√√	-	√√√
Designing special cards to mark patients with MDR bacteria	-	-	√√√	√√√	√√√	√√√	√√√
Sterile oral suction using sterile gas or sterile gloves	-	-	-	-	√√√	√√√	√√√
Cohort ward for positive cases with dedicated staff	-	-	√√√	-	√√√	√√√	√√√

Legend: -, Negative (No action to have been taken); √, Weak; √√, medium; √√√, strict.

MDR Multidrug-resistant bacteria

CRO Carbapenem-resistant organisms

† Period 4 coincided with peak COVID-19 pandemic conditions in our region

The Spearman correlation coefficient test was applied to examine the relationship between mortality incidence rates and HHC. A p-value of less than 0.05 was considered statistically significant.

Results

In the entire hospital, a total number of 346, 166, 315, 199, 233, 270 and 333 Gram-negative isolates were obtained from period 1 to 7, respectively. Overall, in our study among 1862 GNB, *K. pneumoniae* ($n=$ 765;41%) was the most common isolated pathogen followed by *E. coli* ($n=458$; 24.6%), *A. baumannii* complex ($n=$ 261;14%) and *P. aeruginosa* ($n=$ 107;5.7%). The sensitivity pattern to eight antibiotics for GNB is shown separately in each 6-month period in Fig. 2.

The highest rates of antibiotic resistance for all antibiotics were reported in the 4th period, which coincided with the start of the COVID-19 pandemic and hospitalization of patients with COVID-19 in the hospital. The highest rate of antibiotic resistance to meropenem was reported in this period, which was 65.5%. Also, the highest and lowest rates of colistin resistance were reported as 8.2 and 1.5% in the fourth and third periods, respectively.

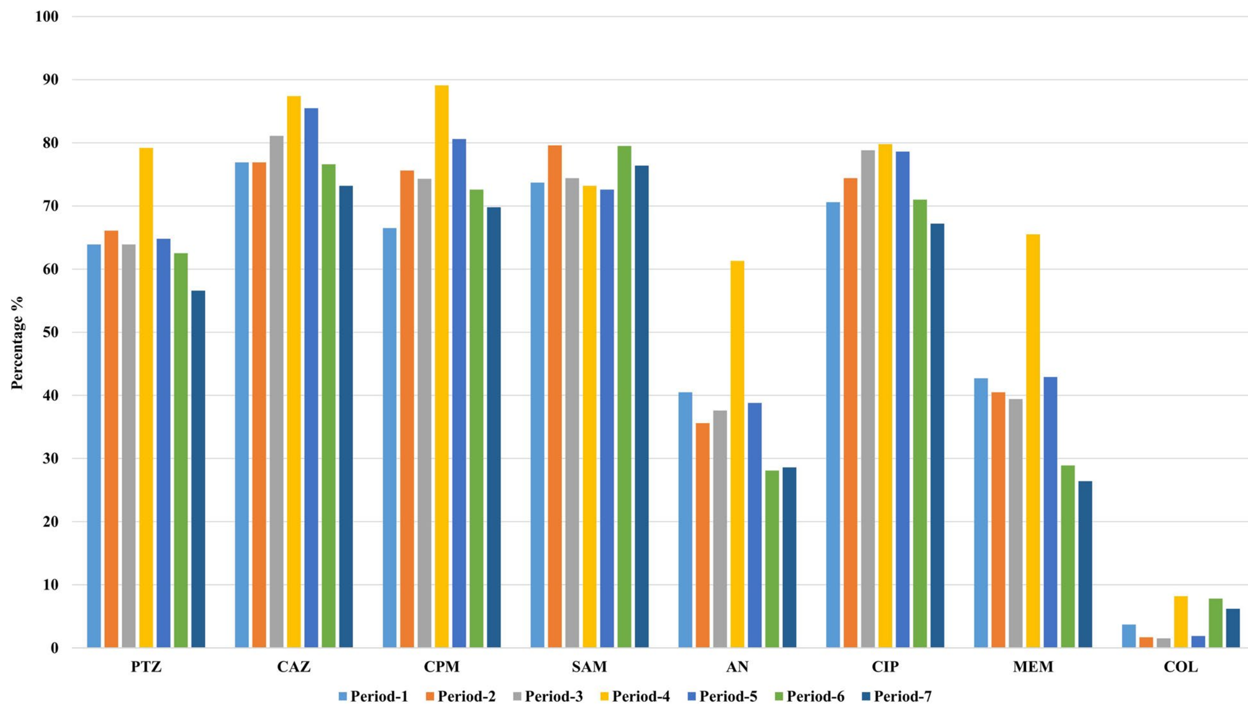

Fig. 2 Trend of antibiotic resistance pattern in gram-negative bacteria isolated from patients in hospital in seven time periods. PTZ, Piperacillin/tazobactam; CAZ, Ceftazidime; CPM, Cefepime; SAM, Ampicillin/sulbactam; AN, Amikacin; CIP, Ciprofloxacin; MEM, Meropenem; COL, Colistin. Note: Period 4 coincided with peak COVID-19 pandemic conditions in our region

Cases of CRKP and CRAB isolates before and after IPC interventions in ICU

Since the information about the infection and colonization of the first and second periods was not available, we examined the percentage of infection/colonization of CRKP and CRAB in general for patients hospitalized in the ICU from period three onwards. As shown in Fig. 3, we identified 178 CRKP and 97 CRAB-positive cases after admission to ICU, from 23 September 2019 to 20 March 2022. Among CRKP and CRAB-positive cases,

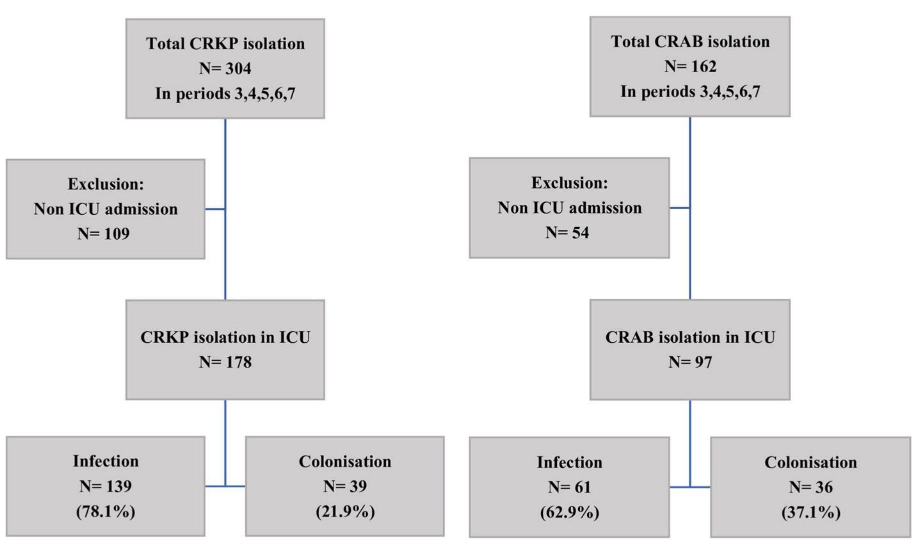

Fig. 3 Study design for CRAB and CRKP isolation among patients admitted to the intensive care units. CRAB, carbapenem-resistant *A. baumannii*; CAKP, carbapenem-resistant *K. pneumoniae*; ICU, intensive care unit

139 (78.1%) and 61 (62.9%) were classified as infection cases, whereas 39 (21.9%) and 36 (37.1%) cases were classified as CRKP and CRAB colonization, respectively.

Figure 4 shows the number of CRKP and CRAB cases in 6-month intervals. The peak prevalence of both cases was observed in the first period (23 September 2018 to 20 March 2019) before the start of infection control activities. After the modified IPC intervention, a significant reduction in the overall prevalence of CRKP and CRAB cases were observed in the second and third periods. As shown in Fig. 4, in fourth period with the outbreak of the COVID-19 pandemic and the allocation of the ICU to COVID-19 patients, as well as the reduction of IPC intervention, the prevalence of CRKP cases increased from 28 to 60 and CRAB from 17 to 44 cases.

From September 2020 until the end of follow-up in March 2022, consistently decreasing prevalence rates were observed and the lowest number of CRKP (26 cases) and CRAB (5 cases) infection/colonisation cases was reported in the seventh period Fig. 5.

During the fifth period and continuing until the end of the study, several IPC interventions were intensified, including sterile oral suction with sterile gas for all intubated and low-consciousness patients, and reinforced staff cohorting for nurses.

A hand hygiene campaign, consisting of training, observation, and feedback, was implemented. This intervention resulted in an increase in hand hygiene compliance from 36.5% in period 1 to 57% in period 3. Subsequently, a lack of frequent training for medical personnel, due to insufficient expert staff, contributed to a decrease in compliance to 23% in period 4. After this period, with weekly training and feedback on adherence results, their performance steadily improved, from 27% in the period 5 to 46% in the period 7.

The incidence rate of mortality and HHC for each period is presented in Table 2. The results of the Spearman's correlation coefficient indicate a moderate inverse relationship of −0.571 between the incidence rate of mortality and HHC, although this result is not statistically significant ($p = 0.180$).

Discussion

The dissemination of CR-GNB is a serious threat to public health. These isolates are usually extensively drug-resistant, resulting in limited antimicrobial treatment options and high mortality [14, 17]. Previous studies have shown that the implementation of an infection-prevention bundle and control interventions led to clinically important and statistically significant decreases in the prevalence of CR-GNB infection/colonization in some countries around the world [13, 18, 19]. To our knowledge, this is the first comprehensive IPC study that aimed at reducing the prevalence of CRKP and CRAB among patients hospitalized in ICU in Iran by enhanced AMS and infection control measures. The main interventions concerned intensified ASCs from various samples of patients, intensified rapid institution of contact isolation, healthcare worker education and adherence monitoring (on multidrug-resistant (MDR) organisms and enhanced awareness and compliance with hand hygiene), daily chlorhexidine mouthwash and environmental cleaning. The results of the study were consistent with previous studies that show that IPC interventions can reduce the colonization and/or infection of ICU-acquired CRKP and CRAB in an ICU ward [1, 2, 19].

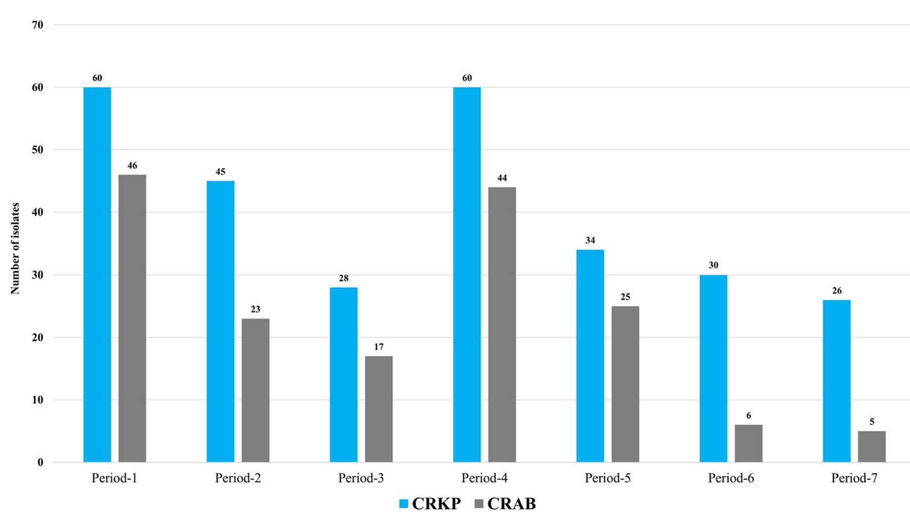

Fig. 4 Six-monthly data from September 2018 to March 2022 about number of carbapenem-resistant *A. baumannii* and carbapenem-resistant *K. pneumoniae*. Note: Period 4 coincided with peak COVID-19 pandemic conditions in our region

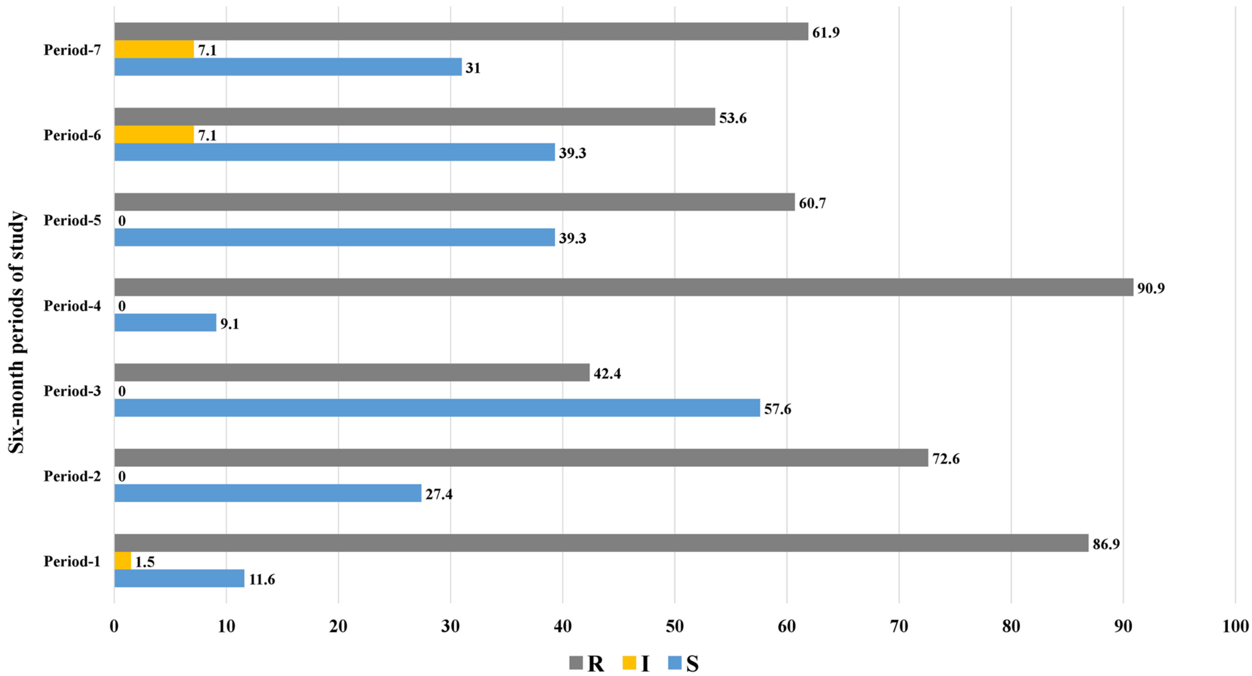

Fig. 5 Meropenem resistance rate in *K. pneumoniae* isolates obtained from the samples of ICU patients based on meropenem resistance by each period. S, sensitive; I, intermediate; R, resistant. Note: Period 4 coincided with peak COVID-19 pandemic conditions in our region

Table 2 ICU mortality rates and hand hygiene compliance before, during, and after COVID-19 pandemic periods

	ICU patient-days	Death (No.)	Incidence Rate (per 1000 ICU patient-days) (%)	Hand hygiene compliance (%)
Period-1 (23 September 2018 to 20 March 2019)	3594	117	32.56	36.5
Period-2 (21 March 2019 to 22 September 2019)	3769	86	22.82	50
Period-3 (23 September 2019 to 20March 2020)	3438	77	22.39	57
Period-4 (21 March 2020 to 21 September 2020)	2898	154	53.19	23
Period-5 (22 September 2020 to 19 March 2021)	3502	89	25.42	27
Period- 6 (21 March 2021 to 22 September 2020)	3777	155	41.06	47
Period- 7 (23 September 2021 to 20 March 2022)	3085	170	55.12	46

The results of the Spearman's correlation coefficient indicate a moderate inverse relationship of −0.571 between the incidence rate of mortality and hand hygiene compliance, although this result is not statistically significant ($p = 0.180$)

Decreasing CRKP and CRAB prevalence was observed only when all interventions were applied. The decrease in the prevalence of CRKP and CRAB was reported at the end of our study in period 7. Our study observed that the reduction in CRKP and CRAB prevalence (accounting for a substantial proportion of GNB isolates) coincided with decreased antibiotic resistance across all GNB, particularly for meropenem and amikacin by period 7. While this association may partly reflect the decline in CRKP (41% of isolates), the concurrent timing with full IPC implementation suggests these interventions likely contributed to the broader resistance trends. Similarly, a 4-year quasi-experimental study in China showed that IPC interventions reduce the prevalence of ICU-acquired CRKP colonization/infections [19].

It should be noted that, in the fourth period, which coincided with the beginning of the first wave of the COVID-19 pandemic in Iran, the ICU became a department dedicated exclusively to COVID-19 inpatients. As shown in Table 1, during this period most of the IPC measures were not implemented due to the following reasons: personnel fear of COVID-19, healthcare workers from other departments without work experience in the ICU setting started working in ICU, changing the job description of the infection control team, especially the two main members, the clinical bacteriologist and the infection control nurse, lack of personnel and lack of quality disinfectants which led to an increase in the prevalence of CRKP and CRAB colonizations/infections in the ICU. These results are similar to a study conducted in Italy that showed the incidence of carbapenem-resistant Enterobacterales acquisition increased from 5% on average in 2019 to 50% during the pandemic [20].

Also, several studies emphasize the role of early detection of CR-GNB carriers for infectious control purposes [14, 21, 22]. Accordingly, the IPC and antibiotic stewardship team instructed physicians to submit active surveillance cultures in a timely manner. Our study revealed that ASCs facilitated early identification of CRKP and CRAB-positive cases, enabling timely de-escalation interventions, including discontinuation of contact precautions and initiation of patient isolation.

In low- and middle-income countries, antimicrobial resistance is a looming crisis, and antibiotic stewardship and implementation of infection control programs are not well implemented in most resource-limited tertiary care centers [23]. Therefore, it is necessary to immediately implement appropriate policies by the Ministry of Health and Medical Education of these countries to control the spread of resistant strains and also to reduce antibiotic resistance in medical centers. In Iran, a lower–middle-income country in the Middle East, the spread of CR-GNB is rampant in healthcare settings. In the previous study [24], we have mentioned the main problems and challenges of the spread of these MDR organisms in medical centers in Iran.

During the entire study period except for the first and fourth periods, we implemented the strict AMS program with a focus on treating patients with colistin and carbapenem based on the patient's clinical and culture results. AMS programs especially de-escalation antimicrobial therapy have been shown to improve patient outcomes and decrease antimicrobial resistance [20]. To implement the AMS program, we implemented two important processes. First, sending microbial cultures from clinical samples based on the opinion of infectious disease specialists and a clinical bacteriologist to prevent empiric colistin use, and second, reading the files of patients who were prescribed broad-spectrum antibiotics for no reason in the stewardship meeting with the presence of various experts and Infection control team members.

It is noteworthy that during all seven peaks, due to the lack of ICU beds, it was not possible to close the ICU, and since daily bathing for inpatients in Iranian hospitals is the task of the nurse's assistant and due to paucity of this service group, daily bathing was not performed for ICU patients in our hospital.

In the present study, the highest and lowest levels of HHC were associated with period 3 (57%) and period 4 (23%), respectively. Therefore, the success of the intervention program carried out for ICU HCWs during periods when the IPC interventions were well implemented showed a significant improvement in the rate of HHC. (Table 2). This finding is in agreement with studies from Egypt and Saudi Arabia reporting improvement from 43–30.9% before intervention to 61.4–69.5% post-intervention [25, 26], and similar to the reported improvement post-intervention from 30.0% to 56.7% in Brazil [27].

Our study had a few limitations. Firstly, this was a single-center study; therefore, the results may not be generalizable to other institutions. Further multicenter, prospective studies are needed to confirm our results. Secondly, we were unable to obtain nasopharyngeal and rectal samples due to the costs and lack of established procedures for sample collection and culture. Thirdly, we were unable to assess interventions separately to decide which intervention was essential and which was the most effective.

Conclusions

In summary, comprehensive IPC interventions and ASP are essential tools for controlling the epidemic spread of CR-GNB colonization/infection and its unexpected association with decreased CRAB and CRKP prevalence at the ICU in our hospital. Our results demonstrate that a multifaceted hospital-wide intervention program could help reduce CR-GNB spread in wards where the possibility of infection is high, such as the ICU. The results of the present study suggest that despite all the deficiencies and problems that exist in the field of infection control in low- and middle-income countries, significant reductions in CR-GNB spread are achievable.

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Not applicable.

Authors' contributions

HS and MA conceived and designed the study. HS, MA, AZ and NK collected the data. MA, CGG, HS and MA interpreted the data and drafted the manuscript. HS supervised the work. All authors were involved in critical revision of the manuscript and approved the final version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to particpate
The study protocol received approval from the Ethical Committee of the Isfahan University of Medical Sciences (approval number IR.ARI.MUI.REC.1402.159).

Human ethics and consent to participate
Not applicable.

Consent for publication
Not applicable.

Permission to reproduce material from other sources
Not required.

Competing interests
The authors declare no competing interests.

Author details
[1]Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. [2]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden. [3]Amin Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.

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