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Carbapenem-resistant gram-negative bacterial prevention practice in nosocomial infection and molecular epidemiological characteristics in a pediatric intensive care unit

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ABSTRACT

Introduction: The increasing prevalence of carbapenem-resistant gram-negative bacilli infection has emerged as a substantial threat to human health.

Methodology: In January 2017, a screening program for carbapenem-resistant gram-negative bacilli colonization was performed in a pediatric intensive care unit (PICU). Subsequently, different strategies for carbapenem-resistant gram-negative bacilli cohorting and patient placements were introduced in January 2018.

Results: The increase in the single room isolation (type A) and the resettlement of the same area placement (type B) resulted in a significant decrease in the nosocomial infection rate from 2.57% (50/1945) in 2017 to 0.87% (15/1720) in 2021 ($P < 0.001$). Notably, the incidence of nosocomial carbapenem-resistant gram-negative bacilli infections decreased in 2019 ($P = 0.046$) and 2020 ($P = 0.041$) compared with that in the respective previous year. During 2019 and 2020, a statistically significant increasing trend of type A and type B placements was observed ($P < 0.05$, each), which may have contributed to the decline of carbapenem-resistant gram-negative bacilli infection. The primary carbapenemase genes identified in carbapenem-resistant isolates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* were $bla_{\text{KPC-2}}$ from sequence type 11 and $bla_{\text{OXA-23}}$ from sequence type 1712.

Conclusion: The integration of various placements for patients with carbapenem-resistant gram-negative bacilli infection with active screening has been demonstrated as an effective preventive strategy in the management of carbapenem-resistant gram-negative bacilli infection.

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1. Introduction

Carbapenem-resistant gram-negative bacilli, including carbapenem-resistant *Enterobacteriaceae*, carbapenem-resistant *Acinetobacter baumannii*, and carbapenem-resistant *Pseudomonas aeruginosa*, are an important contributor to infections acquired through healthcare settings. This poses an emerging threat to global health due to the limited treatment options, elevated mortality rates, high hospitalization costs, and increased challenges in nosocomial infection prevention and control [1]. In children, carbapenem-resistant gram-negative bacilli infections are primarily associated with healthcare and mainly affect critically ill children, particularly those in pediatric intensive care units (PICUs) [2].

The prevalence of carbapenem-resistant gram-negative bacilli infection varies across different regions in China and other countries [3,4]. Additionally, the prevalence has been changing in the Eastern Mediterranean Region over the past decade [3]. A recent survey conducted in 36 hospitals in Shandong Province, China, from 2019 to 2020 showed that the average detection rates of carbapenem-resistant gram-negative bacilli infection varied from 1.91% to 66.04% [4]. The prevalence of carbapenem-resistant gram-negative bacilli differs between pediatric and adult patients. In 2021, the prevalence of carbapenem-resistant *Klebsiella pneumoniae*, carbapenem-resistant *Acinetobacter baumannii*, and carbapenem-resistant *Pseudomonas aeruginosa* in a pediatric hospital in China was 14.8%, 30.7%, and 6.7%, respectively [5], which was comparatively lower than that in general hospitals, presented at 24.4%, 66.5%, and 23.0%, respectively [6]. However, the spread of carbapenem-resistant gram-negative bacilli continues to affect both adult and pediatric patients globally [5,6]. Compared with adults, fewer treatment options are available for children, leading to mortality rates possibly reaching up to 50%, which is still lower than that of adults [7]. Therefore, pediatric patients in tertiary hospitals in China face a significant disease burden, and targeted infection prevention and control interventions are essential under carbapenem-resistant gram-negative bacilli outbreaks and endemic conditions [8].

There is currently a controversy regarding the most effective prevention methods for carbapenem-resistant gram-negative bacilli cross-transmission, especially in resource-limited settings [9]. Due to the typical large-ward settings and relatively insufficient medical resources in China [10], the Chinese guidelines for preventing carbapenem-resistant gram-negative bacilli transmission [11] recommend colonization screening in high-risk departments and various types of isolation of carbapenem-resistant gram-negative bacilli-positive patients. However, relatively few studies have documented the effectiveness of these prevention strategies in reducing carbapenem-resistant gram-negative bacilli infection rates, among pediatric patients in PICUs, with high endemic rates of carbapenem-resistant gram-negative bacilli infections, especially carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa*.

Early detection of carbapenem-resistant gram-negative bacilli and the identification of its underlying carbapenemase gene responsible for inducing resistance are essential for selecting the appropriate antimicrobial therapy, thereby improving clinical outcomes and facilitating the implementation of effective infection control strategies [12]. This article presents a five-year retrospective study of carbapenem-resistant gram-negative bacilli infections with a focus on pediatric patients in the PICU in Shanghai, China. The study includes molecular characteristics of carbapenem-resistant gram-negative bacilli and infection prevention and control strategies, including hand hygiene, contact precautions, and environmental cleaning and disinfection qualification. In addition, the article particularly emphasizes colonization screening and various patient placements against the spread of carbapenem-resistant gram--negative bacilli.

2. Methodology

2.1. Study design and intervention

This retrospective single-center-study was conducted at the Children's Hospital of Fudan University, a tertiary-care teaching hospital with 800 beds. The PICU ward was expanded in March 2019, and the number of single rooms increased from 5 to 12. The remaining patients were accommodated in an open space with a nurse/patient ratio of 1:2.5. As a measure to reduce carbapenem-resistant gram-negative bacilli infections, the screening program of carbapenem-resistant gram-negative bacilli colonization in the intestinal and upper respiratory tracts was performed using pharyngeal and rectal swab cultures in the PICU in 2017. In addition, appropriate placements of carbapenem-resistant gram-negative bacilli-positive patients had been performed since January 2018. Other basic infection prevention bundle measures have been implemented before 2017.

2.2. Basic infection prevention bundle measures

Basic infection prevention bundle measures, such as hand hygiene, contact precautions, environmental cleaning and disinfection, and staff education, had been implemented before 2017. Contact precautions were implemented, including hand hygiene, use of gowns and gloves, along with patient and staff cohorting (for patients with carbapenem-resistant gram-negative bacilli, medical staff were fixed, including the doctors and nurses) upon detection of carbapenem-resistant gram-negative bacilli colonization or infection in a patient. Hand hygiene practice was monitored by collecting data on adherence rates. Similarly, the data regarding environmental cleaning and disinfection qualification rates were collected to monitor environmental hygiene practices. Hospital-associated infections were defined according to the guidelines for the prevention and management of nosocomial infections [13]. The respective guidelines issued by the World Health Organization (WHO) were used to evaluate colonization [14].

2.3. Active surveillance program

An active screening program to detect carbapenem-resistant gram-negative bacilli infections in the intestinal and upper respiratory tracts was conducted in the PICU at the beginning of 2017, which involved basic infection control measures using pharyngeal and rectal swabs. The known carriers were repeatedly screened multiple times. All patients found to be infected or colonized by carbapenem-resistant gram-negative bacilli were placed under contact precautions and patient and staff cohorting.

2.4. Appropriate patient placement program

Placements of carbapenem-resistant gram-negative bacilli-positive patients were performed in January 2018 as a measure of reinforcing management. Given the limited ward facilities, three placement types were implemented based on the situation of the PICU: 'single room placement' (type A) with one patient per room, 'same area placement' (type B) for patients with the same carbapenem-resistant gram-negative bacilli placed in the same area of a common room with partition barriers, and no cohort placement (type C) for patients without carbapenem-resistant gram-negative bacilli or with different carbapenem-resistant gram-negative bacilli placed in the same room without any cohort placement using curtains between the beds. The infection control nurses used a check sheet to record various measures, including contact isolation, medical staff cohort administration, and bundle measures such as hand hygiene, environmental cleaning, and disinfection, use of personal protective equipment, and education.

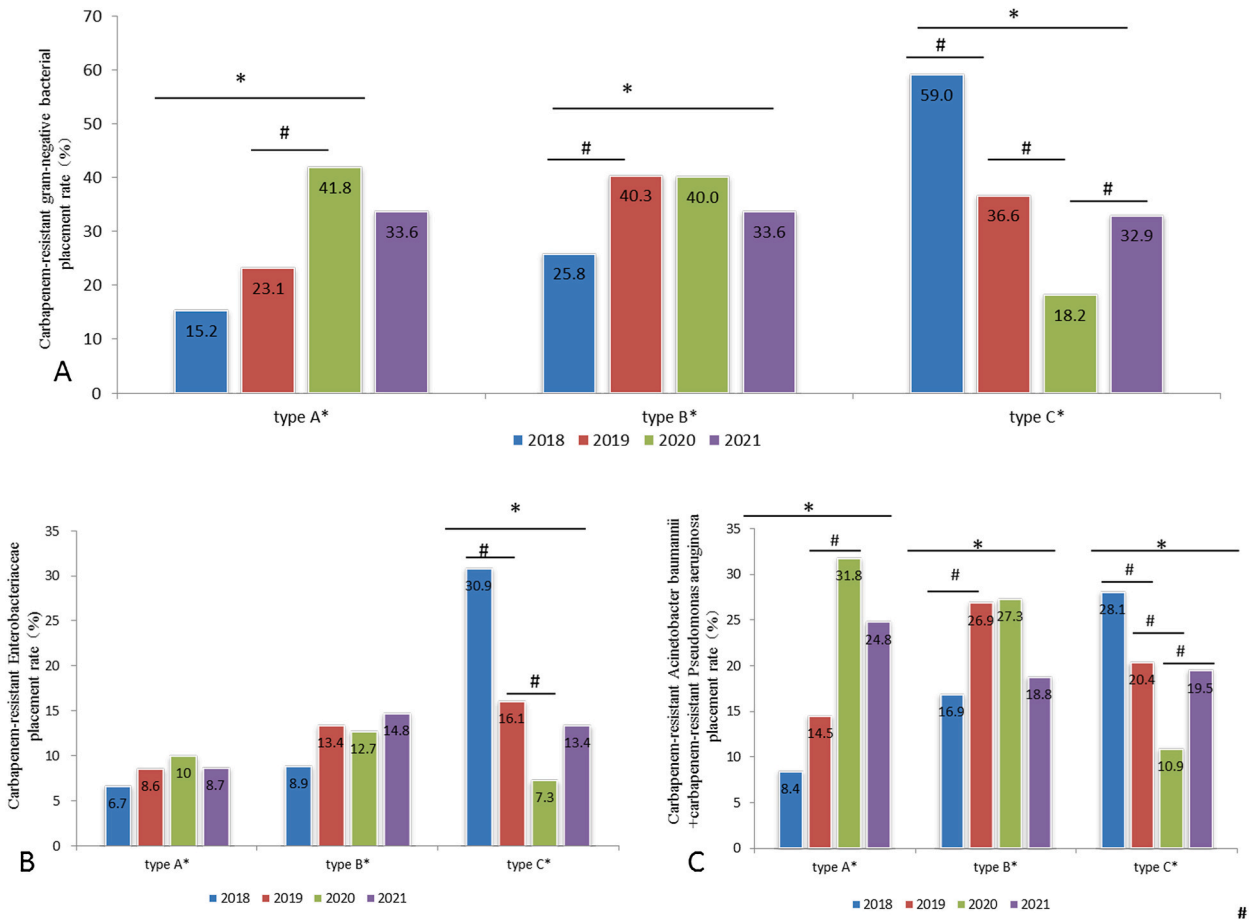

Fig. 1. Patient placement rate (%)
The rates of type A and B resettlement in carbapenem-resistant gram-negative bacilli-positive patients and those in carbapenem-resistant *Acinetobacter baumannii*- and carbapenem-resistant *Pseudomonas aeruginosa*-positive patients showed an upward trend from 2018 to 2020.
'Single room placement' (type A); 'same area placement' (type B); no cohort placement (type C).
*$P < 0.05$, comparison between years.
#$P < 0.05$, comparison with the previous year.

2.5. Microbiological methods

Routine surveillance of carbapenem-resistant gram-negative bacilli infections was conducted using culture and antimicrobial susceptibility tests. Strains were identified by MALDI-TOF biotyper mass spectrometry (Bruker, Germany). For carbapenem-resistant *Acinetobacter baumannii* strains, further confirmation is carried out to detect bla_{OXA-51} using molecular methods. Antimicrobial susceptibility tests were performed using automated Vitek2 compact system, using AST GN13 cards (bioMe irieux) for carbapenem-resistant *Enterobacteriaceae*, AST GN335 cards (biome irieux) for carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa*. For the antimicrobials that weren't covered in AST GN13 card including cefuroxime, cefoperazone/sulbactam and meropenem, we additionally performed KirbyeBauer test to get their susceptibility.

The standard enzyme-producing strains *Escherichia coli* ATCC25922 and *Escherichia coli* ATCC35218 were used as quality control strains for antimicrobial susceptibility tests. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains for drug sensitivity tests based on the disk diffusion method. The clinical information of carbapenem-resistant gram-negative bacilli-positive patients was systematically reviewed based on electronic medical records.

2.6. Molecular detection of resistance genes and multilocus sequence typing

Carbapenemase genes (class A carbapenemase: bla_{KPC}, bla_{GES}, bla_{SME}, and bla_{IMI}; class B carbapenemase: bla_{NDM}, bla_{GIM}, bla_{BIC}, bla_{SIM}, bla_{DIM}, bla_{IMP}, bla_{SPM}, bla_{AIM}, bla_{VIM}, and bla_{DHA}; class D carbapenemase: bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, and $bla_{OXA-143}$) were assessed through polymerase chain reaction based on the methods described previously [15,16]. Multilocus sequence typing analysis was performed according to the standard protocols provided on multilocus sequence typing Pasteur (https://bigsdb.pasteur.fr/index.html) and multilocus sequence typing PubMed website (https://pubmlst.org/). Clonal complexes were defined as

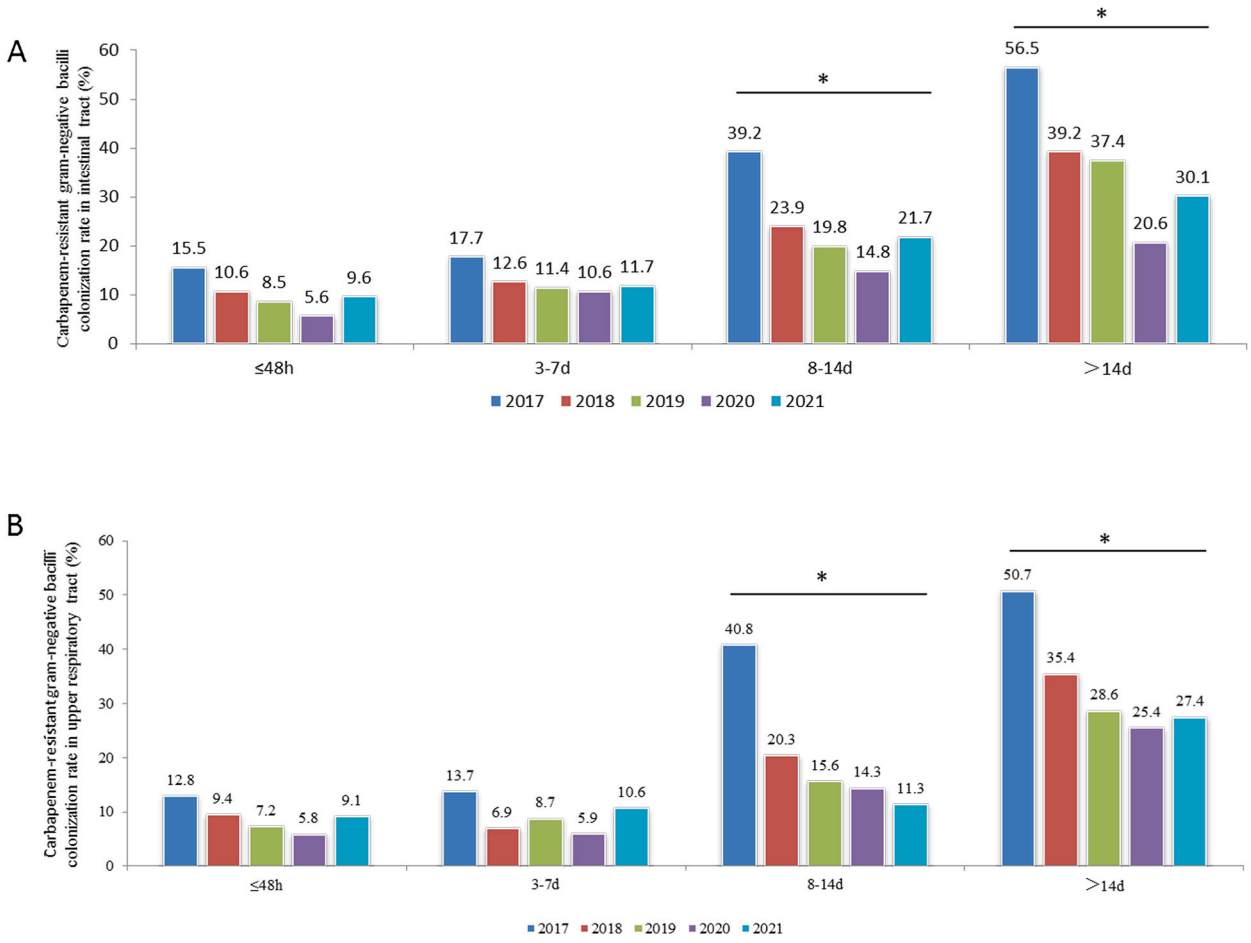

Fig. 2. Carbapenem-resistant gram-negative bacilli colonization incidence (%)
The incidence of carbapenem-resistant gram-negative bacilli colonization in the intestinal tracts (A) and the upper respiratory tracts (B) showed a decreasing trend after seven days of hospitalization from 2017 to 2021 ($P < 0.001$).
*$P < 0.05$, comparison between years.

groups of isolates that differ from each other by one or two alleles.

2.7. Statistical analyses

Categorical variables were presented as rates. Comparisons of categorical variables between groups were performed using Chi-squared tests. All statistical analyses were performed using SPSS 21.0 software (IBM, Armonk, NY, USA), with statistical significance reported at $p < 0.05$.

2.8. Ethics statement

The requirement of obtaining informed consent from patients was waived as only bacterial isolates recovered from routine laboratory tests were assessed. The study was approved by the Ethics Committee of the Children's Hospital of Fudan University, Shanghai, China, with the approval number (2021)372.

3. Results

3.1. Carbapenem-resistant gram-negative bacilli-positive patients and various placements

In total, 623 carbapenem-resistant gram-negative bacilli patients underwent various patient placements between 2018 and 2021: 26.6% (166/623) and 34.5% (215/623) of the carbapenem-resistant gram-negative bacilli-positive patients were isolated using type A and type B placements, respectively. The rates of type A and B resettlement in carbapenem-resistant gram-negative bacilli-positive patients ($P < 0.05$; Fig. 1A) and those in carbapenem-resistant *Acinetobacter baumannii*- and carbapenem-resistant *Pseudomonas aeruginosa*-positive patients showed an upward trend from 2018 to 2020 ($P < 0.05$; Fig. 1B and C).

The incidence of type A placement increased in 2020 compared to that in 2019 ($P = 0.001$), while that of type B increased in 2019 compared to that in 2018 ($P = 0.003$). In contrast, the incidence of type C decreased in 2019 and 2020 compared to that in 2018 and 2019 ($P < 0.001$ and $P = 0.001$, respectively), while it increased in 2021 compared to that in 2020 ($P = 0.008$). Furthermore, the patients positive for carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa* were mainly placed in type A or type B, and their resettlement pattern was consistent with that of carbapenem-resistant gram-negative bacilli-positive patients (Fig. 1B and C).

No significant changes were found from 2017 to 2021 in the hand hygiene compliance rates: 93.3% (126/135), 93.6% (335/358), 96.2% (457/475), 96.8% (390/403), and 95.8% (391/408) ($P = 0.150$). Additionally, the rates of environmental cleaning and disinfection qualification were 98.4% (60/61), 98.2% (325/331), 99.8% (485/486), 99.7% (394/395), 99.1% (213/215) ($P = 0.058$).

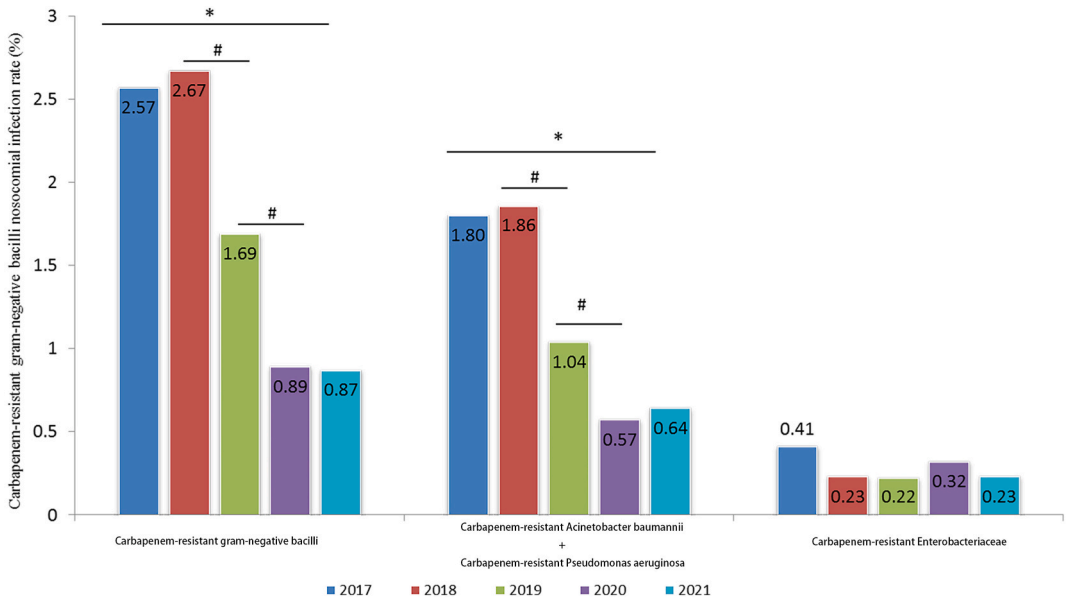

Fig. 3. Nosocomial carbapenem-resistant gram-negative bacilli infection incidence (%)
The incidence of nosocomial carbapenem-resistant gram-negative bacilli infections decreased in 2020 and 2019 compared to that in the respective previous year ($P = 0.041$ and $P = 0.046$, respectively). Similar trends also found in carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa*; however, the decrease in carbapenem-resistant *Enterobacteriaceae* was not statistically significant.
*$P < 0.05$, comparison between years.
#$P < 0.05$, comparison with the previous year.

3.2. Carbapenem-resistant gram-negative bacilli colonization incidences in the PICU

The incidence of carbapenem-resistant gram-negative bacilli colonization was 5.6%–15.5% and 5.8%–12.8% within 48 h in the intestinal and upper respiratory tracts, respectively. After hospitalization for more than two weeks, the incidence increased to 20.6%–56.5% and 25.4%–50.7%, respectively ($P < 0.001$; Fig. 2A and B).

The incidence of carbapenem-resistant gram-negative bacilli colonization in both the upper respiratory and intestinal tracts showed a significant decrease after seven days of hospitalization from 2017 to 2021, with the highest incidence in 2017 and the lowest in 2020 ($P < 0.001$; Fig. 2A and B).

3.3. Incidence of nosocomial carbapenem-resistant gram-negative bacilli infections

In total, 138 patients, including 20 with carbapenem-resistant *Enterobacteriaceae*, 77 with carbapenem-resistant *Acinetobacter baumannii*, and 41 with carbapenem-resistant *Pseudomonas aeruginosa*, developed nosocomial carbapenem-resistant gram-negative bacilli infections. The yearly incidence screening for nosocomial carbapenem-resistant gram-negative bacilli infections revealed a consistent decline from 2.57% in 2017 to 0.87% in 2021 ($P < 0.001$; Fig. 3).

The incidence of nosocomial carbapenem-resistant gram-negative bacilli infections decreased in 2020 and 2019 compared to that in the respective previous year ($P = 0.041$ and $P = 0.046$, respectively). In addition, the nosocomial infection rates of carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa* also decreased significantly in 2020 compared with those in 2019 ($P = 0.048$) and in 2019 compared with those in 2018 ($P = 0.040$); however, the decrease in carbapenem-resistant *Enterobacteriaceae* was not statistically significant ($P = 0.165$; Fig. 3).

3.4. Microbiological data

Carbapenem-resistant *Klebsiella pneumoniae* and carbapenem-resistant *Acinetobacter baumannii* strains were resistant to almost all first- and third-generation cephalosporins and enzyme inhibitors, while the carbapenem-resistant *Pseudomonas aeruginosa* resistance was comparatively low, reaching up to 60%. Except for levofloxacin, carbapenem-resistant *Acinetobacter baumannii* showed high resistance to aminoglycosides and quinolones (>85%), followed by carbapenem-resistant *Klebsiella pneumoniae*, while carbapenem-resistant *Pseudomonas aeruginosa* maintained high sensitivity. The rates of resistance to cefoperazone/sulbactam varied across the different carbapenem-resistant gram-negative bacilli strains, with carbapenem-resistant *Klebsiella pneumoniae* strains showing the highest resistance (Table 1). Carbapenem resistance was observed in more than 90% of all carbapenem-resistant *Klebsiella pneumoniae*, carbapenem-resistant *Acinetobacter baumannii*, and carbapenem-resistant *Pseudomonas aeruginosa* strains (Table 1).

Non-repetitive isolates from non-repetitive patients (35 carbapenem-resistant *Klebsiella pneumoniae*, 35 carbapenem-resistant *Acinetobacter baumannii*, and 35 carbapenem-resistant *Pseudomonas aeruginosa*) were randomly selected for carbapenem resistance gene typing and multilocus sequence typing analysis. Three types of resistance genes were identified in carbapenem-resistant *Klebsiella pneumoniae* isolates, of which $bla_{\text{KPC-2}}$ (80.0%) was the most common, followed by $bla_{\text{NDM-1}}$ (22.9%) and $bla_{\text{DHA-1}}$ (5.7%). Ten sequence types were found in carbapenem-resistant *Klebsiella pneumoniae* isolates, of which sequence type 11 (60.0%) was the most common, followed by sequence type 1883 (8.6%), 883 (5.7%), 15 (5.7%), 1640 (5.7%), and sequence type 883 (5.7%). Additional sequence types included sequence type 290, 304, 495, 846, and sequence type 1322, which occurred in only one isolate (Fig. 4A). Sequence type

Table 1
Drug resistance rate of carbapenem-resistant gram-negative bacilli (%).

Antibiotic name	Carbapenem-resistant *Klebsiella pneumoniae* (n = 82)	Carbapenem-resistant Acinetobacter baumannii (n = 328)	Carbapenem-resistant *Pseudomonas aeruginosa* (n = 166)
Cefoperazone/Sulbactam	93.2	62.8	51.5
Ampicillin/Sulbactam	100	97.6	–
Piperacillin/Tazobactam	86.4	100	53
Ceftazidime	100	100	53.9
Cefepime	95.3	100	59.6
Imipenem	93.2	100	98.8
Meropenem	95.5	99.2	95.1
Ertapenem	100	–	–
Amikacin	61.4	92	49.4
Gentamicin	79.5	85.9	25.9
Ciprofloxacin	86.7	98.7	51.2
Levofloxacin	77.3	25.4	8.3
Trimethoprim/ Sulfamethoxazole	38.6	73.4	–
*Nitrofurantoin	85.7	–	–
Minocycline	–	13.8	97.8
Piperacillin	–	100	54.6

The resistance rates of carbapenem-resistant *Klebsiella pneumoniae* isolates were 100% for cefazolin, cefuroxime, ceftriaxone, cefotaxime, cefoxitin, cefotetan, and aztreonam. The resistance rates were 77.3% and 73.3% for cefmetazoleand and tobramycin, respectively.
-, not available. * Results of urinary tract isolates only.

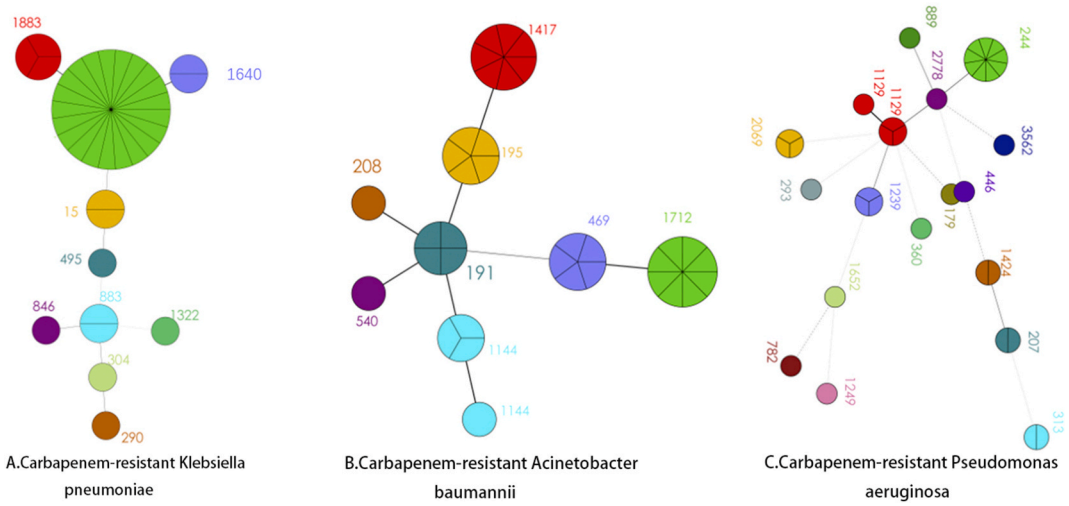

Fig. 4. Minimum spanning tree of carbapenem-resistant *Klebsiella pneumoniae*, carbapenem-resistant Acinetobacter baumannii, and carbapenem-resistant *Pseudomonas aeruginosa* isolates

Each solid circle denotes one sequence type, and the area of the circle is proportional to the number of isolates. The lines connecting the circles indicate the relationships between different STs. Different types of lines represent differences in the number of alleles: one allele (solid lines), two alleles (dashed lines), and three or more alleles (dotted lines). The three identified strains are carbapenem-resistant *Klebsiella pneumoniae* (A), carbapenem-resistant *Acinetobacter baumannii* (B), and carbapenem-resistant *Pseudomonas aeruginosa* (C). Ten, eight and seventeen sequence types were found in carbapenem-resistant *Klebsiella pneumonia,* carbapenem-resistant *Acinetobacter baumannii and* carbapenem-resistant *Pseudomonas aeruginosa* isolates, respectively, of which sequence type 11, 1712 and 244 were the most common in the three identified strains each. Sequence type 11, 1883, and 1640 belonged to clonal complexes 11 in carbapenem-resistant *Klebsiella pneumonia*. All sequence types of carbapenem-resistant *Acinetobacter baumannii* belonged to clonal complexes 92.

11, 1883, and sequence type 1640 differed by one housekeeping gene (rPOB) and belonged to clonal complexes 11 (26, 74.3%). All clonal complexes 11 isolates expressed $bla_{\text{KPC-2}}$. Two isolates belonging to clonal complexes 11 expressed both $bla_{\text{KPC-2}}$ and $bla_{\text{NDM-1}}$, and two isolates belonging to sequence type 15 expressed $bla_{\text{KPC-2}}$ and $bla_{\text{DHA-1}}$. All other isolates expressed $bla_{\text{NDM-1}}$.

$Bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ were detected in all 35 carbapenem-resistant *Acinetobacter baumannii* isolates. Eight sequence types were found in carbapenem-resistant *Acinetobacter baumannii* isolates, of which sequence type 1712 (22.9%) was the most common, followed by sequence type 1417 (20.0%). All sequence types of carbapenem-resistant *Acinetobacter baumannii* belonged to clonal complexes 92 (Fig. 4B). Seventeen sequence types were found in carbapenem-resistant *Pseudomonas aeruginosa* isolates, of which sequence type 244 (25.7%) was the most common, followed by sequence type 1129 (11.4%) (Fig. 4C). Only two $bla_{\text{VIM-2}}$ genes belonging to sequence type 179 were found in the carbapenem-resistant *Pseudomonas aeruginosa* isolates.

Further sequence types are shown in Fig. 4.

4. Discussion

The present study found that active screening and appropriate patient placement intervention measures effectively reduced carbapenem-resistant gram-negative bacilli colonization and nosocomial infections. The predominant carbapenemase genes were $bla_{\text{KPC-2}}$ in carbapenem-resistant *Klebsiella pneumoniae* isolates belonging to sequence type 11 and $bla_{\text{OXA-23}}$ in carbapenem-resistant *Acinetobacter baumannii* isolates belonging to sequence type 1712. Only two $bla_{\text{VIM-2}}$ genes were identified in carbapenem-resistant *Pseudomonas aeruginosa* isolates belonging to sequence type 179. The resistance genes and molecular typing results of carbapenem-resistant gram-negative bacilli were similar to those of adults, suggesting the presence of serious challenges.

Infection prevention and control strategies for carbapenem-resistant gram-negative bacilli highlighted in the literature emphasize the importance of multifaceted approaches and timely interventions [17–19]. The WHO recommends screening, single room isolation, and cohort administration in high-risk departments for early detection of carbapenem-resistant gram-negative bacilli [14]. The gastrointestinal tract, particularly rectal swabs, is the most commonly used site for screening carriage [16]. This type of screening is typically focused on carbapenem-resistant *Enterobacteriaceae* active screening in high-risk departments [20]. In addition to carbapenem-resistant *Enterobacteriaceae* screening, we also conducted active screening of carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa* in the upper respiratory tract and intestine in the current study. Our findings indicated that the overall incidence of carbapenem-resistant gram-negative bacilli colonization significantly increased with prolonged hospitalization, which suggested that the hospital environment may increase the risk of colonization. Following the implementation of patient resettlement measures, the carbapenem-resistant gram-negative bacilli colonization incidence showed a downward trend after 8–14 days of hospitalization, reaching a minimum in 2020. However, no cohort placements or any other types of isolation for carbapenem-resistant gram-negative bacilli-positive patients are commonly implemented in China. In the present study, we found that

the incidence of nosocomial infection of carbapenem-resistant gram-negative bacilli decreased significantly in 2019 and 2020 compared to the respective previous year, only when there was a statistically significant increase in the adoption of single room isolation (type A) and the same area placement (type B) in the same period. Thus, in addition to the most commonly used single-room isolation, the combination of active screening and placement of patients in the same area (type B) is also an effective strategy to prevent nosocomial carbapenem-resistant gram-negative bacilli infections in PICUs. In the PICU of our hospital, carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa* were responsible for the dominant nosocomial carbapenem-resistant gram-negative bacilli infections. Although the infection rates of carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa* decreased significantly, the decrease in carbapenem-resistant *Enterobacteriaceae* was not as pronounced. This finding contrasts with the results of a previous study in a PICU, where the enhanced active surveillance and infection control measures reduced carbapenem-resistant *Klebsiella pneumoniae* infections but not the carbapenem-resistant *Pseudomonas aeruginosa* and carbapenem-resistant *Acinetobacter baumannii* infections, which are inherently more resistant to interventions [21]. Another study also found that active surveillance combined with enhanced infection control measures reduced carbapenem-resistant *Klebsiella pneumoniae* and carbapenem-resistant *Pseudomonas aeruginosa* infection, but conversely led to an increase in carbapenem-resistant *Acinetobacter baumannii* infection incidence [22]. Therefore, according to the epidemic characteristics of pathogens in different regions, it is advisable to conduct pathogen screening targeting different key pathogens. This, combined with infection control measures, could effectively diminish the prevalence of carbapenem-resistant gram-negative bacilli infections in endemic healthcare settings. We conclude that except for the type A contact isolation, type B isolation in PICUs can also effectively block carbapenem-resistant gram-negative bacilli transmission. In resource-limited settings, especially in developing countries such as China with high endemic rates of carbapenem-resistant gram-negative bacilli infection, type B resettlement may be a feasible option when the availability of single rooms is limited.

Investigating the molecular characteristics of carbapenem-resistant gram-negative bacilli strains can provide vital insights into preventing carbapenem-resistant gram-negative bacilli cross-transmission. Carbapenemases are classified into Ambler classes A, B, or D, with class A and D enzymes possessing a serine-based hydrolytic mechanism, and class B enzymes requiring one or two zinc ions for catalytic activity [23]. The class A *Klebsiella pneumoniae* carbapenemase (KPC) has been frequently observed in *Klebsiella pneumoniae*, class B β-lactamases in *Enterobacteriaceae* and *Pseudomonas aeruginosa*, and acquired class D carbapenem-hydrolyzing β-lactamases are commonly reported in *Acinetobacter. Baumannii* [12]. Carbapenem-resistant *Klebsiella pneumoniae* is the most frequently isolated carbapenem-resistant *Enterobacteriaceae*. Unlike the globally common clone sequence type 258, the spread of carbapenem-resistant *Klebsiella pneumoniae* is primarily attributed to organisms producing $bla_{\text{KPC-2}}$, with the majority produced by the sequence type 11 clone in adults and older children in China [15,24]. Previous studies found that the predominant carbapenemase gene was $bla_{\text{NDM-1}}$ in neonatal carbapenem-resistant *Klebsiella pneumoniae* strains and $bla_{\text{KPC-2}}$ in non-neonatal carbapenem-resistant *Klebsiella pneumoniae* strains [15,25]. The results of the present study further confirmed the predominance of sequence type 11 strains expressing KPC-2 in PICU patients. Compared to other carbapenem genes, bla_{KPC} shows more potent virulence and transmission capabilities, which have resulted in numerous hospital outbreaks (often linked with KPN carrying $bla_{\text{KPC-2}}$) [24,26].

Carbapenem-resistant *Acinetobacter baumannii* causes severe nosocomial infections, thus potentially affecting the outcome of hospitalization outcomes, especially in critically ill patients [27]. Further, it causes outbreaks in pediatric and neonatal units [28], and it is ranked first among gram-negative bacteria on the WHO list of antibiotic-resistant bacteria [29]. Resistance to carbapenems is highly prevalent among *A. baumannii* strains, with the class D OXA-type carbapenemases, like $bla_{\text{OXA-23,}}$ being the most prevalent among carbapenem-resistant *Acinetobacter baumannii* isolates [30,31]. In Acinetobacter, carbapenemases of class D OXA-type may be intrinsic ($bla_{\text{OXA-51-like}}$) or acquired ($bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$). Furthermore, carbapenem-resistant *Acinetobacter baumannii* strains harboring OXA-23 and OXA-58 carbapenemases were reported to simultaneously predominate in a single ward of a PICU [32], which were very difficult to eradicate. This study found that the main cause of carbapenem-resistant *Acinetobacter baumannii* infection in children was the strains carrying $bla_{\text{OXA-23}}$. This result was consistent with previous findings in adult patients and hospital environments both domestically and internationally, suggesting that $bla_{\text{OXA-23}}$-carrying carbapenem-resistant *Acinetobacter baumannii* has become endemic [33–35]. Meanwhile, all carbapenem-resistant *Acinetobacter baumannii* sequence types in the present study belonged to clonal complexes 92, suggesting the clonal spread of carbapenem-resistant *Acinetobacter baumannii* clonal complexes 92 isolates in the PICU of our hospital. Clonal complexes 92 is a prevalent variant that has advantages with respect to acquiring resistance determinants and surviving in the nosocomial environments, rendering it preferentially selected under antibiotic pressure [36].

Carbapenem-resistant *Pseudomonas aeruginosa* is also a major healthcare-associated pathogen worldwide. In the United States, *Pseudomonas aeruginosa* is the leading cause of ventilator-associated pneumonia (VAP) in long-term acute care hospitals and hospital wards, and the second most common cause of VAP in intensive care units. Moreover, it is the third most common cause of catheter-related urinary tract infections [37]. The carbapenemases, such as Class A beta-lactamases, KPC and GES, metallo-beta-lactamases NDM and VIM, and the Class D OXA-48 enzymes, exist in *Pseudomonas aeruginosa* strains globally with regional differences [37]. An increasing prevalence of carbapenemases, mainly regarding VIM and NDM, has been reported [38]. However, two isolates expressing $bla_{\text{VIM-2}}$ were found in the carbapenem-resistant *Pseudomonas aeruginosa* isolates in the present study, and carbapenemase genes were less frequent than that observed in other countries [39]. Carbapenem-resistant *Pseudomonas aeruginosa* infection is typically a multifactorial condition caused by several mechanisms, including acquired resistance to carbapenems by acquisition of transferable genes encoding carbapenemases, repression or inactivation of the carbapenem porin OprD, and hyperexpression of the chromosomal cephalosporinase AmpC [39]. Thus, further research is needed to elucidate the underlying mechanisms. Our study found that a large proportion of carbapenem-resistant *Pseudomonas aeruginosa*-positive patients were infected with different sequence types, with sequence type 244 being the major sequence type. This result is consistent with previous results observed in China [40].

The treatment of carbapenem-resistant gram-negative bacilli in pediatric patients poses a challenge due to the limited available antimicrobial options [7,41]. Antimicrobial treatment should be individualized based on several factors, including the severity of infection, the source of infection, and the susceptibility profile of the isolated bacteria. Moreover, the occurrence of resistance to emerging antimicrobials highlights the need for infection prevention and control measures in children [42]. Furthermore, the molecular characteristics of carbapenem-resistant gram-negative bacilli strains showed that the resistance genes and molecular typing results were similar to those of adults, suggesting considerable challenges.

Our study has some limitations. Firstly, this was a single-center study in one department, and therefore, our findings regarding the drug sensitivity of important therapeutic agents, such as ceftazidime/avibactam, polymyxin B, and tigecycline, are constrained by limited data. Furthermore, our analysis was limited to a few variables hypothesized to influence the incidence of nosocomial infection or carriage, focusing specifically on carbapenem-resistant gram-negative bacilli colonization active screening and various carbapenem-resistant gram-negative bacilli patient placements. As a result, we cannot exclude the possibility that other factors may have affected the effectiveness of our intervention, such as the Coronavirus Disease of 2019 in 2020 and 2021. Due to the reduced number of patients admitted to the medical wards, hospitals managed to conduct more comprehensive environmental disinfection and provide more protective equipment. This may lead to changes in the hospital population and the risk factors contributing to carbapenem-resistant gram-negative bacilli.

5. Conclusions

In conclusion, active screening and appropriate patient placement interventions can effectively reduce the colonization of carbapenem-resistant gram-negative bacilli and subsequent nosocomial infections. In addition to single room isolation (type A), the combination of same area placement (type B) and active screening is also an effective approach for preventing nosocomial carbapenem-resistant gram-negative bacilli infection in PICUs. To the best of our knowledge, this is the first report on the dynamic monitoring of infection prevention and control strategy effects and the genetic characteristics of carbapenem-resistant gram-negative bacilli isolates found in PICU patients in China. The predominant carbapenemase genes were $bla_{\text{KPC-2}}$ belonging to sequence type 11 in carbapenem-resistant *Klebsiella pneumoniae* isolates and $bla_{\text{OXA-23}}$ belonging to sequence type 1712 in carbapenem-resistant *Acinetobacter baumannii* isolates, and only two $bla_{\text{VIM-2}}$ genes belonging to sequence type 179 in carbapenem-resistant *Pseudomonas aeruginosa* isolates were found. The findings of this study could contribute to the management of nosocomial carbapenem-resistant gram-negative bacilli infections and improve clinical practice.

Author contribution statement

Lijun Yin: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nana Wu; Lu Lu; Huimin Qian: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Gangfeng Yan: Performed the experiments; Analyzed and interpreted the data.

Weijing Yang: Contributed reagents, materials, analysis tools or data.

Jian Ma; Guoping Lu; Xiaowen Zhai: Conceived and designed the experiments.

Leiyan He: Performed the experiments.

Chuanqing Wang: Conceived and designed the experiments; Analyzed and interpreted the data.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] S. Tomczyk, V. Zanichelli, M.L. Grayson, A. Twyman, M. Abbas, D. Pires, et al., Control of carbapenem-resistant Enterobacteriaceae, acinetobacter baumannii, and Pseudomonas aeruginosa in healthcare facilities: a systematic review and reanalysis of quasi-experimental studies, Clin. Infect. Dis. 68 (5) (2019) 873–884, https://doi.org/10.1093/cid/ciy752.

[2] P.A. Alvares, M.V. Arnoni, C.B. da Silva, M.A.P. Sáfadi, M.J. Mimica, Carbapenem-resistant Gram-negative bloodstream infections in critically ill children: outcome and risk factors in a tertiary teaching hospital in South America, J. Hosp. Infect. 101 (2) (2019) 188–189, https://doi.org/10.1016/j.jhin.2018.10.001.

[3] A. Sleiman, A.G.A. Fayad, H. Banna, G.M. Matar, Prevalence and molecular epidemiology of carbapenem-resistant Gram-negative bacilli and their resistance determinants in the Eastern Mediterranean Region over the last decade, J Glob Antimicrob Resist 25 (2021) 209–221, https://doi.org/10.1016/j.jgar.2021.02.033.

[4] K. Liu, H. Xu, J. Sun, Y. Liu, W. Li, Investigation and analysis of carbapenem-resistant gram-negative bacterial infection rates across hospitals in Shandong Province in China, Front. Public Health 10 (2022), 1014995, https://doi.org/10.3389/fpubh.2022.1014995.

[5] Fu Pan, chuanqing Wang, hui Yu, et al., Antimicrobial resistance profile of climical strains isolated from children in China:A report from ISPED program in 2021, Chinese Journal of Evidence-based Pediatrics 17 (5) (2022) 355–362.

[6] Fupin Hu, Yan Guo, Demei Zhu, et al., CHINET surveillance of antimicrobial resistance among the bacterial isolates in, Chin. J. Infect. Chemother. 2 (5) (2021) 521–530, 022,22.

[7] D. Aguilera-Alonso, L. Escosa-García, J. Saavedra-Lozano, E. Cercenado, F. Baquero-Artigao, Carbapenem-resistant gram-negative bacterial infections in children, Antimicrob. Agents Chemother. 64 (3) (2020), e02183-19, https://doi.org/10.1128/AAC.02183-19.

[8] P. Savard, T.M. Perl, Combating the spread of carbapenemases in Enterobacteriaceae: a battle that infection prevention should not lose, Clin. Microbiol. Infect. 20 (9) (2014) 854–861, https://doi.org/10.1111/1469-0691.12748.

[9] J.A. Otter, N.T. Mutters, E. Tacconelli, A. Gikas, A.H. Holmes, Controversies in guidelines for the control of multidrug-resistant Gram-negative bacteria in EU countries, Clin. Microbiol. Infect. 21 (12) (2015) 1057–1066, https://doi.org/10.1016/j.cmi.2015.09.021.

[10] Q. Li, T. Han, Y. Zhang, Q. Zhang, X. Kong, Y. Yang, Z. Feng, A nationwide survey on neonatal medical resources in mainland China: current status and future challenges, BMC Pediatr. 19 (1) (2019) 436, https://doi.org/10.1186/s12887-019-1780-4.

[11] Bijie Hu, Technical guidelines for prevention and control of carbapenem resistant gram negative bacilli (CRO) infection in China, Chinese Journal of nosocomial infection 29 (13) (2019) 2075–2080, 2019.

[12] Y. Ohsaki, R. Kubo, J. Hobson, et al., MASTDISCS combi Carba plus, a simple method for discriminating carbapenemase-producing Enterobacteriaceae, including OXA-4-type producers, Microbiol. Immunol. 62 (1) (2018) 60–65, https://doi.org/10.1111/1348-0421.12553.

[13] Centers for Disease Control (CDC), Public health focus: surveillance, prevention, and control of nosocomial infections, MMWR Morb. Mortal. Wkly. Rep. 41 (42) (1992) 783–787. PMID: 1406572.

[14] Guidelines for the Prevention and Control of Carbapenem-Resistant Enterobacteriaceae, Acinetobacter Baumannii and Pseudomonas aeruginosa in Health Care Facilities, World Health Organization, Geneva, 2017. PMID: 29630191.

[15] L. Yin, L. He, J. Miao, W. Yang, X. Wang, J. Ma, et al., Actively surveillance and appropriate patients placements' contact isolation dramatically decreased Carbapenem-Resistant Enterobacteriaceae infection and colonization in pediatric patients in China, S0195-6701, J. Hosp. Infect. (20) (2020), https://doi.org/10.1016/j.jhin.2020.03.031, 30130-4.

[16] B. Baljin, G. Baldan, B. Chimeddorj, K. Tulgaa, B. Gunchin, T. Sandag, et al., Faecal carriage of gram-negative multidrug-resistant bacteria among patients hospitalized in two centres in ulaanbaatar, Mongolia, PLoS One 11 (12) (2016), e0168146, https://doi.org/10.1371/journal.pone.0168146.

[17] Y. Carmeli, M. Akova, G. Cornaglia, G.L. Daikos, J. Garau, S. Harbarth, et al., Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control, Clin. Microbiol. Infect. 16 (2) (2010) 102–111, https://doi.org/10.1111/j.1469-0691.2009.03115.x.

[18] N.D. Friedman, Y. Carmeli, A.L. Walton, M.J. Schwaber, Carbapenem-resistant Enterobacteriaceae: a strategic roadmap for infection control, Infect. Control Hosp. Epidemiol. 38 (5) (2017) 580–594, https://doi.org/10.1017/ice.2017.42.

[19] E. Tacconelli, M.A. Cataldo, S.J. Dancer, G. De Angelis, M. Falcone, U. Frank, et al., ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients, Clin. Microbiol. Infect. 20 (Suppl 1) (2014) 1–55, https://doi.org/10.1111/1469-0691.12427.

[20] X. Qin, S. Wu, M. Hao, J. Zhu, B. Ding, Y. Yang, et al., The colonization of carbapenem-resistant Klebsiella pneumoniae: epidemiology, resistance mechanisms, and risk factors in patients admitted to intensive care units in China, J. Infect. Dis. 221 (Suppl 2) (2020) S206–S214, https://doi.org/10.1093/infdis/jiz622.

[21] T. Karampatakis, K. Tsergouli, L. Politi, G. Diamantopoulou, E. Iosifidis, C. Antachopoulos, et al., Polyclonal predominance of concurrently producing OXA-23 and OXA-58 carbapenem-resistant Acinetobacter baumannii strains in a pediatric intensive care unit, Mol. Biol. Rep. 46 (3) (2019) 3497–3500, https://doi.org/10.1007/s11033-019-04744-4.

[22] T. Karampatakis, K. Tsergouli, E. Iosifidis, C. Antachopoulos, A. Karapanagiotou, A. Karyoti, et al., Impact of active surveillance and infection control measures on carbapenem-resistant Gram-negative bacterial colonization and infections in intensive care, J. Hosp. Infect. 99 (4) (2018) 396–404, https://doi.org/10.1016/j.jhin.2018.05.010.

[23] R.A. Bonomo, E.M. Burd, J. Conly, B.M. Limbago, L. Poirel, J.A. Segre, L.F. Westblade, Carbapenemase-producing organisms: a global scourge, Clin. Infect. Dis. 66 (8) (2018) 1290–1297, https://doi.org/10.1093/cid/cix893.

[24] K. Sun, X. Chen, C. Li, Z. Yu, Q. Zhou, Y. Yan, Clonal dissemination of multilocus sequence type 11 Klebsiella pneumoniae carbapenemase - producing K. pneumoniae in a Chinese teaching hospital, APMIS 123 (2) (2015) 123–127, https://doi.org/10.1111/apm.12313.

[25] L. Yin, L. He, J. Miao, W. Yang, X. Wang, J. Ma, et al., Carbapenem-resistant Enterobacterales colonization and subsequent infection in a neonatal intensive care unit in Shanghai, China, Infect Prev Pract 3 (3) (2021), 100147, https://doi.org/10.1016/j.infpip.2021.100147.

[26] P. Nordmann, G. Cuzon, T. Naas, The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria, Lancet Infect. Dis. 9 (4) (2009) 228–236, https://doi.org/10.1016/S1473-3099(09)70054-4.

[27] A. Russo, S. Giuliano, G. Ceccarelli, F. Alessandri, A. Giordano, G. Brunetti, M. Venditti, Comparison of septic shock due to multidrug-resistant acinetobacter baumannii or Klebsiella pneumoniae carbapenemase-producing K. pneumoniae in intensive care unit patients, Antimicrob. Agents Chemother. 62 (6) (2018), e02562-17, https://doi.org/10.1128/AAC.02562-17.

[28] O. Tsiatsiou, E. Iosifidis, A. Katragkou, V. Dimou, K. Sarafidis, T. Karampatakis, et al., Successful management of an outbreak due to carbapenem-resistant Acinetobacter baumannii in a neonatal intensive care unit, Eur. J. Pediatr. 174 (1) (2015) 65–74, https://doi.org/10.1007/s00431-014-2365-8.

[29] E. Tacconelli, E. Carrara, A. Savoldi, S. Harbarth, M. Mendelson, D.L. Monnet, et al., WHO Pathogens Priority List Working Group. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis, Lancet Infect. Dis. 18 (3) (2018) 318–327, https://doi.org/10.1016/S1473-3099(17)30753-3.

[30] R. Rafei, H. Pailhoriès, M. Hamze, M. Eveillard, H. Mallat, F. Dabboussi, et al., Molecular epidemiology of Acinetobacter baumannii in different hospitals in Tripoli, Lebanon using bla(OXA-51-like) sequence based typing, BMC Microbiol. 15 (2015) 103, https://doi.org/10.1186/s12866-015-0441-5.

[31] L. Poirel, P. Nordmann, Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology, Clin. Microbiol. Infect. 12 (9) (2006) 826–836, https://doi.org/10.1111/j.1469-0691.2006.01456.x.

[32] T. Karampatakis, K. Tsergouli, L. Politi, G. Diamantopoulou, E. Iosifidis, C. Antachopoulos, et al., Polyclonal predominance of concurrently producing OXA-23 and OXA-58 carbapenem-resistant Acinetobacter baumannii strains in a pediatric intensive care unit, Mol. Biol. Rep. 46 (3) (2019) 3497–3500, https://doi.org/10.1007/s11033-019-04744-4.

[33] Y. Fu, J. Zhou, H. Zhou, Q. Yang, Z. Wei, Y. Yu, et al., Wide dissemination of OXA-23-producing carbapenem-resistant Acinetobacter baumannii clonal complex 22 in multiple cities of China, J. Antimicrob. Chemother. 65 (4) (2010) 644–650, https://doi.org/10.1093/jac/dkq027.

[34] A. Aliramezani, M. Douraghi, A. Hajihasani, M. Mohammadzadeh, M. Rahbar, Clonal relatedness and biofilm formation of OXA-23-producing carbapenem resistant Acinetobacter baumannii isolates from hospital environment, Microb. Pathog. 99 (2016) 204–208, https://doi.org/10.1016/j.micpath.2016.08.034.

[35] A. Di Popolo, M. Giannouli, M. Triassi, S. Brisse, R. Zarrilli, Molecular epidemiological investigation of multidrug-resistant Acinetobacter baumannii strains in four Mediterranean countries with a multilocus sequence typing scheme, Clin. Microbiol. Infect. 17 (2) (2011) 197–201, https://doi.org/10.1111/j.1469-0691.2010.03254.x.

[36] Z. Ruan, Y. Chen, Y. Jiang, H. Zhou, Z. Zhou, Y. Fu, et al., Wide distribution of CC92 carbapenem-resistant and OXA-23-producing Acinetobacter baumannii in multiple provinces of China, Int. J. Antimicrob. Agents 42 (4) (2013) 322–328, https://doi.org/10.1016/j.ijantimicag.2013.06.019.

[37] F.C. Tenover, D.P. Nicolau, C.M. Gill, Carbapenemase-producing *Pseudomonas aeruginosa* -an emerging challenge, Emerg. Microb. Infect. 11 (1) (2022) 811–814, https://doi.org/10.1080/22221751.2022.2048972.

[38] M. Castanheira, L.M. Deshpande, A. Costello, T.A. Davies, R.N. Jones, Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible Pseudomonas aeruginosa collected during 2009-11 in 14 European and Mediterranean countries, J. Antimicrob. Chemother. 69 (7) (2014) 1804–1814, https://doi.org/10.1093/jac/dku048.

[39] S. Yin, P. Chen, B. You, Y. Zhang, B. Jiang, G. Huang, et al., Molecular typing and carbapenem resistance mechanisms of Pseudomonas aeruginosa isolated from a Chinese burn center from 2011 to 2016, Front. Microbiol. 9 (2018) 1135, https://doi.org/10.3389/fmicb.2018.01135.

[40] J. Ji, J. Wang, Z. Zhou, H. Wang, Y. Jiang, Y. Yu, Multilocus sequence typing reveals genetic diversity of carbapenem- or ceftazidime-nonsusceptible Pseudomonas aeruginosa in China, Antimicrob. Agents Chemother. 57 (11) (2013) 5697–5700, https://doi.org/10.1128/AAC.00970-13. Epub 2013 Aug 12.

[41] M. Paul, E. Carrara, P. Retamar, T. Tängdén, R. Bitterman, R.A. Bonomo, et al., European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant Gram-negative bacilli (endorsed by European society of intensive care medicine), Clin. Microbiol. Infect. 28 (4) (2022) 521–547, https://doi.org/10.1016/j.cmi.2021.11.025.

[42] Y. Wang, J. Wang, R. Wang, Y. Cai, Resistance to ceftazidime-avibactam and underlying mechanisms, J Glob Antimicrob Resist 22 (2020) 18–27, https://doi.org/10.1016/j.jgar.2019.12.009.