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# [Handwashing sinks as reservoirs](https://www.frontiersin.org/articles/10.3389/fpubh.2024.1468521/full)  [of carbapenem-resistant](https://www.frontiersin.org/articles/10.3389/fpubh.2024.1468521/full)  *[Acinetobacter baumannii](https://www.frontiersin.org/articles/10.3389/fpubh.2024.1468521/full)* in the [intensive care unit: a prospective](https://www.frontiersin.org/articles/10.3389/fpubh.2024.1468521/full)  [multicenter study](https://www.frontiersin.org/articles/10.3389/fpubh.2024.1468521/full)

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Introduction: The extent to which sinks are contaminated by carbapenemresistant *Acinetobacter baumannii* (CRAB) in intensive care units (ICUs) and the association between these contaminated sinks and hospital-acquired CRAB infections during the non-cluster period remains largely unknown. Here, we performed a prospective multicenter study in 16 ICUs at 11 tertiary hospitals in Chengdu, China.

Methods: We sampled sinks, collected CRAB clinical isolates, and conducted whole-genome sequencing and analysis.

Results: A total of 789 swabs were collected from 158 sinks, and 16 CRAB isolates were recovered from 16 sinks, resulting in a contamination rate of 10.16%. Twentyseven clinical isolates were collected during the study period. The majority (97.67%, 42/43) of the CRAB isolates belonged to ST2, and 36 (83.72%) of them had both  $bla_{\alpha_{\text{NNA-23}}}$  and  $bla_{\alpha_{\text{NNA-66}}}$ . The 43 strains belonged to 12 clones. One certain clone caused multiple contaminations of seven sinks in one GICU. Two clones of ST2  $bla_{\alpha_{\alpha_{2}}\alpha_{23}}$  and  $bla_{\alpha_{\alpha_{6}}\alpha_{6}}$ -carrying sink strains were likely the sources of the two clusters in the two GICUs, respectively. Five ST2 *bla<sub>OXA-23</sub>-carrying isolates were* found to be common clones but were recovered from two hospitals.

Conclusion: The contamination rate of CRAB in handwashing sinks is high in some local ICUs, and the contaminated sinks can serve as environmental reservoirs for CRAB clusters.

#### **KEYWORDS**

carbapenem-resistant *Acinetobacter baumannii*, intensive care units, handwashing sinks, environmental reservoir, outbreak source

#### 1 Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a common healthcare-associated infection (HAI) pathogen and has been classified as a critical priority by the World Health Organization due to the urgent need for new antibiotics to combat it ([1](#page-7-0)). A systematic review conducted by Antimicrobial Resistance Collaborators showed that the CRAB contributed to 57,700 deaths globally in 2019 [\(2](#page-7-1)). In the intensive care unit (ICU), CRAB is particularly common and is mainly responsible for HAIs and hospital outbreaks  $(3-5)$  $(3-5)$ , causing a wide range of infections, including ventilator-associated pneumonia and bloodstream infections ([6](#page-7-4)[–8\)](#page-8-0).

The most common mechanism by which CRAB strains exert carbapenem antibiotic resistance is the production of oxacillinase (OXA)-type carbapenem-hydrolyzing class D *β*-lactamases, which are encoded by intrinsic *bla*<sub>OXA-51-like</sub> genes (e.g., *bla*<sub>OXA-51</sub>, *bla*<sub>OXA-64</sub>, *bla*<sub>OXA-65</sub>,  $bla<sub>OXA-66</sub>$ , and  $bla<sub>OXA-98</sub>$ , and/or acquired genes, such as  $bla<sub>OXA-23-like</sub>$  (e.g., *bla*OXA-23, *bla*OXA-27, *bla*OXA-49, and *bla*OXA-225), *bla*OXA-24/40-like (e.g., *bla*OXA-24, *bla*<sub>OXA-25</sub>, *bla*<sub>OXA-26</sub>, *bla*<sub>OXA-40</sub>, and *bla*<sub>OXA-72</sub>), *bla*<sub>OXA-58-like</sub> (e.g., *bla*<sub>OXA-58</sub> and *bla*<sub>OXA-164</sub>), *bla*<sub>OXA-235-like</sub> (e.g., *bla*<sub>OXA-235</sub> and *bla*<sub>OXA-237</sub>), and *bla*<sub>OXA</sub>.  $_{143\text{-like}}$  genes ([9](#page-8-1)[–12\)](#page-8-2). The most prevalent acquired carbapenemase gene reported is  $bla_{\text{OX}_A-23}$ , followed by  $bla_{\text{OX}_A-24/40}$  [\(12](#page-8-2)[–16\)](#page-8-3). In addition, class B metal *β*-lactamase genes (e.g., *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>IMP</sub>) have also been reported to exist in a very small number of CRAB strains [\(9](#page-8-1), [10\)](#page-8-4).

Handwashing sinks play an important role in infection prevention and control systems and are common in all hospitals, including in the ICU. In China, a multicenter investigation conducted in 14 provinces showed that the total installation rate of handwashing sinks increased from 69.30% in 2010 to 77.20% in 2016 in 200 hospitals [\(17](#page-8-5)). However, handwashing sinks can be contaminated by multi-drug-resistant organisms and cause outbreaks in hospitals [\(18](#page-8-6)). Previous studies have identified that certain parts of handwashing sinks, such as sink traps and sink splashing [\(19,](#page-8-7) [20](#page-8-8)), sink bowls and drains [\(21,](#page-8-9) [22](#page-8-10)), faucet aerators  $(23)$ , and water taps  $(24)$ , can be contaminated by CRAB during the outbreak period.

However, few studies have focused on the level of handwashing sink contamination by CRAB in different ICUs during the non-outbreak period, and the degree to which contaminated handwashing sinks are associated with hospital-acquired infections in CRAB remains largely unknown. In this study, we conducted a prospective multicenter investigation across 11 hospitals in Chengdu, with the following three objectives: (1) to evaluate the prevalence of CRAB contamination in the sinks of local ICUs during a non-outbreak period; (2) to characterize the carbapenem-resistant genes and molecular epidemiological features of CRAB strains isolated from infected patients and from handwashing sinks in these ICUs; and (3) to assess the potential role of CRAB-contaminated sinks in contributing to patient infections during the non-outbreak investigation period.

## 2 Materials and methods

#### 2.1 Study setting and design

This multicenter prospective investigation was performed in 16 ICUs at 11 hospitals in Chengdu, Sichuan Province, China. The 16 ICUs included 9 general ICUs (GICUs, with a total of 279 beds and 103 handwashing sinks) and 7 neonatal ICUs (NICUs, with a total of 282 beds and 55 handwashing sinks) [\(Table 1](#page-2-0)). From March 2019 to August 2019, all handwashing sinks in the included ICUs were sampled once to screen for CRAB. The clinical CRAB isolates recovered from patients were collected between 2weeks before and 3months after the date of sampling in ICUs. If one or more sinks were positive for CRAB, the clinical isolates from this ICU or from other participating ICUs at the same hospital were recovered and transferred to West China Hospital for identification again using the Vitek II automated system (bioMérieux, Marcy l'Etoile, France). All the isolates were subjected to whole-genome sequencing (WGS) to detect homology.

The study was approved by the Ethical Committee of the West China Hospital, and the need to obtain informed consent was waived.

#### 2.2 Sample sites and sampling methods

The sample points included the surfaces, faucets, bowls, drains, overflows, and drain holes of the handwashing sinks. A total of 100 cm<sup>2</sup> of the sink surface and the bowl were sampled using sterile rayon swabs (Copan, Brescia, Italy) moistened with tryptic soy broth (TSB, Hopebio, Qingdao, China). A  $5 \times 5$  cm caliper was used to confirm the sampling area. The sample points for the faucet included a surface area of 5cm from the outer wall of the water outlet and 3–5cm from the inner wall. For the bubbled faucets, the bubbler was removed, and the samples were sampled using sterile rayon swabs. The drain holes were sampled 3-5cm below the drain by the swabs. All the swabs were immediately placed into 15mL sterile tubes containing 6mL of TSB.

#### 2.3 Microbiological methods

All the tubes were incubated at 37°C overnight and centrifuged as described in our previous study ([25](#page-8-13)). The supernatant was subsequently discarded, and the precipitants were resuspended in 1mL of TSB. A 50μL suspension was inoculated onto *Acinetobacter* selected agar plates (CHROMagar™, Paris, France) containing 4μg/ mL meropenem to screen for CRAB. Isolates that were pink, round, smooth, raised, and moist on the selected agar plates were suspected and identified using a matrix-assisted laser desorption/ionization– time of flight mass spectrometry (Bruker, Billerica, Massachusetts). The minimum inhibitory concentrations (MICs) of meropenem were determined using the broth microdilution method of the Clinical and Laboratory Standards Institute (CLSI) ([26](#page-8-14)).

WGS was performed for all identified isolates using the Illumina HiSeq X10 platform (Illumina, San Diego, California). Subsequent reads were assembled into contigs using SPAdes version 3.14.0 ([27](#page-8-15)). Precise species identification was established based on average nucleotide identity between the strains and type strains of *Acinetobacter* species using JSpeciesWS [\(28\)](#page-8-16). The sequence of the STs was determined using the genome sequence to query the multilocus sequence typing database.<sup>1</sup> Antimicrobial-resistant genes were identified using ResFinder.<sup>2</sup> The draft genomes of the strains have been deposited in GenBank under BioProject number PRJNA977829. Single-nucleotide polymorphisms (SNPs)

<span id="page-1-0"></span><sup>1</sup> [https://pubmlst.org/bigsdb?db=pubmlst\\_abaumannii\\_seqdef](https://pubmlst.org/bigsdb?db=pubmlst_abaumannii_seqdef)

<span id="page-1-1"></span><sup>2</sup> <http://genomicepidemiology.org/>



<span id="page-2-0"></span>TABLE 1 Characteristics of the participating ICUs and CRAB isolates from sinks and clinical samples.

CRAB, carbapenem-resistant *Acinetobacter baumannii*; ICU, intensive care unit.

a In this column, G indicates general ICU; N indicates neonatal ICU; no sink was positive for CRAB at the participating ICUs and not to collect CRAB clinical isolates.

among the same ST strains were analyzed using Snippy version 4.6.0[.3](#page-2-1) Recombination regions were predicted using Gubbins version 2.4.1 [\(29](#page-8-17)) under the GTRGAMMA model with a maximum of 50 iterations. A cutoff of equal to or less than 11 high-quality SNPs was used to define clones within the same ST strains, as described in a previous study [\(30\)](#page-8-18).

#### 3 Results

#### 3.1 The contamination of sinks by CRAB

In total, 789 swabs were collected from 158 handwashing sinks in 16 ICUs at 11 hospitals, and 16 CRAB isolates were recovered from 16 handwashing sinks in 5 ICUs at 5 hospitals [\(Table 1](#page-2-0)). The total contamination rate of CRAB in the handwashing sink was 10.16% (16/158, 95% CI 5.40–14.83%). The contamination rate in different ICUs ranged from 0 to 63.6%, and the sink contamination rate in the GICU was 15.29% (95% CI 7.75–21.38%), while it was 3.57% (95% CI 0.0–5.35%) in the NICU. Of the 16 sink CRAB isolates, 15 were recovered from the GICUs and belonged to ST2 (12 isolates carrying both *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-66</sub> carbapenemase genes, 1 isolate harboring both  $bla_{\rm NDM\text{-}1}$  and  $bla_{\rm OXA\text{-}98,}$  and 2 isolates carrying only  $bla_{\rm OXA\text{-}23}).$  The remaining CRAB isolate was recovered from a NICU and belonged to ST203, and this isolate harbored both *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-66</sub> ([Table 2](#page-3-0)). The 16 sink isolates could be assigned to 6 clones.

#### <span id="page-2-2"></span>3.2 Characteristics of clinical isolates

A total of 27 CRAB clinical isolates were recovered from 4 of the 5 ICUs with CRAB-contaminated sinks. The vast majority of the 27 CRAB clinical isolates were recovered from sputum (*n* =21, 77.8%), whereas the remaining were from bronchoalveolar lavage fluid (BALF, *n*= 5, 18.5%) and cerebrospinal fluid (*n*= 1, 3.7%). All of the clinical isolates belonged to ST2. Twenty-four of them harbored both  $bla_{\text{OXA-23}}$  and  $bla_{\text{OXA-66}}$  carbapenemase genes, while the other three carried only  $bla_{\text{OXA-23}}$  (Table 2). The 27 clinical isolates could be assigned to 12 clones.

#### 3.3 Diverse clonal backgrounds of CRAB isolates and sink as a source of transmission in a GICU

A total of 18 ST2 CRAB isolates were recovered from ICU 1G, including 2 isolates from two sinks, 15 isolates from sputum, and 1 isolate from BALF. The 18 isolates could be assigned to 6 clones. Seven clinical isolates recovered from sputum, along with one sink isolate (090698), harbored both *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-6</sub>. These strains shared 1–7 SNPs, and therefore belonged to a common clone (clone 2b) [\(Figure 1\)](#page-5-0), indicating an outbreak. Four (090713, 090714, 090715, and 090736) of these seven clinical isolates were recovered before the sink sampling date, and the other three clinical isolates (090739, 090740, and 090745) were recovered after the sink sampling date. These three isolates shared smaller SNPs (2, 4, and 2, respectively) with the clinical isolate (090713) than with the sink

<span id="page-2-1"></span><sup>3</sup> <https://github.Com/tseemann/snippy>



<span id="page-3-0"></span>04

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<span id="page-5-0"></span>

isolate (5, 7, and 5, respectively); therefore, the sink isolate (090698) was likely to be contaminated but not the exact route of transmission.

Another isolate (090697) from another sink shared 0–2 SNPs with three clinical isolates (two isolates were recovered from sputum, and one was recovered from BALF) and therefore belonged to a common clone (clone 2c) ([Figure 1\)](#page-5-0), also indicating an outbreak in the ICU 1G. All three clinical isolates were recovered at a later date than the sink sampling date; therefore, the sink was likely to be the route of infection transmission. However, the two sink isolates in this ICU shared 117 SNPs, indicating that they were assigned to two strains. The remaining six clinical isolates belonged to four clones but were not the same as the two sink isolates from ICU 1G.

#### 3.4 A clone of ST2 *bla*<sub>OXA-23</sub> carrying CRAB transmission in two GICUs

There were five isolates (two clinical isolates from ICU 1G, one clinical isolate, and two sink isolates from ICU 4G) that only carried the *bla*<sub>OXA-23</sub> gene and shared 3-10 SNPs (clone 2a) (Figure 1), suggesting interhospital transmission. These two sink isolates (090704 and 090705) were recovered approximately 3months prior to the clinical isolate (090769); therefore, the sinks were likely to constitute the route of patient transmission. Although the two clinical isolates (090704 and 090705) from ICU 1G were recovered approximately 1month prior to the two sink isolates from ICU 4G, the exact route of interhospital transmission remains unclear.

#### 3.5 A high contamination rate of CRAB in the NICU and GICU at a hospital

There were 8 (7 from ICU 2G and 1 from ICU 2N) of the 15 sinks recovered CRAB isolates with a CRAB contamination rate as high as 53.3% in hospital 2. One sink isolate (090694) recovered from ICU 2N belonged to ST203, but no clinical isolate was obtained during the period of the study in this ICU. Therefore, ST203 CRAB was not a common cause of infection among patients. Seven sink isolates (six harboring both  $bla_{\text{OX A-23}}$  and  $bla_{\text{OX A-66}}$  and one isolate harboring both  $bla_{\text{NDM-1}}$  and  $bla_{\text{OVA-98}}$ ) and three clinical isolates (two recovered from sputum and one recovered from cerebrospinal fluid) recovered from ICU 2G belonged to ST2, sharing 1–11 SNPs and belonging to a common clone (clone 2g) ([Figure 1](#page-5-0)). Only one of the three clinical isolates (090718) was recovered earlier date than the sink sampling date and shared 2–5 SNPs with the other two clinical isolates (090767 and 090768). Compared to the seven sink isolates, the two isolates (090767 and 090768) shared 3–7 SNPs and 6–14 SNPs, respectively. Therefore, the sink was likely to be contaminated but not the exact route of the transmission. The remaining clinical isolate (090766) from ICU 2G belonged to a distinct clone (2h) and shared 35–142 SNPs with other isolates.

#### 3.6 A cluster of ST2 *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-66</sub>carrying CRAB in a GICU

In ICU 3G, four sinks were contaminated by CRAB, which belonged to the ST2 type and carried both  $bla_{\text{OXA-23}}$  and  $bla_{\text{OXA-66}}$  genes. Two of the four sink isolates (090700 and 090701) shared 2–7 SNPs with three clinical isolates (090717, 090749, and 090750), indicating the presence of a common clone (clone 2i). Although one (090717) of the three clinical isolates was recovered at an earlier date than the sink sampling date, the other two (090749 and 090750) clinical isolates shared fewer SNPs (3–5 and 2–4) with the two sink isolates than with the clinical isolate (8, 7). Therefore, these two contaminated sinks were likely to be the route of the transmission. The other two sink isolates (090699 and 090703) shared three SNPs and belonged to another common clone (2j) ([Figure 1](#page-5-0)). The remaining clinical isolate (090716) in this ICU belonged to clone (2k) alone, sharing 46–122 SNPs with other isolates.

#### 4 Discussion

To our knowledge, this study was the first multicenter investigation on handwashing sink contamination by the CRAB in China. Unlike most studies in which the handwashing sink was sampled during an outbreak period, this study was conducted during a non-outbreak period in the included ICUs. The results showed that the contamination rate of handwashing sinks ranged from 0 to 63.6% in different ICUs, while other investigations did not find CRAB contamination in handwashing sinks during non-outbreak scenarios ([31](#page-8-19), [32](#page-8-20)). It has been well documented in the literature that the contamination level of handwashing sinks is related to the improper use, the size and depth of the sink, and the frequency of environmental cleaning [\(33](#page-8-21)). This study further revealed that the contamination rate of CRAB in handwashing sinks was significantly higher than that of carbapenem-resistant *klebsiella pneumoniae* (CRKP) or carbapenem-resistant *klebsiella oxytoca* (CRKO), with a rate of 10.1, 3.8, and 3.8%, respectively, during the same period, as we previously reported [\(25\)](#page-8-13). This disparity may be attributed

to the biofilm-forming capability of *Acinetobacter baumannii*, which enables it to survive on various surfaces more effectively than CRKP ([34](#page-8-22)). In addition, the higher prevalence of CRAB compared to CRKP in local settings likely contributed to this difference ([15\)](#page-8-23).

All 27 clinical strains of CRAB obtained in our study were sourced from GICU, and this may be attributed to several factors: Patients in the GICU suffer from more severe illnesses, have longer hospital stays, experience higher rates of CRAB colonization, and are subjected to increased antibiotic usage ([35](#page-8-24), [36\)](#page-8-25). Furthermore, a lack of awareness regarding the prevention and control of HAIs and insufficient cleaning and disinfection practices in GICU environments, including handwashing sinks, may contribute to CRAB infections among GICU patients [\(22,](#page-8-10) [37\)](#page-8-26). In China, hospitals have already possessed relatively good resources for neonatal care and treatment [\(38](#page-8-27)), which likely results in fewer infections occurring in the NICU. It has been reported that CRAB strains carrying class B metallo-*β*-lactamase genes (e.g.,  $bla<sub>NDM</sub>$ ,  $bla<sub>VIM</sub>$ , and  $bla<sub>IMP</sub>$ ) are prevalent in pediatric populations [\(16,](#page-8-3) [39\)](#page-8-28). However, our findings indicate that the dominant strains identified in the GICU sinks carried class D β-lactamase genes, specifically  $bla_{\text{OXA}}$ -<sub>23</sub>, with only one strain carrying  $bla_{NDM-1}$  isolated from a GICU sink.

In this study, we found that sink contamination by CRAB was not the major source of CRAB clinical infections in most ICUs in this study. However, at least three clones (clone 2c in ICU 1G, clone 2a in ICU 4G, and clone 2i in ICU 3G) of ST2 CRAB sink isolates were demonstrated to be the sources of patient infections or outbreaks. The results indicated that these three ICUs should take some proven measures to reduce the microbial contamination rate of sinks, including disinfection, use of drain covers, or even rebuilding the handwashing sink [\(21](#page-8-9), [40,](#page-8-29) [41](#page-8-30)). In addition, we identified a transmission of clone 2a between two hospitals, along with two sink strains that were detected prior to a clinical strain in one of the hospitals. This finding underscores the need to pay close attention to hospital sewage contaminated with antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) ([42](#page-8-31)). Hospital sewage may serve as a potential reservoir for CRAB, highlighting the importance of strengthening disinfection, management, and monitoring protocols for hospital sewage in local settings [\(43](#page-8-32)).

ST2 is the most dominant type of CRAB in the world as well as in China [\(42\)](#page-8-31). The main mechanism of carbapenem resistance in *Acinetobacter baumannii* is the acquisition of carbapenem-hydrolyzing oxacillinase-encoding genes, and *bla*<sub>OXA23</sub> is by far the most widespread in most countries [\(44\)](#page-8-33). In this study, all of the clinical isolates and all but one of the sink isolates belonged to ST2. The *bla*<sub>OXA-23</sub> gene was also the most common (42/43, 97.7%) oxacillinase-encoding gene. In addition, we found that most CRAB isolates (37/43, 86.0%) carried both  $bla_{\text{OXA-23}}$  and  $bla_{\text{OXA-66}}$ , similar to the findings of other studies ([45](#page-8-34)[–48](#page-8-35)). One ST2 CRAB strain isolated from a sink, carrying both *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-51-like</sub> gene *bla*<sub>OXA-98</sub>, was not reported previously, which was alarming.

A strain of ST203 CRAB was recovered from a sink in a NICU. Although ST203 *Acinetobacter baumannii* has been found in cats in France and Japan [\(49,](#page-8-36) [50](#page-8-37)), this is the first report of ST203 CRAB recovered from a hospital environment and harboring both  $bla<sub>OXA-23</sub>$  and  $bla<sub>OXA-66</sub>$ . Although there were no patients infected with this strain in our study, rigorous monitoring is needed to prevent the spread of ST203 CRAB from the sink to humans, especially neonates.

This study also has several limitations. First, the P traps of handwashing sinks were not sampled in our study, which means potential colonization by CRAB in these areas could have been missed. However, we sampled the drainage pipe 3–5cm below the drainage tube,

and the results can reflect the contamination degree of the drainage pipe to a certain extent. Second, the clinical strains were collected only within 2weeks before and 3months after sink sampling. There might have been additional cases of CRAB associated with sinks, which could have been missed. Third, sinks were only sampled once, and other environmental surfaces were not sampled; additional isolates from the sinks may have been missed. Despite these limitations, this study revealed the level of handwashing sink contamination by CRAB in different ICUs and the association of contaminated CRAB with hospital-acquired infections.

## 5 Conclusion

This study revealed that the contamination rate of CRAB in handwashing sinks was high in some local ICUs, and the contaminated sinks could serve as environmental reservoirs for CRAB clusters.

## Data availability statement

The data presented in the study are deposited in the GenBank under BioProject repository, accession number PRJNA977829, available at [https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA977829.](https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA977829)

#### Ethics statement

The studies involving humans were approved by the Ethical Committee of the West China Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because The study only used clinical strains and did not require sample collection for patients.

#### Author contributions

LW: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. YF: Writing – review & editing, Software, Formal analysis. JL: Writing – review & editing, Resources, Investigation. XK: Writing – review & editing, Resources,

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Investigation. HZ: Writing – review & editing, Resources, Investigation. HW: Writing – review & editing, Resources. SR: Writing – review & editing, Investigation. LZ: Writing – review & editing, Investigation. YZ: Writing – review & editing, Investigation. QX: Writing – review & editing, Investigation. YL: Writing – review & editing, Investigation. XW: Writing – review & editing, Investigation. XD: Writing – review & editing, Investigation. WZ: Writing – review & editing, Investigation. QL: Writing – review & editing, Investigation. HG: Writing – review & editing, Investigation. CT: Writing – review & editing, Methodology, Conceptualization. FQ: Writing – review & editing, Writing – original draft, Methodology, Investigation.

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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