



Low-temperature laminar flow ward for the treatment of multidrug resistance *Acinetobacter baumannii* pneumonia

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Abstract

This study was designed to investigate the effect of low-temperature laminar flow ward (LTLFW) on the *Acinetobacter baumannii* pneumonia (MDR-ABP) in neurosurgical intensive care unit (NICU) patients. We evaluated whether patients in a LTLFW had significantly improved clinical outcomes as compared to those in nonconstant-temperature NICU (room temperature). The association of temperature with the prevalence of ABP and *A. baumannii* isolates (ABI) found in NICU patients was specifically investigated. In vitro microbiological experiments were conducted to measure the proliferation, antibiotic sensitivity, and genomic profiles of *A. baumannii* (AB) that grew in variable temperatures. MDR-ABP patients in LTLFW had significantly improved outcomes than those in the room temperature NICU. In addition, the numbers of ABI were positively associated with mean ambient outdoor temperatures ($P = 0.002$), with the incidence of ABP and average numbers of ABI among NICU patients being substantially lower in the winter as compared to other seasons. However, there were no significant seasonal variations in the other strains of the top five bacteria. Consistent with these clinical observations, AB growing at 20°C and 25°C had significantly reduced viability and antibiotic resistance compared to those growing at 35°C. The expression of genes related to AB survival ability, drug resistance, and virulence also differed between AB growing at 20°C and those at 35°C. LTLFW is effective in promoting the recovery of MDR-ABP patients because low temperatures reduced the density and virulence of AB and enhanced the efficacy of antibiotics, likely at the genetic level.

Keywords *Acinetobacter baumannii* · Pneumonia · Temperature · Variation · Treatment

Zhitao Gong, Jin Li and Hongliang Luo contributed equally to this work.

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Abbreviation

AB	<i>Acinetobacter baumannii</i>
ABI	<i>Acinetobacter baumannii</i> isolates
ABP	<i>Acinetobacter baumannii</i> pneumonia
AOTs	Ambient outdoor temperatures
ATBI	Average of the total bacterial isolates
BAL	Broncho-alveolar lavage
Bap	Biofilm-associated protein
bfs	Biofilm synthesis gene
cfu/mL	colony-forming units per milliliter
Fsr	Ferric siderophore receptor
GCS	Glasgow coma scale
GNB	Gram-negative bacteria
ICH	Intracerebral hematoma
ICU	Intensive care units
intI1	Class 1 integron
LTLFW	Low-temperature laminar flow ward
MDR	Multidrug resistance

NICU	Neurosurgical intensive care units
OmpA	Outer membrane protein A
PABTBI	The percentage of AB in total bacterial isolates
qRT-PCR	Quantitative real-time reverse transcriptase polymerase chain reaction
RND	Resistance–nodulation–division
SCF	Cefoperazone/Sulbactam disk
TBI	Traumatic brain injury
TMUGH	Tianjin Medical University General Hospital

Introduction

Acinetobacter baumannii (AB) is a major cause of nosocomial infection of patients in intensive care units (ICU) and often develops resistance to antimicrobials [1]. *A. baumannii* pneumonia (ABP) is a significant risk for the mortality of patients in ICU [2, 3]. However, an optimal therapy for the treatment of multidrug resistance (MDR) ABP remains unclear [4].

Most respiratory infections such as influenza viruses and pneumococcus are more prevalent during the winter, and their outbreaks have been inversely correlated with environmental temperatures [5, 6]. However, several studies have shown that hospital-acquired infections with Gram-negative bacteria (GNB), especially AB, but not those with Gram-positive bacteria, are more common in the summer and are associated with high ambient outdoor temperatures (AOTs) [7–10]. The underlying mechanism for this seasonal variation remains poorly understood [10]. Studies reported in the literature are limited to the ward-based continuous surveillance, which could not determine whether *A. baumannii* isolates (ABI) are infected or colonized. Information on patient-specific analysis is scarce, and the independent association between bacterial infections and temperature is inconclusive, primarily due to the presence of considerable confounding variables [5, 8]. These reports also fail to provide practical temperature-related guidance for improving clinical outcomes of ICU patients.

We have conducted a retrospective clinical study and a microbiological trial to evaluate the impact of low-temperature laminar flow ward (LTLFW) and temperature variations on AB infections of patients admitted to NICU. To mechanistically understand this temperature-influenced AB infections, we also conducted *in vitro* experiments to characterize the impact of temperature on biological characteristics of AB.

Materials and methods

Patients

The retrospective collection of data from patients and their analyses have been approved by the ethics committee of Tianjin Medical University General Hospital (TMUGH), approval number IRB2018-YX-128. This study included patients admitted to NICU of TMUGH from 1 January 2013 to 31 December 2017.

We collected the information on demographics (sex and age) and primary diseases (e.g., intracerebral hematoma, traumatic brain injury). Patient's conditions before ABP diagnosis and treatment situation, including Glasgow coma scale on admission, last white blood cell (WBC) counts before ABP diagnosis, surgeries and mechanical ventilation, types and duration of antibiotics (e.g., meropenem, cefoperazone/sulbactam, tigecycline), hospital stays prior to ABP diagnosis, NICU stays, and total hospital stays of the patients, were also included in the study through medical chart review. The recovery of MDR-ABP was defined as the primary outcome measure. And the secondary outcome variables for this study included last WBC before hospital discharge, the overall prognosis at hospital discharge, and in-hospital mortality.

For patients who developed multiple ABPs, we included only the first episode. To control for the total number of patient-days as a confounding variable, monthly or seasonal bacterial isolates and new cases of ABP were calculated as per 500 or 1500 patient-days, respectively. The date of diagnosis was defined as the time of first positive bacterial culture. MDR in AB was defined as resistance to three or more of the following five classes of antibiotics that are typically prescribed to treat AB: antipseudomonal fluoroquinolones, antipseudomonal cephalosporins, antipseudomonal carbapenems, β -lactam– β -lactamase inhibitor combinations, and aminoglycosides [11, 12]. All patients were continuously monitored until hospital discharge or death.

The inclusion criteria for ABP (diagnostic criteria) were as follows: (1) one or more positive respiratory secretion culture (sputum, tracheal aspirate, or broncho-alveolar lavage [BAL]) for AB according to the positive criteria defined in the next paragraph; (2) acute pulmonary infiltrations on chest x-ray (new, progressive or persistent infiltrate or cavitation); (3) two or more of the following findings: fever $> 38^{\circ}\text{C}$, leukocytosis ($\geq 12,000$ WBC/ μL) or leukopenia (< 4000 WBC/ μL), altered mental status in the elderly with no identifiable causes; new-onset purulent sputum or change in sputum character; worsening pulmonary gas exchange; new onset or worsening cough or dyspnea or tachypnea; rales or bronchial breathing; and (4) infections that could not be attributed to other sources [2, 13, 14].

The respiratory secretions were quantitatively cultured to meet the following criteria for the diagnosis of ABP: bacterial

counts $\geq 10^3$ colony-forming units per milliliter (cfu/mL) in the protected specimen brush and/or 10^4 or 10^5 cfu/mL in a BAL fluid specimen or 10^6 cfu/mL in a tracheal aspirate [2, 13]. For the semiquantitative culture, the cfu/mL was determined by the four-quadrant method and classified as follows: 0 = no growth; 1+ = 10^2 cfu/mL; 2+ = 10^3 cfu/mL; 3+ = 10^4 cfu/mL; and 4+ = 10^6 cfu/mL [3, 15].

The exclusion criteria: Patients who received less than three days of anti-infective treatment were excluded. These patients did not receive effective anti-infective treatment due to death of acute cerebral hernia or discharging automatically from the hospital without medical advice.

Treatments

Upon ABP diagnosis, patients were immediately quarantined, and strict precautions were taken to prevent cross-transmission [16]. The empirical antimicrobial regimen was maintained or adjusted on the basis of microbiological culture results [2, 17]. The common antibiotics used for AB were meropenem (2 g/8 h), cefoperazone/sulbactam (3 g/8 h), and tigecycline (100 mg twice a day in the first 48 h and then at 50 mg twice a day) as previously reported [1, 18].

As part of the NICU, there is a three-bed LTLFW that admitted highly contagious patients with uncontrollable fever to control fever and prevent cross-infection after diagnosed as MDR-ABP. Due to the limited beds, only a portion of MDR-ABP patients were admitted in LTLFW. LTLFW was equipped with an independent temperature control system that maintained the room temperature at 19°C, while the temperature of our regular NICU is non-constant which fluctuated from 22°C to 30°C with the seasons. The patients included in this study were divided into two groups: those who were treated in regular NICU during their hospital stay (non-LTLFW group) and those who were transferred from regular NICU to LTLFW (LTLFW group). Patients transferred to LTLFW were still treated by the team of doctors who previously managed them. For these patients in the NICU, every three patients were managed by a nurse and all patients share other types of staff. Standard precautions and strict hand hygiene practices were executed by all the staff.

The clinical recovery of ABP patients was defined as simultaneous normalizations of highest body temperature ($\leq 38^\circ\text{C}$), leukocyte count ($\leq 10,000$ WBC/ μL), $\text{PaO}_2/\text{FIO}_2$ ratio (> 187), and no or 1+ in AB culture of respiratory secretion [3, 17].

Environmental temperature data

AOTs data were collected from the publicly database and presented as monthly averages in the city of Tianjin [19]. Four seasons were defined as the spring (March 1 to

May 31), the summer (June 1 to August 31), the autumn (Sept. 1 to Nov. 30), and the winter (Dec. 1 to the end of Feb).

Bacterial isolates

Microbiological results were obtained from the patients recruited between January 2013 and December 2017. Clinical isolates were identified using the Vitek 2 Compact System (bio-Merieux, Marcy-L'Etoile, France). We analyzed the first isolate from the same body location of a given patient during his or her hospital stay, regardless of whether it was colonization or infection. Repeatedly isolated strains were excluded [20]. The percentage of AB in total bacterial isolates (PABTBI) were calculated to exclude outbreaks or screening, which might exaggerate the number of AB.

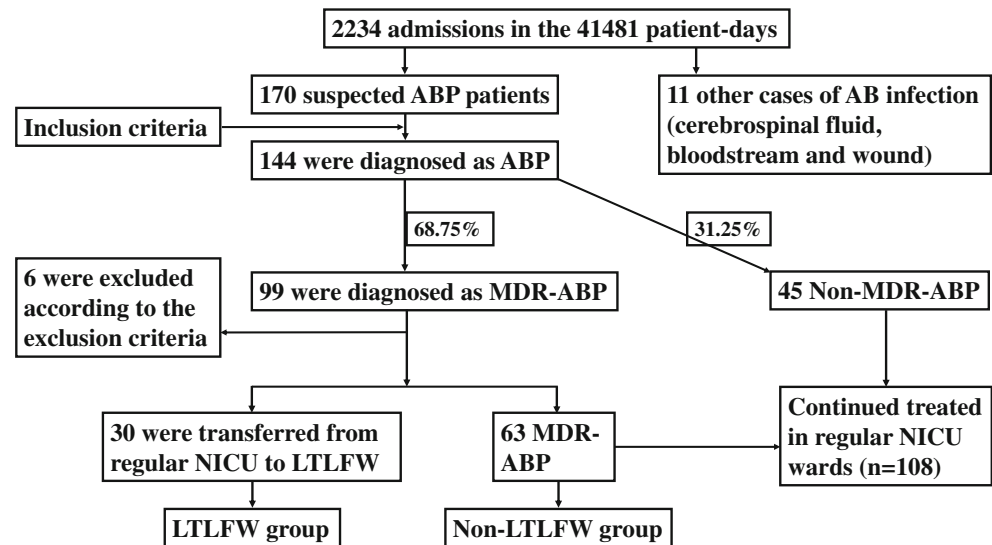
AB culture and antibiotic susceptibility

The AB strains of 62,000 and 63,165 were cultured and analyzed with *Klebsiella pneumoniae* 62,907–2 as the control strains. All strains were randomly selected from clinical isolates. The impact of temperatures on the antimicrobial susceptibility was measured using a standardized disk-diffusion method (Kirby-Bauer) following the guidelines of the Clinical and Laboratory Standards Institute [21]. Briefly, bacterial strains were diluted to a final density of 0.5 and 0.005 McFarland units with phosphate-buffered saline, plated on Columbia blood agar plates (bio-Merieux, Marcy-L'Etoile, France) and cultured for 18 h at 20°C, 25°C, 30°C, or 35°C to allow the formation of bacterial colonies. In parallel, the bacterial broth was evenly spread onto Mueller-Hinton agar plates (0.5 McFarland, bio-Merieux, Marcy-L'Etoile, France). A cefoperazone/sulbactam (SCF) disk (75/30 mg per disk) (Oxoid Ltd., Basingstoke, Hampshire, UK) was then placed in the center of an inoculated plate and incubated at 20°C, 25°C, 30°C, or 35°C for 18 h. The size of the inhibition zone surrounding a SCF disk was then measured.

Quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR)

mRNAs for the genes encoding resistance–nodulation–division (RND) pump (*adeB*, *adeJ*, and *adeG*), outer membrane protein A (*OmpA*), biofilm-associated protein (*Bap*), biofilm synthesis gene (*bfs*), and Class 1 integron (*intI1*) were quantitatively amplified in bacteria grown at 20°C or 35°C using qRT-PCR. These factors were chosen because of their demonstrated roles in the viability, resistance, and virulence of AB [22, 23]. *OmpA* is a major AB virulence factor, essential for killing host cells, whereas both *Bap* and *Bfs* support the formation of biofilm, which protects the bacteria from host immune response. The formation of biofilms results in persistent and difficult-to-treat chronic infections [23]. RND pumps and

Fig. 1 Schematic illustration of the patient inclusion and grouping. AB, *Acinetobacter baumannii*; ABP, *A. baumannii* pneumonia; LTLFW, low temperature laminar flow ward; MDR, multidrug resistance; NICU, neurosurgical intensive care units



intI1 play a crucial role in intrinsic and acquired antibiotic resistance and are also vital for exhausting biocides, dyes, detergents, and organic solvents from AB. Three RND pumps have been reported in AB: AdeABC, AdeIJK, and AdeFGH [22, 24]. In addition, Fsr (Ferric siderophore receptor), which has been shown by De Silva et al. to upregulation at low temperature, was used as the control gene [23]. Table S1 lists the primers used for qRT-PCR. The expression of target genes was normalized against 16SrRNA, which served as the baseline control, using the $2^{-\Delta\Delta C_T}$ method. Total RNA was extracted using an RNA Purification Kit (Biomiga, San Diego, USA) according to the manufacturer's instructions.

Statistical analysis

Continuous variables were expressed as mean \pm standard deviation or median (25th–75th percentile) as indicated in each specific data set. Categorical variables were presented as frequencies and percentages [no (%)]. Categorical variables were compared between groups using the Pearson chi-square test or continuity-adjusted chi-square test. Shapiro–Wilk test was used to determine whether the data was normally distributed. Unpaired Student's t test, one-way ANOVA analysis of variance with Bonferroni correction, Mann–Whitney U test, or Kruskal–Wallis H test were conducted for continuous variables according to data distribution. We also evaluated the effect of the LTLFW on the recovery of MDR-ABP (primary outcome) using multivariate logistic regression models (Stepwise) to adjust for confounding. The demographics, primary diseases, patient's condition before ABP diagnosis, and treatment situation were included in the model. Pearson's correlation and linear regression were used to estimate the association among monthly average AOTs and numbers of ABL. Data were analyzed using SPSS version 22.0 (SPSS Inc.,

Chicago, IL, USA) and R (version 3.6.1). Statistically significant level was defined at a P value of < 0.05.

Results

LTLFW improved clinical outcomes of MDR-ABP patients

During the 5-year study period, a total of 170 suspected ABP patients were enrolled among the total of 2234 admissions in the 41,481 patient-days; only 11 other cases of AB infection (cerebrospinal fluid, bloodstream and wound) were documented. Among them, 26 patients were excluded as they were determined not to be AB infected. And the remaining 144 were diagnosed as ABP on the basis of clinical symptoms and microbiological tests, leading to an overall incidence of 6.45% or 3.47/1000 patient-days. And 99 patients of them were diagnosed as MDR-ABP (MDR rates, 68.75%), with an overall incidence of MDR-ABP being 4.43% or 2.39/1000 patient-days. Six MDR-ABP patients were excluded according to the exclusion criteria. Thirty patients with MDR-ABP were transferred from non-LTLFW to LTLFW wards, with the rest 63 MDR-ABP and 45 Non-MDR-ABP patients continued treated in non-LTLFW during their hospital stays (Fig. 1).

The clinical characteristics were comparable between the MDR-ABP patients in LTLFW and those in non-LTLFW groups. There was no statistical significance observed in terms of the demographics, primary diseases, patient's condition before ABP diagnosis, and other treatment situations between the two groups (Table 1). Patients in LTLFW group had significantly improved outcomes as compared to those in non-LTLFW group, defined by high rates of recovery from MDR-ABP (73.33% vs. 50.79%, $P = 0.039$) and overall good prognosis (66.67% vs. 39.68%, $P = 0.015$), and by a low last WBC counts before hospital discharge (6.91 vs. 10.14, $P = 0.045$) or in-

Table 1 Characteristics of MDR-ABP patients

MDR-ABP patients	LTLFW (n = 30)	Non-LTLFW (n = 63)	P
Demographics			
Sex: male	21(70.00%)	50(79.37%)	0.320 ^a
Age (years)	62.00(47.50,74.75)	59.00(50.00,69.00)	0.521 ^c
Primary diseases			
Intracerebral hematoma	18(60.00%)	35(55.56%)	0.686 ^a
Traumatic brain injury	9(30.00%)	17(26.98%)	0.762 ^a
Others	3(10.00%)	11(17.46%)	0.528 ^b
Patient’s condition before ABP diagnosis			
Glasgow coma scale on admission	7.00(4.00,10.25)	8.00(6.00,13.00)	0.067 ^c
Last WBC before ABP, 10 ⁹ /L	12.42(9.62,16.39)	11.46(9.21,17.27)	0.440 ^c
Surgery rates before ABP	16(53.33%)	43(68.25%)	0.163 ^a
Trachea intubation rates before ABP	19(63.33%)	42(66.67%)	0.752 ^a
Tracheotomy rates before ABP	20(66.67%)	34(53.97%)	0.246 ^a
Ventilator rates before ABP	19(63.33%)	43(68.25%)	0.638 ^a
Hospital stays before ABP	12.00(8.00,18.50)	14.00(8.00,21.00)	0.559 ^c
Types of using antibiotics before ABP	1.00(1.00,2.00)	2.00(1.00,3.00)	0.106 ^c
Total duration of using antibiotics before ABP	8.50(0.75,14.50)	6.00(2.00,18.00)	0.905 ^c
Treatment situation			
Patient rates using meropenem	21(70.00%)	47(74.60%)	0.640 ^a
Days of using meropenem	11.00(6.50,19.00)	12.00(6.00,22.00)	0.942 ^c
Patient rates using cefoperazone/sulbactam	27(90.00%)	55(87.30%)	0.973 ^b
Days of using cefoperazone/sulbactam	14.50(8.75,23.50)	12.00(7.00,20.00)	0.298 ^c
Patient rates using tigecycline	12(40.00%)	16(25.40%)	0.151 ^a
Days of using tigecycline	11.00(5.25,16.00)	8.00(6.00,11.75)	0.280 ^c
Hospital stays in NICU	38.50(21.00,61.00)	30.00(19.00,45.00)	0.197 ^c
Total hospital stays	54.50(28.75,82.75)	52.00(34.00,89.00)	0.767 ^c
Primary outcome			
Rate of MDR-ABP recovery	22(73.33%)	32(50.79%)	0.039 ^{a*}
Secondary outcome			
Last WBC before hospital discharge, 10 ⁹ /L	6.91(5.14,11.15)	10.14(6.76,15.39)	0.045 ^{c*}
Rate of overall good prognosis	20(66.67%)	25(39.68%)	0.015 ^{a*}
In-hospital mortality	3(10.00%)	20(31.75%)	0.044 ^{b*}

NOTE. ABP, A. baumannii pneumonia; LTLFW, low-temperature laminar flow ward; MDR, multidrug resistance; NICU, neurosurgical intensive care units; WBC, white blood cell; ^a Pearson chi-square test, ^b continuity-adjusted chi-square test, ^c Mann–Whitney U test

hospital mortality (10.00% vs. 31.75%, P = 0.044) on univariate analysis. Multivariate logistic regression analysis also revealed that LTLFW is effective in promoting the recovery of MDR-ABP (odds ratio, 2.8956 [95% confidence interval,

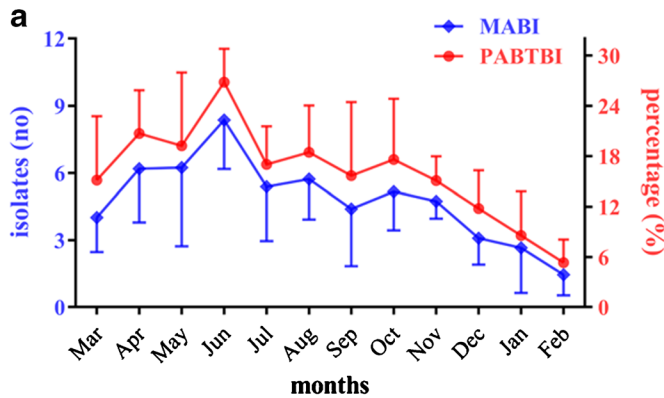
1.0513–8.6976], P = 0.462), after adjustment for the demographics, primary diseases, patient’s condition before ABP diagnosis, and other treatment situations (Table 2). The influence of other factors is very small compared with LTLFW.

Table 2 Outcomes of multivariable logistic regression analyses of the independent influence factors on the recovery from MDR-ABP

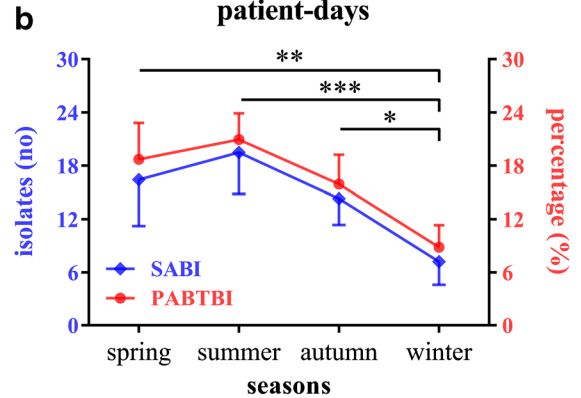
Characteristics	Recovery of MDR-ABP		
	β	OR (95%CI)	P
Intercept	0.2325	1.2618 (0.3101,5.2149)	0.7444
LTLFW	1.0632	2.8956 (1.0513,8.6976)	0.0462 [*]
Last WBC before ABP	−0.0492	0.9520 (0.8874,1.0171)	0.1484
Hospital stays before ABP	−0.0514	0.9499 (0.9005,0.9962)	0.0436 [*]
Days of using cefoperazone/sulbactam	0.0518	1.0531 (1.0054,1.1125)	0.0436 [*]
Total Hospital Stays	0.0085	1.0085 (0.9996,1.0209)	0.1215

Note; ABP, A. baumannii pneumonia; CI, confidence interval; LTLFW, low temperature laminar flow ward; MDR, multidrug resistance; OR, odds ratio; WBC, white blood cell; ^{*} Bold denotes statistical significance (P < 0.05)

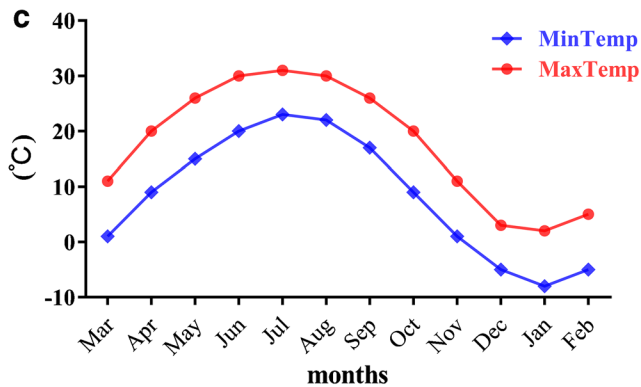
Monthly average ABI and PABTBI per 500 patient-days



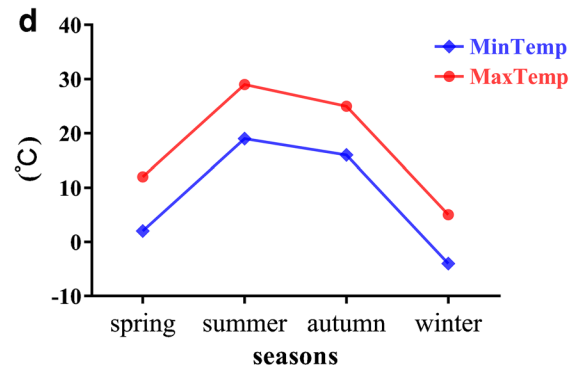
Seasonal average ABI and PABTBI per 1500 patient-days



average daily temperatures per month



average daily temperatures per season



Correlation between monthly average ABI and AOTs

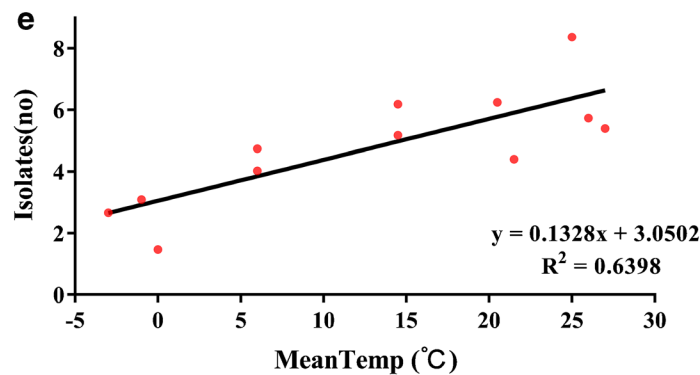


Fig. 2 The average ABI were substantially low in the winter months and positively associated with mean AOTs. (a) The monthly average ABI (one-way ANOVA analysis, $P < 0.001$) and the PABTBI (one-way ANOVA analysis, $P < 0.001$) per 500 patient-days were substantially low in the winter months. (b) The average ABI in the winter were significantly lower than these in the other three seasons per 1500 patient-days, one-way ANOVA analysis, $P = 0.001$. Bonferroni test, *** $P = 0.001$, ** $P = 0.013$, * $P = 0.078$. The PABTBI was also lower in the winter than in the other three seasons, One-way ANOVA analysis, $P < 0.001$. Bonferroni test, *** $P < 0.001$, ** $P = 0.001$, * $P = 0.021$. (c, d)

The monthly and seasonal average daily temperatures in the city of Tianjin. (e) The numbers of ABI were positively associated with monthly AOTs, $y = 0.1328x + 3.0502$, $R^2 = 0.6398$, $P = 0.002$. ABI, *A. baumannii* isolates; AOTs, ambient outdoor temperatures; MABI, monthly *A. baumannii* isolates; MaxTemp, the average daily maximum temperatures; MeanTemp, the average daily minimum temperatures; no, number; PABTBI, the percentage of AB in total bacterial isolates; SABI, seasonal *A. baumannii* isolates; °C, degree Celsius

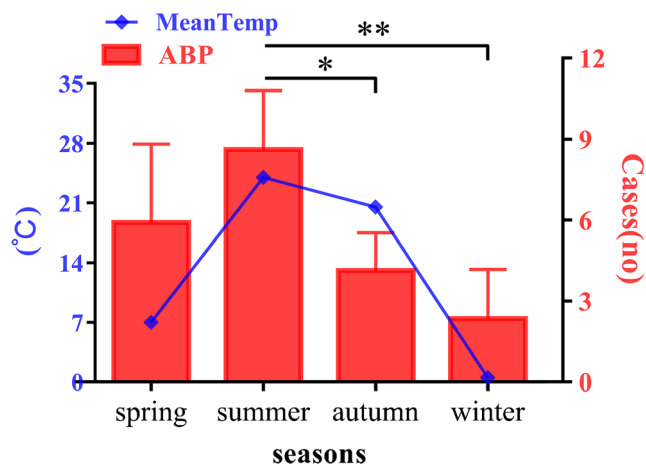


Fig. 3 The new ABP cases in summer were significantly higher than those in winter and autumn. One-way ANOVA analysis, $P = 0.002$. Bonferroni test, $**P = 0.002$, $*P = 0.027$. ABP, the new cases of ABP patients in each season; MeanTemp, the average daily temperatures of each season; no, number; °C, degree Celsius

Seasonal and temperature trends in ABI and ABP patients

A total of 2447 bacterial isolates were selected from 2013 to 2017. The top ten bacteria isolates were the Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis* and the Gram-negative *K. pneumoniae*, AB, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Serratia marcescens*. AB was the second most common and was identified as the pathogen in 401 (16.39%) of the 2447 strains. The monthly average total patient-days were 691.35 ± 89.98 , which were relatively consistent during the entire study period (one-way ANOVA analysis, $P = 0.334$, Table S14). The average numbers of ABI among NICU patients were substantially lower in the winter months than in other seasons and were associated with mean AOTs (Fig. 2).

There is no significant difference among the monthly or seasonal average of the total bacterial isolates (ATBI) from 2013 to 2017 (Fig. S1), but the average ABI and PABTBI were substantially lower in the winter months than in the rest seasons and increased during the summer, reaching a plateau in June (Fig. 2a, b). The highest monthly average ABI was in June, 4.73 times higher than the lowest in February (8.37 vs. 1.46; $P < 0.001$; Fig. 2a). Figure 2c and Fig. 2d show that the monthly and seasonal average daily temperatures in the city of Tianjin. There was a close correlation between monthly AOTs and monthly average numbers of ABI (Pearson's $r = 0.800$, $P = 0.002$). Linear regression showed that temperature was linearly predictive of increasing numbers of ABI. The numbers of ABI were positively associated with monthly AOTs; there is a 4.35% increase in the monthly average ABI for each 1°C increase (Fig. 2e, $R^2 = 0.6398$, $P = 0.002$). The numbers of new ABP cases in the summer were significantly higher than those in the winter and autumn per 1500 patient-days over the 5-year period (Fig. 3, $P = 0.002$).

However, there is no significant difference among the seasonal average of the other strains in the top five bacteria tested over the 5-year study period, including *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, and *S. maltophilia* (Tables S2–S6).

Low-temperature reduced AB viability, virulence, and antibiotic resistance

K. pneumoniae, the top one bacterium tested during the 5-year study period, was selected as the control strain. As shown in Table 1, cefoperazone/sulbactam is the drug with the highest utilization rate in the treatment of multidrug resistance *Acinetobacter baumannii* pneumonia in our NICU. Therefore, cefoperazone/sulbactam was used for testing the impact of temperatures on antibiotic resistance. Consistent with the clinical observations, AB growing at 20°C and 25°C had a significantly reduced viability and were more sensitive to antibiotics as compared to those growing at 35°C ($P < 0.001$). There was no significant difference in AB growth at 30°C and 35°C (Fig. 4). We observed the upregulation of *Fsr* in AB cultured at 20°C compared to those at 35°C ($P = 0.012$). In contrast, the expression of genes encoding *adeB*, *adeG*, *adeJ*, *OmpA*, *Bap*, *bfs*, and *intI1* were considerably reduced at 20°C ($P < 0.005$, Fig. 5). Together, these findings suggest that the vitality, antibiotic resistance, and virulence of AB are significantly reduced in the bacteria growing at 20°C.

Discussion

We have conducted a study to define the influence of environmental temperature on clinical outcomes of MDR-ABP in NICU patients. This clinical study was synergized with a microbiological study of the temperature-related characteristics of AB bacteria. We made several key observations.

First, we demonstrated that patients with MDR-ABP treated in a LTLFW ward had a higher rate of recovery from MDR-ABP and overall good prognosis, resulting in significantly reduced in-hospital mortality (10.00%) as compared to those in regular NICU wards (31.75%). Our finding is also consistent with the previous reports that the crude mortality rates of patients with MDR-AB infections were 26%–47.8%, with 10%–43% attributable mortality [20, 25–27]. In addition, we found that the overall incidence of ABP (6.45%, 3.47/1000 patient-days) in our NICU was obviously lower than the incidences (17.00–27.63%, 4.18–14.26/1000 patient-days) have been reported [27–29]. The PABTBI of 16.39% was also lower than that of 19.1%–38.7% in other ICUs as previously reported [30, 31]. The improved outcomes were observed in LTLFW patients, and the reduced infectious rate suggests that LTLFW provides a more effective way to treat infections of ABP patients in NICU.

Second, we observed seasonal or temperature variations in the incidence of ABI and ABP in China, consistent with previous reports in other countries [32–34]. This may be the

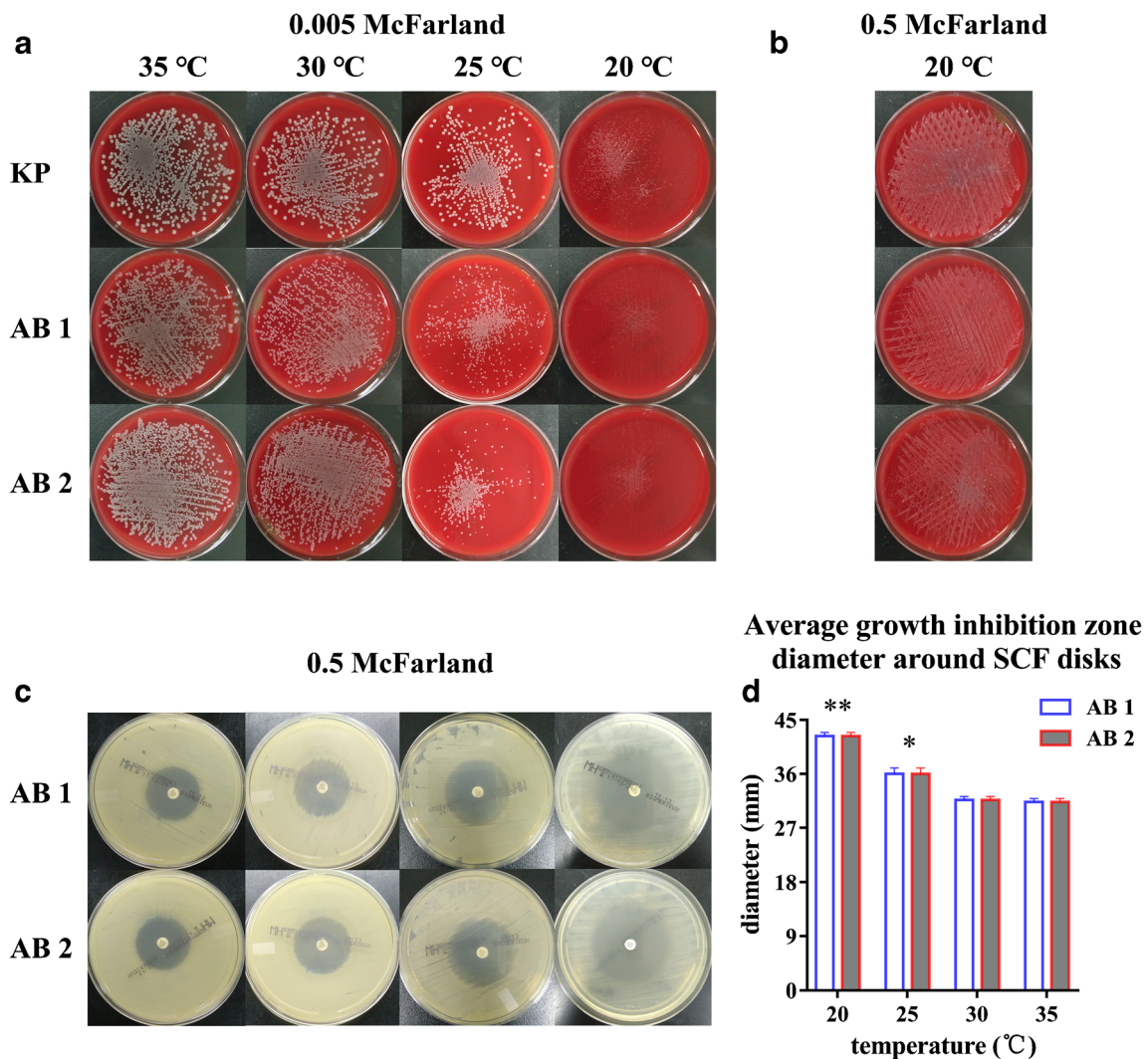


Fig. 4 AB growing at 20°C and 25°C had significantly reduced viability and antibiotic resistance than 35°C. (a, b) AB growing at 20°C and 25°C had obviously smaller and fewer colonies as compared to *K. pneumoniae*. (c, d) The zone diameter in 20°C and 25°C were obviously longer than 30°C and 35°C, one-way ANOVA analysis, $P < 0.001$. Bonferroni test,

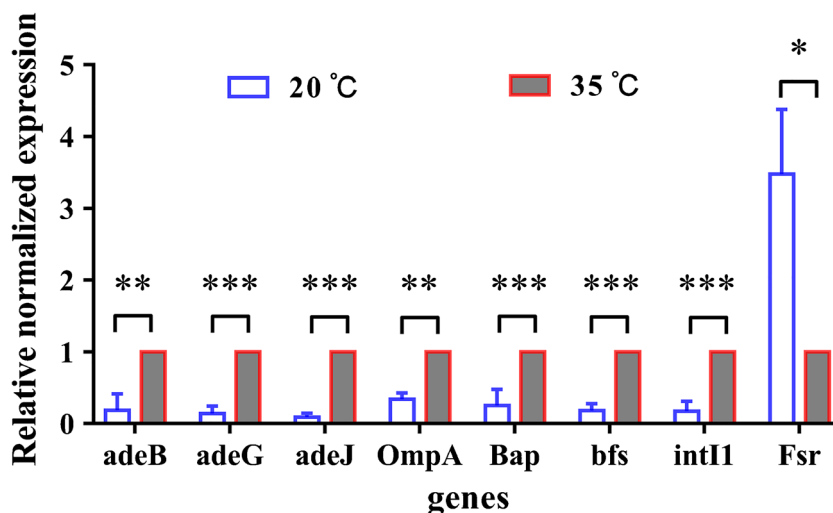
**20°C vs. 35°C (42.33 ± 0.58 vs. 31.33 ± 0.58 , $P < 0.001$), 20°C vs. 25°C (42.33 ± 0.58 vs. 36.00 ± 1.00 , $P < 0.001$), *25°C vs. 35°C (36.00 ± 1.00 vs. 31.33 ± 0.58 , $P < 0.001$). AB 1, AB 62000; AB 2, AB 63165; KP, *K. pneumoniae* 62,907–2; SCF, Cefoperazone/Sulbactam disk; °C, degree Celsius

reason why AB infections are particularly common in the summer or tropical climates [12, 28, 35], where GNB, especially those environmentally acquired, proliferate faster and have enhanced virulence, increasing their colonization and infection [36–39]. High temperatures may also promote the formation of a biofilm “bloom” of *Acinetobacter* species [40]. Similar seasonal variations have also been reported for other GNB such as *K. pneumoniae* and *E. coli* [37, 38, 41–43], as compared to Gram-positive infections that are more common in the winter [9, 44, 45]. Interestingly, we observed the seasonal variations primarily for AB, not for other strains of the top five bacteria tested.

Third, the microbiological trial indicates that AB grew slowly and had reduced antibiotic resistance in low temperature environment ($P < 0.001$). Furthermore, the

expression of genes related to vitality, virulence, and antibiotic resistance of AB is significantly reduced when they were cultured in 20°C compared to those grew at 35°C. De Silva et al. suggested that the upregulation of *Fsr* may be due to lower iron transport efficiency at low temperature [23]. This finding may explain why patients with MDR-ABP treated in LTLFW wards achieved significantly improved outcome. Owing to the ability to survive on environmental surfaces and patients skin, AB strains can cause widespread contamination and cross-infection in the hospital environment [12, 27]. An increased density of environmental AB will increase the probability of human’s exposure. The ability to invade human tissues may increase owing to the expression of virulence factors, and increased resistance makes it difficult to cure.

Fig. 5 Relative expression of genes encoding *adeB*, *adeG*, *adeJ*, *OmpA*, *Bap*, *bfs*, *intI1* and *Fsr* at 20°C and 35°C. *** $P < 0.001$, ** $P < 0.005$, * $P = 0.012$. *Bap*, biofilm-associated protein; *bfs*, biofilm synthesis gene; *intI1*, Class 1 integron; *Fsr*, ferric siderophore receptor; *OmpA*, outer membrane protein A; °C, degree Celsius.



The limitation of this report is that this small sample size, and retrospective study was performed in a single-center, and thus, prospective randomized trials are required to determine the effect of temperature on AB infections. It is important to note that other climatic variables, such as humidity and precipitation that were not examined in this study may confound the correlations between AB infections and temperature.

Conclusions

We reported that substantially decreased AB infectious in the winter months and lower AOTs. LTLFW might be reasonable for promoting the recovery of MDR-ABP patients because a low-temperature environment reduces the density, virulence, and antibiotic resistance of AB at genetic levels. This finding is significant in improving the prognosis of patients with MDR-ABP.

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Authors' contribution RCJ, ZTG and HLL contributed substantially to the collection of literature, the study design, the data interpretation, and the writing of the manuscript. RCJ and HJY reviewed and revised the articles, and performed the quality assessment. JL, ZTG and ZDH performed microbiological trials. DQZ, XHL, CG, JHH, YQ and YMS performed data collection and statistical analyses. WQ, SA, YT, and JS performed the interpretation of data and study oversight. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest All authors report no conflicts of interest relevant to this article.

Ethical approval The retrospective collection of data from patients and their analyses have been approved by the ethics committee of Tianjin Medical University General Hospital (approval number: IRB2018-YX-128).

Statement of informed consent For this type of study, formal consent is not required.

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