Temperature effect on carbapenemase-encoding plasmid transfer

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Abstract

Bacteria that cause human infections can acquire antibiotic resistance, and several factors may play a role. Although temperature is known to affect bacterial growth *in vitro* and regulate the transfer of genes encoding antibiotic resistance, there is little evidence of changes in antibiotic resistance with ambient temperature. We investigated the distribution of antibiotic resistance at different ambient temperatures and evaluated whether temperature affected the transmission of carbapenemase-producing *Enterobacterales* (CPE). The study on *Klebsiella pneumoniae* from eight sentinel hospitals of the Korea Global Antimicrobial Resistance Surveillance System (Kor-GLASS) between 2017 and 2021 was included in the analysis. Conjugation experiments were performed at different temperatures for strains harboring representative carbapenemase genes. The resistance rates to most antibiotics, including carbapenems, varied significantly according to ambient temperature (P< 0.047), except for aminoglycosides. The optimal conjugation temperature for bla_{KPC}-carrying plasmids was 25°C (P= 0.030) and plasmids carrying bla_{NDM} showed the highest conjugation frequency at 30°C (P= 0.007). The bla_{KPC}-IncF showed higher stability at 25°C than at 30°C (P= 0.032) or 37°C (P= 0.047), and the plasmid stability of bla_{KPC}-IncX3 was the lowest at 37°C (P= 0.047). The bla_{NDM}-IncF was very stable at 30°C, and bla_{NDM}-IncX3 was the highest at 30°C and the lowest at 37°C (P= 0.049). In conclusion, we confirmed that carbapenemase gene transmission was optimal at 25–30°C. This suggests that more antibiotic resistance-related genes can be transferred in warmer seasons. Therefore, we suggest that ambient temperature plays an important role in the spread and transfer of CPE.

Introduction

Antibiotic resistance is one of the most important public health threats worldwide and is expected to rapidly promote resistance to existing antibiotics (O' Neill, 2016). Numerous studies have addressed antibiotic resistance, but the effects of many factors, including climate change, remain unclear (Alcorn et al., 2013; MacFadden et al., 2018; Richet, 2012; Schwab et al., 2014). The World Health Organization (WHO) confirmed the important impact of warming on various infectious diseases worldwide. Temperature is a key factor affecting bacterial survival in the presence of antibiotics, and environmental traits are drastically increasing due to climate change (Rodríguez-Verdugo et al., 2020). Infections are associated with environmental factors, such as air, water, temperature, and food, with seasonal changes being particularly important in disease occurrence (Richet, 2012). Temperature affects bacterial growth in vitro and modulates the transfer of genomic material, including genes that encode (or confer) antibiotic resistance (MacFadden et al., 2018). Seasonality has been demonstrated for Gram-negative infections in bloodborne diseases, with peak infection rates in summer being correlated with increasing temperatures. However, the resistance rate to Gram-positive infections has been reported to decrease with increasing temperatures (Alcorn et al., 2013; Richet, 2012; Schwab et al., 2014).

Klebsiella pneumoniae can cause serious nosocomial infections in patients with compromised immune systems, resulting in high mortality rates. In addition, they possess an excellent ability to acquire mobile genetic elements that encode multidrug resistance and high pathogenicity (Yang et al., 2021). Over the last decade, studies of antibiotic-resistant bacteria have primarily focused on their resistance and dissemination mechanisms. Nevertheless, the occurrence of several antibiotic-resistant bacteria are increasing annually, such

as carbapenem-resistant *Enterobacterales* (CRE). The rapid spread of carbapenemase-producing *Enterobacterales* (CPE) is a serious problem for hospitals worldwide, and the prevalence of carbapenemases in *Enterobacterales* is known to be influenced by the environment (Cañada-García et al., 2022). Carbapenem resistance in clinical strains is mediated by carbapenemases encoded by various plasmids (Yang et al., 2021). Carbapenemases are beta-lactamases, including *K. pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), oxacillinase-48 (OXA-48), Verona integron-encoded metallo-β-lactamase (VIM), and imipenemase (IMP), that hydrolyze penicillins, cephalosporins, monobactams, and carbapenems, rendering them ineffective. Frequent exchange of plasmids carrying carbapenemase genes among strains increases the risk of CRE infection (Barbadoro et al., 2021). Among the known groups of genes encoding carbapenemase enzymes, bla_{KPC} and bla_{NDM} are the most prevalent, and the co-occurrence of factors conferring multiple resistance has been frequently reported (Barbadoro et al., 2021).

In this study, we investigated whether ambient temperature affects the antibiotic resistance rate of *K. pneumoniae* and CPE transmission.

Materials and Methods

Data sources and collection

Kor-GLASS is an antimicrobial resistance (AMR) surveillance system that was established by the Korea Disease Control and Prevention Agency (KDCA) in 2016. It provides data for representative non-duplicate clinical isolates of major pathogens collected from sentinel hospitals across the Korean Peninsula, along with patient clinical data (Lee et al., 2018). We selected data on the origin of infection and antibiotic resistance in *K. pneumoniae* from January 2017 to December 2021 from Kor-GLASS. Infection origin was categorized according to the number of hospitalization days at the time of specimen sampling. The monthly average temperature of each region was collected from the Korea Meteorological Administration (http://data.kma.go.kr).

Bacterial isolates & detection of CPE

CPE was collected from 33 general hospitals in the National Laboratory Surveillance System of the KDCA. A total of 2,186 strains of *K. pneumoniae* were collected between 2011 and 2015, of which 749 were CPEs. The isolates were identified using a Bruker MALDI-TOF MS instrument and 16S rDNA sequencing was performed at a national reference laboratory. Antibiotic susceptibility testing (AST) was mainly performed according to minimum inhibitory concentration (MIC) via broth microdilution, following the protocol of the Clinical and Laboratory Standards Institute (CLSI, 2021). All carbapenem-resistant strains were tested for carbapenemase gene expression as previously reported (Poirel et al., 2011).

PCR-based replicon typing (PBRT) and multilocus sequence typing (MLST)

Plasmid characterization was performed by PBRT using a PBRT kit 2.0 (Diatheva, Fano, Italy). This system, consisting of eight multiplex PCR assays, allowed for the identification of the following 30 replicons found in the *Enterobacterales* family: HI1, HI2, I1, I2, X1, X2, X3, X4, L, M, N, FIA, FIB, FIC, FII, FIIS, FIIK, FIB KN, FIB KQ, W,

Y, P1, A/C, T, K, U, R, B/O, HIB-M, and FIB-M. All PCRs were performed according to the manufacturer's instructions and included positive controls (Barbadoro et al., 2021).

Seven targeted housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) were amplified according to Protocol 2 of the MLST Institute Pasteur database (https://bigsdb.pasteur.fr/). This protocol uses primers with universal sequencing tails to amplify all genes at the same temperature and sequences them using the same forward and reverse primers (Barbadoro et al., 2021).

Plasmid transfer by bacterial conjugation

Transconjugants were obtained using carbapenemase-producing *K. pneumoniae* (CP-KP) strains as donors and sodium azide-resistant *Escherichia coli* J53 as recipients. Equal amounts of exponential cultures of the donor and recipient strains were mixed, incubated in Mueller-Hinton broth for 12 h, and spread on brain–heart infusion agar containing sodium azide (10 mg/L) and ceftazidime (1 mg/L). The conjugation frequency according to temperature was measured at 20°C, 25°C, 30°C, 37°C, or 41°C. The presence of CPE was confirmed for each colony using PCR. Plasmid transfer frequency was calculated based on the number of transconjugants per donor (Wang et al., 2018). Conjugation efficiency was measured at least three times for each strain. Experimental data are expressed as the mean ± standard deviation (Liu et al., 2021).

Confirmation of plasmid stability

A single colony of each transconjugant was cultured overnight in Luria–Bertani (LB) broth without antibiotics. Overnight-cultured bacteria were diluted to 1:100 in 10 mL of fresh LB broth; dilution and serial passaging were conducted in a similar manner every 12 h for 120 h. Cultures collected at each step were diluted to 10^{-4}–10^{-6} after a 10-fold serial dilution. The cells were spread on MH plates with or without meropenem (1 μg/mL). Colony-forming units (CFUs) from plates containing antibiotics were considered to be plasmid-positive colonies, whereas CFU from antibiotic-free plates were considered to be the total population in the culture. Plasmid frequency was determined by dividing the plasmid-positive CFUs by the total population CFUs and was representative of the plasmid stability of passaged transconjugants of each type: Higher plasmid frequency suggests higher plasmid stability in the host (Liu et al., 2021).

Statistical analysis

All data were analyzed using SPSS (version 29.0; IBM SPSS, Armonk, NY, USA). Chi-square tests were used to analyze the correlation between temperature and antibiotic resistance. Conjugation frequency and plasmid stability were tested using a *t*-test and analysis of variance (ANOVA), with statistical significance set at $P < 0.05$. The figures were constructed using GraphPad Prism (version 8; GraphPad Software, San Diego, CA, USA).

Results

Antibiotic resistance rates of K. pneumoniae according to ambient temperature

We analyzed the antibiotic resistance rate of *K. pneumoniae* using data from Kor-GLASS. Between January 2017 and December 2021, 9,981 cases of *K. pneumoniae* infections were reported in blood and urine samples from sentinel hospitals in eight regions. For 5 years, the average monthly local temperature in Korea ranged from −4 °C to 29 °C, which we divided into four groups, <0 °C, 0–10 °C, 10–20 °C, and >20 °C, to analyze the

correlation between ambient temperature and antibiotic resistance rates. Significant differences were observed for each temperature group in all antibiotic classes, except aminoglycosides ($P < 0.047$; Table 1). Carbapenems, which have recently become a domestic and international public health problem, also showed a difference in resistance rates with temperature. Our results suggest that ambient temperature affects the antibiotic resistance rate of *K. pneumoniae*.

Genetic characteristics of bla_{KPC}- and bla_{NDM}-encoding K. pneumoniae

We selected 56 CP-KP isolates, including major domestic clones carrying bla_{KPC} (n = 29) and bla_{NDM} (n = 27). They mostly coexisted with extended-spectrum beta-lactamase (ESBL) genes, such as bla_{CTX-M} (data not shown). Isolates carrying bla_{KPC} most often harbored bla_{TEM-1} and bla_{SHV-11} together (37.9%), and isolates carrying bla_{NDM} harbored bla_{TEM-1}, bla_{SHV-11}, and $bla_{CTX-M-28}$ (37.0%). Among the STs of isolates carrying bla_{KPC}, ST258 (34.5%) was the most common, followed by ST307 (24.1%), ST392 (24.1%), and ST11 (10.3%, Table 2). ST14 (26.0%) and ST340 (26.0%) were the most common isolates carrying bla_{NDM}, followed by ST1061 (22.2%), ST307 (7.4%), and ST11 (7.4%, Table 3). Among plasmids carrying the carbapenemase gene, bla_{KPC} belonged to a variety of incompatible groups. IncF was predominant (58.7%), followed by IncX3 (13.8%), IncN (13.8%), and IncH (10.3%). Most bla_{NDM}-carrying plasmids belonged to the IncX3 group (77.8%), along with incompatible groups such as IncF (IncFII and IncFIB) and IncHI.

Effect of temperature on conjugation of plasmids carrying bla_{KPC} and bla_{NDM}

Measurement of the conjugation efficiency of the 56 CP-KP isolates revealed that most strains (82.1%) delivered a plasmid containing the carbapenemase gene to the recipient strain, except for 10 isolates. The carbapenemase gene-harboring plasmids were conjugated using *E. coli* J53 as a recipient at frequencies ranging from 10^{-6} to 10^{-8} transconjugants/donor. The conjugation efficiency of the plasmids carrying bla_{KPC} was $(3.2 \pm 2.5) \times 10^{-7}$ at 20 °C, $(1.1 \pm 0.7) \times 10^{-6}$ at 25 °C, $(7.1 \pm 5.3) \times 10^{-7}$ at 30 °C, $(6.4 \pm 4.8) \times 10^{-7}$ at 37 °C, and $(1.1 \pm 0.8) \times 10^{-7}$ at 41 °C (Table 2 and Supplementary Table 1). The conjugation efficiency of the bla_{NDM}-carrying plasmids was $(2.7 \pm 2.2) \times 10^{-7}$ at 20 °C, $(7.0 \pm 7.3) \times 10^{-7}$ at 25 °C, $(4.5 \pm 3.3) \times 10^{-6}$ at 30 °C, $(2.1 \pm 1.9) \times 10^{-6}$ at 37 °C, and $(3.4 \pm 4.0) \times 10^{-7}$ at 41 °C (Table 3 and Supplementary Table 2). The optimal conjugation temperature for bla_{KPC}-carrying strains was 25 °C ($P = 0.030$), and the conjugation frequencies did not vary at 30 °C and 37 °C ($P = 0.077$, Figure 1A). On the other hand, the bla_{NDM}-carrying strains had the highest conjugation frequency at 30 °C ($P = 0.007$) and were more efficient at 37 °C than at 25 °C ($P < 0.001$, Figure 1B). These findings indicated that the conjugation frequency of the carbapenemase gene-harboring plasmid was not related to specific STs.

Stability of bla_{KPC}- and bla_{NDM}-carrying plasmids according to temperature

Almost 60% of bla_{KPC}- and bla_{NDM}-encoding plasmids were maintained until the 10^{th} passage (Figure 2). The bla_{KPC}- and bla_{NDM}-encoding plasmids showed high stability at 25 °C and 30 °C, respectively. bla_{KPC}-IncF was more stable at 25 °C than at 30 °C ($P = 0.032$) and 37 °C ($P = 0.047$). The plasmid stability of bla_{KPC}-IncX3 was the lowest at 37 °C ($P = 0.047$) and higher at 25 °C than at 30 °C, but with no significant difference ($P = 0.057$). bla_{NDM}-IncF was very stable at 30 °C until the 7^{th} passage, but there was no difference according to

temperature after this passage. The plasmid stability of *bla*$_{NDM}$-IncX3 was the highest at 30 °C and the lowest at 37 °C (P = 0.049), but there was no significant difference between 30 °C and 25 °C (P = 0.387). As shown in Figures 1 and 2, the conjugation frequency and stability of *bla*$_{KPC}$- and *bla*$_{NDM}$-encoding plasmids are dependent on temperature, especially they can be seen that the preferred temperature is different.

Discussion

RM San Lio et al. (2023) reported that while temperatures rise as a consequence of the climate crisis, AMR is increasing, and in fact, increasingly higher temperatures are intimately linked to AMR. A recent study on the distribution of antibiotic resistance across the USA reported that increases in local temperature and population density were associated with increased antibiotic resistance (MacFadden et al., 2018): An increase in temperature of 10°C across regions was associated with an increase in antibiotic resistance of 4.2% and 2.2% for the common pathogens *E. coli* and *K. pneumoniae*, respectively. In addition, Li et al. (2023) reported that a 1°C increase in average ambient temperature was associated with a 1.14- and 1.06-fold increase in carbapenem-resistant *K. pneumoniae* and *Pseudomonas aeruginosa* prevalence, respectively. In contrast, another study found no seasonal variation in *K. pneumoniae* bloodstream infection rates or any association with average temperature (Al-Hasan et al., 2010). Our results do not show that the rate of antibiotic resistance increases with increasing temperature; however, the antibiotic resistance rate of *K. pneumoniae* varied significantly according to temperature over 5 years (Table 1). This indicates that regional temperature has an effect, based on the difference in the antibiotic resistance rate according to ambient temperature. To the best of our knowledge, this is the first study to describe the relationship between antibiotic resistance and ambient temperature in *K. pneumoniae* isolates from South Korea.

According to data from the KDCA, as of 2021, *K. pneumoniae* was the most common cause of CRE infections in Korea (68.6%). The proportion of CPEs that affect the spread of CRE infections has increased from 2019 (57.8%) to 2021 (63.4%), of which more than 90% are KPC (76.2%) and NDM (19.7%) types (Jeong et al., 2022). The carbapenemase gene is usually located on a plasmid, and horizontal gene transfer contributes substantially to the spread of clones in epidemic CPEs to plasmids containing carbapenemase genes (Liu et al., 2021; Zhou et al., 2020).

Among the incompatible plasmid groups associated with carbapenemase genes in *Enterobacterales* (Katlego Kopotsa et al., 2019), the *bla*$_{KPC}$ gene is harbored by IncF, IncI2, IncX, IncA/C, IncR, and ColE1 (Lee et al., 2016), whereas *bla*$_{NDM}$ is mainly located on plasmids of the IncX3 type (Liu et al., 2021). The IncX3 plasmid transports and spreads carbapenemase genes (*bla*$_{NDM}$, *bla*$_{KPC}$, and *bla*$_{OXA-48}$-like), particularly *bla*$_{NDM}$ (Guo et al., 2022). In our study, IncF was dominant for the *bla*$_{KPC}$ gene, and IncX3 was predominant for *bla*$_{NDM}$ (Tables 2 and 3).

IncF plasmids have been shown to participate in the global spread of various antibiotic resistance genes, accounting for nearly 40% of the plasmid-based carbapenemases (Katlego Kopotsa et al., 2019). *bla*$_{NDM}$ is often located on the IncX3 plasmid and is considered the primary vehicle for *bla*$_{NDM}$ transmission. The IncX3 plasmid is highly stable, with a low fitness cost and conjugation efficiency, which can facilitate the rapid and dominant dissemination of antibiotic resistance genes. Various ST strains have been reported to exhibit

different conjugation frequencies within the same species (Guo et al., 2022). As shown in Tables S1 and S2, we revealed that the ST types of the KPC- and NDM-producing isolates were diverse. KPC and NDM have six and seven well-known ST types worldwide, including ST258 and ST11, respectively. In our study, all ST types showed active conjugation frequencies of between 10^{-8} and 10^{-6}.

Several recent studies have reported that plasmid transfer is activated at a specific temperature, suggesting that the spread of antimicrobial-resistant bacteria may be temperature-related (Liu et al., 2021; Wang et al., 2018). Additionally, certain replicon types have been reported to be affected by temperature (Wang et al., 2018; Liakopoulos et al., 2018; Baomo et al., 2021; Anaı¨s Potron et al., 2011), and some plasmids are conjugable only at specific temperatures (Liu et al., 2021). The *bla*$_{NDM-1}$-carrying IncA/C plasmid had the highest transfer rate at 25°C or 30°C, and the transfer efficiency of the IncH1 plasmid was optimal at 22–30°C (Rozwandowicz et al., 2018). Our results showed that the *bla*$_{KPC}$- and *bla*$_{NDM}$-carrying plasmids were best transferred at 25°C for IncF and 30°C for IncX3, respectively, regardless of the ST type. According to Wang et al. (2018), IncX3 plasmids had similar or higher conjugation frequencies than IncFII plasmids at 30°C and 37°C, suggesting that the plasmid replicon types are associated with temperature in the horizontal transfer of antibiotic resistance genes (Wang et al., 2018). IncX3 plasmids can be transferred between various Enterobacterial species over a wide temperature range (Guo et al., 2022). Previous studies reported higher frequencies of plasmid delivery to recipients at 30°C than at 25°C or 37°C (Liakopoulos et al., 2018; Walsh et al., 2011), and that a temperature of 30°C increases conjugation (Anaı¨s Potron et al., 2011). In contrast, another report showed that the IncX3 plasmid has a higher conjugation frequency, higher stability, and lower fitness cost at 37°C (Baomo et al., 2021; Guo et al., 2022). However, the characteristics of the incompatible plasmid group alone cannot sufficiently explain why the optimized transfer temperatures varied between KPC and NDM.

KPC-producing *K. pneumoniae* was first reported in North Carolina, USA, in 1996 (YIGIT et al., 2001); NDM-producing *K. pneumoniae* were detected in New Delhi, India, in 2008 (Yong et al., 2009) and then spread worldwide to become the most common carbapenemase. Despite the worldwide prevalence of KPC and NDM, the incidence of carbapenemases varies both geographically and regionally. In Korea, Spain, and Italy, these two types of carbapenemases are frequently reported (Lee et al., 2016). All three countries are characterized by seasonal climates, with large intra-annual temperature ranges (summer and winter temperatures are often above and below 25°C and 0°C, respectively). The varying occurrence of specific CPEs according to regional temperature are consistent with our results, in which KPC-producing strains showed a high conjugation frequency at 25°C and NDM-producing strains at 30°C. Therefore, ambient temperature would be critical for horizontal gene transfer in *Enterobacterales* (Walsh et al., 2011). Moreover, temperature may affect the worldwide spread of CPE due to high plasmid stability at 25°C and 30°C. This indicates that the spread of genes involved in antibiotic resistance, including CPE, may increase with increasing temperatures.

Our study has some limitations. First, we did not analyze the incidence of infection because data on the total number of hospital patients were not included. Second, we did not consider variables other than temperature (such as humidity). Finally, it was difficult to confirm changes in the resistance rate according to temperature increases because of the brief period of data collection and small sample size. Nevertheless, temperature was clearly related to antibiotic resistance and resistance gene transfer.

Conclusions

Our study revealed clear differences in the occurrence of CPEs according to temperature, with KPC- and NDM-producing strains showing high conjugation frequencies at 25°C and 30°C, respectively. Considering the higher conjugation frequency at lower temperatures than 37°C, environmental transfer may be more important than in the intestine, even for *Enterobacterales* (Walsh et al., 2011). In addition, temperature may affect the worldwide spread of CPE, because of high plasmid stability at 25°C and 30°C. This suggests that, as temperatures increase, the transfer of genes conferring antibiotic resistance, including CPEs, may increase.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ji Woo Yang, Ji-Hyun Nam and Kwang Jun Lee. The first draft of the manuscript was written by Ji Woo Yang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Tables

Table 1. Antibiotic-resistant *K. pneumoniae* isolates according to temperature over a 5-year period.

Antibiotics		No. of resistant isolates (%)				
Class	Drug	<0 °C (N = 795)	0–10 °C (N = 2,644)	10–20 °C (N = 2,928)	≥20 °C (N = 3,614)	X^2
Carbapenems	Meropenem	9 (1.1)	37 (1.4)	61 (2.1)	78 (2.2)	**0.047***
Penicillins	Piperacillin	371 (46.7)	1,125 (42.5)	1,218 (41.6)	1,611 (44.6)	**0.018***
β-lactam-lactamase inhibitors	Ampicillin-sulbactam	345 (43.4)	1,000 (37.8)	1,100 (37.6)	1,477 (40.9)	**0.001****
Cephems	Cefotaxime	307 (38.6)	895 (33.9)	996 (34.0)	1,316 (36.4)	**0.016***
	Ceftazidime	252 (31.7)	710 (26.9)	831 (28.4)	1,076 (29.8)	**0.018***
	Cefepime	291 (36.6)	824 (31.2)	929 (31.7)	1,221 (33.8)	**0.009****
	Cefoxitin	95 (11.9)	286 (10.8)	307 (10.5)	427 (11.8)	0.293
Monobactams	Aztreonam	263 (33.1)	737 (27.9)	871 (29.7)	1,126 (31.2)	**0.009****
Aminoglycosides	Gentamicin	152 (19.1)	511 (19.3)	536 (18.3)	716 (19.8)	0.492
Fluoroquinolones	Ciprofloxacin	287 (36.1)	828 (31.3)	923 (31.5)	1,223 (33.8)	**0.015***

* $P < 0.05$, ** $P < 0.01$

Table 2. Genetic characteristics and conjugation efficiency of *bla*$_{KPC}$-carrying *K. pneumoniae*.

Pathogens	MLST type	Replicon type	Conjugation efficiency	Pathogens	MLST type	Replicon type	Conjugation efficiency
KP-KPC-01	ST258	IncFII	$(9.0 \pm 0.6) \times 10^{-7}$	KP-KPC-15	ST101	IncFIIK	$(3.3 \pm 0.3) \times 10^{-7}$
KP-KPC-02	ST258	IncFII	$(6.5 \pm 0.4) \times 10^{-7}$	KP-KPC-16	ST307	IncFIB	$(4.1 \pm 0.3) \times 10^{-7}$
KP-KPC-03	ST258	IncFIIK	$(1.0 \pm 0.5) \times 10^{-7}$	KP-KPC-18	ST307	IncN	$(14.8 \pm 0.4) \times 10^{-7}$
KP-KPC-04	ST258	IncFIIK	$(4.8 \pm 0.8) \times 10^{-7}$	KP-KPC-19	ST307	IncN	$(2.6 \pm 0.5) \times 10^{-7}$
KP-KPC-05	ST258	IncX3	$(7.8 \pm 0.6) \times 10^{-7}$	KP-KPC-21	ST307	IncN	$(1.1 \pm 0.2) \times 10^{-6}$
KP-KPC-06	ST258	IncX3	$(7.0 \pm 1.4) \times 10^{-7}$	KP-KPC-23	ST307	IncFIB	NT
KP-KPC-07	ST258	IncX3	$(6.8 \pm 3.9) \times 10^{-8}$	KP-KPC-25	ST307	IncFIB	NT
KP-KPC-08	ST258	IncFIB	NT	KP-KPC-26	ST307	IncHI1	NT
KP-KPC-10	ST258	IncX3	$(2.1 \pm 0.3) \times 10^{-7}$	KP-KPC-17	ST392	IncHI2	$(3.5 \pm 0.3) \times 10^{-7}$
KP-KPC-13	ST258	IncFIIK	$(4.4 \pm 0.3) \times 10^{-7}$	KP-KPC-20	ST392	IncFIIK	$(3.4 \pm 0.3) \times 10^{-8}$
KP-KPC-09	ST273	IncN	$(1.0 \pm 0.7) \times 10^{-6}$	KP-KPC-22	ST392	IncFIB	$(2.0 \pm 0.6) \times 10^{-7}$
KP-KPC-11	ST11	IncHI2	$(2.2 \pm 0.4) \times 10^{-7}$	KP-KPC-24	ST392	IncFIB	$(4.8 \pm 1.1) \times 10^{-7}$
KP-KPC-12	ST11	IncL	$(3.9 \pm 0.3) \times 10^{-7}$	KP-KPC-27	ST392	IncFIIK	NT
KP-KPC-14	ST11	IncFIB	$(1.3 \pm 0.1) \times 10^{-6}$	KP-KPC-28	ST392	IncFIB	NT
				KP-KPC-29	ST392	IncFIIK	NT

* NT: not transferred

Table 3. Genetic characteristics and conjugation efficiency of *bla*$_{NDM}$-carrying *K. pneumoniae*.

Pathogens	MLST type	Replicon type	Conjugation efficiency	Pathogens	MLST type	Replicon type	Conjugation efficiency
KP-NDM-01	ST340	IncX3	$(3.7 \pm 1.4) \times 10^{-6}$	KP-NDM-16	ST1061	IncX3	$(1.1 \pm 0.1) \times 10^{-6}$
KP-NDM-02	ST340	IncX3	$(1.7 \pm 0.9) \times 10^{-8}$	KP-NDM-19	ST1061	IncA/C	$(1.8 \pm 1.2) \times 10^{-7}$
KP-NDM-03	ST340	IncX3	NT	KP-NDM-08	ST14	IncX2	$(3.6 \pm 1.5) \times 10^{-6}$
KP-NDM-04	ST340	IncX3	$(7.5 \pm 5.9) \times 10^{-7}$	KP-NDM-14	ST14	IncX3	$(3.6 \pm 0.8) \times 10^{-6}$
KP-NDM-05	ST340	IncX3	NT	KP-NDM-17	ST14	IncX3	$(1.7 \pm 0.6) \times 10^{-6}$
KP-NDM-06	ST340	IncX3	NT	KP-NDM-18	ST14	IncX3	$(1.8 \pm 0.4) \times 10^{-6}$
KP-NDM-07	ST340	IncFIB	$(2.1 \pm 0.5) \times 10^{-7}$	KP-NDM-20	ST14	IncX3	$(1.8 \pm 0.6) \times 10^{-6}$
KP-NDM-09	ST11	IncX3	$(2.3 \pm 1.1) \times 10^{-6}$	KP-NDM-21	ST14	IncX3	$(1.4 \pm 0.2) \times 10^{-6}$
KP-NDM-10	ST11	IncX3	$(3.5 \pm 1.1) \times 10^{-6}$	KP-NDM-22	ST14	IncHI	$(2.1 \pm 0.8) \times 10^{-6}$
KP-NDM-11	ST1061	IncX3	$(1.3 \pm 0.6) \times 10^{-6}$	KP-NDM-23	ST307	IncX3	$(5.7 \pm 2.3) \times 10^{-7}$
KP-NDM-12	ST1061	IncX3	$(8.5 \pm 3.9) \times 10^{-7}$	KP-NDM-24	ST307	IncX3	$(1.0 \pm 0.4) \times 10^{-6}$
KP-NDM-13	ST1061	IncX3	$(9.9 \pm 4.1) \times 10^{-7}$	KP-NDM-25	ST147	IncFII	$(4.9 \pm 0.4) \times 10^{-7}$
KP-NDM-15	ST1061	IncA/C	$(2.2 \pm 1.9) \times 10^{-7}$	KP-NDM-27	ST147	IncX3	$(2.8 \pm 0.5) \times 10^{-6}$
				KP-NDM-26	ST789	IncX3	$(8.4 \pm 2.2) \times 10^{-7}$

* NT: not transferred

Figures

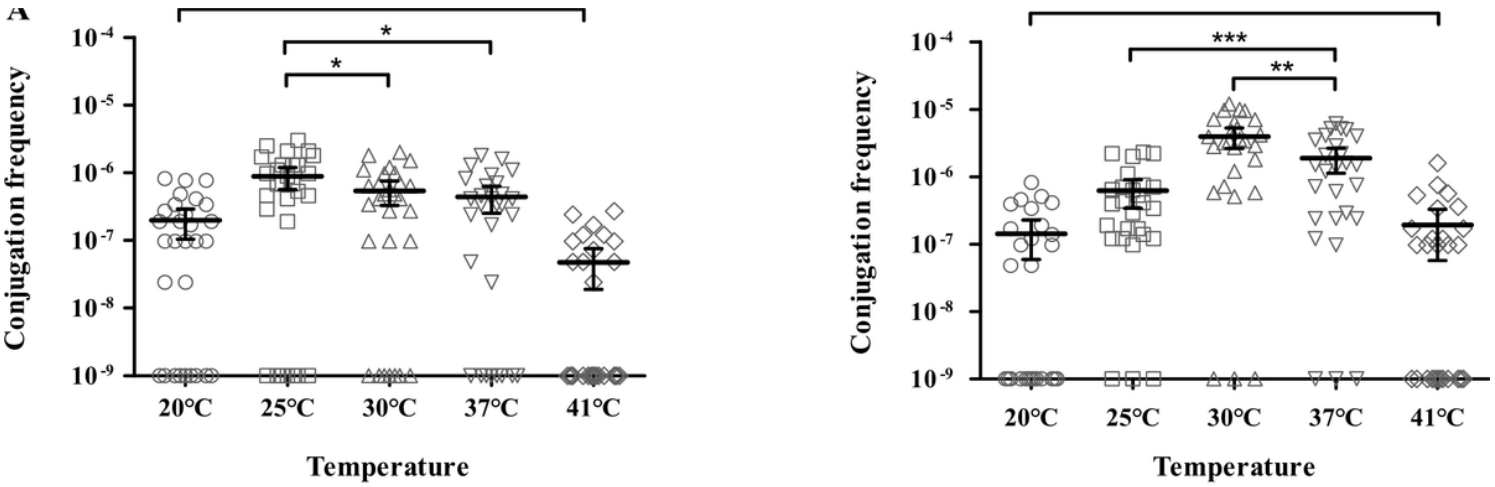

Figure 1

Conjugation efficiency according to temperature. (A) *bla*$_{KPC}$- and (B) *bla*$_{NDM}$-carrying *K. pneumoniae.* Each data point represents an individual. Each bar indicates the average value for a group and error bars represent the 95% CI for the ratio. Differences between different temperature groups are shown as *P < 0.05, **P < 0.01, and ***P < 0.001.

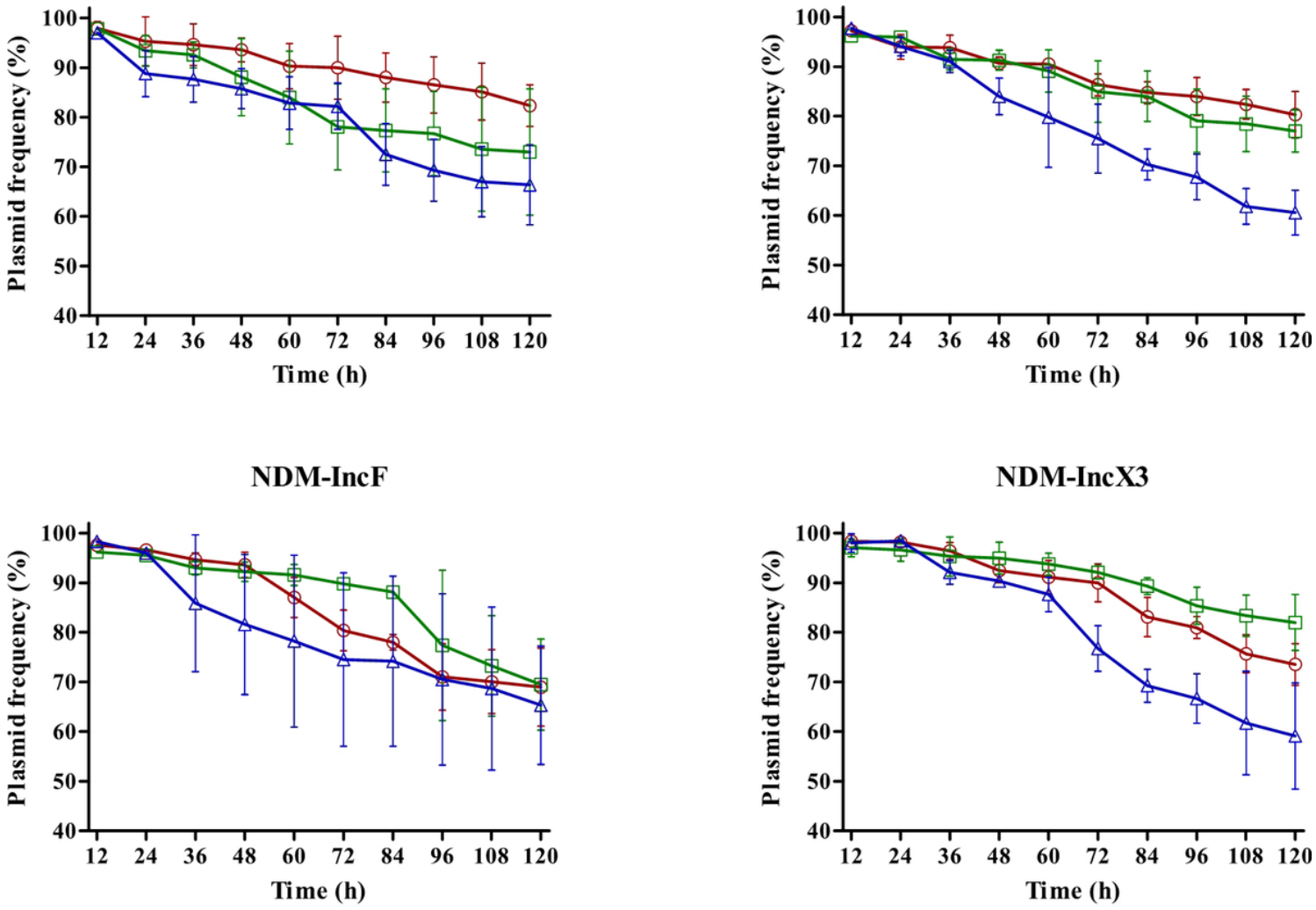

Figure 2

Impact of temperature on plasmid stability. Lines and symbols indicate values at each temperature; 25 °C: red, 30 °C: green, and 37 °C: blue. Error bars represent standard deviation.

Supplementary Files

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