

Table 1. Advantages of Type-2 Chemical Indicator Used in Every Autoclave Cycle

Purpose	Advantages
Sufficient temperature and steam penetration in every cycle	Avoids any uncertainty of air and NCGs gases in the chamber
For porous and hollow items sterilization	Maximum air entrapment so that the type-2 chemical indicator can confirm the total elimination of air and NCGs from the item
A secondary option after physical monitoring	Gives more accuracy by both physical and chemical monitoring and cross checks it
Condition of type-5 or type-6 chemical indicator inside the pack	To avoid sterility check in every package, type-2 chemical indicator in a worst-case load can authorize the entire load.
Incur minimal cost in every load (Type-2 chemical indicator cost is divided by number of items sterilized in a load)	Gives maximum output by assuring complete dismissal of air and NCGs for optimum sterility assurance

Note. NCG, noncondensable gas.

cycle. Every delivery of steam to the sterilizer can have different characteristics that change in every cycle.

To ensure the air and NCG removal process and to control cost, we strongly recommend the use of a type-2 chemical indicator in every cycle (Table 1) by a reusable test device according to the European standard for nonbiological systems for use in sterilizers part 5, specification for indicator systems and process challenge devices for use in performance testing for small sterilizers type B and type S (EN 867-5). This process can confirm that the

sterilizer chamber and packages are free from air and NCGs to assure optimum sterility according to the European standard requirements for medical devices to be designated sterile (EN 556).⁸

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Enhanced survival of ST-11 carbapenem-resistant *Klebsiella pneumoniae* in the intensive care unit

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To the Editor—*Klebsiella pneumoniae*, a gram-negative bacterium of the Enterobacteriaceae, is a well-known and major pathogen. Carbapenems are the mainstream agent of choice against *K. pneumoniae*-producing extended-spectrum β -lactamases (ESBLs). However, carbapenem-resistant *K. pneumoniae* (CRKP) has increased rapidly worldwide and has become an urgent threat to public health. CRKP belongs to various sequence types (STs),

and ST-11 is the dominant type of CRKP in China.¹ However, the factors contributing to the dominance of ST-11 remain largely unknown. Admission to the intensive care unit (ICU) and prolonged length of stay in the ICU are major risk factors for CRKP infection.^{2–4} We hypothesized that ST-11 may have better survival in the ICU than other types of CRKP. We investigated the survival of ST-11 CRKP, a few other types of CRKP, and carbapenem-susceptible *K. pneumoniae* (CSKP) on a polyvinyl chloride (PVC) surface at the room temperature and humidity in a real ICU situation.

We selected 6 ST11 CRKP strains, 4 CRKP of other STs (ST1, ST15, ST37, and ST45) and 1 CSKP strain (ST1229) for study. Bacteria from frozen stocks were cultured on Luria-Bertani (LB) agar overnight at 37°C. A single colony of each strain was incubated in 5 mL LB broth for ~3–5 hours to midexponential growth

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Table 1. Strains and Their Survivals in the Intensive Care Unit

Strain	Recovery Date	Resource	Ward	ST Type	Meropenem MIC, µg/mL	Survival, Days	Carbapenemase Gene	K Type	Biofilm Formation (OD=590 nm)
WCHKP015093	2017/4/5	Rectal swab	ICU	11	128	14	<i>bla</i> _{KPC-2}	KL64	0.056±0.021
WCHKP015521	2017/7/4	Rectal swab	ICU	11	256	32	<i>bla</i> _{KPC-2}	KL64	0.558±0.066
WCHKP015572	2017/7/10	Rectal swab	ICU	11	256	26	<i>bla</i> _{KPC-2}	KL64	0.170±0.071
WCHKP015625	2017/7/24	Rectal swab	ICU	11	64	14	<i>bla</i> _{KPC-12}	KL64	0.259±0.066
WCHKP115016	2018/3/16	Urine	Neurology	11	>512	29	<i>bla</i> _{KPC-2}	KL64	0.017±0.021
WCHKP095845	2016/5/20	Sputum	RICU	1	128	3	<i>bla</i> _{NDM-1}	KL45	0.553±0.051
WCHKP095092	2015/5/13	Blood	ICU	15	128	9	<i>bla</i> _{KPC-2}	KL28	0.370±0.043
WCHKP015642	2017/7/24	Rectal swab	ICU	37	4	14	...	KL38	0.162±0.030
WCHKP015234	2017/5/3	Rectal swab	ICU	45	256	14	<i>bla</i> _{KPC-2}	KL62	0.467±0.051
WCHKP030320	2016/7/21	Blood	Hematology	1,229	≤0.25	11	...	KL125	0.276±0.055

Note. ST, sequence type; OD, optical density; ICU, intensive care unit; RICU, respiratory ICU.

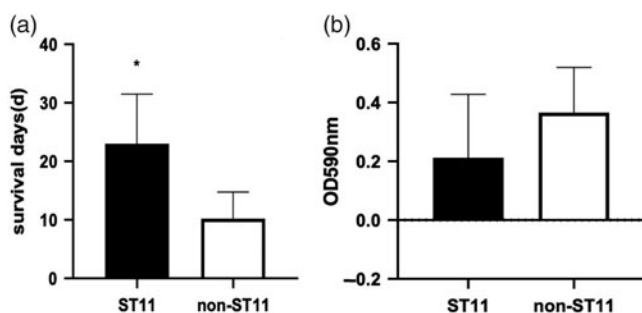


Fig. 1. Survival and biofilm formation of ST11 and non-ST11 *K. pneumoniae*. Panel A, duration of survival days of ST11 and non-ST11 *K. pneumoniae*. Panel B, biofilm formation of ST11 and non-ST11 *K. pneumoniae*.

phase; the mixture was then centrifuged, washed, resuspended in 1 mL PBS, and finally adjusted to 0.5 MacFarland turbidity-equivalent standard (10^8 CFU/mL). Aliquots (10 µL) of each strain were added to wells (3 wells per strain) of a sterile PVC 96-well plate (Corning, NY). The plates were dried in ambient air overnight in a clean biosafety cabinet. Afterward, the plates were put into a paper box, which was placed in a corner of the corridor in an ICU ward of our hospital. This location was away from patient-care areas, and ICU staff were notified of the location to avoid any potential contact. The temperature and relative humidity of the ICU were maintained at $25 \pm 2^\circ\text{C}$ and $54 \pm 4\%$, respectively. To recovery bacterial cells, 200 µL PBS was added into each well and shaken at 85 ± 5 rpm for 60 minutes in a miniature orbital shaker (BETS-M1; Qilinbeier, Haimen, Jiangsu, China). The 100-µL bacterial suspension was streaked on LB agar, which was incubated at 37°C overnight, and the number of colonies was counted manually. The biofilm formation of these strains was facilitated by aerobic incubation at 37°C in a microtiter plate system, as described previously.⁵ All statistics were determined using GraphPad Prism version 8.0.2 software (GraphPad

Software, San Diego, CA). The statistical techniques employed for analysis were descriptive statistics and unpaired *t* test with a Welch correction.⁵ *P* values < .05 were considered statistically significant.

All samples survived for >3 days on the PVC surface in the ICU (Table 1). ST-11 strains exhibited longer survival than non-ST-11 strains ($P = .024$) (Fig. 1, panel A). No significant difference was observed in biofilm formation between ST-11 and non-ST-11 strains in this study ($P = .235$), suggesting that the prolonged survival of ST11 strains was not due to biofilm formation (Fig. 1, panel B). A previous study also found no relationship between biofilm formation of *K. pneumoniae* and the transmission in ICU.⁶

Therefore, ST-11 CRKP shows an advantage in survival days compared to non-ST-11 CRKP, which may contribute to its wide spread in healthcare settings such as the ICU. To our knowledge, this is the first study to demonstrate that ST-11 *K. pneumoniae* strains can survive longer than several other types of CRKP in a real ICU environment. These findings support the essential role of environmental cleaning in preventing the in-hospital transmission of CRKP.

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Transmission of human immunodeficiency virus (HIV) to a family caregiver through a conjunctival blood splash

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To the Editor—Reports of human immunodeficiency virus (HIV) transmission resulting directly from a conjunctival blood splash are extraordinarily rare and are limited by an inability to exclude other exposures and an absence of any phylogenetics, linking the source and recipient's viruses.^{1–6} The risk of acquiring HIV infection from a nongenital mucosal blood splash, is based on distant and very limited data and is stated to be <0.09%.^{2,7} We have, however, identified and confirmed, through phylogenetic analysis and supportive laboratory evidence, an acute HIV infection following conjunctival blood exposure. This report is provided with informed patient consent (caregiver) and their full support to increase knowledge to reduce the future risk to others from such exposures.

The source patient, an adult female, with severe nonverbal autism, had acquired HIV infection through blood transfusions during early childhood. Following confirmation of the source patient's HIV diagnosis, education was provided to family members with ongoing discussion on the importance of avoiding needle sticks, bites, and all blood exposures on open skin. After 20 years of medical care, due to the irreversible nature of multiple medical conditions, intolerance to and unwillingness to take multiple different antiretroviral regimens and following extensive consultations, the source patient was placed on comfort-focused medical care that did not include antiretroviral therapy (ART). In the home, no needles were present, and gloves were used when handling blood or providing hygiene. All razors, toothbrushes, sponges, and other hygiene implements were kept specific to the source patient and were never shared.

An elderly family caregiver had provided full support to the source patient for >20 years. She presented to her family physician complaining of a 7-day history of increasing headache, confusion, backache, profound lethargy, dysphagia, abdominal pain and

weight loss. HIV testing demonstrated an acute HIV seroconversion pattern progressing over 10 days from antigenemia and viremia to antibody seroconversion. Following initiation of ART, HIV replication in the caregiver became suppressed and immunity returned to normal. The source patient's viral load was 113,000 copies/mL and her CD4 count was 81 cells/mm³ (13%) at the time of the caregiver's HIV diagnosis. The HIV serology of the caregiver's husband was negative.

At presentation, when questioned about possible HIV risk exposures, the caregiver reported that she had been regularly providing oral hygiene twice daily to the source patient who, after a dental extraction, had experienced ongoing gum bleeding. Gloves were used on every occasion when blood was visible and/or when fingers were in the mouth, but no eye protection was used. She clearly recalled that ~15 days earlier, she had experienced a single small blood splash to one eye while providing oral hygiene to the source patient. She had not considered the exposure to be significant at the time and did not seek medical attention or postexposure HIV prophylaxis.

To investigate the transmission event using phylogenetics, HIV sequences obtained from the source patient (for earlier genotypic resistance testing and soon after the transmission event) were compared to the caregiver's presenting HIV sequence to determine genetic similarities. Multiple sequence alignment and comparison, including RT Protease sequences of other patients stored in our clinic database revealed nearly perfect identity in the nucleic acid sequences of the source (3 sequences from 3 time points: 2014, 2015, and 2019) and caregiver patient's HIV (1 sequence at presentation). Only 10 unique nucleotide substitutions (with 2014/2015 sequences of the source) and 7 unique nucleotide substitutions (with the 2019 sequences of the source, only one of these changed the amino acid sequence at either time point) were detected between the 2 patients' sequences. Phylogenetic analysis (maximum likelihood method,) general time reversible model (γ distribution with invariable sites), with bootstrap analysis with 1,000 replicates) using sequences of 140 reverse transcriptase (RT) protease region sequences from the clinic data clearly indicated a phylogenetic relationship between the sequences (bootstrap value, 99). Repeating this analysis with >1,000 sequences from

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