Original Article

Determining the resistance of carbapenem-resistant *Klebsiella pneumoniae* to common disinfectants and elucidating the underlying resistance mechanisms

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Introduction: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection is a serious problem in hospitals worldwide, posing a particular risk to immunocompromised patients. Elimination strategies may prevent these drug-resistant bacteria from spreading within hospital environments. Here, the susceptibility of patient-derived CRKP strains to common chemical disinfectants and possible correlations between the presence of drug-resistance genes and increased resistance to disinfectants were investigated.

Methods: The minimum inhibitory (MIC) and the minimum bactericidal concentrations (MBC) of common chemical disinfectants against each CRKP strain were determined using agar dilution; *K. pneumoniae* ATCC700603 served as a standard. The presence of the drug-resistance genes *qacΔE*, *qacA*, *acrA* and *qacE* was determined using PCR.

Results: A total of 27 clinically isolated CRKP strains collected in our hospital from 2011 to 2013 exhibited sensitivity to the following common chemical disinfectants in decreasing order of sensitivity: 75% ethyl alcohol > 2% glutaraldehyde > "84" disinfectant > 0.2% benzalkonium bromide > 2% iodine tincture > 1% iodophor > 0.1% chlorhexidine acetate. Of the 27 strains, 59, 41, 19 and 15% contained *qacΔE*, *qacA*, *acrA* and *qacE* resistance genes; 15% carried *acrA*, *qacΔE* and *qacA*, and 26% carried both *qacA* and *qacΔE*. Comparative analysis indicated that drug-resistance genes were correlated with higher MIC values.

Conclusion: These pan-resistant pathogenic CRKP strains contained various drug-resistance genes and exhibited relatively high resistance to ethyl alcohol, chlorhexidine acetate and iodophor. Monitoring the drug-resistance rates of CRKP strains displaying disinfectant resistance may facilitate appropriate and effective sterilisation and thus preventing the spread of these pan-resistant strains

Keywords: Carbapenem, Resistant, *Klebsiella pneumonia*, Disinfectant

Introduction

Hospital-acquired infections have been steadily increasing over the last few decades due, in part, to the emergence of drug-resistant bacterial strains, which has likely stemmed from the broad use of antibiotics.[1] Among the most prevalent bacteria causing nosocomial infections, *Klebsiella pneumoniae* can lead to serious infections, including urinary tract infections, hospital-acquired pneumonia, intra-abdominal infections, wound infections and primary bacteraemia,[2] especially in immunocompromised patients. Relatively recently, *K. pneumoniae* infection has become a much greater concern, because the bacteria have acquired antibiotic resistance to carbapenem-containing antibiotics, making the infections much more difficult to treat.[3] The first carbapenem-resistant *Klebsiella pneumoniae* (CRKP) case was reported by MacKenzie *et al.* in 1997.[4] Thus far, sporadic cases involving CRKP have been found in USA and China as well as in many other parts of the world.[5,6] Moreover, the incidence of CRKP infection is increasing yearly.[7] Carbapenem-resistant *Klebsiella pneumoniae*-infected patients often have multiple underlying diseases and lower systemic immunity, and effective drugs to eliminate this infection from the patients are limited at present. Carbapenem-resistant *Klebsiella pneumoniae* infections have thus become an independent risk factor for in-hospital death, and strategies to prevent initial infection by eliminating or at least reducing the presence of this bacteria in the clinical environment is of critical

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importance and should be given a high priority by clinicians. Indeed, there is a known high correlation between infected environmental surfaces in hospitals and the spread of hospital-acquired infections.[8] Eliminating bacteria from the hospital environment is especially pertinent for *K. pneumoniae*, as these particular bacteria are easily spread via contact with surfaces on which they have been shown to live for a long time (sometimes >30 months).[8]

The broad application of chemical disinfectants is used as a standard infection control strategy to eliminate pathogens from the hospital environment, especially in the intensive care unit (ICU) and other departments housing critically ill or immunocompromised patients. However, like the emergence antibiotic resistance, it is very likely (and possibly inevitable) that drug-resistant bacteria will also gradually become resistant to the chemical disinfectants commonly used in the clinic, especially since the mechanisms-of-action between antibiotic resistance and disinfectant resistance may be similar.[9] If disinfection resistance does occur but remains unrecognised by hospital staff, inadequate disinfection may lead to ineffective prevention and treatment of infectious diseases, potentially resulting in serious consequences such as outbreaks. Therefore, whether the current sterilisation and disinfection regimens can meet the standard and eliminate the particular CRKP strains present in the hospital environment is an urgent problem that needs to be addressed in each individual hospital.

In the present study, CRKP samples were isolated from patients at the Beijing Tiantan Hospital affiliated with Capital Medical University, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the commonly used chemical disinfectants against each strain of CRKP were evaluated. Moreover, resistance rates were analysed, and the presence of relevant resistance genes was assessed in each CRKP strain. The results from the present study will provide guidance in terms of equipping hospitals and other care-giving institutions with the ability to select which disinfectants can be used within the hospital, which can prevent and avoid ineffective disinfection, reduce the spread of the highly resistant strains and decrease the occurrence of infectious diseases by strictly controlling in-hospital infections. The advantages of this strategy are improving the prognosis of patients with infectious diseases, saving medical expenses and preventing the potential spread of drug-resistant bacterial strains.

This study was approved by the institutional review board of the Beijing Tiantan Hospital and was carried out in compliance with the Helsinki Declaration.

Methods

Bacterial strains

This study was approved by the Ethics Committee of Beijing Tiantan Hospital, and all aspects of the study complied with the Declaration of Helsinki. The Ethics Committee of Beijing Tiantan Hospital specifically approved that only verbal informed consent was required because data were going to be analysed anonymously. We obtained verbal informed consent from each participant and recorded the verbal consents in case report form (CRF). Bacterial strains were isolated from human clinical samples that were collected by the Microbiology Service at Beijing Tiantan Hospital following approved procedures.

K. pneumoniae samples were isolated from 2011 to 2013 from inpatients admitted to Beijing Tiantan Hospital affiliated with Capital Medical University (Beijing, China). Strains were positively identified as carbapenem-resistant using the ATB Bacteria Identifier kit (BioMerieux, France). The standard *K. pneumoniae* strain ATCC700603 and the quality control strain *Escherichia coli* ATCC25922 were purchased from the National Institutes for Food and Drug Control (Beijing, China).

Specimen collection and processing methods

Clinical specimens were collected from blood, urine, stool, wound secretion, sterile body fluid and sputum, among others, and were taken from the same location on each patient within the same time period. *K. pneumoniae* was then isolated from the collected patient samples and cultured.

All specimens were inoculated on blood agar base and Chinese Blue Agar (blood and other body fluids must do enrichment culture), cultured for 24 hours at 35°C. *Klebsiella pneumoniae* colony is usually larger, convex, grey white, bright and adjacent colonies were prone to fusion. If the suspected strains show Gram-negative coccobacillus, double exists, no flagella, non-spore forming, and has obvious capsule, oxidase negative, catalase positive by Gram staining, often preliminary judgement for *Klebsiella pneumoniae*. The strains were identified to species by ATB slab ID32E (purchased from France bioMerieux Company).

Disinfectants and neutralisers

The following disinfectants were used in this study: 2% glutaraldehyde (Chinese Shanghai Likang Disinfection Technology Co., Ltd, Shanghai, China), 2% iodine tincture (Chinese Shandong Lierkang Disinfection Technology Co., Ltd, Shandong, China), 1% iodophor (Chinese Shandong Lierkang Disinfection Technology Co., Ltd.), 75% ethyl alcohol (Chinese Fujian Putian Pharmaceutical Alcohol

Co., Ltd, Fujian, China), "84" disinfectant (Chinese Henan Hualong Pharmaceutical Co., Ltd, Henan, China), 0.2% benzalkonium bromide (Chinese Shandong Rui Teige Washing Disinfection Technology Co., Ltd, Shandong, China) and 0.1% chlorhexidine acetate (Chinese Jinzhou Jiutai Pharmaceutical Co., Ltd, Jinzhou, China). Neutralising agents used to inactivate each disinfectant are listed in Table 1.

MIC test for K. pneumoniae against imipenem and meropenem

The MIC test for *K. pneumoniae* samples against imipenem and meropenem was performed using the agar dilution method. To prepare the inoculum, a CRKP suspension at a concentration equivalent to a 0.5 McFarland standard was made and diluted at a 1 : 10 ratio. The prepared suspension (1–2 μl) was inoculated onto the surface of the drug-containing agar plates using a multipoint inoculator. Plates were then incubated at 35°C for 16–20 hours. To determine the MIC values, the plates were placed onto the surface of dark and non-reflective objects to evaluate endpoints, and the minimum drug concentration inhibiting bacterial growth was recorded as the MIC. Tests in which two or more bacterial colonies grew on agar plates with a higher drug concentration than the endpoint, or in which bacterial colonies grew on a lower drug concentration even though bacterial colonies were observed at higher drug concentrations, were repeated or checked for purity of the CRKP culture. The susceptibility of bacteria to antibacterial agents was assessed according to the 2011 Clinical and Laboratory Standards Institute (CLSI) standard for MIC: sensitive at ≤ 1 μg/ml; moderately sensitive at 2 μg/ml; and drug-resistant at ≥ 4 μg/ml. The *E. coli* ATCC25922 was used as the quality control strain.

Preparation of bacterial suspension

Carbapenem-resistant *Klebsiella pneumoniae* strains and standard strains collected from blood nutrient agar were inoculated onto an inclined surface of the nutrient agar and cultured for approximately 20 hours. The solutions were washed with phosphate-buffered saline (PBS) (pH 7.2) and diluted to a bacterial concentration of 10^8 CFU/ml in 1% peptone-PBS for use.

MIC determination for the effectiveness of each disinfectant against CRKP

Various concentrations of each disinfectant were prepared by making two-fold serial dilutions in distilled water. Each dilution (2.5 ml) was added into tubes containing 2.5 ml of 2 × nutrient broth. Each CRKP specimen or the standard strain (0.1 ml containing a bacterial count of approximately 10^8 CFU/ml) was inoculated into the disinfectant-containing nutrient broth; nutrient broth alone without disinfectant was inoculated with the bacteria and used as the positive-control group, while two tubes containing nutrient broth alone was used as the negative-control group. All tubes were placed in an incubator at 37°C and cultured for 48 h. If the positive-control group was turbid (indicating bacterial growth) and the negative-control group was transparent (indicating no bacterial growth), the concentration of the disinfectant corresponding to the maximum dilution of the tested group without bacterial growth was determined to be MIC of the disinfectant against the tested bacterial strain.

MBC determination for the effectiveness of each disinfectant against CRKP

Minimum inhibitory concentration determination was performed as a continuation of the MIC experiment for the disinfectants and CRKP strains described above. After the 48-h incubation for MIC determination, the reactions from the above MIC tests that did not exhibit bacterial growth were selected, and 0.5 ml of the sterile reaction was transferred into 10 ml tubes containing 4.5 ml of the neutraliser specific for the particular disinfectant used in each test. The solution was mixed thoroughly and incubated at room temperature for 10 minutes as the final reaction solution. This final reaction solution and 2 × nutrient broth were both added into a 10 ml tube at a 1 : 1 ratio. The positive- and negative-control groups were prepared as described above in the MIC experiment, and solutions containing 2.5 ml of a neutraliser plus 2.5 ml of the 2 × nutrient broth were used as the controls for the neutralisers. Samples were incubated at 37°C for 24 hours. If both the negative- and neutraliser-control groups were transparent, and the positive-control group was turbid due to bacterial growth, the minimum

Table 1 Neutralisers specifically used to neutralise each chemical disinfectant in this study

Disinfectants	Neutralisers
2% glutaraldehyde	500 ml phosphate-buffered saline (PBS) + 2.5 g glycine + 2.5 g lecithin + 2.5 g Tween-80
2% iodine tincture	1.0% sodium thiosulfate
1% iodophor	1000 ml PBS + 10 g sodium thiosulfate
75% ethyl alcohol	500 ml PBS + 1.5 g Tween-80
"84" disinfectant	1.0% sodium thiosulfate
0.2% benzalkonium bromide	5.0% Tween-80
0.1% chlorhexidine acetate	5.0% Tween 80

concentration of the disinfectant corresponding to transparent reactions in the test tubes was determined to be the MBC of the disinfectant against the tested bacterial strain.

For all MIC and MBC determinations, inoculation was performed from the lowest to the highest disinfectant concentration, and each test was performed three times. The MIC and MBC of the disinfectants between the experimental strains and the standard strains were compared to determine the sensitivity of the clinically isolated CRKP specimens, with the classification criteria stipulated as follows: if MIC and MBC values were greater than the standard strain, the experimental strain was deemed to be resistant to the disinfectant; if MIC and MBC values were less than or equal to the standard strains, the experimental strain was deemed to be sensitive to the disinfectant.

PCR detection of relevant drug-resistance genes in CRKP

Carbapenem-resistant *Klebsiella pneumoniae* colonies under pure culture conditions were placed into a 0.5 ml centrifuge tube already containing 200 μg/l proteinase K solution. Samples were incubated in a 56°C water bath for 2 hours and then in a 95°C water bath for 10 minutes, followed by centrifugation at 15 000 rpm for 30 minutes. The supernatant was removed, and samples were stored at −20°C until use. Primers for the well-known drug-resistance genes *qacA*, *qacΔE*, *qacE* and *acrA* were designed by Primer5.0 software (PREMIER Biosoft, Palo Alto, USA) according to the various gene sequences published in the GenBank database (www.ncbi.nlm.nih.gov/nucleotide), as shown in Table 2. To perform PCR, denaturation was performed at 93°C for 2 minutes, and each cycle consisted of 93°C for 30 s, 55°C for 30 s, and 72°C for 1 minute for a total of 35 cycles. The final extension step was performed at 72°C for 5 minutes. PCR amplification products were run on a 2% agarose gel and visualised under a UV gel imager.

Results

Clinical data

A total of 35 CRKP strains were isolated from patients within the clinical departments of Beijing Tiantan Hospital from 2011 to 2013. Of these, 27 strains were confirmed to be resistant to carbapenem using a commercially available identification kit. Indeed, the MIC for imipenem and meropenem against each of these 27/35 strains was ≥4 μg/ml (Table 3), further indicating resistance to these drugs. Since the MIC of imipenem and meropenem against the remaining 8/35 strains was <2 μg/ml (data not shown), indicating sensistivity to these drugs, they were formally excluded from the study. These CRKP strains were isolated from the ICU within the neurology department (13/27), the angiopathy ward of the neurology department (5/27), the neurosurgery department (3/27), the general ICU (1/27), the emergency department (3/27), the neurointervention department (1/27) and the cardiology department (1/27). From the patients, the CRKP strains were isolated from sputum (20/27), urine (2/27), cerebrospinal fluid (2/27), blood (1/27) or other locations (2/27). The patient demographics included 21 males and 6 females with a mean age of 60.81 ± 20.27 years old (range: 7–90 years old). The underlying diseases mainly included cerebral infarction, cerebral haemorrhage, subarachnoid haemorrhage, cerebral trauma, Parkinson's disease, brain tumour, intracranial infection, cardiac insufficiency and chronic obstructive pulmonary disease, among others.

Sensitivity of the clinically isolated strains to each of the disinfectants

To determine the disinfectants that would be most effective against each of the patient-derived CRKP strains in our study, we measured the MIC and MBC for each strain and compared the values to the MIC and MBC values obtained from testing with each of the various disinfectants against the standard bacteria *K. pneumoniae* ATCC700603 strain, which were used as the threshold to interpret the sensitivity and resistance of the test strains to the disinfectant (see Table S1, see supplementary material online for this article at www.maneyonline.com/doi/suppl/10.1179/2047773215Y.0000000022). From the MIC and MBC values obtained (Table 4), we determined that the overall population sensitivity of the clinically isolated CRKP strains to each of the different disinfectants was as follows

Table 2 PCR primer sequences

Gene name	Primer sequences (5′ → 3′)	Product length (bp)
qacA	Pl: GCTGCATTTATGACAATGTTTG P2: AATCCCACCTACTAAAGCAG	629
qacΔE	Pl: GCCCTACACAAATTGGGAGA P2: CTGCGGTACCACTGCCACAA	370
qacE	Pl: GCCCTACACAAATTGGGAGA P2: TTAGTGGGCACTTGCTTTGG	350
acrA	Pl: CCTCAAGTTAGCGGGATTAT P2: ACCGTCCTGCGGGAACTTAA	567

Table 3 Patient characteristics and minimum inhibitory concentration (MIC) values for Carbapenem-resistant *K. pneumoniae* to imipenem and meropenem

Strain No.	Sample ID	Imipenem MIC (μg/ml)	Meropenem MIC (μg/ml)	Patient age	Patient sex	Sample location	Hospital location
1	1315414	32	32	90	F	Urine	Cardiology
2	1315814	32	32	46	M	Sputum	Neurosurgery
3	1313627	16	32	67	M	Sputum	Neurology
4	1305982	>32	>32	68	M	Sputum	Neurology
5	1311300	>32	>32	25	M	Sputum	Neurology
6	1304584	32	>32	44	M	Sputum	Emergency
7	1316688	>32	>32	77	M	Sputum	Neurology
8	1307371	32	32	69	M	Sputum	Neurology
9	1205778	>32	>32	51	M	Blood	Neurology
10	1318356	32	32	62	M	Sputum	Neurology
11	1310011	32	32	23	M	Sputum	Emergency
12	1318293	32	32	71	F	Sputum	Neurology
13	1312490	32	32	88	M	Sputum	Neurology
14	1310360	32	>32	60	M	Sputum	Neurology
15	1318071	32	32	77	M	Sputum	Neurology
16	1317144	32	32	46	M	Sputum	Neurology
17	1305835	32	>32	60	M	Sputum	Neurology
18	1315410	32	32	7	F	Urine	Invasive
19	1109947	32	32	65	F	Cerebrospinal	Intensive care unit (ICU)
20	1317434	32	32	69	M	Sputum	Neurology
21	1311326	8	8	82	F	Sputum	Neurology
22	1317145	16	16	65	M	Sputum	Neurology
23	1305432	32	>32	38	M	Sputum	Neurology
24	1309554	32	>32	67	M	Secretion	Neurology
25	1221282	32	32	85	M	Sputum	Neurology
26	1309658	>32	>32	67	F	Secretion	Neurology
27	1307669	16	16	73	M	Cerebrospinal	Neurology

Table 4 Minimum inhibitory concentration (MIC) of the disinfectants compared to the expression of the drug-resistant genes

			Disinfectant-resistance genes			
Disinfectants	MIC (μg/ml)	#/27 of strains	*qacA*	*qacΔE*	*qacE*	*acrA*
Ethyl alcohol	4	2				
	8	5	1			
	16	6	2	2		1
	32	13	7	13	3	3
	64	1	1	1	1	1
Glutaraldehyde	4	11			1	1
	8	13	9	13	2	4
	32	3	2	3	1	
Chlorhexidine acetate	8	1				
	16	5				
	32	19	10	14	3	3
	64	2	1	2	1	2
Iodine tincture	8	9			1	1
	16	5	1	3		
	32	13	10	13	3	4
Iodophor	4	1				
	8	1				2
	16	5				
	32	16	8	12	2	2
	64	4	3	4	2	1
Benzalkonium bromide	4	3				
	8	7		1		
	16	10	5	9	1	3
	32	5	4	5	2	2
	64	2	2	1	1	
“84” disinfectant	8	2				
	16	8		1	1	
	32	16	10	14	3	4
	64	1	1	1		1

(of most-to-least effective disinfectant): 48% (13/27), ethyl alcohol >41% (11/27), glutaraldehyde >37% (10/27), "84" disinfectant=37% (10/27), benzalkonium bromide >33% (9/27), iodine tincture >26% (7/27), iodophor >22% (6/27), chlorhexidine acetate. Interestingly, 3/27 strains in our study population showed resistance to all of the tested disinfectants. These results indicated that while most strains in our study were sensitive to at least one of the disinfectants, many of the CRKP strains exhibited resistance to many of the tested disinfectants.

Detection of drug-resistance genes in 27 clinically isolated CRKP

Several genes, including *qacA*, *qacΔE*, *qacE* and *acrA*, are well known in the literature to confer drug resistance upon *K. pneumoniae* and other bacteria. While these mutations confer resistance to carbapenem-based drugs and other antibiotics, we wondered whether the presence of any of these drug-resistance genes also correlated with resistance of CRKP to any or all of the disinfectants. To address this question, we looked for the drug-resistance genes *qacA*, *qacΔE*, *qacE* and *acrA* in the 27 CRKP strains by PCR analysis and then compared these genotypes to the MIC values obtained for the disinfectants tested against each of the strains. The 27 CRKP strains included the following occurrence of genotypes within the population: *qacΔE* (16/27, 59%), *qacA* (11/27, 41%), *acrA* (5/27, 19%) and *qacE* (4/27, 15%) (Table 4). In some cases, a single strain carried multiple drug-resistance genes as follows: 4/27 (15%) carried *acrA*, *qacΔE*, and *qacA*; and 7/27 (26%) carried *qacA* and *qacΔE*. Comparing the number of strains in each tier of the MIC values for the disinfectants (where higher values corresponded with higher resistance of the bacteria to the disinfectant) with the number of strains carrying drug-resistance genes, we observed that CRKP strains carrying drug-resistance genes correlated with higher MIC values than those without drug-resistance genes. Thus, CRKP strains carrying drug-resistance genes had higher tolerance to killing or growth inhibition by disinfectants than those strains not carrying any drug-resistance genes.

Discussion

Disinfection and sterilisation is an important method to control nosocomial infections within the hospital environment. With the wide use of disinfectants, especially in daily life as well as in the livestock, bird and aquatic breeding industries, various bacteria can potentially become resistant to commonly used disinfectants over time. Moreover, drug resistance is a current issue facing human medicine, especially in the spread of nosocomial infections within the hospital environment.

In the present study, resistance to chemical disinfectants by 27 patient-derived CRKP strains collected between 2011 and 2013 was initially evaluated along with the presence of drug-resistance genes to understand the micro-ecological environment of the Beijing Tiantan Hospital. Since our hospital is a general hospital specialising in neurology, patients with nervous system disease account for a large proportion of our inpatients. These patients have weakened or dysregulated immunity in their central nervous system due to damaged nervous system function; therefore, they can acquire infectious diseases related to decreased immune function. This risk of acquiring infectious diseases are further increased due to their need for long-term bed rest and from undergoing dysphagia.[10,11] Thus, the increase in probability that these patients will receive anti-infective drugs (especially broad-spectrum antibiotics with strong antibacterial activity) and the length of time they receive these drugs may be a high-risk factor in screening for pan-resistant pathogens. In the 27 CRKP strains collected in the study, 23 were collected from patients in the neurology and neurology-related departments, and the primary diseases mainly included cerebrovascular disease, brain tumour and cerebral trauma, among others. These strains all exhibited resistance to carbapenem antibiotics (imipenem and meropenem) as well as the third and fourth generation of cephalosporins. At present, tigecycline and polymyxin are the only remaining antibiotic drugs available for treating patients with these CRKP strains in the clinic. Thus, the consequences of an outbreak may be dire, and effective strategies to combat the presence of CRKP in the hospital environment are necessary to control the spread of this infection.

In order to block the spread of CRKP in the hospital, cutting off the transmission route is a particularly important strategy along with actively treating the infection source based on the sensitivity of the particular strain to certain drugs, which is determined by characterising the strain for drug resistance. In terms of strategies to cut off the transmission routes, disinfectants that effectively kill bacteria must be used to treat the hospital environment, including caregiver hands, desktop, medical devices and other materials that may be contaminated by CRKP. However, the results of the present study showed that the clinically isolated CRKP were highly resistant not only to anti-infective drugs but also to many of the disinfectants commonly used in the clinic. Indeed, the resistance rate of the CRKP strains to the common disinfectants chlorhexidine acetate, iodophor, iodine tincture, benzalkonium bromide, "84" disinfectant, glutaraldehyde and ethyl alcohol was 78, 74, 67, 63,

63, 59 and 52%, respectively (see Supplementary Table S1). These data serve to alert clinicians that the use of any one of the disinfectants listed above may not effectively eliminate all CRKP strains from the environment, which may threaten patient prognosis and safety, and even promote the spread of such pathogens in endemic areas and within hospitals. Therefore, disinfectants to which bacteria have good sensitivity should be selected specifically for the pan-resistant strains found within a particular environment, which requires clinicians to identify what strains are present in their own hospital and which disinfectants are effective against these specific strains. Moreover, an appropriate extension of disinfection time and even the combined use of a variety of disinfectants may improve the effectiveness of disinfection, and comprehensive measures should be adopted, especially in strengthening hand hygiene before and after contact with infected patients or contaminated materials.

In the literature, bacterial resistance to disinfectants is often associated with the overexpression of genes associated with antibiotic drug resistance.[12] This makes sense when considering the mechanism-of-action underlying the functions of the proteins encoded by these genes. For example, the drug-resistance genes found within our CRKP study population, namely *qacΔE*, *qacA*, *acrA* and *qacE*, are components of proteins well known to function as gate molecules controlling efflux out of bacterial cells. *qacA* was first isolated in the 1980s from a multi-drug resistant plasmid, pSK5, carrying β-lactamase and metal ion-resistance genes. *qacA* encodes a multi-drug-resistance efflux protein, Mr55017, containing 514 amino acids (QacA) that discharges drugs absorbed by a bacterium to the outside via an ion pump and mediates resistance to more than 30 different organic compounds, including monovalent cationic (e.g. quaternary ammonium compounds) and divalent cationic (e.g. diamidino and propionamidine hydrazone) compounds.[13] *qacE* and *qacΔE* were isolated from a class I integron in the R751 plasmid, which were first isolated from *K. pneumoniae*. Both genes mediate resistance by a proton pump, and both confer bacterial resistance to quaternary ammonium disinfectants (e.g. benzalkonium bromide, benzalkonium chloride and domiphen bromide), biguanide compounds (such as chlorhexidine) and hydrazones. *qacΔE* resistance to quaternary ammonium disinfectants is lower than that of *qacE*, and studies have shown that these differences are due to changes in the fourth transmembrane segment and in the C-terminal tail of the qacΔE protein, which both do not contain any highly conserved residues.[14] The *acrA* gene encodes for an important component of the AcrAB–Tolc bacterial efflux pump, and its role may be to maximise the combination of acrB and Tolc when the relevant composite protein is being assembled. In addition, this efflux pump has a wide range of substrates and can discharge acridine orange, crystal violet, ethidium bromide and many other agents from the bacterial cell.[15,16]

From the 27 CRKP strains, we analysed in the our study that the resistance rates for the potential chemical disinfectant-resistant genes *qacΔE*, *qacA*, *acrA* and *qacE* were 59, 41, 19 and 15%, respectively. Compared to reports from hospitals in other countries, *qacΔE* and *qacE* gene detection rates were higher in our study, while the gene detection rate for *qacA* was lower.[17,18] Therefore, the distribution and transmission of bacterial resistance to disinfectants are likely affected and constrained by biological, physical and socio-economic factors, among many other factors. Since drug-resistance genotypes and phenotypes vary among different countries, regions, cities and communities, accurate and timely determination of the sensitivity of the particular bacterial population to disinfectants should be routinely assessed in individual hospital settings. Moreover, conducting further research, developing uniform standards and strengthening how and how often these bacteria are monitored in the hospital environment will also help to control the spread of these drug-resistant infections.

There has been some previous precedence to support that drug-resistant bacterial strains with co-resistance to a particular disinfectant could have a survival advantage in hospital settings,[9] potentially leading to an outbreak. As one case in point, methicillin-resistant *Staphylococcus aureus* (MRSA) strains carrying the *qacA/B* genes have survival advantage when chlorhexidine – a widely applied and normally effective disinfectant against MRSA – is used to decolonise the skin of an MRSA-infected patient.[19,20] Indeed, one study showed that MRSA carrying this genotype persisted in patients after chlorhexidine treatment,[19] and another showed that the MBC of the *qacA/B*-containing MRSA for chlorhexidine was three times higher than other MRSA strains.[20] Interestingly, we observed in the present study that our CRKP population had the highest resistance rates against chlorhexidine (78%), which is consistent with previous reports showing high MIC values for chlorhexidine against MRSA.[18] These findings underscore the importance of consistently being aware of the types of strains present in an individual clinic over time as well as monitoring the disinfectants that are most effective against the specific strains. Neglecting this type of monitoring may lead to more serious consequences as bacteria acquire more drug-resistant genes or become otherwise tolerant to disinfectants used in hospital settings.

We observed here that the MIC values exhibited by strains carrying drug-resistance genes were significantly higher than those without drug-resistance genes, indicating that *K. pneumoniae* strains carrying one or more drug-resistance genes (some carried two to three different drug-resistance genes) were highly tolerant to disinfectants. Therefore, the molecular basis for how different CRKP strains can be resistant to a single disinfectant and a single CRKP strain can be resistant to several different disinfectants may be that multiple strains of bacteria may carry a single drug-resistance gene, or a single strain may carry multiple drug-resistance genes.

One limitation of the present study is that the efficacy of the tested disinfectants in eliminating each of these 27 clinically isolated CRKP strains from the real-world hospital settings was not evaluated (i.e. on surfaces or medical equipment). Indeed, similar studies have been criticised for evaluating MIC and MBC levels that use lower effective concentrations of disinfectants than that which is used in practice or conditions that do not accurately reflect what occurs in real-world settings (i.e. the disinfectant seeing the bacteria in agar rather than directly on a hard surface, like a counter or floor),[21] which may underestimate the true effectiveness of a disinfectant against bacterial strains in the clinic. Indeed, some studies report the eradication of CRKP from the hospital environment after an outbreak using conventional disinfectants,[22] although the genotypes of the particular strains and efficacy of the disinfectant alone with regard to the other measures simultaneously undertaken to eliminate the pathogen were not accounted for. Moreover, a recent study showed that several common disinfectants were equally effective in killing both standard and clinically isolated CRKP strains, although they used a different assessment tool, a much smaller sample size and different antibiotics than what we used here, and they did not evaluate their strains to identify whether they contained any particular drug-resistance genes.[23] Despite that the conclusions reached from these studies contrast with our own, the possibility that disinfectant-resistant bacteria can arise and become problematic in the future should be taken into serious consideration, and the resistance of clinically isolated CRKP to disinfectants we show here by MIC and MBC analysis provides evidence to support this potential outcome.

The emergence of disinfectant-resistant bacteria strains may lead to a failure in disinfecting environmental surfaces and materials of certain pathogens and may accelerate the spread of 'antibiotic- and disinfectant-resistant strains,' which has the potential to result in an epidemic infection. In order to regain the control of the continually increasing disinfectant resistance and to avoid a situation like widespread antibiotic resistance, we propose the following suggestions: (1) *scientific-based application of disinfectants.* Disinfectants should be used at a sufficient concentration and time (and other relevant factors) to kill all pathogenic bacteria in a single administration using a dose greater than MBC. In addition, when bacteria are no longer exposed to a certain disinfectant, their disinfectant resistance may disappear, therefore necessitating the use of several disinfectants interchangeably. (2) *Strictly controlled use of disinfectants.* Disinfectants should be prudently and rationally used to reduce any "blind" application of disinfectants. Moreover, belief in the concept that increased application of disinfectants that leads to "cleaner" environments should be actively discouraged to reduce unnecessary use of disinfectants. The use of non-medical disinfectants should also be strictly controlled, and we should reduce or prohibit the more generalised application of disinfectants that are used to clean hospitals, such as in food production and in anticorrosion applications. (3) *Developing standards and strengthening monitoring*: regional and national uniform disinfectant-resistance detection standards can be established by referring to antibiotic-resistance monitoring measures. Meanwhile, a disinfectant-resistance monitoring network should be established to regularly monitor and report disinfectant resistance. When hospitals test bacteria for antibiotic sensitivity, they can also test for 'disinfectant sensitivity' to determine whether any particular disinfectant resistance is occurring in the bacteria, providing the basis for effective elimination of disinfectant-resistant bacterial strains and for the appropriate selection and use of disinfectants for that particular hospital. (4) *Strengthening research*: research into disinfectant-resistance mechanisms should be increased in order to better understand these mechanisms and to propose measures to control disinfectant resistance aimed specifically at these mechanisms. Moreover, high-efficient and low-toxic disinfectants should be developed.

Conclusions

The pan-resistant pathogenic CRKP strains isolated from patients in our hospital carry various drug-resistance genes and exhibit relatively high resistance to the commonly used chemical disinfectants, such as ethyl alcohol, chlorhexidine acetate and iodophor. These results suggest that monitoring drug-resistance rates of CRKP strains that display resistance to disinfectants should be placed as a high priority in hospital environments in order to ensure appropriate and effective sterilising measures within the hospital environment to prevent the spread of these pan-resistant strains.

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Disclaimer statements

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