

# Investigation and invention in carbapenem-resistant *Klebsiella pneumoniae* infection cases associated with Endoscopic retrograde cholangiopancreatography operation

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## Research

**Keywords:** Endoscopic retrograde cholangiopancreatography (ERCP), Carbapenem-resistant *Klebsiella pneumoniae* (CRKP), Contaminated environment, Disinfection

**Posted Date:** December 18th, 2019

**DOI:** <https://doi.org/10.21203/rs.2.19285/v1>

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## Abstract

**Background:** Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is associated with nosocomial infections that poses a serious threat to public health. According to a previous study, endoscopic retrograde cholangiopancreatography (ERCP) is considered a risk factor of CRKP transmission in the hospital.

**Methods:** In this study, two cases infected with CRKP after ERCP were investigated. The origin of CRKP was determined by collecting the isolates from patients and screening the environment for ERCP units and the specific endoscopes. The antimicrobials susceptibility testing and molecular typing were performed for these CRKP. After post-ERCP infection happened, the procedure of endoscope disinfection was changed and hydrogen dioxide disinfection of ERCP unit was performed.

**Results:** A total of five CRKP isolates were obtained from patients and screening the environment for ERCP units and the specific endoscopes, including three from the patients and two from the ERCP operating room. The CRKP from the patients and environment were both ST11, and the pulsed-field gel electrophoresis results showed that they shared identical bands, which indicated that the contaminated environment was associated with the nosocomial CRKP infections. After the control measures of endoscopes and hydrogen dioxide disinfection, post-ERCP infection decreased in the next six months.

**Conclusion:** Early warning and response system should be established to control the spreading of CRKP in ERCP operation unit.

## Background

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is considered an epidemic worldwide and poses a serious threat to public health (1). CRKP is associated with nosocomial and systemic infections, such as bloodstream, intra-abdominal, and urinary infections (2, 3). With limited treatment, CRKP infections are associated with high morbidity and mortality rate (4). The prevalence of CRKP in China rapidly increased in the past decade and reached 26.3% in 2018 (5). The promiscuous plasmids and clone outbreak exacerbate the worldwide spread of CRKP (6), which indicated that the hospital infection control was considerable for the prevention and control of CRKP transmission.

Endoscopic retrograde cholangiopancreatography (ERCP) is widely performed in the management and treatment of several pancreatic and biliary disorders (7). According to a previous study, infectious complications after ERCP operation were relatively low, and it was generally considered to be an effective and safe procedure. In 16,855 patients undergoing ERCP, the infection rate was 1.4% (8), and 7.57% of healthcare-associated infections were identified in a total of 1743 ERCP operations according to a 4-year surveillance study in China (9). However, for the individual patient, the risk for post-ERCP infection may still exist, with inadequate disinfection of the endoscopes as the major reason (10, 11). Considering the above reasons, the Food and Drug Administration (FDA) consistently reminds healthcare facilities the importance of reprocessing the endoscopes and requests the healthcare facilities to label their endoscopes with their actual contamination rate to reduce the risk of patient infection (FDA announcement release in August 29, 2019).

In this study, biliary tract infection was observed in two patients after undergoing ERCP. We suspended the specific endoscopes, screened the ERCP operation unit environment and the endoscopes, and conducted an investigation for these CRKP cases. The antimicrobial susceptibility testing, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE) were performed to determine the cause of post-ERCP infection.

## Methods

### Clinical Carbapenem-resistant *Klebsiella pneumoniae* isolates

Two patients who underwent ERCP in Sir Run Run Shaw Hospital were included in this study, and the CRKP isolates were isolated with routine hospital laboratory procedure. All isolates were identified and tested for susceptibility by the VITEK 2 system (bioMérieux; Inc., USA) using the Gram-Negative identification card (GN) and Gram Negative Susceptibility card (AST-GN13). The susceptibility of tigecycline, meropenem, and cefoperazone/sulbactam was tested using the Kleihauer–Betke (K-B) method. The Ethics Committee of the Sir Run Run Shaw Hospital approved this study as it mainly focused on bacteria, and not the patients. The clinical information was obtained from the hospital electronic system.

### Environmental screening

The specific environmental screening was conducted twice after post-ERCP infection, in October 27, 2017, and November 17, 2017, respectively, by the Department of Infection Control with the support of Hangzhou Center for Disease Control and Prevention. The environmental specimens were collected from the ERCP operation unit, including the operating and washing rooms, where the patients might have contact during ERCP, and the maps of the ERCP unit are demonstrated in Figure 1. All the object surfaces were smeared using 5-cm x 5-cm sponge sticks (3M) soaked with 0.03 mol/L saline solution, and specimens were plated to blood agar and incubated at 37°C for 48 hours considering the national hygienic standard for disinfection in hospital (GB15982-2012).

### Endoscopic retrograde cholangiopancreatography endoscope screening

The specific endoscopes (TJF-1 and TJF-4) used for these two patients were suspended immediately, and the screening was conducted 5 times from October to December 2017 by the Department of Infection Control. The exact screening time is shown in Table 1. Ortho-phthalaldehyde (OPA), peroxyacetic acid (PAA), or ethylene oxide (EO) was used for endoscope disinfection for different purposes; subsequently, neutralizing solution was inoculated and cultured at 37°C for 48 hours considering the national hygienic standard (GB15982-2012). According to WS 5017-2016, less than 20 colony forming units (CFUs)/endoscope was considered as a qualified disinfection. Before October 27, OPA was used for the routine disinfection of endoscope at our hospital; however, the post-ERCP infection indicated that it was not effective. Subsequently, based on a previous study (12, 13), we changed the disinfection procedure. PAA was used for

disinfection every day, and EO was used for conventional sterilization every month. When the post-ERCP infection was observed, EO was immediately used for sterilization after the operation.

#### Hydrogen dioxide disinfection

After the first environmental screening, hydrogen dioxide ( $H_2O_2$ ) disinfection was performed. Vaporized hydrogen peroxide (VHP) sterilizer (MZ-V200) generates  $H_2O_2$  vapor using a stabilized aqueous solution of 35%  $H_2O_2$  and can cover an area up to 500 m<sup>3</sup>. The VHP cycle phases include dehumidification, conditioning, decontamination, and aeration. ATCC 12980 *Geobacillus stearothermophilus* was used for quality control (decreased by 6 log fold).

#### Multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE)

MLST of *K. pneumoniae* was performed as previously described (14). Briefly, seven housekeeping genes were aligned using polymerase chain reaction and sequencing (15). PFGE pattern of CRKP was performed by comparing the *Xba*I/restriction profiles, according to the criteria of Tenover et al. (16).

## Results

### Patients' clinical information and isolates

In this study, two patients (Patients A and B) were included, and both patients underwent two ERCP operations. According to the operation records, the coherent endoscopes (TJF1-1 and TJF-4) were used in the operation sequentially; subsequently, these endoscopes were immediately suspended. The length of hospital stay of each patient is demonstrated in Figure 2.

**Patient A** was a 59-year-old male who was admitted following the complaint of **abdominal pain** on September 25, 2017 (Day 0). The patient was diagnosed with choledocholithiasis and **cholecystitis**. The patient underwent ERCP combined with endoscopic sphincterotomy, endoscopic papillary balloon dilatation, and **nasobiliary drainage** on Day 3 (TJF-1). Emergent ERCP was performed to stop the **alimentary tract hemorrhage** (Day 5, TJF-4). CRKP was isolated from the bile (Day 10, Isolates 295), blood (Day 11, Isolates 293) and **nasobiliary duct** (Day 11). Patient A was discharged at Day 24.

**Patient B** was a 70-year-old male who was admitted following the complaint of recurrent fever and abdominal distension on October 5, 2017 (Day 0). The patient was diagnosed with **choledocholithiasis** and **acute cholangitis**. The patient underwent ERCP twice to remove the calculus (Day 4, TJF-1 and Day 7, TJF-4). CRKP was isolated from the blood (Day 6, Isolates 299) and bile sample (Days 9 and 15), and carbapenem-resistant *Pseudomonas aeruginosa* was also isolated from the bile. Patient B was discharged at Day 27.

The two patients were both discharged from the hospital after an active and effective treatment.

### Isolates from the environment and endoscope screening

The environment of the ERCP operation unit and the specific endoscope were screened 2 and 5 times for CRKP, respectively. The results of the first environmental screening are presented in Table 2, which showed that the environment was contaminated with CRKP. CRKP was isolated from one patient bed in the operating room (Isolate 23-2) and one touch screen of the high frequency electric knife (ERBE) (Isolate 10-2). Both isolates were stocked. Some environmental bacteria such as *Bacillus* spp. were identified in the operating room, specifically in the air outlet, lead curtains and clothes, touch screen, and pressure gage. Opportunistic pathogens such as *Acinetobacter baumannii* and *Serratia marcescens* were identified in the patient's bed and water tank. The specimen obtained from the washing room was negative for bacteria.

Subsequently, VHP disinfection was performed after the first environmental screening, and the second environmental screening did not identify multidrug-resistant microbacteria. The environmentally contaminated bacteria such as *Bacillus* spp. disappeared, as presented in Table 2, and the other bacteria were not detected in the past positive location. Only the air outlet was still positive for bacteria (not identified), and this device should be paid careful attention because it is difficult to be disinfected.

To determine whether the endoscopes were contaminated, the TJF-1 and TJF-4 endoscope were screened for multidrug-resistant (MDR)-negative bacteria (Table 1). In October 27, the result showed that *P. aeruginosa* was identified from the TJF-1 endoscope, but *K. pneumoniae* was not detected. The OPA was used for routine endoscope disinfection before October 27, which indicated that the OPA was not effective. Subsequently, the disinfectant was changed to PAA and EO, and bacteria were already not detected during the four-time screening.

### The results of MLST and PFGE

The isolates from the patients and environment were identified as CRKP, according to the susceptibility test by VITEK (Table 3). The results showed that the isolates were resistant to  $\beta$ -lactams,  $\beta$ -lactams/ $\beta$ -lactamase inhibitors, ciprofloxacin, and gentamycin. Amikacin, gentamycin, tobramycin, and tigecycline were susceptible *in vitro*. To determine the association between infectious patients and ERCP operation, all the isolates were detected by MLST and PFGE, and all of these five isolates belonged to ST11. The results of PFGE showed that the clinical isolates from the patients and the environmental isolates shared identical bands (Figure 3), which indicated that these isolates were closely associated with each other, and the CRKP infection might be due to these contaminated environments.

### Retrospective case review

Considering these two cases, disinfection in the ERCP unit was significantly enforced, and subsequent cases of infection from November to May of the following year were monitored. One post-ERCP infection case was observed in November, December, and January, respectively, and none of the patients experienced infection after ERCP from February to May 2018.

## Discussion

ERCP is a valuable technique that is widely performed worldwide (7), and more than 18.6 million gastrointestinal endoscopies were performed in the USA (17). Noronha et al. revealed that the endoscopes would be considered as the source of nosocomial infection in the twenty-first century (18). Hence, microbiological culturing, EO sterilization, liquid chemical sterilization, and repetition of high-level disinfection were recommended to reduce the risk of infection transmission associated with ERCP (19, 20).

CRKP is increasingly isolated from patients in healthcare settings and transmitted worldwide (1). The prevalence of CRKP in China was increasing rapidly, from 4.0–26.3% in the past decade (5). Endoscope-associated bacterial infection outbreak has recently been reported. For example, Alrabaa et al. reported CRKP transmission to 7 patients in 2 hospital healthcare facilities by a contaminated ERCP equipment (21). Epstein et al. revealed that New Delhi metallo-beta-lactamase (NDM)-producing Escherichia coli was recovered from a reprocessed duodenoscope, which shared more than 92% similarity to all patients' isolates (12). The studies above showed that a large proportion of endoscopy-associated outbreaks were associated with contaminated endoscopes. In this study, during the surveillance screening of endoscopes, only *P. aeruginosa* was found on the endoscope; however, these could not rule out the possibility that our patients were infected with endoscope colonized with other MDR bacteria such as CRKP. The past routine disinfection agent was OPA, but it was inefficient with *P. aeruginosa* identified in the neutralizing solution of TJF-1. Subsequently, we changed the decontaminant to PAA and EO, and the bacteria were not isolated anymore, emphasizing that the reprocessing procedure of endoscopes in our hospital should be adjusted. Recently, the FDA recommended that the healthcare facilities and manufacturers should begin transitioning to ERCP with disposable components to reduce the risk of patient infection, which is a better way to control and prevent post-ERCP infections (22).

Since the environment of the ERCP operation unit screening showed that it was CRKP positive and PFGE results showed that the isolates from contaminated environment were associated with nosocomial infection, in this study, the environment, such as the patient's bed and touch screen, can act as reservoirs for post-ERCP infections. The air outlet was still positive for bacteria after VHP disinfection, indicating that routine disinfection could be insufficient to some settings in the operating room.

The expert consensus on safe operation of digestive endoscopy center in China was updated in 2016; however, standard protocols regarding the duration of disinfection for the environmental surface of the ERCP unit do not exist (23). Routinely, only the bedrail will be sampled after infection outbreaks. Other surfaces, which are considered bacterial intermediary agents and reservoirs, with high risk of contamination such as touch screen, pillows, and mattresses are often overlooked (24).

## Conclusion

It is necessary to decontaminate the beds when patients are discharged and to monitor the efficacy of disinfection in the environment, specifically the high-touch surfaces. The surveillance screening of endoscopes was beneficial for the early detection of bacteria and adjustment of the disinfection procedure. Early warning and response system can provide timely measures to control the spread of the emerging pathogen (25).

## Declarations

**Ethics approval and consent to participate:** This study was approved by the ethics committee of Sir Run Run Shaw hospital (No. 20191213-11) and the informed consents were waived.

**Consent for publication:** Not Applicable.

**Availability of data and materials:** The datasets used and/or analyzed during the current study available from the corresponding authors on reasonable request.

**Competing interests:** The authors have no conflicts of interest to declare.

**Funding:** This work was supported by research grants from the National Natural Science Foundation of China (81830069) and the Zhejiang Province Key Research and Development Program (2015C03046). The funder had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

### Authors' contributions:

YY and QS participated in the study design, collected the specimens, carried out laboratory work, analyzed the data and drafted the manuscript. LF analyzed the data and edited the manuscript. JZ, FT and ZW collected the specimens. YN collected the specimens and drafted the manuscript. FZ carried out laboratory work. DC collected the specimens. YC\* participated in its design and edited the manuscript. YY\* conceived the study, participated in its design and coordination, edited the manuscript, and received the majority of funding needed to complete the research.

**Acknowledgements:** We thank the support of Hangzhou Center for Disease Control and Prevention.

## Abbreviations

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP), Endoscopic retrograde cholangiopancreatography (ERCP), The Food and Drug Administration (FDA), Multilocus sequence typing (MLST), Pulsed-field gel electrophoresis (PFGE), Kleihauer–Betke (K-B) method, Ortho-phthalaldehyde (OPA), Peroxyacetic acid (PAA), Ethylene oxide (EO), Colony forming units (CFUs), Vaporized hydrogen peroxide (VHP), Multidrug-resistant (MDR), New Delhi metallo-beta-lactamase (NDM).

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## Tables

Table 1 The results of Endoscopic retrograde cholangiopancreatography endoscope screening

Endoscope Number	Screening Time (CFU/dish, isolates)				
	October 27	November 1	November 14	November 29	December 20
TJF1	<i>Pseudomonas aeruginosa</i>	Negative	Negative	NA	Negative
TJF4	NA	Negative	Negative	Negative	NA

NA: Not available

Negative: less than 20 CFUs/dish

Table 2 The results of environmental screening

specimen location	October 27		November 17
Patient bed	<i>Klebsiella pneumoniae</i> (23-2) <i>Acinetobacter baumannii</i>		Negative
The adjustment panel of bed	<i>Stenotrophomonas maltophilia</i>		Negative
Air outlet (bed head)	<i>Bacillus megatherium</i>		Positive
Air outlet (bed end)	<i>Bacillus megatherium</i>	<i>Bacillus circulans</i>	Positive
Protective lead curtains	<i>Bacillus megatherium</i> <i>dispersa</i>	<i>Bacillus cereus</i>	<i>Pantoea</i>
Protective lead clothes	<i>Pseudomonas oryzae</i> <i>Micrococcus luteus</i>	<i>Bacillus megatherium</i>	Negative
Reporting desk (computer, keyboard, and mouse)	<i>Pseudomonas putida</i>		Negative
ERCP machine (keyboard and handle)	Positive		Negative
The touch screen of electric knife (Valloy)	<i>Bacillus</i> spp.		Negative
The touch screen of high frequency electric knife (ERBE)	<i>Klebsiella pneumoniae</i> (10-2) <i>Acinetobacter baumannii</i>		Negative
Pressure gage	<i>Bacillus</i> spp. <i>epidermidis</i>	<i>Staphylococcus cohnii</i> <i>Staphylococcus</i>	Negative
Water tank in operating room	<i>Serratia marcescens</i>		Negative
Filter water of disinfectant No.1	Negative		NA
Filter water of disinfectant No.2	Negative		NA
Filter water of disinfectant No.3	Negative		NA
Filter water of disinfectant No.4	Negative		NA

NA: Not available

Negative: less than 20 CFUs/dish

Positive: more than 20 CFUs/dish

Table 3 The antimicrobial susceptibility testing results of the carbapenem-resistant *Klebsiella pneumoniae*

Isolate	specimen location	Imipenem	Meropenem	Ertapenem	Ampicillin	Amikacin	Aztreonam	Ciprofloxacin	Ceftriaxone	Cefazolin	antimi Ce
293	patient A blood	R	R	R	R	S	R	R	R	R	R
295	patient A bile	R	R	R	R	S	R	R	R	R	R
299	patient B blood	R	R	R	R	S	R	R	R	R	R
10-2	patient bed	R	NA	R	R	S	R	R	R	R	R
23-2	touch screen of high frequency electric knife (ERBE)	R	NA	R	R	S	R	R	R	R	R

## Figures



**Figure 1**

The maps of the ERCP unit, which contained the control room, operating room and washing room.



**Figure 2**

The length of hospital stay of each patient including the time of ERCP operation and isolates identified



**Figure 3**

The results of PFGE of five carbapenem-resistant *Klebsiella pneumoniae*, H9812: marker: 293, 295 and 299: isolates from patients; 10-2, 23-2: isolates from environment.