according to the updated CLSI guidelines,6 but in the susceptibility range when considering EUCAST guidelines. It was resistant to ertapenem, with an MIC value of 6 mg/L. It was also resistant to tobramycin, netilmicin, gentamicin, sulphonamides, tetracycline, chloramphenicol, rifampicin, trimethoprim, nitrofurantoin and fluoroquinolones, and remained susceptible to amikacin, tigecycline, fosfomycin and colistin.

Molecular testing showed that both isolates harboured the \textit{bla\textsubscript{OXA-48}} gene together with \textit{bla\textsubscript{TEM-1}} and the \textit{bla\textsubscript{CTX-M-15}} ESBL gene. No gene encoding 16S RNA methylase was identified. Mating-out assays allowed identification of the \textit{bla\textsubscript{OXA-48}} gene on a 70 kb plasmid that was positive for RepP PCR typing and that did not carry any additional resistance marker. Interestingly, a similar RepP-type 70 kb plasmid was shown to be at the origin of the spread of the \textit{bla\textsubscript{OXA-48}} gene among \textit{K. pneumoniae} and \textit{E. cloacae} isolates in Turkey. Analysis of the sequences surrounding the \textit{bla\textsubscript{OXA-48}} gene revealed that it was part of transposon Tn1999.2 in isolates 501 and BOU, whereas the two OXA-48-producing \textit{E. cloacae} clones previously identified in Turkey harboured a Tn1999.1 structure, which differs by the absence of the IS1R element, which has been shown to increase the expression of \textit{bla\textsubscript{OXA-48}}.2

PFGE analysis was performed as described previously 2 in order to evaluate the possible clonal relationship between the two \textit{bla\textsubscript{OXA-48}}-positive \textit{E. cloacae} isolates identified in this study, and also with two previously identified in Turkey.3 A total of four pulsortypes were obtained, showing that diverse clones were at the origin of the dissemination of OXA-48-producing \textit{E. cloacae} isolates. Our data suggest that the current dissemination of the \textit{bla\textsubscript{OXA-48}} gene is linked to the spread of a single plasmid and not to a single clone.

Our findings suggest that the \textit{bla\textsubscript{OXA-48}} gene might have already disseminated in Morocco (after Turkey), and further reinforce the relevance of an extensive screening of carbapenemase producers.

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Transparency declarations
None to declare.

References

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Arrival of Klebsiella pneumoniae carbapenemase (KPC)-2 in Taiwan
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Keywords: ventilator-associated pneumonia, otitis externa, colistin

Sir,
Carbapenem-resistant Gram-negative bacteria represent an emerging threat to the management of hospital-acquired infections.1 Recently, Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae strains have been reported worldwide.1 In China, KPC-producing Enterobacteriaceae strains are widely distributed nationwide, particularly in Zhejiang Province.1,2,3 This report confirms, to our knowledge for the first time, the arrival of KPC-2-producing \textit{K. pneumoniae} in Taiwan.

A middle-aged man, who did business in Zhejiang Province in China, was admitted to the intensive care unit (ICU) in a hospital in Zhejiang after successful resuscitation for cardiac arrest due to an unknown cause in 2010. He developed a sustained fever, together with leucocytosis, thrombocytopenia and anaemia after resuscitation. Empirical antibiotic therapy with cefoperazone/sulbactam was given. Three days after admission he was transferred to the medical ICU at National Taiwan University Hospital (NTUH).

On admission to NTUH, the patient was in a deep coma and put on a ventilator. His body temperature was 38.2°C. Laboratory examinations revealed leucocytosis, anaemia, thrombocytopenia and abnormal renal and liver function. Chest
radiography initially did not show a pneumonia patch on the first hospital day. Intermittent haemodialysis was performed for acute renal injury. Intravenous ceftriaxone (1 g every 12 h) was given empirically. However, fever persisted along with increased purulent sputum production. Chest radiography on the third hospital day showed consolidation in the right lower lobe. Ventilator-associated pneumonia was suspected and intravenous colistin (1.3 MU daily) was given empirically because the sputum culture obtained on the first hospital day yielded imipenem-resistant and colistin-susceptible Pseudomonas aeruginosa. The blood culture (isolate A) and sputum culture (isolate B) obtained on the third hospital day both grew imipenem-resistant K. pneumoniae. Cultures of an anal swab (isolate C) on the fifth hospital day also grew imipenem-resistant K. pneumoniae.

On the 10th hospital day, the patient developed acute, left-side otitis externa, with purulent otorrhoea. The pus culture from the left ear also yielded imipenem-resistant K. pneumoniae (isolate D). The patient received vancomycin for 14 days due to suspected concurrent methicillin-resistant Staphylococcus aureus pneumonia and colistin for 21 days and recovered well on continuous ventilator and haemodialysis support.

All four carbapenem-resistant K. pneumoniae isolates (isolates A to D) were positive for the modified Hodge test using imipenem and ertapenem discs.\(^2\)\(^,\)\(^5\) PFGE profiles of XbaI-digested genomic DNAs from the four isolates were identical. All four isolates of carbapenem-resistant K. pneumoniae harboured a bla\(_{\text{KPC-2}}\) gene [accession number GQ086225.1; identity 100% (750/750)], but did not have bla\(_{\text{VIM}}\) and bla\(_{\text{IMI}}\) genes.\(^2\) The bla\(_{\text{KPC-2}}\)-containing plasmid could be detected from electrot transformation of Escherichia coli DH5\(_{\text{a}}\).\(^5\) All four isolates exhibited high MICs of four carbapenems (64–256 mg/L) but were susceptible to colistin (1–2 mg/L) and tigecycline (1 mg/L) using the broth microdilution method (Table 1).\(^5\) The electrotransformant of E. coli DH5\(_{\text{a}}\) resulted in the following MICs: imipenem, 1 mg/L; meropenem, 2 mg/L; doripenem, 0.5 mg/L; and ertapenem, 2 mg/L. These findings were in accordance with those previously reported.\(^6\) All four isolates did not belong to serotypes K1, K2, K5, K20, K54, K57 or N1.\(^7\)

A total of seven anal swabs collected from seven patients hospitalized in the same ICU and sputum and anal swabs obtained from the patient 14 days after the isolation of the KPC-2-producing K. pneumoniae were negative for KPC-2-producing K. pneumoniae.

We report the first case of severe infection (ventilator-associated bacteraemic pneumonia and otitis externa) due to KPC-2-producing K. pneumoniae in a businessman working in Zhejiang Province, the epicentre of the KPC-2 endemic in China.\(^1\)\(^,\)\(^3\) This case suggests that infections due to KPC-producing K. pneumoniae could become an emerging problem in Taiwan, particularly given the frequency of travel between Taiwan and China in recent years. The optimal treatment for KPC-producing isolates is unknown.\(^8\) Although in vitro activity of tigecycline against KPC-producing K. pneumoniae is reported, tigecycline is not suitable for bloodstream infections due to its low serum concentrations.\(^8\) Colistin is another therapeutic option, but reduced susceptibility to colistin in KPC-producing K. pneumoniae has been reported.\(^8\) In our patient, colistin alone successfully eradicated KPC-2-producing K. pneumoniae in the bloodstream, respiratory tract and external ear, but exerted limited effect on clearance of the colistin-susceptible P. aeruginosa isolates.

In conclusion, we report the first case of ventilator-associated bacteraemic pneumonia caused by KPC-2-producing K. pneumoniae in Taiwan. Although carbapenemase resistance in K. pneumoniae is currently rare in Taiwan, it could be an emerging problem in the future, and continued antimicrobial resistance surveillance is needed.

### Funding
This study was carried out as part of our routine work.

### Transparency declarations
None to declare.

### References

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**Table 1.** MICs of 10 antimicrobial agents for the four KPC-2-producing K. pneumoniae isolates, the electrotransformant of E. coli DH5\(_{\text{a}}\) and E. coli DH5\(_{\text{a}}\), determined using the broth microdilution method

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC (mg/L)</th>
<th>K. pneumoniae isolates</th>
<th>electrotransformant of E. coli DH5(_{\text{a}})</th>
<th>E. coli DH5(_{\text{a}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertapenem</td>
<td>≥256</td>
<td>2</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>128</td>
<td>1</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>64–128</td>
<td>2</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Doripenem</td>
<td>64–128</td>
<td>0.5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Ceftobiprole</td>
<td>128</td>
<td>128</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥256</td>
<td>0.06</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Nemonoxacin</td>
<td>≥256</td>
<td>0.12</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥256</td>
<td>≥256</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥256</td>
<td>128</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1</td>
<td>0.03</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>1–2</td>
<td>1</td>
<td>1</td>
<td></td>
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<tr>
<td>Fosfomycin</td>
<td>≥256</td>
<td>0.12</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>
Identification of KPC-2-producing Pseudomonas aeruginosa isolates in China

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Keywords: pan resistance, genetic localization, genetic context, Ambler class A β-lactamase

Sir,

KPCs have been reported in different parts of the world since the first identification of KPC-positive Klebsiella pneumoniae in the USA.1,2 The increasing emergence and spread of KPCs leaves fewer available therapeutic options due to their broad-spectrum hydrolytic activity and high mobility.2 The KPC-producing bacteria are mostly Enterobacteriaceae, but also rarely Pseudomonas aeruginosa and Acinetobacter spp.2 The first KPC-producing P. aeruginosa isolates were identified in Colombia.3 Although there have been several reports of KPC-2-producing Enterobacteriaceae in China in recent years, this is the first report of KPC-producing P. aeruginosa in China.

In a surveillance study for assessing asymptomatic colonization with carbapenem-resistant organisms in the intensive care unit (ICU) of a 2200-bed teaching hospital (our hospital, i.e. First Affiliated Hospital, College of Medicine, Zhejiang University) in Hangzhou, 291 faecal specimens or rectal swabs were collected from 139 patients on admission and at 5 day intervals after hospitalization and cultured using MacConkey agar plates (bioMérieux, France) containing 1 mg/L meropenem. Species identification was performed using the VITEK 2 system (bioMérieux, France). Ambler class A and B β-lactamase genes were detected for carbapenem-resistant organisms by PCR. Three KPC-2-producing P. aeruginosa isolates (P. aeruginosa 1–3) were recovered from three patients (patients 1–3) who occupied adjacent beds.

Patient 1 was transferred from a regional hospital to our hospital’s ICU with severe pneumonia in August 2009 and was discharged in September 2009. Patients 2 and 3 were admitted to our hospital’s ICU with severe acute pancreatitis in August and September 2009, respectively. Patient 2 was discharged, but patient 3 died in September 2009. Mechanical ventilation was required for each patient. Neither patient 2 nor 3 had a recent history of hospitalization. None of the patients had a history of travel abroad. Each patient had extensive antibiotic exposure, including levofloxacin, cefepime, cefoperazone/sulbactam, piperacillin/tazobactam, meropenem and vancomycin. P. aeruginosa 1 and a carbapenem-resistant Acinetobacter baumannii (CRAB) were recovered from patient 1’s rectal swabs 36 h after his admission to our hospital. For patient 2, a CRAB and a carbapenem-resistant Escherichia coli (CREC) were isolated from rectal swabs 21 days after admission and P. aeruginosa 2 was recovered from stools 25 days after admission. From the stools of patient 3, P. aeruginosa 3, a CRAB and a CREC were selectively isolated 7 days after admission. CRAB and CREC recovered from these three patients were KPC-negative.

Susceptibility testing by Etest (bioMérieux, France) showed that all three P. aeruginosa isolates were pan resistant (resistant to all β-lactams, all aminoglycosides, all tetracyclines, all fluoroquinolones and polymyxin) and shared the same antimicrobial susceptibility patterns for the carbapenems (imipenem, meropenem and ertapenem; MICs >32 mg/L), polymyxin B (MIC═16 mg/L) and colistin (MIC═8 mg/L).

blaKPC, aac(3)-II, aac(6)-II and rmtB genes were co-identified from each P. aeruginosa isolate by using PCR assays followed by sequencing of Ambler class A, B, C and D β-lactamase genes, 16S rRNA methylase genes and aminoglycoside acetyltransferase genes. No metallo-β-lactamase (MBL) genes were detected. MBL (VIM-2, IMP-9 and IMP-1)-producing P. aeruginosa isolates are disseminated in different areas of China; however, the prevalence of MBL-positive P. aeruginosa isolates is lower than that of some developed countries.4

PFGE patterns of SpeI-digested genomic DNA showed the close relatedness of these three P. aeruginosa isolates.5 Multilocus sequence typing also revealed that these three isolates shared the same sequence type of ST463 (http://pubmlst.org/ P aeruginosa), which had not been identified in our hospital previously (Z. Ruan and T. Qu, Zhejiang University, personal communication).

Conjugation experiments performed in mixed-broth cultures with the recipient strain of rifampin-resistant EC600 or sodium azide-resistant J53 failed. An ~50 kb plasmid was extracted from each P. aeruginosa isolate using a Qiagen Plasmid Midi Kit (Qiagen, Germany). Repeated transformation experiments with the recipient strain of E. coli TOP 10 or E. coli DH5α also failed. Southern blotting experiments using the [α-32P]dCTP (DuPont, USA) labelling probes were performed to clarify the localization...