Emergence of ST39 and ST656 Extensively Drug-resistant *Klebsiella pneumoniae* Isolates in Wenzhou, China

Dear Editor,

The emergence and dissemination of carbapenemase-producing extensively drug-resistant (XDR) *Klebsiella pneumoniae* has limited effective therapies and posed a tremendous challenge to public health.\(^1\)\(^2\) *K. pneumoniae* strains belonging to sequences types 11 (ST11), and ST258 are continually reported as pathogens that can cause epidemic infections in Asian and European countries.\(^3\) Nevertheless, ST39 and ST656 had been detected only in sporadic cases so far. Here, we investigated the molecular characteristics of two ST39 and ST656 XDR *K. pneumoniae* strains recovered from a teaching hospital in Wenzhou, China.

Two *K. pneumoniae* clinical isolates (FK541 and FK2180) were isolated from the First Affiliated Hospital of Wenzhou Medical University during 2014–2015. They were collected from sputum and blood samples of two senior male patients at Intensive Care Units, respectively. The modified Hodge test (MHT) was positive for FK541, ethylenediaminetetraacetic acid (EDTA)-disk synergy test was negative. Nevertheless, the MHT and EDTA-disk synergy test were both positive for FK2180. They were proven as carbapenemase producers by utilising the phenotypic methods. Antimicrobial susceptibility testing was conducted by the agar dilution method. Two isolates were resistant to ceftriaxone, ceftazidime, cefotaxime, imipenem, meropenem, ertapenem, levofloxacin, ciprofloxacin, tobramycin, gentamicin, amikacin, chloramphenicol and fosfomycin, but remained susceptible to polymyxin B and tigecycline. Furthermore, polymerase chain reaction amplification and sequencing demonstrated that two strains harboured *bla*\(_{KPC-2}\), *bla*\(_{CTX-M-9}\), *bla*\(_{TEM-1}\), *bla*\(_{SHV}\), *rmtB* and *aac(6')-Ib-cr* genes simultaneously, along with single nucleotide mutations within the quinolone resistance-determining regions for strain FK541 in GyrA (Ser83Ile and Asp87Gly) and ParC (Ser80Ile), and for strain FK2180 in GyrA (Ser83Ile) and ParC (Ser80Ile). Mutations within the *gyrA* and *parC* genes could confer increased resistance to quinolones.\(^4\)

Taken together, the coexistence of multiple resistant genes in XDR *K. pneumoniae* isolates may contribute to high-level resistance to the majority of clinically available antimicrobial agents. Moreover, the plasmid location of the carbapenemase gene *bla*\(_{KPC-2}\) was manifested on an agarose gel following electrophoresis [Figure 1a] and Southern blot. A plasmid of ~54.2 kb in size for both strains was observed to hybridize to the *bla*\(_{KPC-2}\) probe in a Southern blot [Figure 1b], which could be transferred to *Escherichia coli* J53 through conjugation (transconjugants were designated as J541 and

**Figure 1:** Plasmid analysis and southern blot hybridization of the two KPC-2-producing *K. pneumoniae* isolates and transconjugants. (a) Plasmid DNA of two *Klebsiella pneumoniae* isolates and transconjugants was separated by agarose gel electrophoresis; *Escherichia coli* V517 as the marker. (b) Southern blot hybridization of the gel with the *bla*\(_{KPC-2}\) probe. (c) Southern blot hybridization of the gel with the *bla*\(_{CTX-M-9}\) probe. (d) Southern blot hybridization of the gel with the *rmtB* probe.
Moreover, other resistance gene, such as bla_{CTX-M-9} for FK541, was also transferred successfully. The results of bla_{CTX-M-9} and rmtB probes were shown in Figure 1c and 1d. Susceptibility tests revealed that both the transconjugants exhibited resistance or decreased susceptibility to cephalosporins and carbapenems, compared to E. coli J53. These data displayed the resistance determinant was transferable among species. With regard to genotypic relatedness, multilocus sequence typing revealed that two KPC-2-producing K. pneumoniae strains were typed into two rare genotypes. Strain FK541 belonged to ST39 and strain FK2180 belonged to ST656. To the best of our knowledge, this is the first description of ST656 K. pneumoniae carrying bla_{KPC-2} in China.

In conclusion, our study certified that KPC-2-producing K. pneumoniae strains of ST39 and ST656 are emerging in Wenzhou, the two K. pneumoniae isolates harbouring plasmids carrying the bla_{KPC-2} gene can be transmitted among isolates by horizontal transfer. Effective surveillance should provide guidance on the utility of antimicrobial agents to treat XDR K. pneumoniae infections.

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Conflicts of interest
There are no conflicts of interest.

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