Homology analysis between clinically isolated extraintestinal and enteral Klebsiella pneumoniae among neonates

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Abstract

Background: *Klebsiella pneumoniae* is a leading cause of hospital-associated (HA) infections. It has been reported that gastrointestinal colonization (GI) is likely to be a common and significant reservoir for the transmission and infections of *K. pneumoniae* in both adults and neonates. However, the homologous relationship between clinically isolated extraintestinal and enteral *K. pneumoniae* in neonates hasn't been characterized yet.

Results: Forty-three isolates from 21 neonatal patients were collected in this study. The proportion of carbapenem resistance was 62.8%. There were 12 patients (12/21, 57.4%) whose antibiotic resistance phenotypes, genotypes, and ST types (STs) were concordant. Six sequence types were detected using MLST, with ST37 and ST54 being the dominant types. The results of MLST were consist with the results of PFGE.

Conclusions: These data showed that there might be a close homologous relationship between extraintestinal *K. pneumoniae* (EXKP) and enteral *K. pneumoniae* (EKP) in neonates, indicating that the *K. pneumoniae* from the GI tract is possibly to be a significant reservoir for causing extraintestinal infections.

Keywords: *Klebsiella pneumoniae*, Gastrointestinal colonization, Multiple locus sequence typing, Endogenic infection, Antibiotic resistance

Background

*Klebsiella pneumoniae* is part of the healthy human microbiome, providing a potential reservoir for infections. It is known that *K. pneumoniae* could asymptptomatically colonize the skin, mouth, respiratory, and gastrointestinal tracts (GI). *K. pneumoniae* was detected in approximately 10% of Human Microbiome Project samples collecting from the mouth, nasal cavity, and skin, with an addition of 3.8% stool samples [1]. A 2010 study investigated nasopharyngeal colonization rates for adults and children in Indonesian were 15 and 7%, respectively [2], while another study reported that in the Vietnamese adults, the nasopharyngeal and pharynx colonization rates were 2.7 and 14%, respectively [3]. However, among body sites, GI colonization is likely to be a common and significant reservoir in terms of transmission and infection [4]. In addition, it was reported that *K. pneumoniae* GI colonization rates in hospitalized patients were estimated to be 20 to 38% [5–8]. Furthermore, among intensive care unit (ICU) patients, 48% of screened patients with infection were positive for prior GI colonization [9].

Moreover, *K. pneumoniae* has been recognized as one of the most important opportunistic pathogens in the human gut. Studies have demonstrated that trauma, overuse of antibiotics, and inappropriate diet can destroy the intestinal microecology and decrease probiotics in the gut [10]. These factors could lead to the loss of colonization resistance, allowing for the proliferation of opportunistic pathogens such as *K. pneumoniae* and...
Pseudomonas aeruginosa (PA). These opportunistic bacteria can quickly increase in abundance and have the potential to enter the blood, liver, and lungs, thus leading to enteric infections [11, 12]. It was reported that burn injury induces a dramatic dysbiosis of the intestinal microbiome, consequently causing the overgrowth of gram-negative aerobic bacteria, which have the potential to translocate to the extraintestinal sites [13]. Accumulating data [14–16] indicate that K. pneumoniae causing late-onset blood infections are of gut origin. However, in fact, we found that gut K. pneumoniae might be a reservoir for late-onset respiratory and blood infections.

Therefore, screening the characterization of the carriage K. pneumoniae isolates in high-risk patients, could help us predict the probability of potential infections. More importantly, the result of homology analysis of K. pneumoniae will provide more evidence. As a result, our study was designed to analyze the relationship between infections and GI colonization among the neonates.

Results
Clinical characteristics of the patients
The clinical data of neonates was retrospectively reviewed, and the details were partially shown in Table 1. The 43 strains of different types of specimens were isolated from 21 neonates: feces (n = 21, 48.8%), sputa (n = 19, 44.2%), and blood (n = 3, 7.0%). All patients were treated with two or more antibiotics for a long time (The usage of time of each antibiotic was shown in Table 1), such as mezlocillin/sulbactam (MSU), moxalactam (MOX), ceftazidime (CAZ), piperacillin/tazobactam (TZP), cefotiam (CTF), and meropenem (MEM). All neonates except neonate 1 were discharged after long-term treatments. Neonate 1 developed multiple organ failure on account of the septicemia caused by K. pneumoniae. Considering the probability of the treatment failure, the parents of neonate 1 gave up on further treatments. Complete results were shown in Table 2.

Antibiotic sensitivity tests
The results of the antibiotic sensitivity tests in this study showed that the isolates were resistant to different classes of antibiotics (Table 2). All the isolates (43/43) were MDR (multiple drug-resistant) (MDR: Resistant to three or more antimicrobial classes [17]). The proportion of carbapenem resistance was 62.8% among all the isolates (Table 2). In addition, we have compared the resistance rates between the EKP and EXKP. Complete results were shown in supplementary file 1.

Identification of β-lactamase genes and homology analysis of strains
The β-lactamases were divided into four major classes (A to D) by the Ambler scheme [18]. According to the expression of β-lactamase genes, the genotypes were classified into four types (I-IV) I: expressing class A and B β-lactamases; II: expressing class A β-lactamases; III: expressing class A and C β-lactamases; IV: expressing class A and D β-lactamases. The drug resistant phenotypes were divided into five types (A to E) according to the antibiotic sensitive tests. A: resistant to penicillin, penicillin/β-lactamase inhibitors and cephalosporins, sensitive to monobactams and intermediate to carbapenems; B: resistant to penicillin, penicillin/β-lactamase inhibitors, cephalosporins, monobactams and carbapenems; C: resistant to penicillin, penicillin/β-lactamase inhibitors, cephalosporins, monobactams and carbapenems; D: resistant to penicillin, penicillin/β-lactamase inhibitors, cephalosporins and carbapenems and sensitive to carbapenems; E: resistant to penicillin and penicillin/β-lactamase inhibitors and sensitive to cefalosporins, monobactams and carbapenems. Detailed classifications were shown in Table 2.100% of the isolates (43/43) produced SHV (100%), and most produced CTX-M-I5 (79.1%, 34/43) and CTX-M-1 (69.8%, 30/43). Three isolates were identified as NDM-1 positive isolates. There were 12 patients (12/21, 57.1%) whose antibiotic resistance phenotypes, genotypes and the ST types were concordant (When the antibiotic resistance phenotypes, genotypes, and the ST types of the strains were concordant, the paired isolates might be homologous.) Complete results were shown in Table 2.

The STs of the isolates were determined and numbered using the international database of the Institute Pasteur website, which showed an immense diversity with the results presented in Table 2. The isolates were distributed in six types of STs (ST37, 54,70, 29,1083, 1436), among which ST37 and ST54 were the most frequently seen STs. Besides, our data indicated that the ST37 was the main ST type in both the extraintestinal and enteral isolates. The concatenated sequences of all seven loci were used to draw a phylogenetic tree. The results showed that ST37 and ST1083 were homologous, which belong to CC37 clone complex [19]. The result of PFGE also demonstrated that ST37 and ST1083 were homologous. Complete results were shown in Fig. 1 and Fig. 2.

Pulsed-field gel electrophoresis analysis
Among five patients, the pulsed-field gel electrophoresis analysis (PFGE) showed the paired isolates from patients 12,15 and 21 had identical and > 90% similarity in PFGE patterns (Fig. 2).

Discussion
K. pneumoniae is known as the common cause of respiratory tract infections, urinary tract infections (UTIs),
and bloodstream infections (BSIs) [20]. *K. pneumoniae* typically colonize human mucosal surfaces, including nasopharynx and GI tract. The colonization rate varies among different body sites, and also is different between the community-acquired (CA) *K. pneumoniae* and the hospital-acquired (HA) *K. pneumoniae*. It is estimated that the rate of CA nasopharynx colonization was about 11%. The rate in adults is typically higher than that in children [20]. However, the rate of HA nasopharynx colonization is slightly higher, up to 19% [21]. Compared to the nasopharynx, the CA GI colonization rate is estimated to be around 3.9 ~ 5.9% [9]. Furthermore, the HA

| Case | Sex | Age | Ward | Diagnosis            | Antimicrobial therapy | Use time of antibiotic | Clinical outcome | Isolates | Sample |
|------|-----|-----|------|----------------------|-----------------------|------------------------|------------------|----------|--------|
| 1    | M   | 15 days | Neonate | Premature infant HIE | MSU, MOX, MEM    | Unchanged  | 15 days | K1      | Blood  |
| 2    | M   | 15 days | Neonate | Premature infant HIE | MSU, CAZ, MOX    | Improvement | 24 days | K2      | Sputum |
| 3    | M   | 0 days  | Neonate | Respiratory failure | MSU, CAZ, MEM, MOX | 50 days  | Improvement | K3      | Feces  |
| 4    | F   | 4 days  | Neonate | Neonatal pneumonia  | MSU, MOX, MEM   | 56 days   | Improvement | K4      | Feces  |
| 5    | M   | 0 days  | Neonate | Acute bronchopneumonia | MSU, CAZ, MEM, TZP | 75 days   | Improvement | K5      | Sputum |
| 6    | M   | 0 days  | Neonate | Neonatal encephalopathy | MSU, CAZ, MEM | 13 days | Improvement | K6      | Sputum |
| 7    | M   | 0 days  | Neonate | Neonatal encephalopathy | MSU, CTF, MEM | 13 days | Improvement | K7      | Feces  |
| 8    | M   | 0 days  | Neonate | Neonatal pneumonia  | MSU, TZP, MEM   | 16 days   | Improvement | K8      | Blood  |
| 9    | M   | 0 days  | Neonate | Respiratory failure | MSU, TZP, MEM   | 45 days   | Improvement | K9      | Feces  |
| 10   | M   | 0 days  | Neonate | Neonatal encephalopathy | MSU, TZP, CTF | 42 days | Improvement | K10     | Sputum |
| 11   | M   | 0 days  | Neonate | Neonatal pneumonia  | MSU, TZP, CTF, MEM, CAZ | 42 days | Improvement | K11     | Feces  |
| 12   | F   | 6 days  | Neonate | Neonatal pneumonia  | MSU, TZP, CTF, MEM | 42 days | Improvement | K12     | Sputum |
| 13   | M   | 10 days | Neonate | Neonatal pneumonia  | MSU, TZP, MEM   | 29 days   | Improvement | K13     | Feces  |
| 14   | M   | 5 days  | Neonate | Neonatal pneumonia  | MSU, CTF, MEM   | 51 days   | Improvement | K14     | Sputum |
| 15   | F   | 25 days | Neonate | Neonatal pneumonia  | MSU, MOX, CAZ, MEM | 41 days | Improvement | K15     | Sputum |
| 16   | M   | 0 days  | Neonate | Neonatal pneumonia  | MSU, TZP, MEM, MOX | 51 days | Improvement | K16     | Sputum |
| 17   | M   | 30 days | Neonate | Neonatal pneumonia  | MSU, CTF, MEM   | 24 days   | Improvement | K17     | Sputum |
| 18   | M   | 0 days  | Neonate | Respiratory failure | MSU, CAZ, TZP, MEM | 31 days | Improvement | K18     | Feces  |
| 19   | M   | 17 days | Neonate | Respiratory failure | MSU, CAZ        | 48 days   | Improvement | K19     | Sputum |
| 20   | M   | 20 days | Neonate | Respiratory failure | CAZ, TZP, SCF | 48 days   | Improvement | K20     | Sputum |
| 21   | M   | 7 days  | Neonate | Respiratory failure | MSU, SCF        | 11 days   | Improvement | K21     | Sputum |

* M male; F female; MSU mezlocillin/sulbactam; MOX moxalactam; MEM meropenem; CAZ ceftazidime; TZP piperacillin/tazobactam; CTF cefotiam; SCF cefoperazone/sulbactam
| Cases | Isolates | STs       | Presence of β-lactamase genes | AMP ≥32R | PRL ≥128R | AMS ≥32/16R | AMC ≥32/16R | TZP ≥128/4R | KZ ≥8R | CAZ ≥16R | CTX ≥4R | FEP ≥16R | AZT ≥16R | IPM ≥4R | MEM ≥4R | Phenotypes |
|-------|----------|-----------|-------------------------------|----------|-----------|-------------|-------------|-------------|---------|-----------|--------|---------|---------|---------|--------|-----------|-------------|
| Patient 1 | K1 | 54 SHV, TEM-1, NDM-1 | >16 >64 >16/8 >16/8 >32/4 >16 >16 >32 >16 <2 2 2 A<sup>1</sup> | | | | | | | | | | | | | |
| K2 | 54 SHV, TEM-1, NDM-1, CTX-M-15 | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 2 >8 B | | | | | | | | | | | | | | |
| K3 | 54 SHV, TEM-1, NDM-1, CTX-M-15 | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 2 >8 B | | | | | | | | | | | | | | |
| Patient 2<sup>b</sup> | K4 | 54 SHV, TEM-1, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K5 | 54 SHV, TEM-1, CTX-M-1 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 3 | K6 | 37 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 <1 C | | | | | | | | | | | | | | |
| K7 | 70 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >2 <8 D | | | | | | | | | | | | | | |
| Patient 4<sup>b</sup> | K8 | 37 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K9 | 37 SHV, CTX-M-1, CTX-M-14, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 5 | K10 | 70 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K11 | 37 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 6 | K12 | 37 SHV, CTX-M-1, CTX-M-1, CTX-M-14, CTX-M-15 III | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K13 | 70 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 7 | K14 | 37 SHV, CTX-M-14, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K15 | 70 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 8 | K16 | 37 SHV, CTX-M-1, CTX-M-15, OXA-1 IV | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 <1 <1 C | | | | | | | | | | | | | | |
| K17 | 37 SHV, CTX-M-1, CTX-M-14, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 9<sup>b</sup> | K18 | 37 SHV, CTX-M-14, CTX-M-15, OXA-1 III | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K19 | 37 SHV, CTX-M-1, CTX-M-14, CTX-M-15, OXA-1 III | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 10<sup>b</sup> | K20 | 1083 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 <1 <1 C | | | | | | | | | | | | | | |
| K21 | 37 SHV, CTX-M-1, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 <1 <1 C | | | | | | | | | | | | | | |
| Patient 11 | K22 | 37 SHV, CTX-M-1, CTX-M-14, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K23 | 37 SHV, CTX-M-1, CTX-M-14, CTX-M-15 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 <1 <1 C | | | | | | | | | | | | | | |
| Patient 12<sup>b</sup> | K24 | 37 SHV, CTX-M-1, CTX-M-15, TEM-1 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| K25 | 37 SHV, CTX-M-1, CTX-M-15, TEM-1 II | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
| Patient 13<sup>b</sup> | K26 | 37 SHV, CTX-M-14, CTX-M-14, | >16 >64 >16/8 >16/8 >64/4 >16 >16 >32 >16 >16 >8 >8 B | | | | | | | | | | | | | | |
Table 2 The ST types, resistant genotypes and resistant phenotypes of the 43 *Klebsiella pneumoniae* isolates K. pneumoniae isolates (Continued)

| Cases | Isolates | STs | Presence of β-lactamase genes | AMP ≥32R | PRL ≥128R | AMS ≥32/16R | AMC ≥32/16R | TZP ≥128/4R | KZ ≥8R | CAZ ≥16R | CXT ≥4R | FEP ≥16R | AZT ≥16R | IPM ≥4R | MEM ≥4R | Phenotypes |
|-------|----------|-----|-------------------------------|---------|-----------|-------------|-------------|-------------|--------|---------|--------|---------|---------|---------|--------|--------|------------|
| Patient 14<sup>b</sup> | K27 37 | SHV, CTX-M-1, CTX-M-14, CTX-M-15, OXA-1 IV | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | > 8 | > 8 | B |
| Patient 15<sup>b</sup> | K28 37 | SHV, CTX-M-1, CTX-M-15, TEM-1 II | > 16 | > 64 | 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| Patient 16 | K29 37 | SHV, CTX-M-1, CTX-M-15 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| K30 37 | SHV, CTX-M-1, CTX-M-15, TEM-1 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| K31 37 | SHV, CTX-M-1, CTX-M-15 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| K32 1083 | SHV, CTX-M-1, CTX-M-15, TEM-1 III | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| K33 1436 | SHV, CTX-M-1, CTX-M-15 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| Patient 17<sup>b</sup> | K34 1083 | SHV, CTX-M-14, CTX-M-15, TEM-1 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| K35 37 | SHV, CTX-M-15 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| Patient 18<sup>b</sup> | K36 37 | SHV, CTX-M-1, CTX-M-14, CTX-M-15, OXA-1 IV | > 16 | > 64 | > 16/8 | 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | > 8 | > 8 | B |
| K37 37 | SHV, CTX-M-14, OXA-1 IV | > 16 | > 64 | > 16/8 | 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | > 8 | > 8 | B |
| Patient 19<sup>b</sup> | K38 37 | SHV, CTX-M-1, CTX-M-14, CTX-M-15, OXA-1, TEM-1 IV | IVAAA+DA + D | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | > 8 | > 8 | B |
| K39 37 | SHV, CTX-M-15, OXA-1, TEM-1 IV | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | > 8 | > 8 | B |
| Patient 20 | K40 29 | SHV, TEM-1 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | <= 4 | < 1 | E |
| K41 37 | SHV, TEM-1 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| Patient 21<sup>b</sup> | K42 29 | SHV, TEM-1 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |
| K43 29 | SHV, TEM-1 II | > 16 | > 64 | > 16/8 | > 16/8 | > 64/4 | > 16 | > 16 | > 32 | > 16 | > 16 | < 1 | < 1 | C |

ST sequence type; AMP ampicillin; AMS ampicillin/sulbactam; TZP piperacillin/tazobactam; AMC ampicillin/clavulanic acid, PRL piperacillin; KZ cefazolin; CAZ ceftazidime; CXT cefotaxime; FEP cefepime; AZT aztreonam; IPM imipenem; MEM meropenem.

<sup>a</sup>The breakpoints: ≥32R means resistant breakpoints

<sup>b</sup>This symbol means that antibiotic resistance phenotypes, genotypes and the ST types of the paired isolates from the same patient were consistent.
GI colonization rate varies from 23 to 30% [22, 23]. It was reported that the GI carriage of *K. pneumoniae* was related to the subsequent HA infections [6]. In 2017, a study which explored the association between GI colonization and infections. Showed that the rate of *K. pneumoniae* infections was much higher for the GI colonization patients compared with the patients who were culture-negative (16% vs 3%) [9]. However, for the neonates, intestinal colonization occurred immediately after birth [24]. When some pathogens colonize the gut, it might result in the later subsequent infections. Compared to the neonates who were non-colonized, the likelihood of the colonized-neonates developing subsequent infections was remarkably higher (24.8% VS 1.9%). The percentages of the nasopharynx and GI *K. pneumoniae* colonization were respectively 29 and 36.8% in the hospitalized neonates [25]. Furthermore, a study showed that the GI *K. pneumoniae* could invade and penetrate the intestinal epithelium, which indicated that GI *K. pneumoniae* could cause extraintestinal infections. This transcellular translocation mechanism is exploited by *K. pneumoniae* strains from the gut caused systematic infections by this transcellular mechanism [26]. Although there was a close relationship between colonization and infections, the homologous relationship between the GI colonized isolates and extraintestinal isolates has not been reported yet.

In our study, all the isolates (43/43) were MDR *K. pneumoniae*, and 27 strains were resistant to carbapenems with a drug resistance rate of 62.8%. The proportion was moderately higher than 54% in adult that published by World Health Organization [27], while considerably higher than the proportions of 24.7 and 29.8% found in previous studies in the neonates [28, 29].

![Fig. 1 The UMPGA dendrogram, sequence types (STs), and genotypes of 43 *Klebsiella pneumoniae* isolates from 21 patients. The tree shows that ST37 and ST1083 were related. In our previous study, ST37 and ST1083 belong to the same clone complex CC37 [19]](image-url)
One hypothesis indicating that GI colonization was likely to be a significant reservoir in terms of transmission and infections [4]. Furthermore, some drug-resistant genes which were mediated by plasmids could be acquired or lost during bacterial translocation [20]. Based on this hypothesis, the drug-resistant phenotypes might be affected by the loss or acquisition of the β-lactamase genes. In our study, the CTX-M-1, CTX-M-14, CTX-M-15, and TEM-1 were expressed differently between feces and other samples. These genes belong to plasmid-mediated ESBLs [18]. In this case, the drug resistant genotypes and phenotypes were divided into different groups as per the antibiotic sensitivity tests and the expressions of the β-lactamase genes. The results showed that there were 12 patients (12/21, 57.1%) whose paired isolates might be homologous. The data demonstrated that the GI tract might be a significant reservoir for causing extraintestinal infections.

The majority of the isolates were resistant to β-lactam antibiotics. The resistance observed in the present study might be attributed to the expression of resistance genes such as β-lactamase genes. NDM-1 appeared in 7% of isolates, which was first identified in 2006. After it was first identified, it was predominantly found in K. pneumoniae and E. coli. Since 2010, the bacteria producing NDM-1 had been reported worldwide. In China, NDM-1 producing K. pneumoniae has been frequently reported in neonates [30, 31]. The STs of blaNDM-1-producing K. pneumoniae mainly included ST11, ST16, ST20, ST37, ST70, ST147, and ST1419 [32–35]. But our data indicated that the ST54 was the only NDM-1 producing type. In most cases, bacteria with NDM-1 were resistant to almost all antibiotics. Moreover, the dissemination has been facilitated by horizontal gene transfer. That being so, reliable detection and surveillance are of great importance in preventing the clonal outbreaks.

Although these isolates showed high drug resistance and high rates of resistance genes, just one neonate (patient 1) acquired a poor prognosis upon treatment with antibiotics.

To confirm whether the isolate pairs were homologous or not, a UMPGA tree was drawn by employing MEGAX to further analyze the homology among the different isolates from the same patients. Excluding the completely concordant strains, the analysis of the homology among ST37 and ST1083 should be confirmed. According to the analyses, ST37 and ST1083 were in the same cluster (two alleles of the 7 housekeeping genes differed), concluding that the two were closely related and the results validated a great deal of our previous research [19]. The PFGE also indicated that ST37 and ST1083 were homologous. Moreover, our data indicated that ST37 were the main epidemic clones in the Neonate Ward, which showed consistency with what found in other studies [19, 30]. It is discovered that ST37 are presumably to be a potential high-risk MDR K. pneumoniae clonal lineage [36]. In our study, the results of MLST were consist with PFGE. Furthermore, it is reported that carriage of carbapenem-resistant K. pneumoniae (CRKP) in the GI tract may precede and possibly serve as a source for subsequent clinical infections in approximately 9% of carriers [37, 38]. And these carriers may act as a significant reservoir for the dissemination of CRKP in the healthcare facilities [39–41]. Combined with our study, active surveillance for detecting CRKP colonization is critical for preventing the CRKP from spreading. Besides, according to the guidance of CDC for control of Carbapenem-Resistant Enterobacteriaceae (CRE), screening rectal cultures of CRE is an important strategy for CRE prevention [42].

The main strength of our study is the use of multiple approaches to characterize the isolates and their
similarity to one another in the neonates. However, there are several limitations. First, neonatal cases are difficult to collect, only 21 neonatal patients were collected for analyzing. Second, because of the limitation of experimental conditions, only 10 paired strains from 5 patients were selected randomly for PFGE.

**Conclusion**

In this study, we found there was an apparently close phylogenetic relationship between the extraintestinal and enteral strains. This conclusion is a reminder that *K. pneumoniae* which colonizes in the intestine can also induce infections in other parts of the body. Once the Amp C, KPC, and NDM-1 genes are successfully transferred, acquired resistance will potentially cause severe infections. Therefore, the hospital should screen the CRKP which colonized in the gut to limit and prevent current and future outbreaks.

### Methods

**Bacterial strains and clinical characteristic**

Samples isolated from the feces, sputa, and blood were collected from the neonates infected with *K. pneumoniae*. All the sputum samples were collected from the neonates who were diagnosed with neonatal pneumonia, acute bronchopneumonia, and bronchitis. Diagnoses were made based on both clinical and radiologic findings. The strains isolated from the same patient were paired. Forty-three isolates of *K. pneumoniae* were collected from feces, sputa, and blood of 21 neonates. All the neonates were admitted to the Second Xiangya Hospital of Central South University, China, from July 2014 to April 2015. All the data of the neonates were collected by chart review from the hospital’s unified electronic database. These isolates were identified by using the BD Phoenix 100 Automated Microbiology System (BD Diagnostic Systems, MD, USA).

**Table 3 Primers used in this study**

| Primers | Primers sequence (5′-3′) | Annealing temperature (°C) | Length of products (bp) | Ref. |
|---------|--------------------------|---------------------------|------------------------|------|
| NDM-1   | Sense: 5′-CGCAACACTCCCTC-3′ Anti: 5′-CAGCAACACTTCTTACCTC-3′ | 53 | 888 | This study |
| KPC-2   | Sense: 5′-GGCGCTTTTGGCCTCA-3′ Anti: 5′-ATGATTTTACAGGCTTACT-3′ | 52 | 103 | This study |
| OXA-1   | Sense: 5′-CTGTGTTTTGTTGCTGAG-3′ Anti: 5′-CTTGCTTTATGCCCTGAT-3′ | 55 | 440 | This study |
| OXA-2   | Sense: 5′-TAAGCAACACCGAGAG-3′ Anti: 5′-TGCTGATGATGCTTCACT-3′ | 51.2 | 879 | This study |
| OXA-9   | Sense: 5′-AGGCGGAAGACTGAA-3′ Anti: 5′-GGGAAAAAGCGAAGA-3′ | 52.6 | 348 | This study |
| OXA-48  | Sense: 5′-TTTCCTCTGTTGCGACT-3′ Anti: 5′-TACCCGAGCATCTTCTT-3′ | 50 | 586 | This study |
| OXA-181 | Sense: 5′-SGTCTATGCGTATGAC-3′ Anti: 5′-GCACCTTTTGATAGGC-3′ | 51 | 775 | This study |
| CTX-M-1 | Sense: 5′-CAGGCTTTTGCGCGCTTAAG-3′ Anti: 5′-GGCCCATGTTTTAAATCACTGC-3′ | 60 | 945 | [44] |
| CTX-M-2 | Sense: 5′-TCCTGACACCTGCACCCTCA-3′ Anti: 5′-CCGCGCAACCAATCC-3′ | 61.5 | 843 | [44] |
| CTX-M-8 | Sense: 5′-ACTTGACACGACCCGATTCA-3′ Anti: 5′-CGAGTACGTCGAGCAC-3′ | 52.5 | 1024 | [44] |
| CTX-M-14| Sense: 5′-GCAGATAATACGCAGGTG-3′ Anti: 5′-GCTGGTTAAATAGGT-3′ | 55.1 | 640 | This study |
| CTX-M-15| Sense: 5′-ATTAGGCGCGAGTCGG-3′ Anti: 5′-AAGGAAACCGAAGA-3′ | 55.1 | 843 | This study |
| CMY-4   | Sense: 5′-CGCGGTGTGTGCCTCAT-3′ Anti: 5′-CCAATGCACCTTCTTGT-3′ | 55.2 | 796 | [45] |
| CMY-8   | Sense: 5′-AGCGTAAACGAGTGA-3′ Anti: 5′-AGATAAGCCCTTTGTG-3′ | 52 | 1042 | [45] |
| TEM-1   | Sense: 5′-TCCTGTCGCCTTCTTCTT-3′ Anti: 5′-ACGCCTGTGGCTTCTTAT-3′ | 55 | 512 | This study |
| SHV     | Sense: 5′-GCCCTTTATCGCGCTTCAATCAAG-3′ Anti: 5′-TTAGCGTGTGCGATCTCA-3′ | 60 | 898 | [44] |
ATCC 25922 and K. pneumoniae ATCC 700603 were used as quality control strains.

**Antibiotic susceptibility testing**
All bacterial isolates were subjected to antibiotic sensitivity tests using the agar dilution method following the standard antibiotic susceptibility test chart from the CLSI guidelines [43]. The results were interpreted by measuring the minimum inhibitory concentrations (MICs) which were determined as the lowest concentration of antibiotics at which the strains showed no visible growth after overnight incubation at 37 °C. The isolates resistant to carbapenems were verified with the Kirby-Bauer/disk diffusion method following the CLSI guidelines [43].

**PCR and sequencing for resistant genes**
Genomic DNA from the isolates was prepared for PCR and genetic analyses using the TIAN amp Bacterial DNA Kit (Tian Gen Biotech, Beijing, Co., Ltd.). The β-lactamase antibiotic resistance genes which were prevalent in K. pneumoniae were mainly detected (including NDM-1, KPC-2, OXA-1, OXA-2, OXA-9, OXA-48, OXA-181, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-14, CTX-M-15, CMY-4, CMY-8, TEM-1, and SHV; Table 3). These resistance genes were screened through PCR assays, and the PCR products were sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing analysis. The entire sequence of each gene was compared to the sequences in the Gen-Bank nucleotide database at http://www.ncbi.nlm.nih.gov/blast/.

**Multiple locus sequence typing**
The MLST assay was performed as previously described [43]. Briefly, seven K. pneumoniae housekeeping genes (infB, tonB, pgi, gapA, phoE, rpoB, and mdh) were amplified and sequenced. Alleles and STs were assigned using the K. pneumoniae MLST database (http://pubmlst.org/klebsiella/).

**Phylogenetic relationship**
The products of the housekeeping genes were compared and analyzed by utilizing the program BLAST. To explore the phylogenetic relationship among the isolates, the seven loci (rpoB, gapA, mdh, pgi, infB, phoE, and tonB) of each isolate were concatenated and aligned using the Clustal X program. An evolutionary tree for the data set was formed by the UMPGA tree using the software MEGA X. The stability of the phylogenetic relationship was evaluated by bootstrap analysis based on 1000 replicates [46]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree [47].

**PFGE**
We performed PFGE analysis using Bio-Rad syste m [48]. First, bacterial suspension was prepared, and then the restriction enzyme XbaI was used. Second, the electrophoretic gel was imprinted, and stained with ethidium bromide. Finally, electrophoretic images were analyzed with the software BioNumerics (Applied Maths, Inc.). A similarity coefficient > 80% was selected to define a major cluster.

**Statistical analysis**
All data were analyzed with SPSS 19.0 statistical software. Categorical variables were evaluated by the Fisher’s exact test. Values were presented as percentages of the group from which they were derived (categorical variables). A p value of < 0.05 was considered statistically significant. Bio Numerics 5.10 software was used for PFGE.

**Supplementary Information**
The online version contains supplementary material available at https://doi.org/10.1186/s12866-020-02073-2.

**Additional file 1**.

**Abbreviations**
HA: Hospital-associated; GI: Gastrointestinal colonization; PCR: Polymerase chain reactions; MLST: Multiple Locus Sequence Typing; ESKP: Extraintestinal K. pneumoniae; ET: Enteral K. pneumoniae; STs: ST types; ICU: Intensive care unit; PA: Pseudomonas aeruginosa; MICs: Minimum inhibitory concentrations; MSU: Mezlocillin/sulbactam; MOX: Moxalactam; CAZ: Ceftazidime; TZP: Piperacillin/tazobactam; CTF: Cefotiam; MEM: Meropenem; MDR: Multiple drug-resistant; PFGE: Pulsed-field gel electrophoresis analysis; UTIs: Urinary tract infections; BSIs: Bloodstream infections; CA: Community-acquired; HA: Hospital-acquired; CRKP: Carbapenem-resistant K. pneumoniae; CRE: Carbapenem-resistant Enterobacteriaceae; NDM: New Delhi metallo-beta-lactamase; KPC: Klebsiella pneumoniae carbapenemase; OXA: Oxacillinase; CTX-M: Cefotaxime; CMY: Cephemycinase; TEM: Temoneira; SHV: Sulphhydril Variable

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**Authors’ contributions**
CMC performed the test, analyzed data, drafted, and writing the manuscript. MW obtained funding for the project and helped to interpret the data and contributed to drafting the manuscript. XPL assisted in the data analysis. PLL helped us to collect the data. JJT, KZ and CL edited and read the manuscript. All authors approved the final manuscript.

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

**Ethics approval and consent to participate**
We obtained written informed consent both at recruitment and soon after delivery from each mother. This study was approved by the Second Xiangya Hospital of Central South University ethics committee (20200313).
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