Emergence of mobilized colistin resistance-1 in multidrug-resistant Klebsiella pneumoniae and Escherichia coli isolates from the Henan province in China: a multicentre study

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Research

Keywords: polymyxin, E. coli, K. pneumoniae, mcr-1, Henan province

Posted Date: March 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-285784/v1

Abstract

Background

The increased clinical use of polymyxin led to the emergence of polymyxin-resistant strains, especially those carrying plasmid-borne mobilized colistin resistance (*mcr*) gene variants. In this study, we aimed to evaluate the prevalence and characteristics of polymyxin-resistant *Klebsiella pneumoniae* and *Escherichia coli* isolates from the Henan province, China.

Methods

A total of 16 polymyxin-resistant isolates among 2301 *E. coli* and *K. pneumoniae* isolates collected in 6 local hospitals in the Henan province were studied. The isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and the minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique. Polymyxin-resistant isolates were further analysed for *mcr-1* and carbapenemase-mediated resistance using the modified carbapenem inactivation method, the ethylenediaminetetraacetic acid-modified carbapenem inactivation method, and polymerase chain reaction. Pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) were performed to disclose the phylogenetic relationships of the polymyxin-resistant isolates. The clinical characteristics of patients infected with the polymyxin-resistant isolates were also retrospectively analysed.

Results

5/1499 (0.3%) and 11/802 (1.4%) *E. coli* and *K. pneumoniae* isolates, respectively, were polymyxin-resistant. The MICs of polymyxin were in the range of 4–64 μg/mL and all of the 16 polymyxin-resistant isolates were susceptible to tigecycline. Additionally, four of the five *E. coli* polymyxin-resistant isolates were *mcr-1* positive; one of them was also carbapenem-resistant, carrying bla_{NDM-5}. Conversely, only 1/11 *K. pneumoniae* isolates was *mcr-1* positive, while 9 polymyxin-resistant isolates were also carbapenem-resistant (PRCRKP), carrying bla_{KPC-2} but not *mcr-1*. MLST results showed that the five *E. coli* isolates belonged to four sequence types (STs), including ST2, ST132, ST632, and ST983, while all PRCRKP isolates belonged to ST11. However, all 16 isolates showed different PFGE types using a genetic similarity of ≥ 95%. Furthermore, 33.3% (5/15) of the patients carrying polymyxin-resistant *K. pneumoniae* isolates showed a history of polymyxin use, and 10/15 (66.7%) patients displayed good clinical outcomes.

Conclusion

The polymyxin resistance rate of *K. pneumoniae* was slightly higher than that of *E. coli* in the Henan province; however, *mcr-1* was only detected in one *K. pneumoniae* isolate. Thus, close monitoring is needed to prevent and control the spread of PRCRKP.

Background

Antibiotic resistance has become a global public health priority in recent years. Colistin, also known as polymyxin, is one of the few therapeutic options available for the treatment of infectious diseases caused by multidrug-resistant gram-negative bacteria [1]. In China, injectable polymyxin was approved for the treatment of bacterial infections in January 2017. However, because of the increased usage of polymyxin in clinical settings, polymyxin-resistant strains, especially those carrying plasmid-borne mobilized colistin resistance (*mcr*) gene variants have emerged in China and various countries worldwide [2]. Moreover, the intraspecies transmission of resistant isolates has already been reported [1, 3].

Since its discovery in southern China in late 2015 [4], *mcr-1* has spread to over 40 countries and regions, implying that it plays a prevalent role in the transferability of polymyxin resistance. Of note, *mcr-1*-positive strains have also emerged in the Henan province, including in pig-derived *Escherichia coli* isolates [5]. In fact, clinical *E. coli* isolates co-producing bla_{NDM} and *mcr-1* were previously reported by our laboratory [6], and a novel conjugative mcr-8.2-bearing plasmid was identified in an almost pan-resistant hypermucoviscous *Klebsiella pneumoniae* ST11 isolate [7]. However, overall, the reports of *mcr* in human-derived *E. coli* and *K. pneumoniae* isolates are mainly centred outside of Henan.

Additionally, colistin resistance in *K. pneumoniae* can be mediated by chromosomal mutations in genes involved in lipopolysaccharide synthesis, namely *phoPQ*, *pmrAB*, and *crrA*/*crrB* as well as the *mgrB* regulatory gene [8-10].

To better understand the epidemiological trends and characteristics of polymyxin-resistant clinical strains, here, we looked for polymyxin resistance among isolates collected at 6 hospitals in Henan from 2018 to 2019. A total of 16 polymyxin-resistant strains were collected, and their molecular resistance characteristics were analysed. To the best of our knowledge, this is the first multi-centre study screening for polymyxin-resistant isolates among *E. coli* and *K. pneumoniae* in the Henan province, China.

Methods

Sample collection

Non-duplicated *E. coli* and *K. pneumoniae* strains were obtained from routine microbiological cultures of clinical samples including blood, urine, sputum, bronchoalveolar lavage fluid (BAL), bile, hydrothorax, ascites, and various other specimens. A total of 2301 strains were isolated from 6 hospitals in Henan. Identification at the species level was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany).

Susceptibility testing and determination of the minimum inhibitory concentrations (MICs)

Susceptibility to polymyxin was screened using Etest strips (Antu, Zhengzhou, China); only isolates with MICs higher than 2 μg/mL were subjected to further susceptibility testing for validation using the microbroth dilution method based on the clinical breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2018) [11].

Additionally, susceptibility to ampicillin (AMP), meropenem (MEM), imipenem (IPM), ceftazidime (CAZ), cefotaxime (CTX), cefazolin (KZ), ampicillin/sulbactam (SAM), aztreonam (ATM), cefepime (FEP), piperacillin/tazobactam (TZP), levofloxacin (LEV), amikacin (AK), gentamicin (GN), trimethoprim/sulfamethoxazole (SXT), ceftazidime/avibactam (CZA), and tigecycline (TGC) was determined only in the context of polymyxin-resistant strains using the microbroth dilution method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12].

Multi-locus sequence typing (MLST)

Polymyxin-resistant *K. pneumonia* and *E. coli* isolates were typed using MLST following the scheme established by the Pasteur Institute (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html; https://bigsdb.pasteur.fr/ecoli/ ecoli.html).

Characterization of *mcr-1*- and carbapenemase mediated resistance

The modified carbapenem inactivation method (mCIM) and ethylenediaminetetraacetic acid-modified carbapenem inactivation method (eCIM), which are recommended by the CLSI, were used for the phenotypic detection of carbapenemase production. The presence of carbapenem resistance genes (bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{NDM}, and $bla_{oxa\text{-}48\text{-}like}$) and of the polymyxin resistance gene *mcr-1* in polymyxin-resistant isolates were screened by polymerase chain reaction using the methods described previously [13, 14].

Pulsed-field gel electrophoresis (PFGE)

Molecular epidemiology of all polymyxin-resistant strains was determined by PFGE after total chromosomal DNA digestion with XbaI in accordance with a previous report [15]. The PFGE patterns were analysed using the BIONUMERICS software (Applied Maths NV, Sint-Martens-Latem, Belgium) using the Dice similarity coefficient. Isolates were considered as the same strain (PFGE type) if they possessed a genetic similarity of ≥ 95%.

Results

Overall prevalence of polymyxin-resistant strains

Over the course of the study, 16 out of the 2301 *E. coli* and *K. pneumoniae* isolates (0.7%) were found to be polymyxin-resistant: 5 *E. coli* and 11 *K. pneumoniae* isolates, collected from 6 different hospitals. The prevalence of polymyxin resistance in *E. coli* and *K. pneumoniae* was 0.3% and 1.4%, respectively (Table 1).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing showed that all of the isolates (100%) were resistant to AMP, KZ, and CTX; 93.3% were resistant to LEV; 86.6% were resistant to CAZ, FEP, and ATM; 80% were resistant to SAM and TZP; 66% were resistant to GN and AK; 62.5% were resistant to IPM and MEM; and 60% were resistant to SXT. Only one isolate was resistant to CZA (6.3%), and all of them were susceptible to TGC (100%) (Figure 1).

The MICs of polymyxin in the context of these 16 strains ranged from 4–64 μg/mL: the full range in the context *K. pneumoniae* isolates (median: 64 μg/mL) and 4 μg/mL in the context of *E. coli* isolates (Table 2).

Detection of antimicrobial resistance genes

Among the 16 polymyxin-resistant isolates, 5 carried the *mcr-1* gene, including 1 *K. pneumoniae* and 4 *E. coli* isolates. In addition, 9 *K. pneumoniae* and 1 *E. coli* isolates were carbapenemase-positive. The mCIM and eCIM results showed that the 9 *K. pneumoniae* isolates were serine carbapenemase-positive, and the *E. coli* isolate was metallo-carbapenemase-positive. Furthermore, the PCR results showed that the 9 *K. pneumoniae* isolates were bla_{KPC-2}-positive, but none of them carried *mcr-1*. On the other hand, the *E. coli* isolate was both bla_{NDM-5}- and *mcr-1*-positive. Of note, no other carbapenemase genes, such as bla_{IMP}, bla_{VIM}, and $bla_{OXA48\text{-}like}$, were detected (Table 2).

Epidemiological characterization

MLST analysis showed that the nine *K. pneumoniae* carbapenem-resistant isolates all belonged to sequence type (ST) 11. On the other hand, among the five *E. coli* isolates, two belonged to ST132 and the other three belonged to ST2, ST983, and ST632 (Table 2).

Of note, dendrogram analysis of PFGE at 95% similarity revealed that homology among the 5 *E. coli* and 11 *K. pneumoniae* isolates was low and sporadic, suggesting a very low likelihood of clonal spread (Figure 2).

Clinical characteristics of the patients infected with polymyxin-resistant isolates

The 16 polymyxin-resistant isolates were collected within 1 year from 15 patients aged 2 months to 93 years old, in 6 hospitals. Two strains were isolate from a single patient, from blood and urine samples, while each of the other 14 patients showed only 1 strain, isolated from urine (n = 4), BAL (n = 3), blood (n = 3), secretion (n = 2), peritoneal puncture fluid (n = 1) and sputum (n = 1) samples. The underlying diseases in these patients included cerebrovascular disease (n = 3), urinary tract disease (n = 3), pneumonia (n = 2), sepsis (n = 1), fever (n = 1), acute coronary syndrome (n=1), pregnancy-induced hypertension (n = 1), premature baby (n = 1), infection around the prosthesis (n = 1), and Guillain-Barre syndrome (n = 1). Of note, five patients received polymyxin treatment before the isolation of polymyxin-resistant strains. Importantly, ten patients displayed positive clinical outcomes (Table 3).

Discussion

Polymyxin has been used against aggressive infections caused by multidrug-resistant bacteria; however, its use has been severely compromised by the emergence of plasmid-mediated polymyxin resistance in Enterobacteriaceae. Hence, in this study, we surveyed the polymyxin resistance rates in *E. coli* and *K. pneumoniae* isolates from hospitalized patients at six local hospitals in the Henan province, China.

Among the total 2301 *E. coli* and *K. pneumoniae* isolates, 16 (0.7%) strains were polymyxin-resistant, 5 of which carried the *mcr-1* gene. Of note, of the 1499 *E. coli* isolates, 5 (0.3%) were polymyxin-resistant and 4 were *mcr-1*-positive; on the other hand, of the 802 *K. pneumoniae* isolates, 11 (1.4%) were polymyxin-resistant, 1 of which carried *mcr-1*. Previously, 0.88% (34/3854) of the *E. coli* isolates and 0.21% (5/2410) of the *K. pneumoniae* isolates carrying *mcr-1* were reported in the China Antimicrobial Resistance Surveillance Trial [16]. Additionally, another study found that 1% (20/1495) of the *E. coli* isolates and 0.18% (1/571) of the *K. pneumoniae* isolates recovered from bloodstream infections in China were *mcr-1*-positive [17]. Our results are, therefore, in line with the previous ones, with rates not exceeding 1.5%. Although *mcr-1* was more common in *E. coli* isolates than in *K. pneumoniae* isolates, the polymyxin resistance rate of *K. pneumoniae* was slightly higher than that of *E. coli* in our study, which is presumably due to antibiotic selection because the detection rate (32.8%) of carbapenem-resistant *K. pneumoniae* (PRCRKP) in Henan ranked first among all Chinese provinces in 2019 (http://www.carss.cn/Report/Details?aId=770). Of note, compared to polymyxin-resistant *E. coli*, polymyxin-resistant *K. pneumoniae* were associated with 8–64 times higher MICs, suggesting chromosomal mutations in related genes, such as *phoP*/*phoQ*, *pmrA*/*pmrB*, and *mgrB*. Additionally, other intrinsic mechanisms might also play important roles in increasing polymyxin resistance in *K. pneumoniae* [17].

Two carbapenemase genes, bla_{KPC} and bla_{NDM}, are responsible for the phenotypic resistance of 90% of carbapenem-resistant Enterobacteriaceae strains in China [18]. The co-existence of *mcr* and carbapenemase genes, such as bla_{NDM-5} [19], bla_{NDM-4} [20], bla_{KPC} [21], and bla_{OXA} [22], has been sporadically reported in different countries. In the national monitoring data from China, one report showed that the *mcr-1* gene was detected in 4.6% (13/282) of carbapenem-resistant *E. coli* isolates and coexisted with the New Delhi metallo-enzyme (NDM)-5 in one strain [23]. In another study, the *mcr-1* prevalence among carbapenem-resistant *E. coli* and PRCRKP isolates was 3.7% (14/376) and 0% (0/1134), respectively, and 14 carbapenem-resistant *E. coli* isolates coproduced $bla_{NDM4/5/9}$ with *mcr-1* [24]. In this study, only one *E. coli* isolate coproduced *mcr-1* and bla_{NDM-5}.

An *E. coli* isolate belonging to ST167 that co-expressed bla_{NDM} and *mcr-1* was previously reported in Henan [6, 25], but in our study, the aforementioned coproducing *E. coli* isolate belonged to ST2. The other *E. coli* strains in this study belonged to ST132, ST983, and ST632. Additionally, nine PRCRKP isolates belonged to ST11, but PFGE showed different types, suggesting these strains, all isolated from the same hospital, were unrelated. Altogether, our results demonstrate that polymyxin-resistant isolates are non-clonal and have different virulence and resistance potentials.

The patients carrying polymyxin-resistant isolates had varying severities of illness, and 33.3% of them had a history of polymyxin use. Moreover, 66.7% of them were cured, and these positive outcomes could be explained by the finding that most polymyxin-resistant isolates remained susceptible to other antimicrobials, such as CZA, SXT, and TGC.

In conclusion, here, we show that polymyxin resistance rate of *K. pneumoniae* is slightly higher than that of *E. coli*, while the presence of *mcr-1* is lower in polymyxin-resistant *K. pneumoniaeversus E. coli* in the Henan province, China. Further molecular investigations and studies are warranted to elucidate the polymyxin resistance mechanism of PRCRKP. In addition, continuous and close monitoring is required to prevent the dissemination of polymyxin-resistant *K. pneumoniae* and *E. coli* strains.

Abbreviations

polymyxin and carbapenem resistant *K. pneumoniae* (PRCRKP), Minimum inhibitory concentrations (MICs), pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), sequence types (STs), mobilized colistin resistance (mcr), bronchoalveolar lavage fluid (BAL), ampicillin (AMP), meropenem (MEM), imipenem (IPM), ceftazidime (CAZ), cefotaxime (CTX), cefazolin (KZ), ampicillin/sulbactam (SAM), aztreonam (ATM), cefepime (FEP), piperacillin/tazobactam (TZP), levofloxacin (LEV), amikacin (AK), gentamicin (GN), trimethoprim/sulfamethoxazole (SXT), ceftazidime/avibactam (CZA), tigecycline (TGC), carbapenem inactivation method (mCIM), ethylenediaminetetraacetic acid-modified carbapenem inactivation method (eCIM)

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Henan Provincial People's Hospital, Henan, China (20190050). The requirement for informed consent from patients was waived.

Consent for publication

No personally identifiable information was collected in this study.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding Information

This work was supported by the Science and Technology Research Project of Henan Province [grant number SBGJ2018084 and 202102310355], the Joint Program of Medical Science and Technology Research of Henan Province (LHGJ20190611) and the Collaborative Innovation Major Project of Zhengzhou [grant number 20XTZX05015]. The funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

WJY and YL contributed to conception and design of the article. YZ, SYR, DMH, WMZ, CQS and XJZ were responsible for microbiological tests. NJ and YHY carried out the molecular genetic studies. WJY and QZ wrote the first version of the manuscript. MYW and YL conceived and supervised the project. All authors critically revised the manuscript, and read and approved the final manuscript.

Acknowledgments

We would like to thank Editage (www.editage.cn) for English language editing.

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Tables

Table 1. Prevalence of polymyxin-resistant isolates in the six participating hospitals

	Hospital	No. of isolates	No. of polymyxin-resistant isolates (%)
Escherichia coli			
	Hospital 1	326	3 (0.9)
	Hospital 2	231	1 (0.4)
	Hospital 6	942	1 (0.1)
Total		1499	5 (0.3)
Klebsiella pneumoniae			
	Hospital 3	141	1 (0.7)
	Hospital 4	133	1 (0.8)
	Hospital 5	78	2 (2.6)
	Hospital 6	450	7 (1.6)
Total		802	11 (1.4)
Overall total		2301	16 (0.7)

Table 2 Phenotypic and genotypic characteristics of the polymyxin-resistant strains

e	Isolate	Polymyxin MIC (μg/mL)	mCIM	eCIM	KPC	NDM	mcr-1
spital5	Kpn1	4	+	-	KPC-2	-	-
spital4	Kpn2	32	+	-	KPC-2	-	-
spital6	Kpn3	64	+	-	KPC-2	-	-
spital5	Kpn4	8	+	-	KPC-2	-	-
spital6	Kpn5	64	ND	ND	-	-	mcr-1
spital3	Kpn6	64	ND	ND	-	-	-
spital6	Kpn7	16	+	-	KPC-2	-	-
spital6	Kpn8	32	+	-	KPC-2	-	-
spital6	Kpn9	64	+	-	KPC-2	-	-
spital6	Kpn10	64	+	-	KPC-2	-	-
spital6	Kpn11	64	+	-	KPC-2	-	-
spital1	Eco1	4	+	+	-	NDM-5	mcr-1
spital1	Eco2	4	ND	ND	-	-	mcr-1
spital1	Eco3	4	ND	ND	-	-	mcr-1
spital2	Eco4	4	ND	ND	-	-	mcr-1
spital6	Eco5	4	ND	ND	-	-	-

Kpn, *Klebsiella pneumoniae*; Eco, *Escherichia coli*; ND, data was not collected; MIC, minimal inhibitory concentration; mCIM, modified carbapenem inactivation method; eCIM, ethylenediaminetetraacetic acid-modified carbapenem inactivation method; KPC-2, *K. pneumoniae* carbapenemase-2; NDM-5, New Delhi metallo-enzyme-5; mcr-1, mobilized colistin resistance-1

Table 3 Clinical characteristics of the patients carrying polymyxin-resistant isolates

Patient	Gender/age (years)	Isolate	Source	Clinical diagnosis	Underlying disease	Indwelling devices	Antimicrobial use within 30 days prior to culture	Outcome
1	Male/87	Kpn	Blood	Cerebral infarction	Diabetes	Tracheal cannula	TGC, Carbapenems	Discharge
2	Female/33	Kpn	Secretion	Pneumonia, AFE	No	No	Clindamycin, Quinolones	Discharge
3	Male/38	Kpn	Sputum	Septic shock	No	CVC, Tracheal cannula	β-lactam, Quinolones	Die
4	Female/69	Eco/Eco	Blood/urine	Acute coronary syndrome	Diabetes, CHD	No	β-lactam, Quinolones	Discharge
5	Male/81	Eco	Blood	Fever	Haemodialysis	No	β-lactam, Quinolones,	Discharge
6	Male/93	Eco	BAL	Cerebral haemorrhage	Diabetes	CVC, Tracheal cannula	Carbapenems	Discharge
7	Female/69	Kpn	Urine	Urinary retention	No	CVC	β-lactam	Get worse
8	Male/62	Kpn	BAL	Cerebral haemorrhage	No	Tracheotomy	TGC, Polymyxin	Get worse
9	Female/89	Eco	Urethral secretions	cUTI	Hypertension, CHD	Urethral catheter	β-lactam, Quinolones	Die
10	Male/56	Kpn	Urine	Guillain-Barre syndrome	Hypertension	Tracheal cannula	TGC, Polymyxin, Carbapenems	Discharge
11	Male/67	Kpn	Urine	Urethral injury	Hypertension	Urethral catheter	β-lactam	Discharge
12	Female/2 months	Kpn	BAL	Premature baby	No	Tracheal cannula	Polymyxin, Fosfomycin, fluconazole	Discharge
13	Female/28	Kpn	Peritoneal puncture fluid	Pregnancy-induced hypertension	SLE	Peritoneal drainage tube	TGC, Polymyxin, Carbapenems	Discharge
14	Male/89	Kpn	Blood	Severe pneumonia	No	Tracheal cannula	Polymyxin, Carbapenems, fluconazole	Die
15	Female/54	Kpn	Secretion	Infection around the prosthesis	No	No	Carbapenems, Quinolones	Discharge

Kpn, *Klebsiella pneumoniae*; Eco, *Escherichia coli*; CHD, coronary heart disease; SLE, systemic lupus erythematosus; AFE, amniotic fluid embolism; CVC, central venous catheter; BAL, bronchoalveolar lavage fluid; cUTI, complicated urinary tract infection; TGC, tigecycline.

Figures

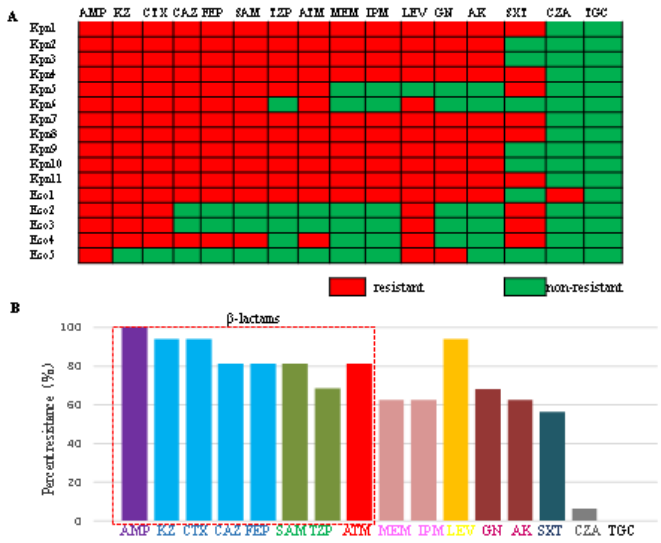

Figure 1

Antimicrobial susceptibility profiles of the 16 polymyxin-resistant isolates. (A) Heatmap showing the resistance phenotypes of each of the isolates. (B) Percentage of strains resistant to the tested antibiotics. AMP, ampicillin; KZ, cefazolin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; ATM, aztreonam; MEM, meropenem; IPM, imipenem; LEV, levofloxacin; GN, gentamicin; AK, amikacin; SXT, trimethoprim/sulfamethoxazole; CZA, ceftazidime Avibactam; TGC, tigecycline.

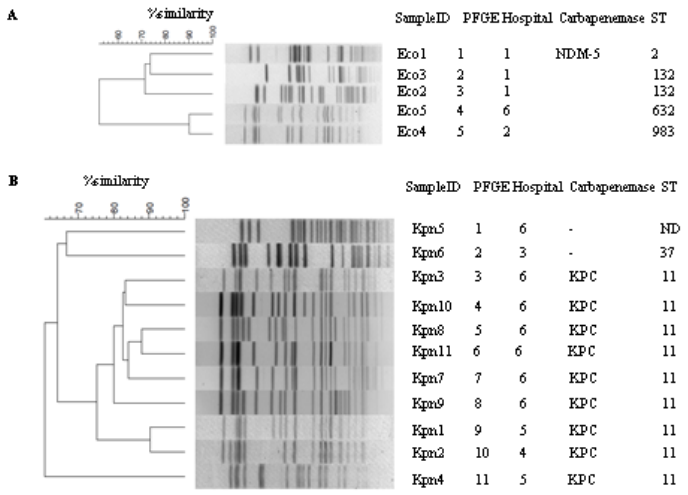

Figure 2

Pulsed-field gel electrophoresis (PFGE)-based dendrogram of polymyxin-resistant Escherichia coli (A) and Klebsiella pneumoniae (B) strains. Kpn, K. pneumoniae; Eco, E. coli; NDM-5, New Delhi metallo-enzyme-5; ST, sequence type; KPC, K. pneumoniae carbapenemase.