

Diverse genetic characteristics of ESBL-producing *Klebsiella pneumoniae* isolates from obstetrics & gynaecology settings in some major hospitals, China

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Background: We investigated the susceptibility patterns and genotype of extended-spectrum-beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* isolates. **Methods:** A prospective survey of *K. pneumoniae*-infected patients was conducted in obstetrics & gynaecology settings in three associated hospitals of a university in China from 2017 to 2019. All isolates were identified as *K. pneumoniae* by the conventional standard procedures and confirmed by use of the VITEK GNI system. Susceptibility to 16 antimicrobial agents of ESBLs was determined by way of a screening procedure using discs. PCR amplification of *bla* genes, and sequencing of PCR products were performed to determine their molecular types. **Results:** A total of 518 of *K. pneumoniae* were isolated from sputum specimens in three affiliated hospitals. Out of these, 158 strains were ESBL producers and 64 strains were non-ESBL producers from the first hospital; 114 strains were positive for producing ESBLs and 60 strains were negative for ESBLs in the second hospital, and 86 strains were ESBL producer and 36 strains were negative from the third hospital. *bla* gene contents showed that 88 strains (24.6%) carried the *bla*_{TEM-2} gene, and 74 strains (20.1%) contained the *bla*_{SHV-3} gene, 44 strains (12.3%) had the *bla*_{CTX-M-2} gene, 42 strains (11.7%) had the *bla*_{CTX-M-4} gene, 38 strains (10.6%) harboured *bla*_{CTX-M-5} and *bla*_{CTX-M-8} genes, and 29 (9.5%) strains contained *bla*_{CTX-M-15} gene. Also, 20 (5.6%) strains contained *bla*_{OXA-1}. The co-existence of *bla*_{CTX-M-15} and *bla*_{SHV-3} was identified in 20 strains (5.6%), 12 strains (3.3%) co-existed with *bla*_{SHV-3} and *bla*_{TEM-2}, 14 isolates (3.9%) harboured *bla*_{TEM-2} and *bla*_{CTX-M-15}, eight isolates (2.2%) possessed *bla*_{SHV-3}, *bla*_{TEM-2}, and *bla*_{CTX-M-15}. **Discussion:** The high prevalence of ESBLs in *K. pneumoniae* isolates with diverse enzyme gene types indicated the need for screening the changes in ESBL-producing isolates in this region.

Keywords

ESBLs; *Klebsiella pneumoniae*; Molecular epidemiology

1. Introduction

Resistance of *Enterobacteriaceae* to extended-spectrum-beta-lactamase (ESBL) has spread rapidly worldwide and poses a serious threat in many regions [1]. *Klebsiella pneu-*

moniae (*K. pneumoniae*) is one of the most common ESBL-producing *Enterobacteriaceae* in healthcare-associated infections (HAI) including hospital infection (HA) and community infection (CA). In recent years, the prevalence of ESBL-producing *K. pneumoniae* in clinical settings has been accelerated by the inappropriate application of antibiotics leading to a significant public health concern [2, 3]. ESBLs are transmitted by a resistance plasmid or integron in, and between, hospitals among *Enterobacteriaceae*, frequently resulting in multiple antimicrobial resistance and clinical treatment failures. The nosocomial infections of *K. pneumoniae* frequently occur due to horizontal transfer of resistant mobile genetic elements in hospitals and in the community, which contributes to multiple mechanisms of transmission of antimicrobial agents [4, 5]. Therefore, understanding the molecular mechanism of resistance to antibiotics and predicting the clinical characteristics of antimicrobial drugs, we investigated the occurrence and rapid dissemination of *K. pneumoniae* producing ESBLs from obstetrics and gynaecology settings in three associated hospitals of a university over a three-year period in China to make a better choice of antimicrobial agents and improve patient survival.

2. Clinical data and methods

This retrospective study was based on a survey of 6147 in-patients under obstetrics & gynaecology settings in three associated hospitals of a university over a three-year period in China. Patients with concurrent HAIs were classified into respiratory tract infections including pneumonias, bloodstream infections including catheter-related infections; urinary tract infections, surgical site infections, and other infections were excluded. For purposes of data analysis, we collected data for each HAI including age, date of onset, and microbes. The pregnant women admitted to obstetrics settings had an average age of 36.7 ± 3.5 years with high maternal age for caesarean section operations. The women admitted to gynaecology settings had a median age of 63 years for treatment or surgery of a cancer or tumour. Only gram-negative

aerobic and facultative anaerobic isolates from infection sites were considered clinically significant. The pathogenic bacteria associated with HAI were collected within 48 h or after 48 h of hospitalisation according to local hospital criteria. The research was approved by the institutional ethics committees of the participating hospitals and written informed consent was obtained from all participants enrolled in the study.

A single specimen was obtained from sputum or bronchial washing. All isolates were identified as *K. pneumoniae* by the conventional standard procedures and confirmed by VITEK GNI system (bioMérieux Vitek Inc., USA). No repetitive isolates from a single patient were included. Susceptibility to 16 antimicrobial agents was determined and interpreted by an agar doubling dilution method akin to that in the Clinical and Laboratory Standards Institute (CLSI) criteria. ESBL screening was determined by using discs (Bio-Rad, Hercules, CA, USA) according to CLSI guidelines [6]. Control strains were *E. coli* ATCC 25922, *Streptococcus pneumoniae* ATCC 49619, and *P. aeruginosa* ATCC 27853.

A series of *bla* genes including *bla_{TEM}*, *bla_{SHV}*, *bla_{OXA}*, and *bla_{CTX-M}* were identified by PCR amplification, primers and PCR reactions were performed as described elsewhere [7, 8]. PCR products of *bla* genes were sent to Sangon Biotech Co., Ltd (Shanghai, China) for sequencing, and DNAMAN software was used to analyse the sequencing results.

3. Results

From January 2017 to December 2019, we calculated that a total of 1564 patients would be recorded to estimate an anticipated HAI prevalence of 21.8%. A total of 1564 isolates, which were obtained from respiratory tract and sputum, frequently included *Klebsiella* species (33.1%), *P. aeruginosa* (27.8%), *Acinetobacter* species (17.8%), *Enterococcus* species (10.9%), and *Escherichia coli* (9.3%). Besides that, others were gram-positive including *Staphylococcus* species (9.8%, including 3.7% *S. aureus*).

In total, out of 518 *K. pneumoniae* isolates, 124 strains were isolated from pregnant patients with an average hospitalisation length of 10 days for caesarean section operations, and 394 strains were separated from patients for treatment or surgery of a cancer or tumour. The average duration of hospitalisation exceeded 15 days. 358 isolates (69.2%) produced ESBLs, and 160 isolates (30.8%) did not produce ESBLs based on antimicrobial susceptibility testing. In view of the distribution of the three hospitals, 222 *K. pneumoniae* isolates were identified in the first affiliated hospital. 158 (158/222, 71.2%) were ESBL producers and 64 (64/222, 28.8%) were not; 114 isolates (114/174, 65.5%) were ESBL producers and 60 strains (60/174, 34.5%) were not in 174 isolates of *K. pneumoniae* from the second affiliated hospital; additionally, out of 122 isolates of *K. pneumoniae*, 86 (86/122, 70.5%) were ESBL producers and 36 (36/122, 29.5%) were not in the third affiliated hospital.

All ESBL-producing *Klebsiella pneumoniae* isolates were resistant to aztreonam and cefpodoxime, and the least resis-

Table 1. Comparison of antimicrobial susceptibility of *K. pneumoniae* with and without ESBLs from 2017 to 2019.

Antibiotic	ESBLs (n = 358)		Non-ESBLs (n = 160)	
	Strain (n)	Rate (%)	Strain (n)	Rate (%)
AMP	358	100.0	160	100.0
CXM	298	83.3	0	0
FOX	0	0	0	0
CRO	298	83.3	78	48.3
CTX	298	83.3	34	20.8
CAZ	242	67.8	58	36.8
FEP	310	86.7	68	42.7
AZM	208	58.3	0	0
IMP	0	0	0	0
MEM	0	0	0	0
GEN	310	86.7	58	36.7
KAN	12	50.0	56	35.4
SXT	268	75.0	40	25.0
AMC	180	50.0	0	0
CIP	274	76.8	60	37.7

AMC, Ampicillin/clavulanic acid; AMP, Ampicillin; AZM, Azithromycin; CAZ, Ceftazidime; CIP, Ciprofloxacin; CRO, Ceftriaxone; CTX, Cefotaxime; CXM, Cefuroxime; ESBLs, extended-spectrum-beta-lactams; FEP, Cefepime; FOX, Cefoxitin; GEN, Gentamycin; IMP, Imipenem; KAN, Kanamycin; MEM, Meropenem; SXT, Sulfamethoxazole.

tant antibiotic was ceftazidime (67.8%). Besides gentamycin, as a non-beta-lactam, imipenem was also found to confer effectiveness as an antibiotic. In non-ESBL *K. pneumoniae* strains, resistance to aztreonam was not observed, however, the highest resistance to cefotaxime and ceftazidime were observed (more than 50%) (Table 1).

All ESBL-producing isolates were subjected to PCR for detection of the most widely spread ESBLs: genes and amplicons were subjected to sequencing. Among *K. pneumoniae*-producing ESBLs, 88 strains (88/358, 24.6%) contained the *bla_{TEM-2}* gene, and 74 strains (74/358, 20.1%) were positive for the *bla_{SHV-3}* gene, 44 strains (44/358, 12.3%) contained the *bla_{CTX-M-2}* gene, 42 strains (42/358, 11.7%) contained the *bla_{CTX-M-4}* gene, 38 strains (38/358, 10.6%) contained the *bla_{CTX-M-5}* and *bla_{CTX-M-8}* genes, respectively, 34 (34/358, 9.5%) strains contained the *bla_{CTX-M-15}* gene. Also, 20 (20/358, 5.6%) strains contained *bla_{OXA-1}*. The co-existence of *bla_{CTX-M}* and *bla_{SHV-2}* was identified in 20 strains (20.9%), 12 strains (13.4%) co-existed with *bla_{SHV-3}* and *bla_{TEM-2}*, 14 isolates (6%) harboured both *bla_{TEM-2}* and *bla_{CTX-M-15}*, eight isolates (6%) contained *bla_{SHV-3}*, *bla_{TEM-2}*, and *bla_{CTX-M-15}* (Table 2).

4. Discussion

ESBL-producing *K. pneumoniae* are common pathogens isolated from various body sites. Our findings showed that the frequency of HAIs and antimicrobial resistance in *K. pneumoniae* isolates posed a considerable threat to public health in the region. In these obstetrics and gynaecology settings,

Table 2. Diverse genotype of ESBLs-producing *K. pneumoniae*.

Genotype	No. 1 hospital	No. 2 hospital	No. 3 hospital
	Positive strains (n)	Positive strains (n)	Positive strains (n)
<i>bla</i> _{TEM-2}	38	26	24
<i>bla</i> _{SHV-3}	28	24	22
<i>bla</i> _{CTX-M-2}	20	14	10
<i>bla</i> _{CTX-M-4}	20	14	8
<i>bla</i> _{CTX-M-5}	16	12	10
<i>bla</i> _{CTX-M-8}	18	14	6
<i>bla</i> _{CTX-M-15}	18	5	6
<i>bla</i> _{OXA-1}	16	2	2
<i>bla</i> _{TEM-2} + <i>bla</i> _{SHV-3}	12	0	0
<i>bla</i> _{TEM-2} + <i>bla</i> _{CTX-15}	14	0	0
<i>bla</i> _{SHV-3} + <i>bla</i> _{CTX-5}	8	2	2
<i>bla</i> _{SHV-3} + <i>bla</i> _{CTX-15}	8	2	2
<i>bla</i> _{TEM-2} + <i>bla</i> _{SHV-3} + <i>bla</i> _{CTX-15}	8	0	0

ESBLs, extended-spectrum-beta-lactams.

pregnant patients for caesarean section operations, female patients treated for cancer or tumour were more susceptible to infection than those in other departments due to the length of hospitalisation, age, use of retention tube, the low patient-to-nurse ratio, and ICU bed occupancy. The findings may be explained by the fact that these patients were administered an excess of antibiotics due to their immunological status. As shown elsewhere, the main sites of infection are the respiratory tract, incision, and urinary tract: these are the most likely sites to cause infection in gynaecological in-patients [9].

Among *in vitro* ESBL-producing susceptibility to the third generation of cephalosporins, *K. pneumoniae* isolates included in the study exhibited the highest resistance to aztreonam, cefpodoxime, aztreonam, ceftriaxone, as well as ceftazidime: however, all isolates were susceptible to imipenem, meropenem, and ciprofloxacin (92.3%). So, imipenem or meropenem prophylaxis can be administrated as an effective antibiotic for *K. pneumoniae*-producing ESBL isolates. Evidence indicated that the number of administrations of third-generation cephalosporins (except imipenem or meropenem) affected incidence of HAIs. This finding may be supported by the fact that these infecting isolates were resistant to the third-generation cephalosporins, while all of them were susceptible to imipenem or meropenem in addition to gentamycin. These findings correlate with those reported previously by authors in China where 59.2% of isolates were ESBL-positive and all isolates were susceptible to imipenem or meropenem [10].

In the present study, we found that the rates of ESBL production were variable among three hospitals. The data ranged from 71.2% in the first hospital to 65.5% in the third hospital, showing the percentage of *K. pneumoniae* isolates from respiratory infection that contributed to different frequencies of ESBL production. The highest rates of *K. pneumoniae* isolates producing ESBLs were exhibited in the first hospital, and the lowest in the third hospital. Our finding showed that the frequency of occurrence

of *bla*_{CTX-M} exceeded that of *bla*_{TEM} and *bla*_{SHV}. The prevalence of *bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV} genes in this study was 54.7%, 23.9%, and 16%, respectively. Our findings are in agreement with the results from a survey in which Guo *et al.* found that 31.3 % of *K. pneumoniae* isolated from one of the largest hospitals in central China were ESBL-positive whereas their detected prevalence of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M-I}, and *bla*_{CTX-M-III} among these isolates was 97%, 71.6%, 73.1%, and 20.9%, respectively [11]. Our results showed high prevalence of *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} and a low frequency of occurrence of *bla*_{OXA-1}. Significantly, *bla*_{CTX-M} was found to be the dominant ESBL in *K. pneumoniae* isolates. They exhibited a diverse genotype consisting of *bla*_{CTX-M-2}, *bla*_{CTX-M-4}, *bla*_{CTX-M-5}, *bla*_{CTX-M-8}, and *bla*_{CTX-M-15}. The increasing proportion of *bla*_{CTX-M} in the different settings suggested that the dissemination of *K. pneumoniae* isolates carried ESBL genes from remote wards to local hospital. Additionally, *bla*_{CTX-M-15} was first reported in India in 1999: this ESBL-type gene has now propagated in various countries and has become globally predominant. Studies demonstrated that the *bla*_{CTX-M} alleles have evolved from *bla*_{CTX-M-2} to *bla*_{CTX-M-15} through point mutation or substitution owing to overuse of cefotaxime and ceftriaxone, resulting in a temporal shift in ESBL type. Similar findings were reported by other authors in Italy, Iran, and Turkey [4, 12, 13]. Another finding was that *K. pneumoniae*-positive for all three genes were found. This may be due to the coexistence of three genes in the same plasmid or mobilisation of genetic elements.

The emergence and spreading dissemination of ESBL-producing strains is of concern to public health officials: the detection rate of ESBL-producing *K. pneumoniae* increased significantly in recent years. One of the reasons for this may be related to inadequate and excessive use of antimicrobial drugs, a lack of adequate antimicrobial surveillance programmes, and such in-patients are in poor health: if nursing

care was not timely or indeed absent, they are easily infected. Moreover, incomplete sterilisation of the ward can cause bacterial accumulation, and patients suffer from respiratory tract infection. So, strengthening the training of obstetrics and gynaecology nursing staff have been deemed essential to improve their awareness of infection prevention and control [14].

Hospital management needs to be given enough attention to formulate effective measures to reduce the incidence of HAIs. The management system of infection should be completed, and each operational process should be standardised; strict aseptic operations should be performed, and the disinfection of various instruments and equipment should be done well during hand surgery. The ward was disinfected regularly to ensure that the patient was in a clean, healthy, and sterile environment [15, 16].

There are some limitations to the current study: we only speculated upon the *K. pneumoniae* isolates and ESBL genetic contents from an obstetrics and gynaecology setting. We lacked evidence from a hospital surveillance study aimed at detecting *K. pneumoniae* isolates. Additional research is needed to explore the specific enzymes and kinetics present in *K. pneumoniae* isolates producing ESBL in the region.

In summary, the high prevalence of ESBLs in *K. pneumoniae* isolates with diverse enzyme gene types indicated the need for screening the changes in ESBL-producing isolates in this region. The data in this study contributed to the increasing recognition of ESBL isolates, enhanced surveillance, and strict control of hospital epidemics in this species.

Author contributions

ZXM and LMC designed the present study. LMC, BXY and CJY performed the assay, analyzed and interpreted the data. WH wrote the draft manuscript. BXY and CJY collected clinical samples. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The research was approved by the institutional ethics committees of the participating hospitals (Protocol Number 2017-02-01), and written informed consent was obtained from all participants enrolled in the study.

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Conflict of interest

The authors declare no conflict of interest.

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