Characterization of Extended-Spectrum β -Lactamases (ESBLs)-Producing *Klebsiella Pneumoniae* Strains in Captive Giant Pandas

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Abstract

Background: Extended-spectrum β -lactamases (ESBLs)-producing strains of *Klebsiella pneumoniae* remain a critical clinical concern in the world. However, limited information is available concerning ESBLs-producing *K. pneumoniae* in giant pandas. The objective of this study was to characterize ESBLs-producing *K. pneumoniae* isolates from the giant panda. A total of 211 *K. pneumoniae* isolates were collected from 108 giant pandas housed at the Chengdu Research Base of Giant Panda Breeding, China. Samples were screened for the ESBLs-producing phenotype via the double-disk synergy test method.

Result: A total of three (1.42%, $n = 211$) ESBLs-producing *K. pneumoniae* strains were identified and subjected to MLST (Multi-locus Sequence Typing) analysis for the characterization of ESBLs-encoding genes, transposons, antimicrobial resistance genes, and antimicrobial susceptibility. Among the three ESBLs-producing isolates, different ESBLs-encoding genes, including $\text{bla}_{\text{CTX-M-1}}$, bla_{SHV} , and bla_{TEM} were detected. Three isolates were found to carry transposons (i.e., aac6-ib and tnpU) and antimicrobial resistance genes (i.e., aac6-ib , tnpU , aac6-1 , qnrA , and qnrB). Furthermore, these three isolates were resistant to at least 15 antibiotics and had different ST isotypes (ST37, ST290, and ST2640).

Conclusion: Effective surveillance and strict infection control strategies should be implemented to prevent outbreaks of ESBLs-producing *K. pneumoniae* in giant panda.

Introduction

Klebsiella pneumoniae is an important human pathogen causing numerous infections in hospitals, long-term care facilities, and the associated communities. The bacteria infect the lungs, urinary tract, and surgical sites, causing soft tissue infections and bacteremia (Zhan *et al.*, 2017). The giant panda, *Ailuropoda melanoleuca*, is one of the world's most recognized and rarest animals and is now only distributed in the mountainous areas of Sichuan, Gansu, and Shaanxi provinces (Wang *et al.*, 2018). The results of the fourth census of giant pandas showed that the population of wild giant pandas reached 1864 (Di, 2015), and the current population of captive giant pandas reached 600. With the growth of the captive population of giant pandas and the in-depth study of giant panda diseases, infections with *K. pneumoniae* have emerged. Wang *et al.* (1998) reported that giant pandas infected with *K. pneumoniae* developed hemorrhagic enteritis. In the same year, a sub-adult giant panda was infected with *K. pneumoniae* and *Escherichia coli* subsequently died from hemorrhagic sepsis, and the isolated *K. pneumoniae* was found to be pathogenic to mice (Wang & Wang, 2003). Subsequent cases of genital hematuria, enteritis, and sepsis caused by *K. pneumoniae* infection in giant pandas were reported (Wang *et al.*, 2006). We conducted an etiological study on a dead giant panda found in a nature reserve in Sichuan and discovered the giant panda died of multiple organ dysfunction syndrome caused by *K. pneumoniae* and *Proteus mirabilis* infection (Chen *et al.*, 2020). This showed that *K. pneumoniae* infection is a serious threat to the life and health of giant pandas. In addition, studies have shown that *K. pneumoniae* can be transmitted through the air (Bolister *et al.*, 1992), therefore captive animals infected with *K. pneumoniae* may infect husbandry staff and even visitors to wildlife centers..

The emergence of antibiotic resistance is an increasingly alarming public health threat (Amaya *et al.*, 2011), since it undermines the efficacy of antibiotic treatment and is expected to be the leading cause of global mortality by 2050, possibly exceeding that of cancer (Neill, 2014). β -lactam antibiotics are among the most frequently prescribed antimicrobials. However, extended-spectrum β -lactamases (ESBLs) have emerged in numerous hospitals worldwide. These enzymes confer resistance to extended-spectrum cephalosporins and related oxyimino- β -lactams (ceftazidime, cefotaxime, and aztreonam) but are predominantly sensitive to carbapenems, cephamycins, and β -lactamase inhibitors such as clavulanic acid (Paterson & Bonomo, 2005). Antibiotics including β -endamide are commonly used in disease prevention and control in captive pandas. Statistics for antibiotics use in giant panda indicate that β -lactam antibiotics account for about 50%, while carbapenem antibiotics included imipenem. In the field of human and livestock medicine, experts and researchers have conducted in-depth studies on the clinical isolation of ESBLs-producing *K. pneumoniae* in regards to drug resistance, genetic toxicity, and molecular epidemiology, and these results have played a positive role in the scientific and effective prevention and control of ESBLs-producing *K. pneumoniae*. However, molecular epidemiology studies of ESBLs-producing *K. pneumoniae* in giant

pandas are currently lacking. Therefore, this study aimed to isolate ESBLs-producing *K. pneumoniae* from captive giant pandas, explore the prevalence and genotype of ESBLs-producing *K. pneumoniae*, provide a scientific basis for the clinical use of antibiotics, and also provide systematic experimental data and a reference basis for preventing and controlling the spread of such bacteria.

Materials And Methods

Bacterial isolates and screening for ESBLs phenotype

Two hundred and eleven nonduplicated *K. pneumoniae* isolates were collected from fresh feces of captive giant pandas from 2018 to 2019 at the Chengdu Research Base of Giant Panda Breeding (Panda Base) in Sichuan, China.. These isolates were identified as *K. pneumoniae* by Gram staining, 16s rDNA, and bacterial biochemical identification. All of the isolates were tested for ESBLs production by the CLSI-recommended confirmatory double-disc combination test (CLSI, 2019). All of the isolates were screened for ESBLs production using cefotaxime (CTX) and ceftazidime (CAZ) alone and in combination with clavulanic acid according to the double-disk synergy test method (DDST) (CLSI, 2016). Phenotypic presence of ESBLs in the isolates was determined by detecting diameter enhancement of the inhibition zone of the clavulanate disk and corresponding β -lactam antimicrobial disk. If the enhancement value was > 5 mm, the isolate was presumed to be an ESBLs producer (Yang *et al.*, 2014a; Yang *et al.*, 2014b).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests of three ESBLs-producing isolates and seven non-ESBLs-producing isolates were evaluated using the disk diffusion method to Penicillin 1 μ g (PEN), Piperacillin 100 μ g (PRL), Ampicillin 10 μ g (AMP), Oxacillin 1 μ g (OX), Amoxicillin 20 μ g (AML), Moxalactam 30 μ g (MOX), Ceftazidime 30 μ g (CAZ), Cefixime 5 μ g (CFM), Cefmatazole (CMZ), Cefepime 30 μ g (FEP), cephalosporine (CTX), Cephalexin 30 μ g (CL), Cefazolin 30 μ g (KZ), Ceftriaxone 30 μ g (CRO), Cefoxitin 30 μ g (FOX), Piperacillin/Tazobactam 100/10 μ g (TZP), Cefuroxime 30 μ g (CXM), Cefaclor (CEC), Ampicillin/Sulbactam 10/10 μ g (SAM), Cefoperazone 75 μ g (CFP), Ceftizoxime 30 μ g (ZOX), Aztreonam 30 μ g (ATM), Meropenem 10 μ g (MEM), Imipenem 10 μ g (IPM), Kanamycin 30 μ g (K), Streptomycin 10 μ g (S), Ofloxacin 5 μ g (OFX), Norfloxacin 10 μ g (NOR), Ciprofloxacin (CIP); Gatifloxacin 5 μ g (GTX), Chloramphenicol 30 μ g (C), Vancomycin 30 μ g (VA), Azithromycin 15 μ g (AZM), Doxycycline (TE), Minocycline 30 μ g (MH), Compound Sulfamethoxazole (SMZ) 23.75/1.25 μ g, and Trimethoprim 5 μ g (TMP) (Oxoid, Basingstoke, United Kingdom). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints. *Escherichia coli* ATCC 25922 was used as a control for antimicrobial susceptibility testing.

Multi-locus Sequence Typing (MLST)

MLST was performed on three ESBLs-producing isolates and 10 non-ESBLs producing isolates by amplifying the seven standard housekeeping loci gapA, infB, mdh, pgi, phoE, rpoB, and tonB as described previously (Diancourt *et al.* 2005). Sequence types (STs) were assigned using the online database on the Pasteur Institute MLST website (<http://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>).

Molecular investigations of antimicrobial resistance

All three ESBLs-producing isolates and seven non-ESBLs-producing isolates were screened by PCR for the presence of ESBL genes (*bla*_{CTM}, *bla*_{CTM-M-1}, *bla*_{CTM-M-2}, *bla*_{CTM-M-8}, *bla*_{CTM-M-9}, *bla*_{CTM-M-25}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{GES}, *bla*_{PER}, *bla*_{VEB}, *bla*_{OXA-1}, and *bla*_{OXA-2}), Transposons (IS26, *int1*, *traA*, IS26, *int1*, *traA*, *trbC*, *tnpU*, and IS903), Aminoglycoside genes (*aac*(3)-I, *aac*(3)-II, *aac*(6')-I, *aac*(2")-I, *aac*(6')-II, *tnp513*, *ant*(3")-I, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *armA*, *aadA5*, and *npmA*), and Quinolones genes (*qnrA*, *aac*(6')-Ib, *qnrS*, and *qnrB*). The PCR amplicons of *bla*_{CTM-M-1}, *bla*_{TEM}, and *bla*_{SHV} from three ESBLs-producing *K. pneumoniae* isolates were sent to Shanghai Sangong Biotech (Shanghai, China), and sequence analysis was performed using the National Center for Biotechnology Information (NCBI) website with the BLASTn tool.

Tab. 1 Primers used for detection and sequencing of target genes in *Klebsiella pneumoniae* isolates.

Target	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)	Annealing temperature (°C)
β-Lactamases and ESBLs-encoding genes				
CTX-M	ATGTGCAGYACCAGTAARGT	TGGGTRAARTARGTSACCAGA	593	52
CTX-M-1	AAAAATCACTGCGCCAGTTC	AGCTTATTCATCGCCACGTT	415	52
CTX-M-2	CGACGCTACCCCTGCTATT	CCAGCGTCAGATTTTTTCAGG	552	52
CTM-M-8	TCGCGTTAAGCGGATGATGC	AACCCACGATGTGGGTAGC	666	52
CTM-M-9	ATGGTGACAAAGAGAGTGCA	CCCTTCGGCGATGATTCTC	869	52
CTX-M-25	GCACGATGACATTCGGG	AACCCACGATGTGGGTAGC	327	52
TEM	CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	800	52
SHV	AGCCGCTTGAGCAAATTAAAC	ATCCCGCAGATAAATCACCAC	713	52
GES	AGTCGGCTAGACCGGAAAG	TTTGTCCGTGCTCAGGAT	399	52
PER	GCTCCGATAATGAAAGCGT	TTCGGCTTGACTCGGCTGA	520	52
VEB	CATTTCCCGATGCAAAGCGT	CGAAGTTTCTTTGGACTCTG	648	52
Transposons gene				
IS26	ATGAACCCATTCAAAGGCCGGCAT	TATGCAGCTTTGCTGTTACGACGG	387	55
intl1	CCGAGGATGCGAACCCTTC	CCGCCACTGCGCCGTTACCA	373	53
traA	CTGCTGTGGCGGCGTTCTTC	AGTAACCGGCGACCGACATACC	246	53
trbC	CGGYATWCCGSCSACRCTGCG	GCCACCTGYSBGCAGTCMCC	255	53
tnpU	GCAAGGAGAAGCGACGAGTGTG	TACATGGCGGTCTCGGCTATCG	367	55
IS903	GCAATACGCACGCTTTCAGGC	ACTGCACGGTTACGGTCTGCA	521	55
Aminoglycoside gene				
aac(3)-I	ACCTACTCCCAACATCAGCC	ATATAGATCTCACTACGCGC	169	55
aac(3)-II	ACTGTGATGGGATACGCGTC	CTCCGTCAGCGTTTCAGCTA	237	52
aac(6')-I	ATGAGTGGCTAAATCGATC	CCCGCTTTCTCGTAGCA	394	55
aac(2'')-I	GCGAAATCTGCCGCTCTGG	GCTGTTACAACGGACTGGCCG	319	55
aac(6')-II	TTCATGTCCGCGAGCACCCC	ACTCTTCCGCCATCGCTCTGGG	178	54
tnp513	CGAGTCAACCTCACACGCTTCC	TGCTCAATGACCTTCGGATCTTCG	269	55
ant(3'')-I	TGATTTGCTGGTTACGGTGAC	CGCTATGTTCTCTTGCTTTTG	284	55
rmtA	CTAGCGTCCATCCTTTCCTC	TTTGCTTCCATGCCCTTGCC	634	55

rmtB	CCCAAACAGACCGTAGAGGC	CTCAAACCTCGGCGGGCAAGC	565	55
rmtC	CGAAGAAGTAACAGCCAAAG	ATCCCAACATCTCTCCCACT	800	55
rmtD	CGGCACGCGATTGGGAAGC	CGGAAACGATGCGACGAT	750	55
armA	AGGTTGTTTCCATTTCTGAG	TCTCTTCCATTCCCTTCTCC	590	55
aadA5	ATGGGTGAATTYTTYCCTGCACAA	TCAACGCAAGATTCTCTCATTCGT	789	55
npmA	CGGGATCCAAGCACTTTCATACTGACG	CGGAATTCCAATTTTGTTCTTATTAGC	334	54
Quinolones gene				
qnrA	AGAGGATTTCTCACGCCAGG	TGCCAGGCACAGATCTTGAC	578	53
aac(6')-lb	TGACCTTGCGATGCTCTATG	TTAGGCATCACTGCGTGTT	497	53
qnrS	GCAAGTTCATTGAACAGGGT	TCTAAACCGTCGAGTTCGGCG	191	53
qnrB	GGMATHGAAATTCGCCACTG	TTTGCGYGYCGCCAGTCGAA	263	55

Results

Prevalence of ESBLs-producing isolates and ESBLs-encoding genes

The prevalence of ESBLs-producing isolates was 1.42% (n = 211) in the giant pandas listed in the Panda Base (Tab.2). ESBLs-producing isolates encoded genes including bla_{SHV} (n = 3), bla_{TEM} (n = 2), and bla_{CTX-M-1} (n = 3). Two isolates were detected co-carrying bla_{SHV}, bla_{TEM}, bla_{CTX-M-1}, and one co-carrying bla_{SHV} and bla_{CTX-M-1} (Tab.3).

Tab. 2 Screening for ESBLs phenotype in isolates

Isolates	inhibition zone /mm			Result
	cefotaxime	cefotaxime / Clavulanic acid	value	
X1	10.43	18.16	7.73	positive
RJ	0.00	17.74	17.74	positive
X2	15.01	24.97	9.96	positive

Tab. 3 Prevalence of ESBLs-encoding genes in ESBLs-producing isolates from giant pandas (n = 211)

Genes	Isolates	Number of isolates	Percentage of isolates
bla _{TEM}	X1, X2	2	0.09%
bla _{SHV}	X1, X2, RJ	3	1.42%
bla _{CTX-M-1}	X1, X2, RJ	3	1.42%

Antimicrobial resistance profiles

The three ESBLs-producing isolates were all resistant to penicillin (100%), piperacillin (100%), cephalosporine (100%), cephalexin, cefazolin, ceftriaxone, cefuroxime, cefaclor, cefoperazone, kanamycin, streptomycin, vancomycin, doxycycline,

and compound sulfamethoxazole (Fig. 1). Isolate X1 was resistant to 18 antibiotics. Isolate X2 was resistant to 15 antibiotics while isolate RJ was resistant to 27 antibiotics, however, seven non-ESBLs-producing isolates were resistant to vancomycin (100%) and imipenem (75%).

Molecular characteristics of ESBLs-producing isolates

ESBLs-producing Isolates X2 and X1 were detected co-carrying three ESBLs encoding genes (CTX-M1, TEM, and SHV), while RJ co-carried two ESBLs-encoding genes (CTX-M1 and SHV). Among the three ESBL isolates, three STs were identified, ST2640, ST290, and ST37. Genetic phylogenetic tree analysis showed that ESBLs-positive isolates X2 and X1 belonged to the same cluster (Fig. 2).

Transposons and antimicrobial resistance genes distribution

All three ESBLs-producing isolates and seven non-ESBLs-producing isolates tested were carrying at least one of the 22 investigated transposons and antimicrobial resistance genes. Three ESBLs-producing isolates were carrying five antimicrobial resistance genes and ~6–8 transposons. Seven non-ESBLs-producing isolates were carrying ~1–3 antimicrobial resistance genes and three transposons. All of the isolates were carrying *aac6-ib* and *tnpU*. Compared with the seven non-ESBLs-producing isolates, the three ESBLs-producing isolates carried *aadA5*, *rmtD*, *IS903*, and *intl1* (Fig. 2).

Discussion

ESBL-producing *K. pneumoniae* have been frequently encountered worldwide, especially in hospitals. However, ESBLs-producing *K. pneumoniae* are rarely found in wild animals such as the giant panda. Therefore, this study intended to analyze the prevalence, genotype, and antimicrobial susceptibility profiles of ESBL-producing *K. pneumoniae* collected from captive giant pandas to provide a scientific basis for the clinical use of antibiotics, and also to provide a systematic data and reference base for preventing and controlling the spread of such bacteria. In this study, the prevalence of ESBLs-producing *K. pneumoniae* among *K. pneumoniae* isolates (n=211) from giant pandas was 1.42%. The prevalence of ESBLs-producing *K. pneumoniae* in giant pandas was lower than in humans. The most common ESBL-producing Enterobacteriaceae detected in a Children's hospital in Japan was *E. coli* (79.8%), followed by *K. pneumoniae* (9.1%) (Yamanaka *et al.* 2020). In Iran, the prevalence of ESBL-producing *K. pneumoniae* was 43.5% (95% CI 39.3–47.9%) among clinical *K. pneumoniae* isolates (Beigverdi *et al.*, 2019a). In a study from Sweden in 2018, Hessman *et al.* (2018) reported that only 2% of *K. pneumoniae* isolates produced ESBLs. In Spain, the prevalence of ESBL-producing *K. pneumoniae* in 2017 was 7.2% (Cubero *et al.*, 2018). In a similar study conducted in Canada from 2010–2012, Karlowsky *et al.* (2013) reported that the prevalence of ESBL producers was 3.6% among *K. pneumoniae* isolates. Studies in Saudi Arabia, United Arab Emirates, and Tunisia between 2009 to 2018 revealed a prevalence of ESBL-producing *K. pneumoniae* between 38% and 55% in different community settings and hospitals (Beigverdi *et al.*, 2019b). A possible explanation for the prevalence of ESBLs-producing *K. pneumoniae* in giant pandas was lower is that the use of antibiotics is less frequent in giant pandas than in humans.

In this study, three ESBL-producing *K. pneumoniae* isolates were detected co-carrying 2–3 ESBLs-encoding genes, including *bla_{TEM}*, *bla_{SHV}*, and *bla_{CTX-M-1}*. This result was similar to that described in previous studies where TEM- and CTX-M- types were the main families of ESBLs (Paterson & Bonomo 2005; Bush & Jacoby 2010). Furthermore, reports have indicated that CTX-Ms have been rapidly disseminated among populations of Gram-negative bacteria in clinical settings in recent years (Salverda *et al.*, 2010; Seiffert *et al.*, 2013).

Three ESBL-producing *K. pneumoniae* isolates showed high resistance to many antimicrobial agents (more than 15 antibiotics). In our study, Meropenem was effective against three ESBLs-producing *K. pneumoniae* isolates, and IPM was effective against about 10% of the strains (n = 10). Three ESBLs-producing *K. pneumoniae* isolates were carrying five antimicrobial resistance genes, such as *aac6-ib*, *tnpU*, *aac6-1*, *qnrA*, and *qnrB*, which are associated with aminoglycoside and quinolones resistance. In China, ESBLs-positive *K. pneumoniae* strains were > 70% susceptible only to IPM, ertapenem, or amikacin (Zhang *et al.*, 2017). Similar studies have reported that although the majority of ESBLs-producing strains

could tolerate high concentrations of most cephalosporins and fluoroquinolones, none exhibited resistance to carbapenems (i.e., imipenem and meropenem) (Qiao *et al.*, 2017). Interestingly, some of these non-ESBLs-producing *K. pneumoniae* strains were detected carrying aminoglycoside and quinolones-encoding genes that are associated with aminoglycoside and quinolones resistance, but none of them exhibited resistance to those antimicrobial agents. The reason may be partly due to the expression levels of aminoglycoside and quinolones-encoding genes being quite low in most cases (Poirel & Nordmann, 2006).

Some studies have reported that about 50% of the antibiotic prescriptions for the treatment of *K. pneumoniae* infection are inappropriate and may cause the emergence of antimicrobial resistance (AMR) (Lee *et al.*, 2013). We recommend that veterinary professionals pay close attention to the choice of antimicrobial agents to prevent widespread AMR emergence in captive giant pandas.

Transposons are transposable genetic elements that harbor resistance genes. Transposons were found to be involved in the spread and development of drug resistance in Gram-negative bacteria. The greater the number and types of transposons, the greater the potential for drug resistance transmission (Wellington *et al.*, 2013; Kelly *et al.*, 2009; Domingues *et al.*, 2012). ESBLs-producing *K. pneumoniae* strains in this study carried eight different types of transposons, while non-ESBLs-producing *K. pneumoniae* strains carried significantly fewer transposons (2–3). The results indicated that ESBLs-producing *K. pneumoniae* strains had a stronger ability to spread drug resistance.

Our study assessed the genotypes of three ESBL-producing *K. pneumoniae* using MLST, and the analysis revealed that three ESBLs-producing isolates had different ST isotypes (ST37, ST290, and ST2640). ST37 and ST290 had a closer phylogenetic relationship.

Conclusions

In summary, three (1.42%) out of the 211 *K. pneumoniae* isolates recovered from giant pandas were confirmed as ESBLs positive. Among the three ESBLs-producing isolates, different ESBLs-encoding genes, including bla_{CTX-M-1}, bla_{SHV}, and bla_{TEM}, were detected. Furthermore, three isolates were found to carry transposons (i.e., aac6-ib and tnpU) and antimicrobial resistance genes, (i.e., aac6-ib, tnpU, aac6-1, qnrA, and qnrB). These three isolates were resistant to at least 15 antibiotics and had different ST isotypes (ST37, ST290, and ST2640). We recommend that clinical veterinarians pay more attention to the choice of antimicrobial agents to prevent widespread ESBLs-producing *K. pneumoniae* emergence in the giant panda.

Declarations

The research in the manuscript has been conducted under the guidance of the Institutional Animal Care and Use Committee of the Chengdu Research Base of Giant Panda Breeding.

Consent for publication

The manuscript is approved by all authors for publication. The work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Ethics approval and consent to participate

The use of materials and all experimental procedures involving animals were approved by Institutional Animal Care and Use Committee of the Chengdu Research Base of Giant Panda Breeding protocol #2018017.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Author Contributions:

All authors read and approved the final manuscript. Xiaoyan Su: Conceptualization, Formal analysis, funding acquisition, methodology, investigation, validation, project administration, resources, writing-original draft, writing-review and editing, project administration; Xia Yan: methodology and Formal analysis; Yunli Li: Investigation; Dongsheng Zhang: Resources; Lin Li: software; Fei Su, Yi Geng and Chanjuan Yue: Methodology; Rong Hou: funding acquisition; Songrui Liu: Writing-review & editing

Conflicts of Interest: Not applicable.

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Figures

Figure 1

Antimicrobial susceptibility profiles of Kp isolates. X1, X2, and RJ are ESBLs- positive *Klebsiella pneumoniae* isolates. YL, GZ, KL, YY, CG, XPP, and LL are non- ESBLs positive *Klebsiella pneumoniae* isolates as controls. PEN, Penicillin; PRL, Piperacillin; AMP, Ampicillin; OX, Oxacillin; AML, Amoxicillin; MOX, Oxycephalosporin; CAZ, Ceftazidime; CFM, Cefixime; CMZ, cefmatazole; FEP, Cefepime; CTX, cephalosporine; CL, Cephalexin; KZ, Cefazolin; CRO, Ceftriaxone; FOX, Cefoxitin; TZP, Piperacillin/Tazobactam; CXM, Cefuroxime; CEC, Cefaclor; SAM, Ampicillin/Sulbactam; CFP, Cefoperazone; ZOX, Ceftizoxime; ATM, Aztreonam; MEM, Meropenem; IPM, Imipenem; K, Kanamycin; S, Streptomycin; OFX, Ofloxacin; NOR, Norfloxacin; CIP, Ciprofloxacin; GTX, Gatifloxacin; C, Chloramphenicol; VA, Vancomycin; AZM, Azithromycin; TE, Doxycycline; MH, Minocycline; SXT, Compound Sulfamethoxazole; TMP, trimethoprim.

Figure 2

Dendrogram and genetic features of 10 isolates from giant pandas recovered in 2018 and 2019 with the respective ESBLs genes, transposons, and antimicrobial resistance genes. X1, X2, and RJ are ESBLs- positive *Klebsiella pneumoniae* isolates. YL, GZ, KL, YY, CG, XPP, and LL are non- ESBLs positive *Klebsiella pneumoniae* isolates as controls.