Antimicrobial and Transconjugants Characteristics of Sul3 Positive Escherichia Coli Isolated from Animals in Nanning, Guangxi Province

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Abstract

Background: Sulfonamides is the second most popular antibiotic in many countries, which leads to the widespread emergence of sulfonamides resistance. *Sul3* is a late sulfanilamide resistance gene, whose research is relatively little.

Result: 46 *sul3* positive *E. coli* strains were separated. A total of 12 ST types were observed, and 1 of those was previously unknown type. The ST350 is the most numerous type. All isolates were multidrug-resistant *E. coli*, with high antimicrobial rates to penicillin, ceftriaxone sodium, streptomycin, tetracycline, ciprofloxacin, gatifloxacin and chloramphenicol (100%, 73.9%, 82.6%, 100%, 80.4%, 71.7% and 97.8%), and with at least 3 resistance genes in addition to *sul3*. The plasmids transferred from 3 *sul3*-positive isolates to C600, the most of which brought 7 antibiotic resistance and increased resistance genes to C600. The transferred *sul3* gene and the plasmid that carries it could be stably inherited in the recipient bacteria for at least 20 days. Those plasmids had no effect on the growth of the recipient bacteria, but it would greatly reduce (at least 60 time) the in vitro competitiveness of the strains.

Conclusions: In Nanning, these *sul3*-positive *Escherichia coli* have strong antimicrobial resistance, and the plasmid carrying *sul3* has the ability to transfer multiple resistance genes, so long-term monitoring is necessary. Since the transferred plasmid will greatly reduce the in vitro competitiveness of the strain, we can consider limiting the spread of antimicrobial in this respect.

Background

The problem of bacterial resistance has a long history, and now it has become a medical problem which we cannot be ignored. Many resistances in bacteria is dominated by mobile genetic elements, including plasmids, integrons and transposons [1]. *Escherichia coli* is a common Gram-negative bacteria and one of the symbiotic bacteria in the intestines and environment of most livestock and poultry. But many studies [2, 3] have also shown that *E. coli* can cause a variety of diseases in humans and animal. And antibiotics have been used to treat bacterial infections and even used as feed additives to promote the growth of livestock and poultry for a long time [1, 4]. These used antibiotics are not completely absorbed or metabolized by the body[5]. After being discharged, these antibiotics can pollute and spread in the environment through a variety of ways, such as agricultural runoff, sewage discharge and nearby farm leaching[6]. As a result, many symbiotic bacteria such as *Escherichia coli* have to live in the environment which is containing antibiotics for a long time, and this kind of environment can provide appropriate selection pressure for the emergence and spread of multi-antibiotic-resistant bacteria and antibiotic-resistant genes.

Sulfonamide is an antibiotic which is not easy to degrade, have low soil adsorption rate and high mobility[7, 8]. It can inhibit bacterial growth and reproduction by competing for binding sites of P-aminobenzoic acid and dihydrofolate synthase (DHPS) [9]. Moreover, the sulfonamide has many advantages, such as wide range of use, low cost, a wide variety and etc. Since the first sulfonamide was

used in clinical practice in 1935, it has been one of the commonly used antimicrobials in the prevention and treatment of aquatic and livestock diseases[10, 11].

Sulfonamide resistance genes (including *floP* and *sul*) can encode a kind of DHPS with low affinity to sulfonamides, which makes bacteria grow and reproduce normally in the environment containing sulfonamides[12, 13]. At present, four kinds of sulfonamides resistant genes (*sul1-4*) have been found in plasmids. *Sul1* and *sul2* were discovered successively in 1985[14, 15]. *Sul4* was recently found in swinery in swiss [16]and was also found in type I integron transmission genes observed in Indus River sediments[9], but it has not been reported in clinical isolates[9]. *Sul3* is a sulfanilamide drug-resistant gene type discovered in 2003[17]. Since its discovery, *sul3* has been successively found in more and more regions, sources and strains[18–21], among which even have human-originated *Escherichia coli* [22]

Nanning is the capital of Guangxi Province, which is located in the southwest of China. The breeding industry that in Nanning is mainly composed of retail investors. The unreasonable use of antibiotics for livestock and poultry diseases, coupled with the lack of effective management measures, perpetuate the problem of bacterial resistance. The purpose of this study is to detect the antimicrobial, multi-locus sequence typing (MLST) and antimicrobial gene characteristics of *sul3* positive *E. coli* from animals in Nanning area. At the same time, to evaluate the influence of *sul3* positive bacteria on host bacteria after conjugation.

Results

Isolates and MLST

From 2015 to 2017, 142 strains of *Escherichia coli* were detected from 150 bacteria samples of animal origin in Nanning, among which 46 strains carried *sul3*, accounting for 32.4% of the total number of *Escherichia coli* samples. The 46 strains of *sul3* positive *Escherichia coli* were divided into 12 ST genotypes in total. Overall, ST350 was the dominant cluster (13, 28.2%), both it and ST156 were identified in chickens. ST10, ST746 and ST641 were detected among isolates from chickens (n = 2, 2, 1) and pig (n = 2, 3, 3). ST101 was identified in pigs(n = 2). ST2178 strains were almost detected in isolates of dogs. Finally, the sample of the unknown type is from pig (Table 1)(NOTE: Table 1 is longer than a page of A4, so we put it at the end of this document. You can put Table 1 at the end of this paragraph).

Table 1
Strain information, MLST typing and antimicrobial gene

Isolates	Year	Source	ST type	Antibiotic resistance genes
EC001	2017	pig	641	<i>tetA-tetM-TEM-flor-oqxA</i>
EC029			641	<i>rmtA- tetA-tetM-TEM-flor-oqxA</i>
EC004			2178	<i>tetA-CTX-MU-CTX-M9- flor-mcr-1-Sul2-FosA3</i>
EC012			2178	<i>aac(3)-II-tetA-tetM-TEM-mcr-1-oqxA-oqxB-Sul1-Sul2</i>
EC026			10	<i>aac(6')-Ib-tetA-flor-mcr-1-oqxA-Sul1-Sul2</i>
EC041			10	<i>aac(6')-Ib-tetA- CTX-MU-flor-oqxA-oqxB-Sul2</i>
EC025			746	<i>tetA-TEM-flor-oqxA-Sul1-Sul2</i>
EC038			746	<i>tetA-TEM-flor-oqxA-Sul1-Sul2</i>
EC009			222	<i>tetA-CTX-MU-CTX-M9-mcr-1-Sul2-FosA3</i>
EC006			unknown	<i>aac(3)-II-tetA-Sul2</i>
EC022		chicken	350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul2</i>
EC028			10	<i>rmtA- aac(6')-1b-tetA-flor-mcr-1-oqxA-Sul2</i>
EC027			156	<i>aac(6')-Ib-aac(3)-II-tetA-tetM-TEM-CTX-MU-OXA-1-flor-oqxA-Sul1-Sul2</i>
EC044			457	<i>aac(3)-II-tetA-tetM-flor</i>
EC042		dog	2178	<i>tetA- CTX-MU-CTX-M9-flor-oqxA-Sul2-FosA3</i>
EC043			2178	<i>tetA-CTX-MU-CTX-M9-mcr-1-Sul2-FosA3</i>
EC014	2016	pig	101	<i>tetA-TEM- CTX-MU-flor-oqxA-oqxB-Sul2-FosA3</i>
EC034			641	<i>tetA-TEM-flor-oqxA-Sul1</i>
EC039			746	<i>rmtA- tetA-TEM-flor-Sul2</i>
EC003		chicken	350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul2</i>
EC005			350	<i>tetA-tetM-CTX-MU-CTX-M9-flor</i>
EC013			350	<i>tetA-tetM-CTX-MU-CTX-M9-flor</i>
EC016			350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul1-Sul2</i>
EC018			350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-Sul2</i>
EC019			350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-flor-oqxA-Sul2</i>
EC023			350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul2</i>

Isolates	Year	Source	ST type	Antibiotic resistance genes
EC036			350	<i>tetA-tetM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul1-Sul2</i>
EC037			350	<i>tetA-tetM-CTX-M9-qnrB-flor-oqxA-Sul1-Sul2</i>
EC035			746	<i>tetA-TEM-CTX-MU-flor-oqxA-Sul2</i>
EC017			156	<i>rmtB-aac(6')-1b-aac(3)-II-tetA-tetM-TEM-CTX-MU-OXA-1-flor-oqxA-oqxB-Sul1-Sul2</i>
EC007		dog	950	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-flor-oqxA</i>
EC040			950	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul2</i>
EC011			457	<i>aac(3)-II-tetA-tetM-TEM-qnrB-flor-Sul2</i>
EC021			457	<i>aac(3)-II-tetA-tetM-TEM-qnrB-flor-oqxA-Sul2</i>
EC010			2178	<i>tetA-TEM-CTX-MU-CTX-M9-flor-mcr-1-oqxA-Sul1-Sul2-FosA3</i>
EC031		pig	101	<i>rmtB-tetA-TEM-qnrA-oqxA-Sul2</i>
EC024	2015	chicken	350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-OXA-1-qnrB-flor-oqxA-Sul2</i>
EC030			350	<i>tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul1-Sul2</i>
EC032			350	<i>aac(6')-Ib-tetA-tetM-TEM-CTX-MU-CTX-M9-qnrB-flor-oqxA-Sul1-Sul2</i>
EC020			457	<i>aac(3)-II-tetA-tetM-TEM-qnrB-flor-oqxA-Sul2-marA</i>
EC002			457	<i>aac(3)-II-tetA-tetM-TEM-qnrB-flor-oqxA-Sul2</i>
EC008			641	<i>tetA-TEM-flor</i>
EC033			746	<i>aac(3)-II-tetB-TEM-CTX-MU-CTX-M9-OXA-1-flor-Sul2</i>
EC045			10	<i>tetM-TEM-CTX-MU-CTX-M9-flor-oqxA-Sul2-FosA3</i>
EC046			23	<i>aac(6')-Ib-tetA-tetM-CTX-MU-CTX-M9-OXA-1-flor-mcr-1-oqxA-oqxB-Sul1-FosA3</i>
EC015		dog	2178	<i>tetA-CTX-MU-CTX-M9-flor-mcr-1-Sul2-FosA3</i>

Antibiotic Resistance And Resistance Gene

The results showed that 46 strains of *sul3* positive *Escherichia coli* were highly resistant to penicillin, ceftriaxone, streptomycin, tetracycline, ciprofloxacin, gatifloxacin and chloramphenicol, which were 100% (46/46), 73.9% (34/46), 82.6% (38/46), 100% (46/46), 80.4% (37/46), 71.7% (33/46) and 97.8% (45/46),

Some strains were also resistant to amikacin and colistin (10.9%, 5/46), only sensitive to meropenem (Table 2).

Table 2
Determination of antimicrobial of *Sul3* positive *E. coli*

Antimicrobial agents	The proportion (%) (Positive number /total)		
	R	I	S
penicillin	100(46/46)	0(0/46)	0(0/46)
ceftazidime	26.1(12/46)	13.0(6/46)	60.9(28/46)
ceftriaxone	73.9(34/46)	2.2(1/46)	23.9(11/46)
meropenem	0(0/46)	0(0/46)	100(46/46)
amikacin	10.9(5/46)	0(0/46)	89.1(41/46)
streptomycin	82.6(38/46)	13.0(6/46)	4.4(2/46)
tetracycline	100(46/46)	0(0/46)	0(0/46)
ciprofloxacin	80.4(37/46)	0(0/46)	19.6(9/46)
gatifloxacin	71.7(33/46)	17.4(8/46)	10.9(5/46)
chloramphenicol	97.8(45/46)	2.2(1/46)	0(0/46)
fosfomycin	21.7(10/46)	0(0/46)	78.3(36/46)
colistin	10.9(5/46)	8.7(4/46)	80.4(37/46)

In addition to *sul3*, 20 kinds of antimicrobial genes were detected, of which *tetA* (95.7%, 44 / 46), *flor* (89.1%, 41 / 46), *oqxA* (76.1%, 35 / 46), *sul2* (80.4%, 37 / 46) were detected of rate higher, and strains carrying *mcr-1* (21.7%, 10 / 46) were also detected, *armA* and *SHV* was not detected (Tables 1 and 3).

Table 3
Prevalence of antimicrobia-resistant genes in *Sul3* positive *E. coli*

Drug-resistant genes	Positive prevalence (Positive number /total)
<i>TEM</i>	67.4% (31/46)
<i>SHV</i>	0.0% (0/46)
<i>CTX-MU</i>	60.9% (28/46)
<i>CTX-M9</i>	52.2% (24/46)
<i>OXA-1</i>	8.7% (4/46)
<i>armA</i>	0.0% (0/46)
<i>rmtA</i>	6.5% (3/46)
<i>rmtB</i>	4.3% (2/46)
<i>aac(6')-1b</i>	15.2% (7/46)
<i>aac(3)-II</i>	21.7% (10/46)
<i>tetA</i>	95.7% (44/46)
<i>tetB</i>	2.2% (1/46)
<i>tetM</i>	58.7% (27/46)
<i>qnrA</i>	2.2% (1/46)
<i>qnrB</i>	32.6% (15/46)
<i>flor</i>	89.1% (41/46)
<i>mcr-1</i>	21.7% (10/46)
<i>oqxA</i>	76.1% (35/46)
<i>oqxB</i>	10.9% (5/46)
<i>Sul1</i>	30.4% (14/46)
<i>Sul2</i>	80.4% (37/46)
<i>FosA3</i>	19.6% (9/46)

Transconjugants And Related Experiments

Three suspected transconjugants were successfully obtained through the conjugation experiment. After *sul3* positive identification and ERIC-PCR (Fig. 1), the 3 suspected transconjugants were all the plasmid

strains obtained from the recipient bacteria (C600) (named as EC027/T, EC035/T and EC038/T according to the donor bacteria name)

In comparison with the recipient bacteria, the MIC of the maximum 7 antimicrobials in transconjugants (EC027/T) showed different degrees elevated, including penicillin, ceftazidime, streptomycin, amikacin, tetracycline, ciprofloxacin and chloramphenicol (Table 4). According to the detection results of resistance genes, in addition to *sul3* gene, E027/T was detected with 6 new resistance genes, while E025/T and E038/T were 2 (Table 5). However, compared with the sensitivity changes of antibacterial drugs, we found that no corresponding resistance genes were detected in the chloramphenicol of streptomycin and chloramphenicol. The reasons for this phenomenon will be explained in our discussion. The plasmid stability experiment showed that the plasmid could be stably and continuously passed for at least 40 generations with strong stability (Fig. 2).

Table 4
Changes in antimicrobia sensitivity of recipient bacteria and transconjugants

Antimicrobial agents	C600	EC027/T	EC035/T	EC038/T	EC027	EC035	EC038
penicillin	8	> 512	> 512	32	512	256	512
ceftazidime	1.25	10	1.25	1.25	80	1.25	1.25
streptomycin	16	256	128	128	> 512	128	512
amikacin	8	128	16	4	> 512	4	4
tetracycline	4	256	128	128	256	256	256
ciprofloxacin	< 0.25	64	< 0.25	< 0.25	128	32	32
chloramphenicol	32	128	128	256	512	256	512

Table 5
Gene detection of conjugation resistance

Isolates	Positive resistance genes
EC027/T	<i>OXA-1, sul3, tetM, flor, aac(6')-Ib, sul2, sul1</i>
EC035/T	<i>TEM, sul3, tetA</i>
EC037/T	<i>TEM, sul3, tetA</i>

The Adaptive Cost Of Plasmid C600

The growth curves of the 3 transconjugants and the recipient bacteria showed that the transconjugants and the recipient bacteria had minor changes only during the logarithmic growth period, and the changes were not obvious after entering the stable period at 8 hours. It is indicating that the transconjugants had little influence on the growth of the recipient bacteria (Fig. 3).

n the competitive test, we observed that the competitive ability of the 3 transconjugants was significantly reduced compared with that of the recipient bacteria C600, among which the most obvious one was EC035/T (0.043), followed by EC027/T (0.058) and EC038/T (0.061) (Fig. 4).

Discussion

Nowday, sulphonamides are rarely used to treat bacterial infections in humans in many regions, but they are still widely used in aquaculture, animal husbandry and veterinary because of the lower price[20]. Massive use plus great potential for penetrate into the environment. For these reasons, the concentration of sulfonamides becomes a priority for sewage treatment, rivers and water sources [20]. Sulfonamine-resistant genes (*sul*) have also spread widely for these reason. Analogously, with regard to the detection rate of *sul*, we compared several recent studies [23–26] found that the detection rate of sulfonamine-resistant genes was high and that the detection rate of *sul1* and *sul2* in sulfamine-resistant genes was generally higher than that of *sul3*. It was suggested that there may be more sulfamine-resistant bacteria in Nanning, and we should pay more attention to them.

After analyzing the genetic environment of *sul* gene, Jang et al. [20] said that compared with the other two genes, the diversity of adjacent genetic transfer elements and the resistance genes of *sul3* were lower, and some *sul3* even existed on chromosomes, which affected the transmission of *sul3*. But the studies also showed that [20, 47], *sul3* is related to type I integron, and can replace *sul1* to form atypical type I integron, which emphasize the potential for widespread in the future. This indicates that there is still a certain transmission problem of *sul3* at present, but this problem may be solved over time. And in our study, the stability test showed that the transferred *sul3* wild plasmid could inheritance in bacteria for a long time, it also indirectly reflects that *sul3* has the potential for long-term transmission. In addition, studies have shown that[27, 28], *sul3*, *tetQ*, *tetO* and other sulfonamides and tetracycline resistance genes are significantly positively correlated with the content of Cu, Zn and other heavy metals ($P < 0.05$ or $P < 0.01$, $r = 0.882–0.992$), which indicates that heavy metals can help *sul3* appear and there may be some heavy metal pollution in the Nanning's farms.

The emergence of multidrug-resistant bacteria seriously affects the cure rate of bacterial infection diseases, becoming a potential threat to the health of human beings and livestock[29]. In the study, all the strains we tested showed multiple antibiotic resistance, which is possible because we tested the *sul3*-positive *E. coli*. Here we need to pay attention to the pathogenicity of these strains. Compare antibiotic resistance and resistance genes. In isolates strains, only quinolones and aminoglycosides had differences in the detection rate of resistance genes and antimicrobial resistance rate, similarly, no resistance genes associated with streptomycin and chloramphenicol were detected in conjugates, it suggests that there may be other related genes mediating the tolerance of the above-mentioned antimicrobials, which may be the efflux pump or the resistance genes of the relevant antimicrobials. Regarding the plasmids in these isolates, we cannot determine the type and quantity of these transfer plasmids, what we can confirm is that after acquiring the plasmid, there are several (at least 4) antibiotics resistance changes to the strains, and corresponding to the resistance genes tested. It indicates that the

transferred drug-resistant genes can be expressed by host cells, it may affect the effective use of antibiotics in Nanning.

And compared with other studies[29–31], the recombinant plasmid had no effect on the growth performance of the strain, just like the wild plasmid in this study, but the wild plasmids reduced the competitiveness of host bacteria in vitro to a greater extent. Although the types and quantities of drug-resistant genes studied are different, this also indicates that the adaptation cost of wild plasmids will bring greater adaptive cost to the recipient bacteria due to multiple drug-resistant genes or other unknown genes.

In the test, the diversity of each *sul3* positive strain is low, but there are still more common types in ST typing. ST23, ST156 and ST10 were reported to be related to human [32–34], Among them, ST10 is the most common pedigree in human urine *Escherichia coli* isolates[32], and these reports also pointed out that these 3 types were also found in other *E.coli* strains. Although these 3 types were rarely detected in this study, it is still necessary to pay attention to the transmission between human and livestock.

Conclusion

Forty-six *sul3* positive strains of *E. coli* carry multiple drug resistance gene and have serious drug resistance, *sul3* wild plasmid can pass a variety of antibiotic resistance genes, enhancing receptor bacteria to antibiotics sensitivity, affect the strain of sports ability and the ability of biofilm formation, lower strain in vitro competition ability, in the future can be in nanning of the potential threat of antibiotic use

Methods

Isolation and identification of *sul3* positive *Escherichia coli*

142 strains of *Escherichia coli* were identified from 150 samples of bacteria collected from farms and pet hospital in Nanning from 2015 to 2017. All the strains were identified by MaConkey's agar and Eosin methylene blue agar, and sequence detection with primers of *16 s rRNA* and *sul3* gene.

Each sample was cultured at 37°C for 16–18 h in McConkey medium and 18–24 h in Eosin methylene blue agar. DNA was extracted by boiling method. The primer of *16 s rRNA* and *sul3* is reference the previous description (Table 1). The PCR product were sent to the company for sequencing, and uploaded to NCBI for BLST confirmation of suspected isolates and *sul3* carrier. The 30% glycerol sample (V/V) and DNA sample of *sul3* positive *E. coli* were stored at -20 °C.

Mlst Typing Detection

A total of 46 strains of *sul3* positive *E. coli* were detected, PCR amplification was conducted using 7 pairs of primers (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) (Table 6). The positive products were sent to the company for sequence determination, and the results were uploaded to the MLST website (<https://pubmlst.org>) to obtain the ST type. (**NOTE:** Table 6 is longer than a page of A4, so we put it at the end of this document. You can put Table 6 at the end of this paragraph)

Table 6
Primers used in this study

Gene	Primer sequence (5'→3')	Product size (bp)	Annealing temp (°C)	References
<i>16Sr RNA</i>	F: AGAGTTTGATCCTGGCTCAG R: ACGGCTACCTTGTTACGACTT	1466	55	[38]
<i>TEM</i>	F: AGGAAGAGTATGATTCAACA R: CTCGTCGTTTGGTATGGC	511	52.5	[38]
<i>SHV</i>	F: GGTATGCGTTATATTCGCCTGTG R: TTAGCGTTGCCAGTGCTCGATCA	56.5	1031	[38]
<i>CTX-M1</i>	F: GGTAAAAAATCACTGCGTC R: TTGGTGACGATTTTAGCCGC	864	56	[39]
<i>CTX-M9</i>	F: ATGGTGACAAAGAGAGTGCA R: CCCTTCGGCGATGATTCTC	870	50	[40]
<i>CTX-MU</i>	F: ATGTGCAGTACCAGTAAAGT R: TGGGTRAAGTARGTCACCAGAA	593	56	[41]
<i>OXA-1</i>	F: TTGAAGGAACTGAAGGTTGT R: CCAAGTTTCCTGTAAGTGCG	651	54	[35]
<i>armA</i>	F: AGGTTGTTTCCATTTCTGAG R: TCTCTTCCATTCCCTTCTCC	591	55	[42]
<i>rmtA</i>	F: CTAGCGTCCATCCTTTCTC R: TTTGCTTCCATGCCCTTGCC	635	60	[43]
<i>rmtB</i>	F: ATCAACGATGCCCTCACCTCC R: TTCCACGCCCCGCCTAAACT	631	61	[42]
<i>aac(6')-Ib</i>	F: CAAGAGTCCGTCACTCCATACA R: ATGGAAGGGTTAGGCATCACT	396	61	[44]
<i>aac(3')-II</i>	F: ACTGTGATGGGATACGCGTC R: CTCCGTCAGCGTTTCAGCTA	237	60	[45]
<i>tetA</i>	F: GCTACATCCTGCTTGCCCTTC	210	60	[46]

Gene	Primer sequence (5'→3')	Product size (bp)	Annealing temp (°C)	References
	R: CATAGATCGCCGTGAAGAGG			
<i>tetB</i>	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	659	65	[46]
<i>tetM</i>	F: GTGGACAAAGGTACAACGAG R: CGGTAAAGTTCGTCACACAC	406	55	[46]
<i>qnrA</i>	F: CAAGAGGATTTCTCACGCCAG R: AATCCGGCAGCACTATTACTCC	628	67	[35]
<i>qnrB</i>	F: ATGACGCCATTACTGTATAA R: GATCGCAATGTGTGAAGTTT	562	57	[35]
<i>flor</i>	F: GTCATTCTCACCTTCATCCTAC R: GACACCAGCACTGCCATTG	243	60	[47]
<i>mcr-1</i>	F: ATGATGCAGCATACTTCTGTG R: TCAGCGGATGAATGCGGTG	1626	65	[48]
<i>oqxA</i>	F: GATCAGTCAGTGGGATAGTTT R: TACTCGGCGTTAACTGATTA	670	56	[49]
<i>oqxB</i>	F: TTCTCCCCCGGCGGGAAGTAC R: CTCGGCCATTTTGGCGCGTA	512	68	[49]
<i>sul1</i>	F: GGCTGGTGGTTATGCACTCA R: CGAGACCAATAGCGGAAGC	263	64	[50]
<i>sul2</i>	F: ACGCAAGCCTATGCCTTGTCG R: TTGCGTTTGATACCGGCACCC	234	62	[50]
<i>sul3</i>	F: CGTAAATATAACCACCGAT R: CCAAGCCTGAATAAATCTCA	326	55	[50]
<i>fosA3</i>	F: GCGTCAAGCCTGGCATTTT R: GCCGTCAGGGTCGAGAAA	258	55	[38]
<i>ERIC-2</i>	AAGTAAGTGACTGGGGTGACGC	Variable	50	[51]
<i>Adk</i>	F: CTCGCCATTAACCGTTTCAG	739	55	[49]

Gene	Primer sequence (5'→3')	Product size (bp)	Annealing temp (°C)	References
	R: CCAGATCAGCGCGAACTTCA			
<i>FumC</i>	F: TCACAGGTCGCCAGCGCTTC R: TCCCGGCAGATAAGCTGTGG	769	64	[49]
<i>gyrB</i>	F: ATCGGCGACACGGATGAC R: GTCCATGTAGGCGTTCAGG	816	66	[49]
<i>lcd</i>	F: CCGGCACAAGGCAAGAAGATC R: GGACGCAGCAGGATCTGTT	857	59.5	[49]
<i>mdh</i>	F: GCCTTCAGGTTCAGAACTCTCTCT R: TTCTGTTCAAATGCGCTCAGG	798	55	[49]
<i>PurA</i>	F: CGCGCTGATGAAAGAGATGA R: CATACGGTAAGCCACGCAGA	817	66	[49]
<i>recA</i>	F: CGCATTCGCTTTACCCTGACC R: GTCGAAATCTACGGACCGGAAT	731	55	[49]

Antibiotic Sensitivity Experimentt

The minimum inhibitory concentration (MIC) of antimicrobial agents against *sul3* positive *E. coli* was used by the broth dilution method recommended which was recommended by Clinical and Laboratory Standards Institut (CLSI). The tested antimicrobial agents included penicillin, ceftazidime, ceftriaxone, meropenem, amikacin, streptomycin, tetracycline, ciprofloxacin, gatifloxacin, chloramphenicol, fosfomycin and colistin. The results of antibiotic sensitivity were also judged according to the break-point standard established by CLSI. The *E. coli* of ATCC 25922 was used for the quality control of antibiotic sensitivity test.

Antimicrobial Gene Detection

There were 24 antimicrobial genes, including the β -lactam (*TEM*, *CTX-M9*, *CTX-MU* and *OXA-1*), aminoglycosides (*armA*, *rmtA*, *rmtB*, *aac(6')-Ib* and *aac(3')-II*), tetracyclines (*tetA*, *tetB* and *tetM*), quinolones (*qnrA* and *qnrB*), sulfonamides (*Sul1* and *sul2*), and other classes (*flor*, *mcr-1*, *oqxA*, *oqxB*, and *fosA3*) (Table 6).

Conjugative Experiment

The conjugative experiment was conducted by filter membrane method. the *Escherichia coli* C600, which did not produce acid and has rifampicin resistance, was used as the recipient bacteria. And *sul3*-positive isolates were used as the donor bacteria. The transconjugants were screened from McConkey medium with a concentration of 6000 µg/mL sulfamethazine and 3500 µg/mL rifampicin.

The suspected transconjugants were subjected to PCR and antibiotics sensitivity tests to confirm whether the plasmid transfer was successful, and then ERIC-PCR was used to determine the correlation between the transconjugants and C600, with the ERIC-primers as described previously[35] (Table 1). It also detects whether other resistant genes are co-transmitted.

Growth Curve

We used absorbance method to observe the change of the growth status of transconjugants and C600, specific as follows. After shaking culture at 37°C overnight, the bacterial solution was added to fresh LB broth according to the ratio of 1:1000. A total of 16 time points, 3 ml was taken from each time point for OD₆₀₀ absorbance detection. The observation lasted for 24 hours and need repeated 3 times in parallel.

In Vitro Competitive Test

In vitro competition experiments refer to previous descriptions[36]. First, two kinds of bacteria were cultured to 0.5 McFarland, then took 100 µL for each mixed in a 1:1 ratio and added to 10 mL LB broth, and incubated for 16 h at 37°C, 220 r/min. After diluted 10⁶ times, 100 µL bacterial solution was respectively coated with streptomycin 60 µg/mL LB agar and streptomycin free LB agar, and cultured overnight at 37°C. The total CFU and streptomycin resistant CFU were counted, and the competition index of without resistant CFU and streptomycin resistant CFU was calculated, and the parallel repetition was 3 times.

Plasmid Stability

According to the previous description of plasmid stability[37], the transconjugants was shaken in LB medium at 37°C for 12 h, and then inoculated in new LB medium and shaken at 37°C for 12 h again, repeat every 12 h. Each time was counted as one generation, and the procedure was repeated for 60 generations. Every 10 generations, part of the bacterial solution was diluted and coated with agar medium, 24 colonies of bacteria were randomly selected. DNA was extracted by boiling method. Then the PCR of *sul3* was performed to determine the positive rate of *sul3*.

Abbreviations

MIC

Minimum Inhibitory Concentration, CLSI: Clinical and Laboratory Standards Institut

Declarations

Ethics approval and consent to participate

All data were published with the written consent of the farmer and pet owner, and all experiments were conducted in accordance with the animal Ethics guidelines approved by the Experimental Animal Committee of Guangxi University.

Consent for publication

Not applicable

Availability of data and materials

The others datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZL and HQ participated in research design, analysis and manuscript writing; GZ and YC participated in manuscript modification and sample testing; QL, JS and YY participated in experimental data sorting and recording

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Figures

Figure 1

The ERIC-PCR result of 3 transconjugants and C600 Note: 1-3 for transconjugants EC027/T, EC035/T and EC038/T, 4 for C600, The band size and combination of the 3 transconjugants were consistent with that of C600, indicating that these transconjugants and C600 were homologous strains

Figure 2

Stability test of sul3 positive wild plasmid The positive rate of sul3 remained above 70% when the transconjugants were passed on to the 20th day(40 generations), indicating that the sul3 plasmid could be inherited stably for a long time in the transconjugants

Figure 3

Growth curves for 3 transconjugants and C600 There was no overall significant difference between the growth curve of zygons and the growth curve of C600 (red) ($P > 0.05$)

Figure 4

The competitive index of extracorporeal competition The competition index indicated that the ratio of CFU of the streptomycin resistant strain to the CFU of the sensitive strain and the ratio of the three zygons were all less than 0.06, indicating that the competition ability of the transconjugants in vitro was greatly reduced