



Article Lineage, Antimicrobial Resistance and Virulence of Citrobacter spp

Liyun Liu ^{1,*,+}, Liyun Qin ^{2,+}, Shuai Hao ^{3,+}, Ruiting Lan ^{4,+}, Baohong Xu ², Yumei Guo ², Ruiping Jiang ², Hui Sun ¹, Xiaoping Chen ¹, Xinchao LV ², Jianguo Xu ¹ and Chuan Zhao ^{2,*}

- ¹ State Key Laboratory of Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China; sunhui@icdc.cn (H.S.); chenxiaoping@icdc.cn (X.C.); xujianguo@icdc.cn (J.X.)
- ² Shijiazhuang Center for Disease Control and Prevention, Shijiazhuang 050011, China; qinliyun-2007@163.com (L.Q.); Wsws1120@163.com (B.X.); Guokexin2199@163.com (Y.G.); Jrpok97@163.com (R.J.); lvxinzhao1986@163.com (X.L.)
- ³ Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University, Beijing 100048, China; haoshuai@btbu.edu.cn
- ⁴ School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia; r.lan@unsw.edu.au
- * Correspondence: liuliyun@icdc.cn (L.L.); shijiazhuangcdc@sina.com (C.Z.)
- ⁺ These authors contributed equally to this work.

Received: 4 February 2020; Accepted: 4 March 2020; Published: 6 March 2020

Abstract: *Citrobacter* spp. are opportunistic human pathogens which can cause nosocomial infections, sporadic infections and outbreaks. In order to determine the genetic diversity, *in vitro* virulence properties and antimicrobial resistance profiles of *Citrobacter* spp., 128 *Citrobacter* isolates obtained from human diarrheal patients, foods and environment were assessed by multilocus sequence typing (MLST), antimicrobial susceptibility testing and adhesion and cytotoxicity testing to HEp-2 cells. The 128 *Citrobacter* isolates were typed into 123 sequence types (STs) of which 101 were novel STs, and these STs were divided into five lineages. Lineages I and II contained *C. freundii* isolates; Lineage III contained all *C. braakii* isolates, while Lineage IV and V contained *C. youngae* isolates. Lineages II and V contained more adhesive and cytotoxic isolates than Lineages I, III, and IV. Fifty-one of the 128 isolates were found to be multidrug-resistant (MDR, ≥3) and mainly distributed in Lineages I, II, and III. The prevalence of quinolone resistance varied with Lineage III (*C. braakii*) having the highest proportion of resistant isolates (52.6%), followed by Lineage I (*C. freundii*) with 23.7%. Seven *qnrB* variants, including two new alleles (*qnrB93* and *qnrB94*) were found with Lineage I being the main reservoir. In summary, highly cytotoxic MDR isolates from diarrheal patients may increase the risk of severe disease.

Keywords: Citrobacter; sequence types; multidrug resistance; adhesion; cytotoxicity

1. Background

The genus *Citrobacter* contained 11 species, most of which are opportunistic human pathogens that can cause nosocomial infections [1], and a range of other infections [2–6]. In this study, we focus on three *Citrobacter* species, *C. freundii*, *C. youngae* and *C. braakii*, as potential foodborne pathogens. *C. freundii* is the most commonly isolated *Citrobacter* species causing diarrhea and other infections [7,8], while *C. youngae* and *C. braakii* are rarely reported to cause infections. Some *C. freundii* strains caused food poisoning or diarrhea in humans which were found to carry virulent factors, such as Shiga-like toxins, heat-stable toxins, or virulence islands [9,10]. *C. youngae* can cause peritonitis [11]. *C. braakii* has been isolated from the peritonea of acute peritonitis patients, as well as from food products [12–

15]. In our previous studies, *C. freundii* and *C. youngae* have been isolated from the fecal samples of diarrheal patients and different types of food samples, and are potential foodborne pathogens [10,16], while *C. braakii* has been isolated from food [10].

Antibiotic resistance of *Citrobacter* has increased, and multidrug-resistant (MDR) isolates have frequently been reported [10,17–21]. Frequent isolation of MDR *C. freundii* with resistance to β -lactams, quinolones and aminoglycosides has been reported by several international surveillance programs [18]. In our previous study, 31.7% of *C. freundii* isolates were MDR that were resistant to β -lactams, quinolones, aminoglycosides, tetracyclines, phenicols, sulfonamides or nitrofuran [10,16]. Furthermore, although not MDR, 4.9% were also resistant to aminoglycosides, β -lactams, and quinolones [10,16].

Antibiotic resistant *Citrobacter* often harbored extended-spectrum β -lactamase (ESBL) [1,19,20], and plasmid-mediated quinolone resistance (PMQR) determinants [21]. The prevalence of ESBL and PMQR *Citrobacter* isolates was reported from several international studies [1,19–21]. In our previous study, we identified four *C. freundii* isolates from clinical sources and foods that were ESBL producing and 21 isolates from clinical sources and foods that harbored PMQR genes, including *aac*(6')-*Ib-cr*, *qnrS1*, *qnrB9*, *qnrB13*, *qnrB16*, *qnrB17*, *qnrB63*, *qnrB76*, *qnrB77*, or *qnrB92* [10,16].

Fluoroquinolone resistance is associated with mutations in DNA gyrase and topoisomerase IV genes, in particular, mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes [22]. It has been reported that *C. freundii* isolates with reduced susceptibility to fluoroquinolones were found to contain Thr83Ile or Asp87Asn mutation in *gyrA* [22,23]. In our previous study, we screened mutations in the QRDRs of *gyrA* and *parC* genes in fluoroquinolone resistant isolates. Four of the six fluoroquinolone resistant isolates were found to carry Thr59Ile, Gln111Arg and Ile134Val mutations of the *gyrA* gene [16]. However, it remains to be determined whether these mutations confer resistance to fluoroquinolones. No mutations in the QRDR region of the *parC* gene was found in the six fluoroquinolones resistant isolates [16].

To further understand the genetic diversity, virulence and antibiotic resistance of *Citrobacter spp*. From different sources, in this study, we isolated 128 *Citrobacter* isolates from diarrheal outpatients, food and environment in Shijiazhuang Hebei Province, China. We performed multilocus sequence typing (MLST) to determine the relationships of the isolates and screened for *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM} and *qnr* genes by PCR and mutations in *gyrA* and *parC* genes by PCR sequencing and assayed the adhesion and cytotoxicity to Hep-2 cells of all isolates.

2. Results

2.1. Multilocus Sequence Typing of Citrobacter Isolates

The 128 *Citrobacter* isolates were typed into 123 STs with the 67 *C. freundii* isolates dividing into 65 STs, the 45 *C. braakii* isolates into 42 STs and the 16 *C. younga*e isolates into 16 STs (Table 1 and Figure 1). Of the 123 STs, 101 were novel STs (from ST269 to ST387). No STs were predominant. Two STs (ST1 and ST100) of *C. freundii* each contained two isolates. ST1 contained one isolate from a diarrheal patient and one from food. ST100 contained one isolates from diarrheal patients or foods. ST297 of *C. braakii* contained two isolates, with one from the environment and one from food.

The concatenated sequence of the seven housekeeping genes for the 128 *Citrobacter* isolates, was used to construct a neighbor-joining tree (Figure 1). *Salmonella* LT2 was used as an outgroup. The 128 *Citrobacter* isolates were divided into five lineages with strong bootstrap support to the lineage divisions. Lineages I and II contained *C. freundii* isolates, while Lineages IV and V contained *C. youngae* isolates exclusively. Lineage III contained all *C. braakii* isolates. *Citrobacter* isolates from different sources were not grouped by source and were dispersed among different lineages (Table 1 and Figure 1).

Table 1. Adherence, cytotoxicity, multidrug-resistant and Genotypes of 128 Citrobacter Isolates.

Clusters and	Isolates	Year	Source	STs	Adhesion	LDH	NDR	ESBLs	qnr
Lipeage	HB2016004	2016	П	17	***	12.4 ± 0.4	2		aur B0
C from dii	HB2016004	2016	р г	17	. /	12.4 ± 0.4	2		qnr B9 ann B0
C. jreunuu	HB2016023	2010	F	204	*	0.3 ± 2.2	9	blaction blatting	and(6') The or our BO
	HB2016023	2010	F	280	*	3.9 ± 0.2 20 5 + 1 3	3	DIUCIX-M-3, DIUIEM-1	uuc(0)-10-cr,qnrD3
	HB2016024	2010	F	260	*	50 ± 1.0	7		anr\$1
	HB2016034	2016	D	298	+/-	0.6 ± 0.2	2		gitt S1
	HB2017002	2010	D	300	+/-	10.3 ± 1.8	1	hlactx-M-9	anrS1 anrB13 include the Lex A hinding site
	HB2017003	2017	D	301	*	89+08	2	of we have a second sec	in origin bio menuice me least oniming one
	HB2017004	2017	D	85	*	12.3 ± 2.2	2		
	HB2017006	2017	D	303	**	27.3 ± 0.6	2		anrB76 include the Lex A binding site
	HB2017009	2017	D	304	*	11.9 ± 0.6	4		<i>anrB</i> 76 include the LexA binding site
	HB2017012	2017	D	306	*	7.1 ± 0.4	1		anrB76 include the LexA binding site
	HB2017016	2017	D	309	*	15.0 ± 1.7	1		
	HB2017017	2017	D	1	*	15.9 ± 1.0	2		
	HB2017026	2017	D	313	**	17.4 ± 1.1	6		aac(6')-Ib-cr
	HB2017031	2017	D	318	+/-	12.7 ± 0.2	2		qnrB9
	HB2017033	2017	Е	320	**	19.1 ± 3.2	0		qnrB76
	HB2017036	2017	Е	322	**	14.1 ± 0.8	3		
	HB2017038	2017	F	324	*	12.4 ± 8.9	4		qnrB94
	HB2017039	2017	F	325	*	19.8 ± 5.7	2		qnrB17
	HB2017040	2017	F	326	*	6.7 ± 1.4	8		
	HB2017042	2017	F	328	-	8.9 ± 7.8	9	blatem-1	
	HB2017045	2017	F	331	*	14.3 ± 1.8	7	blactx-м-9, blateм-1	qnrS1
	HB2017052	2017	F	337	**	15.5 ± 1.5	2		
	HB2017053	2017	F	338	**	21.6 ± 2.5	4		qnrB9
	HB2017054	2017	F	339	***	16.5 ± 3.6	2		qnrB76
	HB2017059	2017	F	343	*	12.8 ± 0.4	7	blaстх-м-з, blaстх-м-9	qnrB93
	HB2017060	2017	F	344	+/-	11.9 ± 1.6	7	blacтх-м-9, blaтем-1	
	HB2017061	2017	F	1	**	17.3 ± 2.4	2		
Lineage II	HB2016001	2016	D	269	**	11.0 ± 2.1	1		
C. freundii	HB2016002	2016	D	216	*	10.4 ± 0.2	3		
	HB2016003	2016	D	270	**	15.6 ± 1.1	1		
	HB2016006	2016	Е	272	**	9.3 ± 2.8	1		
	HB2016008	2016	F	274	***	22.7 ± 7.3	8	blatem-1	aac(6')-Ib-cr,qnrB2
	HB2016010	2016	Е	276	*	8.4 ± 1.9	1		
	HB2016011	2016	F	100	***	22.6 ± 3.0	1		
	HB2016012	2016	F	277	**	16.9 ± 1.5	2		
	HB2016013	2016	F	278	**	15.3 ± 3.9	3		
	HB2016017	2016	F	282	***	21.4 ± 7.3	1		
	HB2016018	2016	F	283	+/-	9.2 ± 1.5	2		
	HB2017001	2017	D	169	***	29.4 ± 5.8	2		
	HB2017008	2017	D	12	**	13.6 ± 0.7	5	11	
	HB2017011	2017		163	**	15.8 ± 0.7	1	OLATEM-1	<i>aac</i> (6)-10-cr
	HB2017013	2017	D	202	**	0.0 ± 0.3	1		
	HD2017014	2017	D D	308 105	**	16.0 ± 13.3	1		
	HB2017018	2017	D	125	**	21.5 ± 7.3	ے 1		
	HB2017019	2017		217	***	14.0 ± 1.4	1		
	HB2017020	2017	D	310	**	22.9 ± 0.9 20.2 + 1.3	2		
	HB2017022	2017	D	219	**	20.2 ± 1.3 18.0 + 3.4	2		
	HB2017023	2017	D	150	**	10.0 ± 0.4 21.2 ± 1.0	1		
	HB2017024	2017	D	312	*	80 ± 50	3		
	HB2017027	2017	D	314	***	24.7 + 2.7	4		
	HB2017030	2017	D	317	**	20.2 + 3.0	1		
	HB2017032	2017	D	319	**	19.7 ± 1.3	5		
	HB2017034	2017	E	100	***	19.4 ± 2.5	1		
	HB2017035	2017	Ē	321	**	21.8 ± 5.7	1		
	HB2017037	2017	Е	323	**	24.8 ± 6.8	1		
	HB2017041	2017	F	327	*	5.4 ± 1.6	1		
	HB2017043	2017	F	329	*	20.0 ± 0.6	1		
	HB2017046	2017	F	332	**	22.9 ± 7.0	2		
	HB2017047	2017	F	333	**	23.5 ± 5.0	6		

	HB2017049	2017	F	335	*	31.2 ± 10.2	2		
	HB2017051	2017	F	214	**	21.0 ± 4.4	2		
	HB2017055	2017	F	161	**	18.2 ± 3.1	6		
	HB2017056	2017	F	340	**	19.2 ± 3.4	4		
	HB2017057	2017	F	341	**	20.3 ± 3.3	4		
Lineage III	HB2016015	2016	F	280	+/-	3.4 ± 0.7	5		qnrS1
C. braakii	HB2016032	2016	Е	295	**	4.2 ± 4.1	5		
	HB2016033	2016	F	296	**	5.3 ± 3.2	2		
	HB2016035	2016	F	297	*	11.1 ± 2.2	6		aac(6')-Ib-cr,qnrB2
	HB2017044	2017	F	330	**	20.4 ± 5.5	2		
	HB2017048	2017	F	334	*	20.6 ± 4.0	2		
	HB2017062	2017	F	345	*	14.8 ± 1.9	2		
	HB2017068	2017	F	351	**	12.5 ± 8.9	2		
	HB2017070	2017	D	353	*	9.5 ± 0.4	2		
	HB2017071	2017	D	354	*	22.2 ± 6.9	2		
	HB2017072	2017	D	355	**	25.2 ± 4.0	1		
	HB2017074	2017	D	356	***	14.9 ± 7.4	2		
	HB2017075	2017	D	357	**	25.2 ± 4.0	1		
	HB2017076	2017	D	358	**	28.9 ± 1.6	4		
	HB2017077	2017	D	357	**	13.6 ± 0.2	1		
	HB2017078	2017	D	359	+/-	13.7 ± 5.7	1		
	HB2017079	2017	E	360	*	15.5 ± 2.0	3		
	HB2017081	2017	E	362	**	17.7 ± 0.8	2		
	HB2017082	2017	F	363	**	13.1 ± 1.9	1		
	HB2017083	2017	F	364	**	10.8 ± 0.6	1		
	HB2017084	2017	F	365	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	13.9 ± 2.0	3		
	HB2017087	2017	F	367	. /	3.5 ± 4.0	8		aac(6)-1b-cr, qnrB2
	HB2017089	2017	Г Г	369	+/-	11.7 ± 9.7	2		
	HB2017090	2017	г	370 271	+/-	4.3 ± 1.7 4.7 ± 0.1	0		
	HB2017091	2017	г Г	371	-	4.7 ± 0.1 11.7 ± 10.7	3		
	HB2017092	2017	г Г	372	±/	11.7 ± 10.7 65 ± 2.0	4		
	HB2017093	2017	F	81	+/-	9.8 ± 5.6	2		
	HB2017094	2017	F	225	**	9.0 ± 3.0	6	hlacty MO	aurB2
	HB2017095	2017	F	374	**	31.7 ± 4.8	6	blacty M 2	<i>qnrb2</i>
	1102017070	2017	1	0/4		51.7 ± 4.0	0	offici A-M-5	
	HB2017097	2017	F	375	**	175 + 19			
	HB2017097 HB2017098	2017 2017	F	375 376	** +/-	17.5 ± 1.9 14 2 ± 6 5	1		
	HB2017097 HB2017098 HB2017099	2017 2017 2017	F F E	375 376 297	** +/- +/-	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1	0 1 6		aac(6')-Ih-cr
	HB2017097 HB2017098 HB2017099 HB2017100	2017 2017 2017 2017	F F E F	375 376 297 377	** +/- +/- *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2	0 1 6 2		aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017099 HB2017100 HB2017101	2017 2017 2017 2017 2017	F F E F	375 376 297 377 378	** +/- +/- *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1	0 1 6 2 2		aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017099 HB2017100 HB2017101 HB2017102	2017 2017 2017 2017 2017 2017 2017	F F F F F	375 376 297 377 378 379	** +/- * ***	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2	1 6 2 2 3		aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017099 HB2017100 HB2017101 HB2017102 HB2017103	2017 2017 2017 2017 2017 2017 2017	F F F F F F	375 376 297 377 378 379 380	** +/- * *** *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4	0 1 6 2 2 3 4		aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017099 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F	375 376 297 377 378 379 380 381	** +/- +/- * *** * * +/-	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7	0 1 6 2 2 3 4 3		aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017099 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F	375 376 297 377 378 379 380 381 382	** +/- * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9	0 1 6 2 3 4 3 9	blaтем-1	aac(6')-Ib-cr aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017099 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F	375 376 297 377 378 379 380 381 382 383	** +/- * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3	1 6 2 3 4 3 9 4	blaтем-1	aac(6')-Ib-cr aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F	375 376 297 377 378 379 380 381 382 383 384	** +/- * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1	1 6 2 3 4 3 9 4 7	blaтем-1	aac(6')-Ib-cr aac(6')-Ib-cr
	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017108	2017 2017 2017 2017 2017 2017 2017 2017	F E F F F F F F F F F F	375 376 297 377 378 379 380 381 382 383 384 385	** +/- * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1	1 6 2 3 4 3 9 4 7 6	blaтем-1 blactx.м.9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2
	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017108 HB2017109	2017 2017 2017 2017 2017 2017 2017 2017	F E F F F F F F F F F F	375 376 297 377 378 379 380 381 382 383 384 385 386	** +/- * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4	1 6 2 3 4 3 9 4 7 6 1	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2
	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017108 HB2017109 HB2017110	2017 2017 2017 2017 2017 2017 2017 2017	F E F F F F F F F F F F	375 376 297 377 378 379 380 381 382 383 384 385 386 375	** +/- * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0	1 6 2 3 4 3 9 4 7 6 1 0	blaтем-1 blacтх-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2
	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017108 HB2017109 HB2017110 HB2017111	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F F F	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387	** +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8	1 6 2 3 4 3 9 4 7 6 1 0 2	blaтем-1 blacтx-м-9	aac(6′)-Ib-cr aac(6′)-Ib-cr aac(6′)-Ib-cr, qnrB2
Lineage IV	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017110 HB2017111 HB2016029	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F F F D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8	1 6 2 3 4 3 9 4 7 6 1 0 2 1	blaтем-1 blacтх-м-9	aac(6′)-Ib-cr aac(6′)-Ib-cr aac(6′)-Ib-cr, qnrB2
Lineage IV C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017108 HB2017109 HB2017110 HB2016029 HB2017007	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F F D D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 386 375 387 292 237	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8	<i>bla</i> тем-1 <i>bla</i> стх-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017110 HB2016029 HB2017007 HB2017007	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F D D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 386 375 387 292 237 74	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3	<i>bla</i> тем-1 <i>bla</i> стх-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB2017010 HB2017007 HB2017021 HB2017029	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F D D D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB2017010 HB2017007 HB2017021 HB2017029 HB2017067	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F C D D D D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5 15.3 ± 2.7	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB20170107 HB2017007 HB2017021 HB2017029 HB2017067 HB2017086	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F C D D D D F	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258	** +/- +/- * * * * * * * * * * * * * * * * * * *	$\begin{array}{c} 17.5 \pm 1.9 \\ 14.2 \pm 6.5 \\ 17.1 \pm 1.1 \\ 17.9 \pm 2.2 \\ 21.1 \pm 5.1 \\ 25.0 \pm 4.2 \\ 0.7 \pm 0.4 \\ 14.0 \pm 1.7 \\ 13.9 \pm 2.9 \\ 18.6 \pm 0.3 \\ 18.5 \pm 1.1 \\ 13.2 \pm 3.1 \\ 20.4 \pm 2.4 \\ 18.0 \pm 3.0 \\ 18.0 \pm 0.8 \\ \hline 6.5 \pm 4.8 \\ 8.4 \pm 2.3 \\ 10.0 \pm 0.5 \\ 16.5 \pm 2.5 \\ 15.3 \pm 2.7 \\ 7.0 \pm 5.1 \\ \end{array}$	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae Lineage V	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB2017007 HB2017007 HB2017021 HB2017029 HB2017067 HB2017086 HB2016026	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F C D D D D F C	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183	** +/- +/- * * * * * * * * * * * * * * * * * * *	$\begin{array}{c} 17.5 \pm 1.9 \\ 14.2 \pm 6.5 \\ 17.1 \pm 1.1 \\ 17.9 \pm 2.2 \\ 21.1 \pm 5.1 \\ 25.0 \pm 4.2 \\ 0.7 \pm 0.4 \\ 14.0 \pm 1.7 \\ 13.9 \pm 2.9 \\ 18.6 \pm 0.3 \\ 18.5 \pm 1.1 \\ 13.2 \pm 3.1 \\ 20.4 \pm 2.4 \\ 18.0 \pm 3.0 \\ 18.0 \pm 3.0 \\ 18.0 \pm 0.8 \\ \hline 6.5 \pm 4.8 \\ 8.4 \pm 2.3 \\ 10.0 \pm 0.5 \\ 16.5 \pm 2.5 \\ 15.3 \pm 2.7 \\ 7.0 \pm 5.1 \\ 17.2 \pm 0.9 \\ \end{array}$	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae Lineage V C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB2017007 HB2017021 HB2017021 HB2017029 HB2017067 HB2017086 HB2016026 HB2016027	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F C D D D D F C D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183 291	** +/- +/- * * * * * * * * * * * * * * * * * * *	$\begin{array}{c} 17.5 \pm 1.9 \\ 14.2 \pm 6.5 \\ 17.1 \pm 1.1 \\ 17.9 \pm 2.2 \\ 21.1 \pm 5.1 \\ 25.0 \pm 4.2 \\ 0.7 \pm 0.4 \\ 14.0 \pm 1.7 \\ 13.9 \pm 2.9 \\ 18.6 \pm 0.3 \\ 18.5 \pm 1.1 \\ 13.2 \pm 3.1 \\ 20.4 \pm 2.4 \\ 18.0 \pm 3.0 \\ 18.0 \pm 3.0 \\ 18.0 \pm 0.8 \\ 6.5 \pm 4.8 \\ 8.4 \pm 2.3 \\ 10.0 \pm 0.5 \\ 16.5 \pm 2.5 \\ 15.3 \pm 2.7 \\ 7.0 \pm 5.1 \\ 17.2 \pm 0.9 \\ 28.3 \pm 0.5 \end{array}$	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1 1 1	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae Lineage V C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017104 HB2017105 HB2017106 HB2017107 HB2017109 HB2017010 HB20170107 HB2017021 HB2017021 HB2017029 HB2017067 HB2017086 HB2016026 HB2016027 HB2016028	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F D D D D D F C D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183 291 187	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5 15.3 ± 2.7 7.0 ± 5.1 17.2 ± 0.9 28.3 ± 0.5 27.4 ± 1.2	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1 1 0	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae Lineage V C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017103 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB20170107 HB2017021 HB2017021 HB2017029 HB2017067 HB2017086 HB2016026 HB2016028 HB2016031	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F C D D D D D F C D F F	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183 291 187 294	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5 15.3 ± 2.7 7.0 ± 5.1 17.2 ± 0.9 28.3 ± 0.5 27.4 ± 1.2 18.7 ± 2.7	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1 1 1 0 2	blaтем-1 blactx-м-9	aac(6')-1b-cr aac(6')-1b-cr aac(6')-1b-cr, qnrB2 aac(6')-1b-cr
Lineage IV C. youngae Lineage V C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017103 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB20170107 HB2017021 HB2017021 HB2017029 HB2017067 HB2017067 HB2016026 HB2016027 HB2016028 HB2016031 HB2017063	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F F D D D D D F D D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183 291 187 294 346	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5 15.3 ± 2.7 7.0 ± 5.1 17.2 ± 0.9 28.3 ± 0.5 27.4 ± 1.2 18.7 ± 2.7 18.7 ± 2.7 18.7 ± 2.7 18.7 ± 1.5	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1 1 1 0 2 2 1 1 1 0 2 2 2 3 4 4 3 9 9 4 4 7 7 6 1 1 0 0 2 2 2 3 4 4 3 9 9 4 4 7 7 6 6 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	blaтем-1 blactx-м-9	aac(6')-1b-cr aac(6')-1b-cr aac(6')-1b-cr, qnrB2 aac(6')-1b-cr
Lineage IV C. youngae Lineage V C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017103 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB2017010 HB2017007 HB2017021 HB2017029 HB2017067 HB2017066 HB2016026 HB2016028 HB2016031 HB2017063	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F F C D D D D C F C D	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183 291 187 294 346 347	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5 15.3 ± 2.7 7.0 ± 5.1 17.2 ± 0.9 28.3 ± 0.5 27.4 ± 1.2 18.7 ± 2.7 18.7 ± 1.5 25.2 ± 1.4	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1 1 1 0 2 2 1 1 0 2 2 0 0	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae Lineage V C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017103 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB20170107 HB2017021 HB2017021 HB2017029 HB2017026 HB2016026 HB2016027 HB2016028 HB2016031 HB2017064 HB2017064	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F F F F C D D D C F C D D C C	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183 291 187 294 346 347 348	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5 15.3 ± 2.7 7.0 ± 5.1 17.2 ± 0.9 28.3 ± 0.5 27.4 ± 1.2 18.7 ± 2.7 18.7 ± 1.5 25.2 ± 1.4 20.4 ± 4.9 20.4 ± 4.9	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1 1 0 2 2 1 1 0 2 2 0 7 7	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr
Lineage IV C. youngae Lineage V C. youngae	HB2017097 HB2017098 HB2017009 HB2017100 HB2017101 HB2017102 HB2017103 HB2017103 HB2017105 HB2017106 HB2017107 HB2017109 HB2017109 HB2017010 HB2017011 HB2017021 HB2017021 HB2017027 HB2017026 HB2016026 HB2016028 HB2016031 HB2017064 HB2017065 HB2017065	2017 2017 2017 2017 2017 2017 2017 2017	F F F F F F F F F F F C D D D D F D D D C D C	375 376 297 377 378 379 380 381 382 383 384 385 386 375 387 292 237 74 316 350 258 183 291 187 294 346 347 348 349	** +/- +/- * * * * * * * * * * * * * * * * * * *	17.5 ± 1.9 14.2 ± 6.5 17.1 ± 1.1 17.9 ± 2.2 21.1 ± 5.1 25.0 ± 4.2 0.7 ± 0.4 14.0 ± 1.7 13.9 ± 2.9 18.6 ± 0.3 18.5 ± 1.1 13.2 ± 3.1 20.4 ± 2.4 18.0 ± 3.0 18.0 ± 0.8 6.5 ± 4.8 8.4 ± 2.3 10.0 ± 0.5 16.5 ± 2.5 15.3 ± 2.7 7.0 ± 5.1 17.2 ± 0.9 28.3 ± 0.5 27.4 ± 1.2 18.7 ± 2.7 18.7 ± 1.5 25.2 ± 1.4 20.4 ± 4.9 19.5 ± 3.2	1 6 2 3 4 3 9 4 7 6 1 0 2 1 8 3 2 1 2 1 1 0 2 2 1 1 1 0 2 2 0 7 0 0 7 0 0	blaтем-1 blactx-м-9	aac(6')-Ib-cr aac(6')-Ib-cr aac(6')-Ib-cr, qnrB2 aac(6')-Ib-cr

HB2017085 2017 F 366 ** 22.1 ± 6.5 1

Adhesion index: ***, >50; **, >1 and <50; *, <1; +/-, ambivalent or no adhesion; -, no adhesion. LDH (% \pm SD): The lactate dehydrogenase released from Hep-2 cells; STs, sequence types; NDR, number of drugs resistance; D, F and E, isolates from diarrheal patients, foods and environment. ESBLs: extended-spectrum β -lactamase.



Figure 1. Phylogenetic relationships of the 128 *Citrobacter* isolates from this study. The phylogenetic tree of the 128 *Citrobacter* isolates was constructed using the concatenated sequences of the seven housekeeping genes by the neighbor-joining algorithm. *Salmonella* LT2 was used as an outgroup. Lineage divisions were marked. Bootstrap values of 50% or more from 1000 replicates were shown.

The STs of *C. freundii* in Lineages I and II from this study were compared with 85 STs of *C. freundii* from Maanshan Anhui Province in our previous study [10,16], only nine STs found in this study shared the same STs from Maanshan city. *C. freundii* from these two regions displayed high diversity. All STs of *C. freundii* from this study and our previous study were used to construct a phylogenetic tree. The tree was divided into six clusters with strong bootstrap support of the cluster divisions (Figure 2 and Supplementary Figure S1). Cluster 1 to 5 were the same as previously defined [16]. However, a group within cluster 1 of our previous study is now a separate cluster and named cluster 6. The STs of *C. freundii* in Lineage I and Lineage II from this study were equivalent to cluster 1 and cluster 2 in our previous study (Figure 2 and Supplementary Figure S1).

			Source	Year	Adhesion	LDH	qnr/ESBLs	NDR	Antibiotic resistance phenotype
Г		- ST298 HB2016036	D	2016	+/-	0.6±0.2		2	(AMP)(CTX,CFX)
•		- ST288 HB2016023	F	2016	•	8.9±0.2	bla CTX-M-3, bla TEM-1, aac(6')-lb-cr,qnrB5	9 9	(AMP)(CTX,CA2,FEP,CTX,TIO)(A2M(N AL, CP,LEV)(GEN,KAN,STR(TET,20X)(CHL)(SUL,SXT)(AM2)
72	Ιг	ST227 AH2016013	D	2016	•	6.6±0.9		2	(AMP) (CFX, CFZ)
1 1		62 C ST173 AH2014040	н	2014	-/+	6.6±1.2		2	(CTX, CFZ) (AK)
1 1		ST318 HB2017031	D	2017	+/-	12.7±0.2	gnrB9	2	(CEX)(AMZ)
69	11	ST199 AH2014016	D	2014	•	4.0±0.5	gnrB9	1	(CAZ, FEP, CFX, CFZ, CRO)
		66 ST303 HB2017006	D	2017		27.3±0.6	qnrB76 include the LexA binding site	2	(CFX)(STR)
		ST222 AH2015007	D	2015		13.4±3.6		1	(CFX, CFZ)
		ST304 HB2017009	D	2017		11.9±0.6	qnrB76 include the LexA binding site	4	(AMP)(CTX,CFX)(TET)(CHL)
	72 0	55 ST175 AH2014025	D	2014	**	10.3±0.6		3	(CTX, CAZ, FEP, CFX, CFZ, CRO) (AZM) (F)
	/2 84	+ CT174 AU2044042		2014		11 1+1 3	bia	7	(AMP) (CTX CAZ EEP, CEX CEZ CBO) (AZM) (TET) (CHL) (SXT) (E)
	IГ		- D	2014 2016	·	140437 30403	cont BB	422	
		S117 AH2014007 AH2014021 HB2016004		2014, 2010	01	14.912.7, 2610.1 of 12.410.4	97720	4,2,2	(MAP) (CTX C A7 C C X T C) (A7 M (C T C)
		59 ST338 HB2017053	F	2017		21.012.0	ym Ba	-	(AMP)(CTACA2,CPA,TIO)(A2IN)(STR)
	- 11	ST331 HB2017045	F	2017	•	14.3±1.8	bla CTX-M-9bla TEM-1, qnrS1	7	(AMP)(CTX,CFX,TID)(NAL)(GEN,STR)(TET)(CHL)(SUL,SXT)
		on ST236 AH2016007	D	2016	-	14.3±1.6		4	(AMP) (CTX, CFX, CFZ, CRO)(TET) (SXT)
	4	ST289 HB2016024	F	2016	•	20.5±1.3		3	(AMP)(CTX,CFX,TID)(NAL,C IP LE V)
		65 ST328 HB2017042	F	2017		8.9±7.8	bla TEM-1	9	(AMP)(CTX,CAZ,FEP,CFX,TID)(MI,MEN)(N.AL,CP,LEV)(GEN,STR(TET,DOX)(CHL)(SUL,SXT)(AME)
		on ST301 HB2017003	D	2017	•	8.9±0.8		2	(AMP)(CFX)
		85 ST326 HB2017040	F	2017	•	6.7±1.4		8	(AMP)(CTX,CFX,TIO)(NAL)(STR)(TET,DOX)(CHL)(SUL,SXT)(AMZ)
		89 • ST172 AH2014042	н	2014	-/+	7.2±2.0		4	(AMP) (CTX,CFX, CFZ) (AZM) (AK)
		ST322 HB2017036	F	2017	**	14.1±0.8		3	(AMP)(CFX)(STR)
	1	ST208 AH2014030	D	2014	-/+	2.6±1.0	anrB17	1	(CTX.CFX, CFZ)
				2017		19.8+5.7	aprB17	2	(AMP)(CEX)
		S1325 HB2017039	5	2016		15.9+0.2	4	2	(AMP) (CTY CAZ CEY CEZ CRO)
		50 S1232 AH2016001	-	2010		155101			
		ST337 HB2017052	F	2017		10.51.5		-	(AMP)(CFA)
		ST1 HB2017017 HB2017061	D or F	2017	- or	15.9±1.0 0F17.3±2.4		2	(AMP)(CFX)
		ST211 AH2014047	н	2014		3.1±0.4	durp.	2	(AMP)(CFX, CFZ)
		87 ST324 HB2017038	F	2017	·	12.4±8.9	qnrB94	4	(CFX)(KAN)(TET,DOX)(SUL,SKT)
		ST45 AH2008007 AH2015011	D or F	2015, 2008	•	5.5±0.6 or 0.7± 0.4	aac(6')+b+cr, qnrB63,	qnrB77	(AMP)(CAZ)(NAL)(TET,DOX) or (CTX, CAZ, CFZ)
		ST260 HB2016034	F	2016	•	5.0±1.4	qnrS1	7	(AMP)(CTXCFXTIO)(NAL)(STR)(TET,DO X)(CHL)(SUL,SXT)
		ST320 HB2017033	E	2017		19.1±3.2	qnrB76	0	
1		ST191 AH2014041	н	2014	-/+	6.3±0.8	qnrB13	4	(AMP) (CTX,CFX, CFZ) (AZM) (AK)
1		ST309 HB2017016	D	2017		15.0±1.7		1	(CFX)
1		ST306 HB2017012	D	2017		7.1±0.4	qnrB76 include the LexA binding site	1	(CFX)
1		ST85_AU2011007_AU2046044.UB201200	D or F	2016,2017, 2011	* m ·/	9.6±2.7, 0.2±0.2 or	a	6 2 2	(CAZU/NAU/(STD)/TETU/CAU/(SUL) (AND) (CEV, CET) (AND)(CEV)
1		- 5105 AH2011007 AH2010011 HE2017004			of +/-	12.3±2.2		0, 2, 2	(GPA, GPA)(GPA)(GPA)(GPA)(GPA)
1		- 31198 AM2014010	D	2014	•	12.0±1.8	qnrB13	2	(AMP)(CTX, CFZ)
1		ST166 AH2014031 AH2014034	н	2014	- or -/+	5.7±0.6 or 7.0±1.4	qnrB76	1, 1	(CFX, CFZ, CRO) or (CTX,CFX, CFZ, CRO)
1		L ST220 AH2015003	D	2015		13.6±2.4	qnrB16	1	(CFX, CFZ)
		ST313 HB2017026	D	2017	**	17.4±1.1	aac(6)-b-cr	6	(AMP)(TET)(NAL,CIP)(TET,DOX)(CHL)(SUL,SXT)
		ST300 HB2017002		2017	+/-	10.3±1.8	big CTX-M9 _ anS1 and13 include the LexA binding site	1	(CEX)
		ST339 HB2017054	-	2017		16 5+3 6	apr876	2	(MP/CEX)
		ST343 HB2017059		2017		12.8+0.4	bis CTV-M-3 bis CTV-M-9 cor/892	7	(AMD)(CTY EEP CEY TIO)(AM7)(GEN KAN STRVT ET)(CHI)(SUI SYT)
		61 - ST201 AH2014019		2017		11.0104			
		ST203 AH2014022	0	2014		4.1±0.3	dur.marz	2	(AMP)(CTX, CFX, CF2, CR0)
			D	2014		4.2±0.9		2	(CFX, CFZ)(A2M)
		31264 HB2016019	F	2016	+/-	6.3±2.2	qnrB9	9	(AMP)(CTX,FEP,CFX,TIO)(MEN)(NAL,LEV)(STR)(TET,DOX)(CHL)(SUL,SXT)(AMZ)
		- 31344 HB2017000	F	2017	+/-	11.9±1.6	bla CTX-M-9,bla TEM-1	7	(AMP)(CTX,FEP,CFX,TIO)(NAL)(GEN,STR)(TET,DOX)(CHL)(SUL,SXT)
	90	ST47 AH2008009	F	2008	•••	20.2± 5.4		0	
		ST332 HB2017046	F	2017	••	22.9±7.0		2	(AMP)(CFX)
		ST341 HB2017057	F	2017	••	20.3±3.3		4	(CFX)(TET)(CHL)(SUL,SXT)
		ST333 HB2017047	F	2017		23.5±5.0		6	(AMP)(CFX,CTX)(GEN,STR)(TET)(CHL)(SUL,SXT)
		- ST33 AH2007014	D	2007		5.3±.08		2	(AMP)(NAL,CIP)
		ST116 AH2014015	D	2014		24.0±3.1	aac/61-ib-cr	5	(AMP) (CTX, CAZ, FEP, CFX, CFZ) (TET) (CHL) (SXT) (F)
4		ST314 HB2017027	D	2017		247+27		4	(AMP)(CEX)(NAL)(TET)
	82	L ST327 HB2017041	-	2017		54416		4	(050)
		ST150 HR2017024	-	2017		5A11.0			(CFA)
		CT140 100047000	D	2017		212±10		1	(CFA)
			D	2017		229±09		1	(CEA)
		S1308 HB2017014	D	2017		18.0±13.5		1	(CFX)
		ST125 HB2017018	D	2017	**	21.5±7.3		2	(CFX)(NAL)
		ST185 AH2014039 AH2016010	H or D	2014, 2016	- or *	4.5±1.4 or 11.9±1.4		5 or 2	(AMP) (CTX, CAZ, CFX, CFZ) (AZM) (CIP, LEV) (GEN, AK) or (AMP) (CFX, CFZ)
		ST214 AH2015009 HB2017051	D or F	2015, 2017	* or **	12.0±2.9 or 21.0±4.4		1 or 2	(CTX, CAZ,CFX, CFZ) or (CFX)(TET)
	90	ST312 HB2017025	D	2017		8.0±5.0		3	(AMP)(TET)(CHL)
		- ST8 AH2015015	D	2015		8.4±2.0		2	(AMP)(CEX)
		ST282 HB2016017	F	2016		21.4±7.3		1	(NAL)
		- ST229 AH2016009	n	2016		17.8+3.6		1	(CEX CEZ)
		ST270 HB2016003		2016		15 6+1 1		4	(CEX)
		ST43_AH2008005	F	2008		14.9+ 7.8			(AND/CAZ/(NAL)/TET DOX)
	5.4	5145 A1200000		2000	_	14.91 7.8			(AMP)(CA2)(NAE)(TET(DOA)
	34	STEA AU20000001		2011		3.5±0.4		1	(AMP)
	99			2009	+)-	22±12	bia IEM-1, ghrS1		(AMP)(CTX,TID)(NAL,CIP,LEV)(STR)(TET,DDX)(CHL)(SDL,SXT)
1	- 1		F	2016		15.3±3.9		3	(AWP)(GPA) (MEM)
1		- 3130 AH200/018	D	2007	•	16.7± 4.1		1	(AMP)
		ST323 HB2017037	E	2017	**	24.8±6.8		1	(CEX)
1		ST176 AH2014048	н	2014	-/+	13.7±0.3		1	(CFX, CFZ)
1		- ST317 HB2017030	D	2017		20.2±3.0		1	(TIO)
1		ST329 HB2017043	F	2017	•	20.0±0.6		1	(CFX)
1		ST340 HB2017056	F	2017	••	19.2±3.4		4	(AMP)(CFX)(NAL)(TET.DOX)
1		ST177 AH2014046	н	2014	-/+	6.8±2.2		3	(AMP)(CTX, CAZ,CFX, CFZ)(AK)
1		ST217 AH2015008 HB2017019	D	2015, 2017	+/- or **	14.5±5.3 or 14.6±1.4		1 or 1	(CFX, CFZ) or (CFX)
1		ST44 AH2008006	F	2008		0.1± 0.4	qnrB63	2	(AMP) (LEV.NAL)
1		ST215 AH2015013	D	2015	-/+	10.5±0.4		1	(CFX, CFZ)
1		ST216 AH2015012 HB2016002		2015 2014	+/- or *	8.8±1.1 or 10.4+0.2		1 or 3	(CTX, CFX, CFZ) or (AMPI/CTXCAZCFXT IO)/AZ MI
1		ST42 AH2008004	F	2008		30+23	bla TEM-1, aac/61-b-cr	8	(AMPI/CTXI/NALCPLEV/(KAN.STR)(TETDOXI/CHI)/SUI SXTI/AM71
1		STR6 AH2011008 AH2011009	F	2011	· or ··	0 1+ 0 1 ~ 4 2+ 1 3		2011	AMP/CA7) or (SXT)
1		ST276 HP2016010			or	0.12 0.1 0F4.2± 1.3		∠ dr 1	/050
1		- 512.7 0 HB2010010	E	2016		0.4±1.9		1	(MIR)
		ST311 HB2017022	D	2017		20.2±1.3		2	(AMP)(CFX)
1		S1186 AH2014014	D	2014		13.5±0.4	qnrSt	4	(AMP)(CIX, CAZ, CFX, CFZ)(AZM) (TET)
1		ST274 HE2016008	F	2016		22.7±7.3	bla TEM-1, aac(6)-ib-cr,qnrB2	8	(AMP)(CTX,FEP,CFX,TIO)(NAL)(GEN,STR)(TET)(CHL)(Sul,SXT)(AMZ)
1	I	ST100 HB2016011 HB2017034	F or E	2016, 2017		22.6±3.0 or 19.4±2.5		1	(CFX)
1		ST163 HB2017011	D	2017	••	15.8±0.7	bla TEM-1, aac(6)-lb-cr	7	(AMP)(CTX,CAZ,CFX)(AZM)(NAL,CIP,LEV)(STR)(TET,DOX)(CHL)(SUL,SXT)
1	- (ľ	L ST272 HB2016006	E	2016		9.3±2.8		1	(CFX)
1	_ I	ST161 HB2017055 AH2014018	D or F	2014, 2017	••	6.2±1.4 or 18.2±3.1		6 or 3	(AMP)(CFX)(NAL)(TET)(SXT)(AMZ) or (AMP) (CFX, CFZ) (AZM)
1	- 18	ST235 AH2016004	D	2016		24.6±3.0		1	(CFX, CFZ)
1	_ <u> </u>	ST212 AH2015005	D	2015		23.6±0.7		1	(CFZ)
1		H ST228 AH2016006	D	2016		8.4±2.7		1	(CFX, CFZ)
1	- fî	= ST307 HB2017013	_	2017		68-03		4	(CE0
1	Ľ	ST321 HB2017035	-	2017		21 8+6.7		4	(CE0
1	19	- SI321 HB2017035	E	2017		21.8±5.7		1	
1	_l⊢	ST210 AH2014043	н	2014	-/+	7.3±0.7		3	(CTX, CAZ, CFZ) (AZM) (AK)
1	−⊪	ST283 HB2016018	F	2016	+/-	9.2±1.5		2	(AMP)(CFX)
1	⊩	ST12 AH2015001 HB2017008	D	2015, 2017	·	6.5±0.1 or 13.6±0.7		2 or 5	(CTX, CAZ, CFX, CFZ) (AK) or (AMP)(CTX,CFX)(TET)(CHL)(SXT)
	. IF	ST46 AH2008008	F	2008		11.0± 2.0		1	(SXT)
1*	Դե	ST169 HB2017001	D	2017		29.4±5.8		2	(CFX)(CT)
111	I Ift	ST277 HB2016012	F	2016	••	16.9±1.5		2	(AMP)(CFX)
111	Isa T	ST219 AH2015006 AH2015017 HB2017023	D or H	2015, 2017	• or ••	13.4±4.6, 22.0±3.4 or 18.0±	3.4	1, 1, 3	(CFZ), (CFZ) or (AMP)(CTX;CAZ;CFX;TIO)(AZM)
1	174	ST269 HB2016001		2016		11.0±2.1		1	(CFX)
	17	ST319 HB2017032	D	2017		19.7+1.3		5	(AMPI(CEX)(TET)(CHL)(SULSXT)
98	11.0		0	AV11				2	(AMP)(CEX)
98	Ľ۲	CT335 U00047040		20.17		212-102		~	
98	"L	ST335 HB2017049	F	2017		312±10.2		-	(AMR) (CEY, CEZ)
98	98 	ST335 HB2017049 ST223 AH2015020	F	2017 2015		312±10.2 10.5±1.8		2	(AMP) (CFX, CFZ)

Figure 2. Phylogenetic relationships of the 123 *C. freundii* isolates from this study and two previous studies [10,16]. Lineages are marked on the node with roman numerals. Bootstrap values from 1000 replicates are shown on or near the nodes if \geq 50%. The presence of ESBL and *qnr* genes, source, year, NDR (number of drugs resistant to), adhesion, LDH and antibiotic resistance phenotype of an isolate is shown on the right. The tree was constructed using the neighbor joining method. ST, D, F, E, H, and LDH denote sequence types, isolates from diarrheal patients, foods, environment and healthy individuals, and lactate dehydrogenase, respectively. Adhesion index: ***, >50; **, >1 and <50; *, <1; +/-, ambivalent or no adhesion; -, no adhesion.

The 16 STs of *C. youngae* in Lineage IV and Lineage V from this study were compared with 32 STs of *C. youngae* from Maanshan, Anhui Province in our previous study. Fifteen STs from this study were novel STs, and there was little overlap of STs. All STs of *C. youngae* from this study and our previous study were used to construct a phylogenetic tree, the tree was divided into two clusters (cluster 1 and cluster 2) with strong bootstrap support of the cluster divisions, and 16 STs of *C. youngae* in Lineage IV and Lineage V from this study were equivalent to cluster 1 and cluster 2 in our previous study [10] (Figure 3),

100 STS A4000015 D 2008 * 15.2.2.2.8 1 (AMF) 98 STS A4000017 F 2008 * 2.2.1.1 5 (MAL CAPLEN)(MAL STR)(TELCOX)(OLL)(SLLSXT) 98 STS A4000013 F 2016 * 13.7.2.7 2 (AMF)(GTX)(AUL)(STR)(TELCOX)(OLL)(SLLSXT) 97 STS A4000704 D 2007 * 3.7.4.1.2 5 (AMF)(GTX)(AUL)(STR)(TELCOX)(OLL)(SLLSXT) 100 STS A40007021(5) D 2007 * 3.7.4.1.2.6 2 (AMF)(GTX) 110 A40007021(5) D 2007 * 3.7.4.2.0.1 1 (AMF) 110 STS A40007021(5) D 2007 * 5.26.0.1 2 (AMF) 110 STS A4000702 F 2010 * 5.22.4.2 1 (AMF) 110 STS A4000702 F 2018 * 2.2.4.2.2 1 (AMF) 110 STS A4000702 F 2018 * 2.
9 5 3TT A 4000017 F 2008 • 2.2±1.1 5 (MAL CPLED/(MALSTR)(TELDOX(CPL)(SLLS.T)) 9 5TT A 4000017 F 2016 • 1.72.7 2 (AMP)(CX) 97 STT A 40000101 F 2016 • 1.72.7 2 (AMP)(CX) 97 STT A 4000702 D 2007 • 3.7±1.2 5 (AMP)(TCXD.2) (STT)(D) 5170 A 40007024 D 2017 • 3.7±1.2.8 2 (AMP)(TXT)D) 5170 A 40007024 D 2018 • 17.2±0.9 1 (CTX) 5170 A 40007024 D 2017 • 5.9±0.1 2 (AMP)(TXT)D) 5170 A 40007024 F 2009 • 5.2±0.2 1 (AMP)(ADP)(ADP)(ADP)(ADP)(ADP)(ADP)(ADP)(AD
T374 HE2010031 F 2016 ** 187.27 2 (AMP) (CN) 97 T374 HE201005 0 2017 ** 204.49.9 7 (AMP) (CN) 157.4 HE201005 0 2007 * 37.1 2.6 2 (AMP) (CNC) (CNL) (IETLOX) 157.4 HE201005 0 2007 * 37.1 2.6 2 (AMP) (CNC) 157.8 HE201025 0 9016 ** 72.8.9 1 (CNC) 157.8 HE201025 0 916 ** 72.8.9 1 (CNC) 157.8 HE201025 0 916 ** 72.8.9 1 (CNC) 10 F137 AH201010 F 2017 * 55.0.1 2 (AMP) (CNC) 10 LSTS AH201000 F 2018 ** 52.0.2 1 (AMP) 10 LSTS AH201000 F 2018 ** 22.2.2 1 (AMP) 10 STA AH201000 F 2018 ** 22.2.3.4
9 TST-84 HE2017065 0 2017 *** 20.44.9 7 (AMP[COX](AU](SRP[(TE:DOX](C4,USL)) 9 TST-84 HE2017065 0 2017 ** 20.44.9 7 (AMP[CX)(AU](SRP[(TE:DOX](C4,USL)) 9 TST-84 HE2017065 0 2007 ** 37.11 ± 2.6 (AMP[CXCA2] (SCT) (ST) (TE:DOX) 9 ST184 HE2017024 0 2007 ** 37.11 ± 2.6 (AMP[CXCA2] (SMP[(TE:DOX)(C4,USC) (MP[C4,MP](C4,Z) (MP[C4,MP] (MP[
TSD A MODIFICIDE F 372 + 12 S (AMP/CTXCA2) (SMT) (TETLODA) - TSD A MODIFICIDE D 2007 ** 372 + 12 S (AMP/CTXCA2) (SMT) (TETLODA) - TSD A MODIFICIDE D 2007 ** 372 + 12.0 S (AMP) (CTXTO) - TSD A MODIFICID D 2016 ** 172.02 1 (AMP) (AZM)
TTZ AL020704 D 2007 * 371 2.6 2 (AMP) (CDX.TO) 1000 TTZ AL020704 D 2007 * TZ AL020704 D 2007 * S Stall AL0207022 (S) D * TZ AL020704 D 2007 * S Stall AL020702 (AMP) (AMP) (AL20 (CAL CTX TD) (TELDOX); (AMP); (AMP) (CAL CTX TD) (TELDOX); (AMP); (AMP); (AMP) (CAL CTX TD) (TELDOX); (AMP);
STI33 HE2015028 D 2016 ** 172.0.5 1 USD - STI33 HE2015028 D 2007 ** 12.4.12 (AMF) (AMF) (AZA) (AMF) (ATA) (ATA) (AMF) (ATA) (AMF) (ATA) (AMF) (ATA) (AMF) (ATA) (AMF) (ATA)
0.000 5T39 A4007022(5) D 0 F 2007.000 *0** 539.6.1 1, 2,4,12 (AMF)(CAZ.)(AMF)(CAZ.)(AMF)(CAZ.)(AMF)(AAZM)(CAZ.CTX.TD)(TET,DOX); (AMF); (AMF)(CAZ.) 5T37 A40007023 D 2007 ** 539.6.1 2 (AMF)(CAZ.) 5T37 A40007024 F 2009 ** 5240.2 1 (AMF) 5T37 A4000004 F 2016 ** 274.12 0 5T38 A40007027 F 2016 ** 274.12 0 5T38 A40007027 F 2016 ** 292.23 qm25 6 (AMF)(CAZ.) 5T48 A4007023 F 2019 ** 0.11 1.1 (AMF) 5T48 A4007023 F 2019 ** 0.11 1.2 (AMF) 5T48 A4007024 F 2019 ** 0.11 1.2 (AMF)(CAZ.) 5T48 A4007023 F 2019 ** 0.11 1.1 (AMF) 5T48 A4007023 F 2019 ** 0.11 1.1 (AMF) 5T48 A4007021 D 2017 ** 0.16 1.3 2 (AMF)(CAZ.) 5T48 A4007022 F 2008 ** 57.02 1 (AMF)(CAZ.) 5T48 A4007023 F 2018 ** 22.14 0 5T48 A400703 D 2017 ** 0.16 1.4 0 5T48 A4007051 F 2008 * 57.02 1 (AMF)(CAZ.) 5T48 A4007052 F 2008 ** 57.02 0 5T48 A4007053 F 2017 ** 0.25 1.4 0 5T48 A4007054 F 2017 ** 0.25 1.4 0 5T48 A4007054 F 2017 ** 0.25 1.4 0 5T48 A4007055 F 2017 ** 0.21 1.5 1.4 0 5T48 A4000051 F 2017 ** 0.25 1.4 0 5T48 A4000051 F 2017 ** 0.25 1.4 0 5T48 A400055 F 2017 ** 0.25 1.4 0 5T48 A400055 F 2017 ** 0.21 1.5 1.4 0 5T48 F 201708 F 2017 ** 0.21 1.5 1.4 0 5T48 F 201708 F 2017 ** 0.21 1.5 1.4 0 5T48 F 2
TSD 4-0007033 D 2007 ** 5.9:0.1 2 (AMP)(PEP) TSD A-0007033 F 2011 * 5.9:0.1 1 (AMP) TSD A-0007034 F 2009 * 5.2:0.2 1 (AMP) TSD A-0000004 F 2009 ** 27.4:1.2 0 TSD A-0000002 F 2016 ** 27.4:1.2 0 TSD A-000002 F 2018 ** 27.4:1.3 2 (AMP)(NALCPLEV)(TST(TEDON)(CH4)(SAL,SXT) TSD A-000002 F 2010 ** 0.1:1.3 2 (AMP)(NALLEV) TSD A-000002 F 2007 * 0.1:1.3 2 (AMP)(NALLEV) TSD A-0000723 D 2007 * 0.1:1.5 2 (AMP)(NALLEV) TSD A-000723 D 2017 * 10.7:1.5 2 (AMP)(NALLEV) TSD A-0000721 D
STEP A42011010 F 2011 • 157.0.1 1 (AMF) 100 _STS7 A42001004 F 2009 ** 52.0.2 1 (AMF) 100 _STS7 A42001004 F 2009 ** 52.0.2 1 (AMF) 100 _STS7 A42001002 F 2009 ** 52.0.2 1 (AMF) 100 _STS7 A42001000 A4200002 F 2009 ***.*. 15.8.3.5, 15.5.2.3.4.8.0.8 2.0.0 (AMF)(AZ) 100 _STS7 A4200100 F 2009 ***.*. 15.8.3.5, 15.5.2.3.4.8.0.8 2.0.0 (AMF)(MALLEP)/(STR)(TELDON)(CH4.)(SLLSXT) 100 _STS7 A42001001 F 2007 * 15.8.1.3 2 (AMF)(MALLEP)/(STR)(TELDON)(CH4.)(SLLSXT) 100 _STS3 H42017023 H 2007 * 15.8.1.3 2 (AMF)(MALLEP)/(STR)(TELDON)(CH4.)(SLLSXT) 100 _STS3 H42017024 H 2007 * 15.8.1.3 1 (AMF) 100 _STS3 H42017056 D
13T5 A40203004 F 2009 ** 5.20.2 1 (MP) 10 5T67 F82016025 P 2168 ** 5.20.2 1 (MP) 10 5T67 F82016025 P 2168 ** 27.41.2 0 10 5T67 F82016025 P 2108 ** 0.11.3 2 (AMP)(CX.2) 5T57 A40203004 F 2008 * 0.11.3 2 (AMP)(NAL.CPLEY)(TRITEDON)(CH.)(SAL.SXT) 10 5T58 A4020102 F 2007 * 6.6.0.4 1 (AMP)(NAL.CPLEY)(TRITEDON)(CH.)(SAL.SXT) 10 5T58 A4020721 D 2017 * 115.1.3 2 (AMP)(NAL.EP) 10 5T58 A4020721 D 2017 * 115.7.15 2 (AMP)(NAL.EP) 10 5T58 A40207031 F 2018 * 57.80.2 1 (AMP)(CR) 5T58 A40207064 2 2017 * 16.51.2 0 * 57.80.40200102 * 57.80.
100 CTIE CTIE 2016 *** 214.1.2 0 100 CTIE 2008 *** 244.1.2 0 100 CTIE 2008 *** 244.1.2 0 100 CTIE 2008 *** 244.1.2 0 100 CTIE 2008 ** 23.2.3.2 20.01 (MMP)(OZ.2) 100 CTIE 2008 ** 23.2.2.3 gwd5 6 (AMP)(NL.CPL)(STR](TELOOK)(CH.)(SULSXT) 100 STIE 51.03 20 (AMP) (AMP) (AMP) 100 STIE 51.03 20 (AMP)(NL.CPL)(STR](TELOOK)(CH.)(SULSXT) (AMP) 100 STIE 51.03 20 (AMP)(NL.CPL)(STR](TELOOK)(CH.)(SULSXLSXT) 100 STIE 51.03 20 (AMP)(NL.CPL)(STR](TELOOK)(CH.)(SULSXLSXT) 100 STIE 51.13 2 (AMP)(NL.CPL)(STR](TELOOK)(CH.)(SULSXLSXT) 100 STIE 51.02 1 (AMP) 100
Construction
Instructure Instructure <thinstructure< th=""> <thinstructure< th=""></thinstructure<></thinstructure<>
Sec TSTA HEQDINGL F 2008 ** 22.2.2 qm/S1 G (AMP(NLLCP)LEV)(STR(TET.DON)(CH4)(SLLSNT) 5 STA HEQDINGL P 2007 * 11.6:1.3 qm/S1 G (AMP(NLLCP)LEV)(STR(TET.DON)(CH4)(SLLSNT) 5 STA HEQDINGL7 D 2007 * 66.0.4 1 (AMP) 100 STAT HEQDINGL7 D 2016 ** 22.3.0.5 1 (AMP) 100 STAT HEQDINGL7 D 2017 * 18.7.1.5 2 (AMP)(CAQ) 98 STAT HEQDINGL4 D 2017 * 15.5.2 0 95 STAM HEQDINGL6 D 2017 * 15.5.2 0 91 STAM HEQDINGL6 D 2017 * 15.5.2 0 91 STAM HEQDINGL5 F 2017 * 15.5.2 0 100 STAM HEQDINGL5 F 2017 * 21.6.5 1 (CFX) 100
Solution F 2007 - 22.22.3 gms/s 6 (MMP(NULLEP))SIN(IELUON(CM-[SGLS.A)) Image: Star Aucontral D 2007 - 115.1.3 2 (MMP(NULLEP))SIN(IELUON(CM-[SGLS.A)) Image: Star Aucontral D 2007 - 115.1.3 2 (MMP(NULLEP)) Image: Star Aucontral D 2007 - 16.6.0.4 1 (AMP) Image: Star Aucontral D 2016 * 23.0.5 1 (CFX) Image: Star Aucontral D 2017 * 18.7.1.5 2 (AMP) Image: Star Aucontral D 2017 * 18.7.1.5 2 (AMP) Image: Star Aucontral D 2017 * 18.7.1.5 2 (AMP) Image: Star Aucontral D 2017 * 18.5.2.2 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Image: Size ARC001/21 D 2007 ** 6 & 6.4 1 (AMP) Image: Size ARC001/23 H 2007 ** 6 & 6.4 1 (AMP) Image: Size ARC001/23 D 2016 ** 28.36.5 1 (AMP) Image: Size ARC001/23 D 2017 * 18.71.5 2 (AMP)(CRX) Image: Size ARC001/23 D 2017 * 18.71.5 2 (AMP)(CRX) Image: Size ARC001/14 D 2017 * 18.56.2 0 (AMP) Image: Size ARC001/06 D 2017 * 18.56.2 0 (AMP)(SiL) Image: Size ARC001/06 D 2017 * 18.24.46 2 (AMP)(SiL) Image: Size ARC001/06 D 2017 * 18.24.46 2 (AMP)(SiL) Image: Size ARC001/06 F 2017 * 18.24.46 2 (AMP)(SiL) Image: Size ARC001/0706 F 2017 * 12.16.5 1
V 65 100 AF200/023 m 2007 ** 6 6 £ 0.4 1 (AMP) 100 5130 AF200/023 D 2016 ** 28.30.5 1 (CFX) 100 5134 AF200/023 D 2017 * 187.15 2 (AMP)(CX) 90 5134 AF200/023 D 2017 ** 25.21.4 0 90 5134 AF200/026 D 2017 ** 25.21.4 0 90 5130 AF200/026 F 2017 ** 105.51.2 0 90 5130 AF2008012 F 2008 ** 9.240.2 0 100 5130 AF2008012 F 2008 ** 9.240.2 0 100 5130 AF2008012 F 2008 ** 9.240.2 0 100 5130 AF2008012 F 2007 ** 85.2.4 3 (AMP)(SUL)
V Start HeadTroad D 2016 ** 28.30.5 1 (CFX) 100 START HEADTROAD D 2017 * 18.71.5 2 (AMP(CFX)) 100 START HEADTROAD D 2017 * 18.71.5 2 (AMP(CFX)) 90 START HEADTROAD D 2017 * 18.51.2 0 90 START HEADTROAD D 2017 * 19.51.2 0 100 START HEADTROAD D 2017 * 19.52.2 0 100 START HADORTROAD F 2017 * 21.16.5 1 (CFX)
100 57349 HE0217063 D 21/7 * 18.74.1.5 2 (AMP)(CPA) 00 57349 HE0217064 D 2017 ** 25.24.1.4 0 00 57349 HE0217064 D 2017 ** 25.24.1.4 0 00 57349 HE0217064 D 2017 * 19.55.3.2 0 00 57359 HE0217069 F 2017 * 19.24.16.6 2 (AMP)(SUL) 00 57359 HE0217069 F 2017 * 19.24.16.5 1 (AP)(SUL) 100 57369 HE0217085 F 2017 ** 22.16.5 1 (AP)(SUL) 100 57369 HE0217085 F 2017 ** 22.16.5 1 (STA) 100 57341 Accounts F 2017 ** 22.16.5 1 (STA)
STR8 A42008011 P 2008 * 5740.2 1 (AMP) *6 TST4 H82017064 D 2017 ** 25.21.4 0 *0 TST4 H82017064 D 2017 ** 19.51.2 0 *0 TST4 H82017086 D 2017 * 19.21.4 0 *0 TST2 H82017086 D 2017 * 19.21.6 2 (AMP)(SUL) *0 TST2 H82017086 F 2017 * 19.21.6 0 *100 TST8 H82017085 F 2017 * 21.46.5 1 (AMP)(SUL) *100 TST4 HA2007026 H 2017 ** 22.16.5 1 (D70)
Image: STAT / HE2017064 D 2017 *** 25.24.4 0 Image: STAT / HE2017066 D 2017 * 155.32 0 Image: STAT / HE2017069 F 2017 * 162.45.6 2 Image: STAT / HE2017069 F 2017 * 162.45.6 2 Image: STAT / HE2017069 F 2017 * 12.42.2 0 Image: STAT / HE20170785 F 2017 * 22.16.5 1 (G7A) Image: STAT / HA2007026 H 2007 ** 32.46.5 1 (G7A)
Image: STS8 HE8017086 D 2017 -* 19.54.2 0
L STSS2 HE0070709 F 2017 4'. 162:44.6 2 (AMF(SLL)) 000 FTSS2 HE0070709 F 2017 4'. 162:44.6 2 0 100 FTSS6 HE0070705 F 2017 4'. 22:16.5 1 (CFX) 100 FTSI6 HE0070705 F 2017 ** 32:6.5 1 (CFX)
Euro p=\$150 AH2030812 F 2008 ** 9.240.2 0 100 L=\$156 H82017085 F 2017 ** 22.16.5 1 (GFX) 100 L=\$156 H82017085 F 2017 ** 32.6.5 1 (GFX) 100 L=\$154 HA2007026 H 2007 *** 36.5.2.4 3 (AMP(CX2)(DDX)
100 L ST366 HB2017085 F 2017 ** 22.1±6.5 1 (CFX) 100 r= ST41 AH2007026 H 2007 *** 36.5± 2.4 3 (AMP(CAZ)(COX))
¹⁰⁰ St41 AH2007026 H 2007 *** 36.5±2.4 3 (AMP(CAZ)(DOX)
78 ST53 AH2008016 H 2008 +/- 4.4±0.7 1 (AMP)
□ 100 F STED AH2009008 F 2009 * 0.1±2.4 1 (SXT)
UUU U ST71 AH2009010 F 2009 *** 60.4±2.7 5 (NAL,CLP,LEV)(KAN,STR)(TET,DOX)(CHL)(SXT)
99 LST72 AH2009011 F 2009 ** 29.4± 3.8 6 (AMP)(NAL,CLP.LEV)(KAN.STR)(TET,DOX)(CHL)(SXT,SUL)
99 T ST37 AH2007019 D 2007 * 4.2±4.2 1 (AMP)
C ST56 AH2009003 F 2009 ** 14±1.6 3 (AMP(AZM)(KAN)
r ST25 AH2007001 AH2007002 D 2007 ** 22.4± 1.1 or 9.8± 0.7 2 or 2 (CAZ)(TET,DOX) or (AMP)(SXT)
ST31 AH2007010 D 2007 ** 0.1±1.3 1 (AMP)
UT L ST350 HB2017067 D 2017 ** 15.342.7 1 (GFX)
ST258 HB2017086 F 2017 T 7.0±5.1 2 (AMP(CFX)
100 99 ST316 H82017029 D 2017 ** 165425 2 (AMP/CFX)
L ST32 AH2007013 D 2007 * 32+0.8 1 (AMP)
STR3 APD11005 F 2011 * 64+19 1 (C47)
69 ST34 AH0007015 D 2007 ** 192764 2 (ANR/GET)
100 x 120 x 100 x
GI ST74 AF00001014 H82017021 D 2009 217 +/ w1 14x0 2 w100 0 5 2 w2 (ANEV/STD w(ANEV/STD W)
39 5778 AP200019 E 2009 04:04 (AIR) (AIR)(16)(300,37)
90 3122 FIDEUTUDZE 2 2016 4/- 0.524.0 1 (CFX)
Sisa Antouruno D 2007 - 1.34 U.S 2 (AMP/SXT)
C STAUT TOBUSTINOV D 2017 * 8.452.3 ase(6)-Ib-cr 8 (AMP)(CTX,CAZ,OFX)(AZM(NAL,CPLEV)(KAN,STR)(TET,DDX)(OHL)(SUL,SXT) Objected 127

Figure 3. Phylogenetic relationships of the 57 *C. youngae* isolates from this study and our previous study [10]. Lineages divisions are marked on the node with roman numerals. The number in bracket after strain name denote number of strains for ST39 which includes AH2007022, AH2007024, AH2007025, AH2008001, AH2008002). Bootstrap values (numbers on or near the nodes) from 1000 replicates are shown if \geq 50%. The presence of ESBLs and *qnr* genes, source, year, NDR (the number of drugs resistant to), adhesion, LDH and antibiotic resistance phenotype of an isolate is shown on the right. The tree was constructed using the neighbor joining method. ST, D, F, H, and LDH denote sequence types, isolates from diarrheal patients, foods and healthy individuals, and lactate dehydrogenase, respectively. Adhesion index: ***, >50; **, >1 and <50; *, <1; +/-, ambivalent or no adhesion; -, no adhesion.

The 42 STs of *C. braakii* in Lineage III from this study were compared with 8 STs of *C. braakii* from Maanshan Anhui Province in our previous study [10], and only one ST was common between the two regions. All STs of *C. braakii* from this study and our previous study were used to construct a phylogenetic tree (Figure 4), and all isolates belonged to the same cluster.

We further analyzed the 123 STs using eBURST [24,25] to identify clonal complexes. In this study we defined CCs as STs shared six of the seven alleles to identify the most closely related STs [24,25]. We also retrieved all *C. freundii* STs from other countries from the public MLST database to identify CCs that include isolates from other countries. It should be noted that there is no *C. youngae* or *C. braakii* isolates from other countries in the MLST database. There were 27, 7 and 5 CCs identified for *C. freundii*, *C. youngae* and *C. braakii* isolates, respectively (Supplementary Table S1). For the 27 *C. freundii* CCs, 17 CCs included isolates from other countries with one CC containing isolates from five different countries, 10 CCs included isolates from two different regions of China

but no isolates from other countries, and only four CCs were restricted in the same region of China. For the seven *C. youngae* CCs, only one was from two different regions of China. For the five *C. braakii* CCs only one was from two different regions of China also.

pm pm<		S	ource	Year	Adhesion	LDH	qnr/ESBLs	NDR	Antibiotic resistance phenotype	
• TSIDE HEQUITION F 2017 ••••••••••••••••••••••••••••••••••••	g	9 ST79 AH2010001	F	2010		0.1±0.5	qnrS1	7	(AMP)(NAL,CLP,LEV)(STR)(TET,DOX)(CHL)(SUL,SXT)	
- 5358 H2017078 D 2017 +1 13.25.7 1 (NALCP) - 5356 H2017078 F 2017 +1 32.20 31 (CSX)(NAL(TET)) - 5356 H2017071 D 2017 +1 32.20 31 (CSX)(NAL(TET)) - 5356 H2017071 D 2017 +1 32.40 31 (CSX)(NAL(TET)) - 5356 H201708 F 2017 +1 18.20.8 2 (AMP)(CSX) - 5356 H201708 F 2017 +1 14.21.9 2 (AMP)(CSX) - 5358 H201708 F 2017 +1 14.21.4 4 (AMP)(CSX) - 5358 H201706 F 2017 +1 14.27.4 2 (CSX)(ST) - 5358 H201706 F 2017 +1 14.27.4 2 (CSX)(ST) - 5358 H2017076 D 2017 +1 15.40.2 2 (CSX)(NAL)(TET.DOX) - 5358 H2017076 D 2017 +1 15.40.2 2 (CSX)(NAL)(TET.DOX)		L ST385 HB2017108	F	2017	**	13.2±3.1	blaCTX-M-9, aac(6)-lb-cr, qnrB2	6	(AMP)(CTX,FEP,CFX,TIO)(AZM)(NAL)(TET,DOX)(SUL,SXT)	
- 505 H2017034 F 2017 * 13.92.0 3 (CX)(HAL)(TET) - 575 A42000016 H201704 F 2009 - 4.0.5 (AMP)(CX)(TEDXOX(NLL)) - 1573 A4200011 F 2007 * 182.06 2 (AMP)(CX)(TEDXOX(NLL)) - 1573 A4200011 F 2017 * 182.06 2 (AMP)(CX) - 5736 H201703 F 2017 * 182.06 2 (AMP)(CX) - 5736 H201703 F 2017 * 182.01 2 (AMP)(CX)(NL)(TET) - 5736 H201704 F 2017 * 112.41 2 (CX)(RL)(TT) - 5736 H201704 F 2017 * 14.927 2 (CX)(RL)(TT) - 5736 H2017074 D 2017 * 14.927 2 (CX)(RL)(TT) - 5736 H2017076 D 2017 * 16.920 3 (AMP)(CX)(RL)(TT)		- ST359 HB2017078	D	2017	+/-	13.7±5.7		1	(NAL,CLP)	
- 5784 AC011001 HE201709 F 2017, 2011 +9.855 e.0.27.0.1 3 (CPX)MMLARC2 (or LARP(CRALLEP) - 5784 AC00109 F 2 2009 - 4.0.5. 5 (AMP)(CPX)(FELDX(NALL) - 5784 AC001709 F 2017 * 2.2.2.6.9 2 (AMP)(CPX) - 5784 AC001708 F 2017 * 1.8.4.1.9 2 (AMP)(CPX) - 5784 AC001708 F 2017 * 1.8.4.1.9 2 (AMP)(CPX) - 5784 AC01708 F 2017 * 1.4.8.1.9 2 (AMP)(CPX) - 5785 HE201708 F 2017 * 1.4.9.7.4 2 (CPX)(FER) - 5785 HE201708 F 2017 * 1.4.9.7.4 2 (CPX)(NAL) - 5785 HE201708 F 2017 * 1.8.4.0.1 (CPX)(NAL) (CPX)(NAL) - 5785 HE201708 F 2017 * 1.8.4.0.1 (CPX)(NAL) (CPX)(NAL) - 5786 HE2017070 F 2017 * 1		- ST365 HB2017084	F	2017		13.9±2.0		3	(CFX)(NAL)(TET)	
0 FTR AFC00016 F 2007 - 4.6.5 5 (AMP)(CX)(TET,DOX)(NAL) 5TR5 / FE0017111 P 2017 - 18.6.0.3 2 (AMP)(CX) 5TR5 / FE0017111 P 2017 - 18.6.0.3 2 (AMP)(CX) 1 5TR5 / FE001710 F 2017 - 18.6.0.3 2 (AMP)(CX) 1 5TR5 / FE001710 F 2017 - 18.4.1.1 2 (CX)(SKT) - 5TR5 / FE001704 D 2017 - 11.7.10.7 4 (CX)(SKT) - 5TR5 / FE001707 D 2017 - 20.4.2.4 1 (CX)(SKT) - 5TR5 / FE001707 D 2017 - 20.4.2.4 1 (CX)(NL)(STR) - 5TR5 / FE001707 D 2017 - 16.6.0.3 4 (CX)(NL)(STR) - 5TR5 / FE001707 D 2017 - 16.5.2.0 3 (AMP)(CX)(RL)(STR) - </td <td></td> <td>- ST81 AH2011001 HB2017094</td> <td>F</td> <td>2017, 2011</td> <td>+/- 9.8±</td> <td>5.6 or 0.7±</td> <td>0.1</td> <td>3</td> <td>(CFX)(IMI)(AMZ) or (AMP)(NAL,LEV)</td> <td></td>		- ST81 AH2011001 HB2017094	F	2017, 2011	+/- 9.8±	5.6 or 0.7±	0.1	3	(CFX)(IMI)(AMZ) or (AMP)(NAL,LEV)	
• • 1 22.64.9 2 (AMP)GPX • • • 18.0.0.0 2 (AMP)GPX • • • 18.0.0.0 2 (AMP)GPX • • • 0.7.0.4 4 (AMP)GPX • • 0.7.0.4 4 (AMP)GPX • • 0.7.0.4 4 (AMP)GPX • • 0.7.0.7.0.4 (AMP)GPX • • 0.7.0.7.0.4 (AMP)GPX • • 0.7.0.7.0.4 (AMP)GPX • 0.7.0.7.0.4 (AMP)GPX (CPX)(FE) • • 0.7.0.7.0.4 (AMP)GPX • 7.0.0.4.2.0.7.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0		- ST78 AH2009018	F	2009	_	4± 0.5		5	(AMP)(CAZ)(TET,DOX)(NAL)	
F378 H2017111 F 2017 * 14.8±1.9 2 (AMP(CX)) F378 H2017103 F 2017 * 0.14.8±1.9 2 (AMP(CX)) F378 H2017103 F 2017 * 0.14.8±1.9 2 (AMP(CX)) F378 H2017101 F 2017 * 0.14.8±1.9 2 (CX)(TET) F378 H2017101 F 2017 * 1.1.7±1.7 2 (CX)(TET) F378 H201702 F 2017 * 2.4.2.4 1 (CX)(NL) F378 H201708 F 2017 * 2.4.2.4 1 (CX)(NL) F378 H201708 F 2017 * 2.4.2.4 1 (CX)(NL) F378 H201708 F 2017 * 2.4.4.2.4 1 (CX)(NL) F378 H2017076 D 2.017 * 2.4.4.1.7 3 (AMP(CX)(NL)(ET) F378 H201708 F 2.017 * 1.4.4.1.7 3 (AMP(CX)(NL)(ET) F378 H2	02	L ST354 HB2017071	D	2017		22.2±6.9		2	(AMP)(CFX)	
First First <th< td=""><td></td><td>- ST387 HB2017111</td><td>F</td><td>2017</td><td>**</td><td>18.0±0.8</td><td></td><td>2</td><td>(AMP)(CEX)</td><td></td></th<>		- ST387 HB2017111	F	2017	**	18.0±0.8		2	(AMP)(CEX)	
-5780 HE201703 F 2017 + 0.72.0.4 4 (AMP(CSX)(NAL)(TELDOX) -5780 HE201708 F 2017 + 1.12.97 2 (CSX)(SKT) -5787 HE201702 D 2017 * 1.12.97 2 (CSX)(SKT) -5787 HE201702 D 2017 * 1.12.107 4 (AMP(CSX)(NAL)(TELDOX) -5787 HE201706 F 2017 * 2.042.4 1 (CSX)(NAL) -5788 HE201706 F 2017 * 1.86.03 4 (CSX)(NAL)(TELDOX) -5788 HE201706 F 2017 * 1.86.03 4 (CSX)(NAL)(TELDOX) -5788 HE201706 F 2017 * 1.86.03 4 (CSX)(NAL)(TELDOX) -5780 HE201707 F 2017 * 1.86.01 1 (CMP(CSX)(NAL)(STR)(TELDOX)(CAL)(SLLSXT) -5787 HE201708 F 2017 * 1.86.01 1		1 F ST345 HB2017062	F	2017		14.8±1.9		2	(AMP)(CEX)	
Image: ST389 H201708 F 2017 # 11.759.7 2000000000000000000000000000000000000		L ST380 HB2017103	F	2017		07+04		4	(AMP)(CEX)(NAL)(TET DOX)	
ST378 HE2017101 F 2017 *** 21.15.1 2 (GFX)(TET) ST378 HE201702 F 2017 ** 21.15.1 2 (GFX)(TET) ST378 HE201702 F 2017 * 20.42.4 1 (GFX) ST378 HE201706 F 2017 * 20.42.4 1 (GFX) ST378 HE201706 F 2017 * 15.84.0.3 4 (GFX)(NL1) ST388 HE2017076 D 2017 * 15.84.0.3 4 (GFX)(NL1) ST388 HE2017076 D 2017 * 15.82.0 3 (AMP(GFX)(NL1) ST388 HE2017076 F 2017 * 15.84.0 4 (GFX)(FX)(NL1) ST388 HE201708 F 2017 * 16.84.0 1 (GFX)(CFX)(NL1) ST388 HE201708 F 2017 * 3.54.0 aac(0'-b-c, qm22 3 (AMP(GFX)(NL1)(STF)(TELDX)(OL1)(SLL,ST) ST378 HE201708 F 2017 * 3.54.0 a	5	ST369 HB2017089	F	2017	+/-	11.7±9.7		2	(CFX)(SXT)	
ST356 H2217074 D 2017 *** 14.9±7.4 2 (AMP)(CFX)(NL)(STR) ST356 H2201709 F 2017 * 11.7±10.7 4 (AMP)(CFX)(NL)(STR) ST356 H2201703 F 2016 * 5.3±2 2 (CFX)(NL)(STR) ST356 H22017076 D 2017 * 16.8±0.3 4 (CFX)(NL)(TET,DOX) ST356 H22017076 D 2017 * 16.8±1.1 7 (AMP)(CFX)(NL)(ARANSTR)(TET,DOX)(CHL)(SLL,SXT) ST356 H22017076 E 2017 * 16.8±1.1 7 (AMP)(CFX)(NL)(KANSTR)(TET,DOX)(CHL)(SLL,SXT) ST356 H22017077 F 2017 * 16.8±1.1 7 (AMP)(CFX)(NL)(STR)(TET,DOX)(CHL)(SLL,SXT) ST356 H2201707 F 2017 * 16.8±1.6 1 (AMP)(CFX)(NL) ST356 H2201707 F 2017 * 15.8±0.8 ac(GF)-D-cr, qm22 3 (AMP)(CTX)(CAL,FEP,OCK,TO)(AZM,(NL)(STR)(TET,DOX)(SLL,SXT) ST376 H2201708 F 2017 * 17.9±0.3 (AMP)(CTX)(CAL,FEP,OCK,TO	1	ST378 HB2017101	F	2017		21.1±5.1		2	(CFX)(TET)	
ST372 H2017002 F 2017 11.7±10.7 4 (AMP)(GSX)(N4L)(STR) ST372 H2017002 F 2017 2.04±2.4 1 (CP) ST386 H2017103 F 2017 * 18.4±0.3 4 (CP)(N4L)(STR) ST386 H2017076 D 2017 * 18.4±0.3 4 (CP)(N4L)(TET,DOX) (ST) ST386 H2017076 D 2017 * 18.5±0.3 4 (AMP)(CF)(N4L)(TET,DOX) (ST) ST386 H2017076 E 2017 * 18.5±0.3 4 (AMP)(CF)(N4L)(TET,DOX) (ST) ST386 H2017076 F 2017 * 18.5±0.3 1 (C4L) ST386 H2017083 F 2017 * 18.5±0.3 1 (C4L) ST376 H201708 F 2017 * 18.5±0.3 4 (AMP)(CFX)(NLL)(STR)(TET,DOX)(C4L)(SL,SXT) ST376 H201708 F 2017 * 19.5±0.4 1 (AMP)(C7X)(NLL)(STR)(TET,DOX)(C4L)(SL,SXT) ST376 H201708 F 2017 * 17.2±0.3		ST356 HB2017074	D	2017	***	14 9+7 4		2	(AMP)(CEX)	
ST389 H2017109 F 2017 * 20424 1 (GFX) ST389 H2017109 F 2016 * 5.33.2 2 (GFX)(HAL)(TETLOX) (SXT) ST389 H2017107 D 2017 * 28.94.16 4 (AMP)(GFX)(HAL)(TETLOX) (SXT) ST389 H2017107 F 2017 * 15.54.20 3 (AMP)(GFX)(HAL)(TETLOX) (SXT) ST389 H2017107 F 2017 * 15.54.20 3 (AMP)(GFX)(HAL)(TETLOX) (SLL)SXT) ST389 H2017107 F 2017 * 16.54.1 7 (AMP)(GFX)(HAL)(KAL)(STR)(TETLOX)(C4L)(SLL,SXT) ST380 H2017068 F 2017 * 3.54.0 aac(67.4b.cr; qm/82 8 (AMP)(GCX)(HAL)(STR)(TETLOX)(C4L)(SLL,SXT) ST387 H201702 F 2017 * 3.54.0 aac(67.4b.cr; qm/82 8 (AMP)(GCX) ST387 H201702 F 2017 * 3.54.0 aac(67.4b.cr; qm/82 8 (AMP)(GCX) ST387 H201700 F 2017 * 1.7.9.8 (GCX) (AMP)(GCX) ST387 H201700 F 2017 *		ST372 HB2017092	F	2017		11 7+10 7		4	(AMP)(CEX)(NAL)(STR)	
Bit Stape Heading033 F 2016 ** 5.33.2 (CPS)(N4L) ST32e Heading033 F 2017 ** 18.64.0.3 4 (CPS)(N4L)(TELDOX) (SKT) ST32e HEADINTOR D 2017 ** 18.64.0.3 4 (CPS)(N4L)(TELDOX) (SKT) ST32e HEADINTOR F 2017 * 15.54.2.0 3 (AMP)(CPS)(N4L)(TELDOX)(C4L)(SULSXT) ST32e HEADINTOR F 2017 * 16.54.1 7 (AMP)(CPS)(N4L)(KAN STR)(TELDOX)(C4L)(SULSXT) ST32e HEADINOR F 2017 * 3.54.0 aac(6)-Ib-cr, gmB2 8 (AMP)(C7X)(CAL)(SAL)(STR)(TELDOX)(C4L)(SULSXT) ST32e HEADINOR F 2017 * 3.54.0 aac(6)-Ib-cr, gmB2 8 (AMP)(C7X)(CAL)(STR)(TELDOX)(C4L)(SULSXT) ST32e HEADINOR F 2017 * 17.90.8 2 (AMP)(C7X)(CAL)(STR)(TELDOX)(C4L)(SULSXT) ST374 HEADINOR F 2017 * 17.90.8 2 (AMP)(C7X)(CAL)(STR)(C4C)(STR)(C4L)(SULSXT) ST374 HEADINOR F 2017 *		C ST386 HB2017109	F	2017		20 4+2 4		1	(CEX)	
USDA RED/TORG F 2013 CONTROL ST38 HE201700 F 2017 ** 18.8.0.3 (CPS)(N4L)(TET,DOX) (SXT) ST38 HE201707 D 2017 ** 18.6.0.3 (AMP/CFX)(N4L)(TET,DOX) ST38 HE201707 F 2017 * 18.5.1.1 7 (AMP/CFX)(N4L)(TET,DOX)(C4L)(SLL,SXT) ST38 HE2017107 F 2017 * 18.5.1.1 7 (AMP/CFX)(N4L)(N4L)(STR)(TET,DOX)(C4L)(SLL,SXT) ST38 HE2017108 F 2017 * 3.5.4.0 asc(6)-1.0-cr, qmB2 8 (AMP/CAX)(N4L)(STR)(TET,DOX)(C4L)(SLL,SXT) ST37 HE2017087 F 2017 * 3.5.4.0 asc(6)-1.0-cr, qmB2 8 (AMP/CAX) ST37 HE2017087 F 2017 * 3.7.4.8 bia CTK-M3 6 (AMP/CAX) ST37 HE2017086 F 2017 * 1.7.4.8 bia CTK-M3 6 (AMP/CAX) ST37 HE2017086 F 2017 * 1.7.4.8 bia CTK-M3 6 (AMP/CAX) ST37 HE2017006 <td>60</td> <td>- ST296 HR2016033</td> <td>F</td> <td>2016</td> <td></td> <td>53+32</td> <td></td> <td>2</td> <td>(CEX)(NA1)</td> <td></td>	60	- ST296 HR2016033	F	2016		53+32		2	(CEX)(NA1)	
ST38 HE2017078 D 2017 * 28.91.6 4 (AMP)(CF)(N4L)(TETLOOX) ST38 HE2017078 E 2017 * 15.52.0 3 (AMP)(CF)(N4L)(TETLOOX) ST38 HE2017078 E 2017 * 15.52.0 3 (AMP)(CF)(N4L) ST38 HE2017107 F 2017 * 16.52.1 7 (AMP)(CF)(N4L)(TETLOOX) ST38 HE2017107 F 2017 * 16.52.1 7 (AMP)(CF)(N4L)(STR)(TETLOOX)(C4L)(SLL,SXT) ST37 HE2017102 F 2017 * 25.04.2 3 (AMP)(CAZ) ST37 HE2017008 F 2017 * 17.9.0.8 2 (AMP)(CAZ) ST37 HE2017008 F 2017 * 17.9.0.8 2 (AMP)(CAZ) ST37 HE2017008 F 2017 * 17.9.1.8 2 (CF)(STR) ST37 HE2017008 F 2017 * 17.9.1.2 (CF)(STR) </td <td></td> <td>ST383 HR2017106</td> <td>F</td> <td>2017</td> <td></td> <td>18.6+0.3</td> <td></td> <td>4</td> <td>(CEX)(NAL)(TET DOX) (SXT)</td> <td></td>		ST383 HR2017106	F	2017		18.6+0.3		4	(CEX)(NAL)(TET DOX) (SXT)	
- 5730 H2017070 E 2017 * 1522.0 3(AMP)(CP)(N4L) - 5730 H2017070 F 2017 * 1522.0 3(AMP)(CP)(N4L) - 5730 H2017070 F 2017 * 1552.1 3(AMP)(CP)(N4L)(SALSTR)(TET)(CPL)(S4L)SLST) - 5737 H2017027 F 2017 * 10.8±0.6 1 (C4L) - 5737 H2017027 F 2017 * 3.5±4.0 aec(9)-Ib-cr. gm/22 3(AMP)(CRX,TO)(AZM)(NAL)(STR)(TET,DOX)(C4L)(SL,SXT) - 5737 H2017026 F 2011 - 0.1±0.3 3(AMP)(CAZ)(TCX,CPX,TO)(AZM)(STR)(TET,DOX)(C4L)(SL,SXT) - 5737 H2017026 F 2017 * 17.9±2.2 2(GP)(STR) - 5737 H2017006 F 2017 * 17.9±2.2 2(GP)(N4L)(TET,DCX)(SUL)	- 1	- ST359 HB2017076	D	2017		28.9+1.6		4	(AMP/(CEX)(NAL)(TET DOX)	
Construction E District Source Sou		ST360 UP2017070	5	2017		15 5+2 0		2	(AMD)(CEV)(NAL)	
Bit State Local J The Model J Construction 1 State Action J 1 1 1 Action J 1 State Action J 1 1 Action J Action J 1 State Action J 1 1 (CHJ) Action J 1 State Action J 3 5:4:0 ase(6)-lb-cr.qm/B2 8 (AMP)(CFX)(NAL)(STR)(TELDOX)(CHL)(SLL,SXT) 1 State Action J 1 1:0:4:0:3 3 (AMP)(CFX)(NAL) 5 State Action J 1:0:4:0:3 3 (AMP)(CFX)(NAL) State State 5 State Action J 1:0:4:0:3 3 (AMP)(CFX)(CAZ) State State State State Action J State State State		- CT391 LID2017104	5	2017	*/	14.0±1.7		3		
1001 F 2017 * 10281 (AMP)(CAT)(CAT, TO(A, ZM)(NAL)(STR)(TET, DAX)(CAL)(SL, SAT) 5738 H 20017087 F 2017 * 1028.0 1 (CH-1) 5738 H 20017087 F 2017 * 25.04.2 3 (AMP)(CAT, ZM)(NAL)(STR)(TET, DAX)(CH_1)(SL, SAT) 5737 H 20017087 F 2017 * 10.40.3 3 (AMP)(CAT, ZM)(NAL)(STR)(TET, DAX)(CH_1)(SL, SAT) 5737 H 2001708 F 2017 * 17.74.0.8 2 (AMP)(CAZ) 5737 H 2001709 F 2017 * 17.74.8 0.a CT/AM3 6 (AMP)(CAZ) 5737 H 2001700 F 2017 * 17.48 0.a CT/AM3 6 (AMP)(CAZ) 5737 H 2001700 F 2017 * 17.20.1 5 (AMP)(CAZ)(SUL) (AMP)(CAZ)(SUL) 5737 H 2001700 F 2017 * 4.54.1 (AMP)(CAZ)(CAZ, TP)(AZM)(NAL, CLP, LEV)(GBASTR)(TET, DOX)(SUL, SXT)(AMZ) 5737 H 201700 F 2017 * 4.54.1 (AMP)(CAZ, CAZ, TP)(AZM)(NAL, CLP, LEV)(GBASTR)(TET, DOX)(SUL, SXT)(AMZ) 5737 H 2017070 D 2017 <td>1</td> <td>67304 HD2017104</td> <td>-</td> <td>2017</td> <td>.,-</td> <td>10 5 1 1 1</td> <td></td> <td>7</td> <td></td> <td></td>	1	67304 HD2017104	-	2017	.,-	10 5 1 1 1		7		
Istate H2017087 F 2017 - 10280.0 F ST37 HE2017102 F 2017 * 25.04.2 3 (AMP)(CTX,CPX,TD)(A2M)(NAL)(STR)(TET,DDX)(O4.)(SUL,SXT) ST37 HE2017102 F 2017 * 25.04.2 3 (AMP)(CTX,CPX,TD)(A2M)(NAL)(STR)(TET,DDX)(O4.)(SUL,SXT) ST37 HE2017102 F 2017 * 11.74.8 bit CTX-H3 (AMP)(CTX,CPX,TD)(A2M)(STR)(TET,DDX)(SUL,SXT) ST37 HE201706 F 2017 * 11.74.8 bit CTX-H3 (AMP)(CTX,CPX,TD)(A2M)(STR)(TET,DDX)(SUL,SXT) ST37 HE201706 F 2017 * 11.74.1 5 (AMP)(CTX,CPX,TD)(A2M)(STR)(TET,DDX)(SUL,SXT) ST37 HE201706 F 2009 * 1.74.1 5 (AMP)(CTX,CPX,TD)(A2M)(STR)(TET,DDX)(SUL,SXT) ST37 HE201706 F 2009 * 1.74.1 5 (AMP)(CTX,CPX,TD)(A2M)(NAL,CLPLEV)(GEM,STR)(TET,DDX)(SUL,SXT)(AMZ) ST38 HE201708 F 2017 * 4.52.1 8 (AMP)(CTX,CPX,TD)(A2M)(NAL,CLPLEV)(GEM,STR)(TET,DDX)(SUL,SXT)(AMZ) ST37 HE2017070B F 2017		* S1384 HB2017107	-	2017		18.5±1.1			(AMP)(CFX)(NAL)(KAN,STR)(TET,DOX)(CHL)(SUL,SXT)	
Star H221/106/ F 2017 3358.0 aac(f)-bcr, qrd2 6 (AMP)(CA2, ID)(A2M)(NL, (CA2)(SL, SXT)) = 5137 H2201702 F 2017 - 0.1e 0.3 3 (AMP)(CR)(NL) = 5137 H2201702 F 2017 * 17.9.0 2 (AMP)(CR)(NL) = 5137 H2201702 F 2017 * 17.9.4.8 bia CTX-M3 6 (AMP)(CR)(NL) = 5137 H2201700 F 2017 * 17.9.4.8 bia CTX-M3 6 (AMP)(CR)(NL) = 5137 H2201700 F 2017 * 17.9.4.8 bia CTX-M3 6 (AMP)(CR)(TR)(TL)(AUX)(STR)(TE), DOX)(SUL,SXT) = 5137 H2201700 F 2008 + 17.0.1 5 (AMP)(CR)(TR)(CR)(SUL) 5 = 5137 H22017070 D 2017 + 9.55.0.4 (CFX)(NL)(TE)(CAL)(SUL)(SUL) 5 = 5137 H22017070 D 2017 + 9.55.0.4 (CFX)(NL)(TE)(CAL)(SUL)(SUL) 5 = 5137 H22017061 F 2017 * 2.52.4.0		S1364 HB201/083	-	2017		10.8±0.6		1	(CHL)	
Stray Prezint 1/102 F 2017 - 2 Stray Prezint 1/102 F 2017 - 0 16 0.3 (AMP)(CA2) STR2 A PRO11/002 F 2017 * 117.40.8 2 (AMP)(CA2) STR2 HE2017/08 F 2017 * 117.40.8 2 (AMP)(CA2) STR3 HE2017/09 F 2017 * 117.40.8 2 (AMP)(CA2) STR3 HE2017/09 F 2017 * 17.94.22 2 (CPA)(STR) STR3 HE2017/00 F 2017 * 17.94.22 2 (CPA)(STR) STR3 HE2017/00 F 2017 * 1.94.23 2 (AMP)(CA2)(TELDOX)(SUL) STR3 HE201700 F 2017 * 4.54.17 8 (AMP)(CA2/M)(NAL, CLPLEV)(GBMSTR)(TET, DOX)(SUL, SXT)(AMZ) STR3 HE2017070 D 2017 * 5.50.4 2 (CPX)(NL1) STR3 HE2017075 F 2017 * 2.66.40 2 (CPX)(TELDOX) STR3 HE2017075 F		CT070 HB201/08/	-	2017		3.314.0	aac(6')-lb-cr, qnrB2	•	(AMP)(C1X,CFX,1IO)(AZM)(INAE)(STR)(TET,DOX)(CHE)(SOE,SXT)	
ST82 FR2017021 F 2011 0.1E 0.3 3 (AMP/CX) ST82 FR2017061 E 2017 * 17.90.8 2 (AMP/CX) ST82 FR2017066 F 2017 * 17.91.8 2 (CPX)(CTX, CA2_FEP,CPX, TD)(AZM)(STR)(TET, DOX)(SUL, SXT) ST87 HE2017066 F 2017 * 17.91.2 2 (CPX)(STR) ST87 HE2017066 F 2009 * 1.74.1 5 (AMP/CX)(TET, DOX)(SUL) ST85 HA2008014 F 2008 * 1.104.6 2 (AMP/CTX, CA2_TET, DOX)(SUL) ST83 HE2017070 D 2017 * 9.550.4 2 (CPX)(TET, DOX)(SUL) ST83 HE2017070 D 2017 * 9.550.4 2 (CPX)(TET, DOX) SUL, SXT) ST83 HE2017074 F 252.40 1 (CPX)(TET, DOX) ST83 SUL 2017075 F 2017 * 252.40 1 (CPX)(- 1	5- S13/9 HB201/102	-	2017	-	25.0±4.2		3	(AMP)(CFX)(NAL)	
 ST32 HE201708 F 2017 T T		S182 AH2011002	-	2011	_	0.1±0.3		3	(AMP)(CAZ)	
ST37 H B201706 F 2017 T 31.24.8 0 is CI-M43 6 CMM/CIA22-PE/CX, ID/JA2/M(SHQ,IE), LDX/SULS,XI) ST37 H B201706 F 2017 T 17.92.2 2 (CPX)(STR) 1001 ST55 AH2008014 F 2008 - 4.44.1.8 1 (AMP)(CA2)(TELDX)(SUL) 1575 AH2008015 H 2008 + 1.160.4.6 2 (AMP)(CA2)(TELDX)(SUL) 1575 AH200700 F 2017 + 4.54.1.7 8 (AMP)(CA2/MCRXTO)(AZM)(NAL, CLPLEV)(GBM,STR)(TET,DX)(SUL,SXT)(AMZ) 5735 AH2017070 D 2017 + 5520.4 2 (CPX)(NAL) 5737 HB2017075 HB2017070 D 2017 * 26.64.0 2 (CPX)(TELDX) 5737 HB2017075 HB2017075 HB2017075 HB2017075 HB2017077 2017 * 26.64.0 2 (CPX)(TELDX) 5733 HB2017082 F 2017 * 26.64.0 2 (CPX)(TELDX) 5733 HB2017075 HB2017071 h 2017 * 26.64.0 2 (CPX)(TELDX)		S1362 HB201/081	E	2017		17.7±0.8		2	(AMP)(CFX)	
FIRST HEQ17100 F 2017 * 1.19222 2 (L5X)(11) Tog TST H2000014 F 2009 - 1.44:1.8 1 (AMP) Tog TST AH2000012 F 2009 - 1.72:0.1 5 (AMP)(CTX)(TELDOX)(SUL) TSTS AH2000012 F 2009 + 1.10:44.6 2 (AMP)(CTX,CPX,TO)(A2M)(NAL,C2,PLEV)(SBM.STR)(TET,DOX)(SUL,SXT)(AMZ) TSTS AH20017070 D 2017 + 9.56:04 2 (CPX)(NAL) TSTS AH2017070 D 2017 * 9.56:04 2 (CPX)(NAL) TSTS HE2017070 D 2017 * 9.56:04 2 (CPX)(NAL) ST33 HE2017070 D 2017 * 2.52:4:0 1 (CPX)(TELDOX) ST33 HE2017082 F 2017 * 3.4:0:2 (CPX)(TELDOX) 5 ST33 HE2017082 F 2017 * 3.9:2:9 <i>bia</i> TEM+1, aac(G):4:-cr 9 (AMP)(CTX,CCX,TD)(AZM)(NAL,CLP,LEV)(GBM,STR)(TET)(CH_L)(SUL,SXT)(AMZ) ST33 HE201	- 1		F	2017		31.7±4.8	bla CTX-M-3	6	(AMP)(CIX,CAZ,FEP,CFX, IIO)(AZM)(STR)(TET,DOX)(SUL,SXT)	
100_TST3 A42008014 F 2008 4.4 ± 1.8 1 (AMP) 100_TST3 A42008014 F 2008 4.4 ± 1.8 1 (AMP)(CA2)(TET,DOX)(SUL) 100_TST3 A42008015 H 2008 +/- 11.0±4.6 2 (AMP)(CA2)(TET,DOX)(SUL) 100_TST3 A42008015 H 2008 +/- 11.0±4.6 2 (AMP)(CA2)(TET,DOX)(SUL) 100_TST3 HE2017090 F 2017 +/- 4.5±1.7 8 (AMP)(CA2,T)(TET,DOX)(SUL) 100_TST3 HE2017080 F 2016 +/- 3.4±0.7 qmrS1 5 (CFX)(NAL)(TET)(CAL)(SUL,SXT) 110_TST3 HE2017081 F 2017 * 2.0±4.0 1 (CFX) 110_TST3 HE2017083 F 2017 * 0.6±2.0 2 (CFX)(TET,DOX) 110_TST3 HE2017083 F 2017 * 1.0±7.5 2 (AMP)(CTX,CFX,TD)(A2M)(NAL,CLP,LEV)(GBM,STR)(TET)(CHL)(SUL,SXT)(AMZ) 111_TST3 HE2017081 F 2017 * 1.0±7.5 2 (AMP)(CTX,CFX,TD)(A2M)(NAL,CLP,L	- 1	ST377 HB2017100	F	2017	•	17.9±2.2		2	(GFX)(STR)	
IDD ST55 AF200902 F 2009 * 1.740.1 5 (AMP/CSX)(FELDXX(SU.) ST55 AF2009015 H 2008 * 1.740.1 5 (AMP/CSX)(FELDXX(SU.) ST55 AF2009015 H 2008 * 1.740.1 5 (AMP/CSX)(FELDXX(SU.) ST351 H2017070 F 2017 * 4.5±1.7 8 (AMP/CSX)(AL2M)(NAL.CLPLEV)(GBM.STR/TET.DDX)(SUL,SXT)(AMZ) ST351 H2017070 F 2017 * 25.64.0 2 (CPX)(NAL)(TET/COX) ST351 H2017075 H2017075 H2017075 2017 * 25.24.0 1 (CPX) ST353 H2017083 F 2017 * 25.24.0 1 (CPX) ST353 H2017085 F 2017 * 13.14.19 1 (STR) ST353 H2017084 F 2017 * 13.92.29 blaTEM-1, aac(6)-do-cr 9 (AMP/CTX.CPX.TD)(A2M/(NAL.Q.PLEV/(GBM.STR/(TET)(CH_L)(SUL,SXT)(AMZ)) ST371 H2017091 F 2017 * 47.26.1 3 (CPX)(NAL)(TET.DOX)		100 ST51 AH2008014	F	2008	_	4.4± 1.8		1	(AMP)	
L STS2 AF2003015 FH 2008 +/- 11.04.6 2 (AMP)(CTX,CTX,TO)(AZM)(NAL,CLPLEY)(GBM,STR)(TET,DOX)(SULS,XT)(AMZ) - ST35 HE2017070 D 2017 + 9.5±0.4 2 (GPX)(NAL)(TET)(CAL)(SULS,XT)(AMZ) - ST35 HE2017070 D 2017 + 9.5±0.4 2 (GPX)(NAL)(TET)(CAL)(SULS,XT) - ST35 HE2017070 D 2016 +/- 3.4±0.7 qmrS1 5 (GPX)(NAL)(TET)(CAL)(SULS,XT) - ST33 HE2017070 D 2017 * 25.2±4.0 1 (GPX)(TET)COX) - ST33 HE2017078 F 2017 * 3.5±2.0 2 (GPX)(TET)COX) - ST33 HE2017062 F 2017 * 1.5±2.0 2 (GPX)(TET)COX) - ST33 HE2017062 F 2017 * 1.5±5.5 2 (AMP)(CTX,CTX,TO)(AZM)(NAL,CLPLEY)(GBM,STR)(TET)(CH_1(SULS,ST)(AMZ) - ST33 HE2017061 F 2017 * 1.5±5.5 2 (AMP)(CTX,CTX,RO)(AL,QTET,DOX) - ST33 HE2017061 F 2017 * 4.7±0.01 0<	- [100 ST55 AH2009002	F	2009		1.7±0.1		5	(AMP)(CAZ)(TET,DOX)(SUL)	
Image: Stratube 2017080 F 2017 +/- 4.5±1.7 8 (AMP/GTXCRXT00/AZM)(NAL_C2PLEV)(GBM.STR)(TET,D0X)(SUL,SXT)(AMZ) Image: Stratube 10000 F 2017 +/- 4.5±1.7 8 (AMP/GTXCRXT00/AZM)(NAL_C2PLEV)(GBM.STR)(TET,D0X)(SUL,SXT)(AMZ) Image: Stratube 10000 F 2017 + 9.5±0.4 2 (CFX)(NAL)(TET)(CAL)(SUL,SXT) Image: Stratube 10000 F 2017 * 20.6±0.0 2 (CFX)(NAL)(TET)(CAL)(SUL,SXT) Image: Stratube 100000 F 2017 * 20.6±0.0 2 (CFX)(NAL)(TET)(CAL)(SUL,SXT) Image: Stratube 100000 F 2017 * 25.2±4.0 1 (CFX) Image: Stratube 100000 F 2017 * 13.1±1.9 1 (STR) Image: Stratube 1001704 F 2017 * 13.9±2.9 biaTEM-1, aac(6)-d-or 9 (AMP)(CTX,CTX:T0)(A2M)(NAL:CLPLEV)(GBM.STR)(TET)(CHL)(SUL,SXT)(AMZ) Image: Stratube 1000000 F 2017 * 4.7±0.1 3 (CFX)(MAL:TET,DOX) Image: Stratube 1000000 <		L ST52 AH2008015	н	2008	+/-	11.0±4.6		2	(AMP)(SXT)	
Image: Strass He2017070 D 2017 * 9.550.4 2 (CFX)(N4L) Tg ST30 He2017075 F 2016 + 3.440.7 qmrS1 5 (CFX)(N4L) (CFX)(N4L) (TFX) 1		C ST370 HB2017090	F	2017	+/-	4.5±1.7		8	(AMP)(CTX,CFX,TIO)(AZM))(NAL,CLP,LEV)(GEM,STR)(TET,DOX)(SUL,SXT)(AMZ)	
τp ST20 H82010015 F 2016 +/- 3.46.7 qnrS1 5 (CFX)(H1L)(FIL)(SULSXT) ST334 H820170704 F 2017 * 20.54.0 1 (CFX)(H1L)(SULSXT) ST334 H82017075 F 2017 * 25.24.0 1 (CFX)(H1L)(SULSXT) ST335 H82017075 F 2017 * 5.52.40 1 (CFX)(H1L)(SULSXT) ST335 H82017082 F 2017 * 13.1±1.9 1 (STR) ST335 H82017062 F 2017 * 13.9±2.9 <i>JiaTEM</i> +1, <i>aac(6)-dx-cr</i> 9 (AMP)(CTX,CFX,TD)(AZM)(NALCZ,PLEV)(GBM,STR)(TET)(CHL)(SULSXT)(AMZ) ST335 H82017061 F 2017 * 13.9±2.9 <i>JiaTEM</i> +1, <i>aac(6)-dx-cr</i> 9 (AMP)(CTX,CFX,TD)(AZM)(NALCZ,PLEV)(GBM,STR)(TET)(CHL)(SULSXT)(AMZ) ST335 H82017091 F 2017 * 20.45.5 2 (AMP)(CTX,CFX,RD)(NAL)(TET,DOX) ST371 H82017091 F 2017 * 6.22.5 <i>JiaCTX-MA9, qnrB2</i> 6 (AMP)(CTX,CFX,RD)(NAL)(TET,DOX)(CHL)(SULSXT) 5 <td>d</td> <td>ST353 HB2017070</td> <td>D</td> <td>2017</td> <td>·</td> <td>9.5±0.4</td> <td></td> <td>2</td> <td>(CFX)(NAL)</td> <td></td>	d	ST353 HB2017070	D	2017	·	9.5±0.4		2	(CFX)(NAL)	
■ 5733 H8201705 H82017075 H82017083 F 2017 ** 25.24.0 (CFX) ● 5737 H82017093 F 2017 ** 15.24.0 (CFX)(TELDOX) ● 57373 H82017093 F 2017 ** 15.24.0 (CFX) ● 5738 H82017093 F 2017 ** 13.14.9 (STR) ● 5737 H82017094 F 2017 ** 13.94.2 blaTEM-1, aac(6)-t0-cr 9 (AMP)(CTXCRXTD)(AZM)(NALCLPLEV)(GBM.STR)(TET)(CHL)(SULSXT)(AM2) ● 5737 H82017094 F 2017 * 20.45.5 2 (AMP)(CTXCRXTD)(AZM)(NALCLPLEV)(GBM.STR)(TET)(CHL)(SULSXT)(AM2) ● 57375 H82017095 F 2017 * 6.242.5 bla CTX-M9, qr/B2 6 (AMP)(CTXCRXCRD)(NAL)(TELDOX) ● 57375 H82017086 F 2017 * 6.242.5 bla CTX-M9, qr/B2 6 (AMP)(CTXCRXCRD)(NAL)(TELDOX)(CHL)(SULSXT) ● 57375 H82017086 F 2017 * 6.242.5 bla CTX-M9, qr/B2 6 (AMP)(CTXCRXCRD)(NAL)(TELDOX)(CH		79 ST280 HB2016015	F	2016	+/-	3.4±0.7	qnrS1	5	(CFX)(NAL)(TET)(CHL)(SUL,SXT)	
Image: Start HE2017075 P2017 ** 252:24.0 1 (GFX) > 5737 HE2017076 2017 ** 252:24.0 1 (GFX) > 5737 HE2017076 F 2017 ** 13:11.9 1 (STR) > 5736 HE2017062 F 2017 ** 13:92.2 0 fat EML 1, aac(6):40-cr 9 (AMP)(CTX,CPX,T0)(AZM(NAL,C2,PLEV)(GBM,STR)(TET)(CH_J(SUL,SXT)(AMZ)) > 5733 HE2017062 F 2017 * 13:92.2 0 fat EML 1, aac(6):40-cr 9 (AMP)(CTX,CPX,T0)(AZM(NAL,C2,PLEV)(GBM,STR)(TET)(CH_J(SUL,SXT)(AMZ)) > 5733 HE2017064 F 2017 * 24:25.5 2 (AMP)(CTX,CPX,R0)(NAL,(TET,DOX) > 5737 HE2017056 F 2017 * 6:24:5.5 1 (CFX) > 05 1225 HE2017068 F 2017 * 6:24:5.5 1 (CFX) > 1737 HE2017088 F 2017 * 14:24:5.5 1 (CFX) > 1737 HE2017088 F 2017 * 12:24:5.9 2 (AMP)(CTX,CFX,R0)(NAL,(TET,DOX)(CH_J(SUL,S		ST334 HB2017048	F	2017	•	20.6±4.0		2	(CFX)(TET,DOX)	
B ST33 H82017093 F 2017 +/- 6.5±2.0 2 (CRX)(TELDXX) ST383 H82017082 F 2017 *' 13.1±1.9 1 (STR) ST383 H82017082 F 2017 *' 13.1±1.9 1 (STR) ST383 H82017062 F 2017 *' 13.9±2.9 <i>biaTEM</i> .1, <i>aac(6).4b-cr</i> 9 (AMP)(CTX,CFX,TD)(AZM)(NAL,CLPLEV)(GBM,STR)(TET)(CH_J(SUL,SXT)(AMZ)) ST330 H82017091 F 2017 *' 20.455.5 2 (AMP)(CTX,CFX,TD)(AZM)(NAL,CLPLEV)(GBM,STR)(TET)(CH_J(SUL,SXT)(AMZ)) ST371 H82017091 F 2017 *' 4.7±0.1 3 (CFX)(NAL)(TET,DOX) ST375 H82017095 F 2017 *' 6.2±2.5 <i>biaCTX.4b.9, qn/B2</i> 6 (AMP)(CTX,CFX,RO)(NAL)(TET,DOX)(CH_J(SUL,SXT) ST375 H82017086 F 2017 *' 14.2±6.5 1 (CFX) ST375 H82017087 F 2017 *' 14.2±6.5 1 (CFX) ST375 H82017087 F 2017 *' 14.2±6.5		ST357 HB2017075 HB2017077	7 D	2017	**	25.2±4.0		1	(CFX)	
ST383 H82017082 F 2017 ** 13.11.9 1 (STR) ST382 H82017065 F 2017 ** 13.11.9 (AMP)(CTX,CFX,TO)(AZM)(NAL,Q.P.LEV)(GBM,STR)(TET)(CHL)(SUL,SXT)(AMZ) ST383 H82017044 F 2017 * 20.455.5 2 (AMP)(CTX,CFX,TO)(AZM)(NAL,Q.P.LEV)(GBM,STR)(TET)(CHL)(SUL,SXT)(AMZ) ST371 H82017044 F 2017 - 4.720.1 3 (CFX)(NAL)(TET,DOX) ST371 H82017095 F 2017 - 6.222.5 bla CTX.4H.9, qmB2 6 (AMP)(CTX,CFX,RO)(NAL)(TET,DOX)(CHL)(SUL,SXT) OTST25 H82017095 F 2017 * 6.222.5 bla CTX.4H.9, qmB2 6 (AMP)(CTX,CFX,RO)(NAL)(TET,DOX)(CHL)(SUL,SXT) OTST25 H82017095 F 2017 * 6.224.5 1 (CFX) ST375 H82017086 F 2017 * 14.246.5 1 (CFX) ST375 H82017086 F 2017 * 12.549.9 2 (AMP)(CTX,CFX,RO)(NAL	8	ST373 HB2017093	F	2017	+/-	6.5±2.0		2	(CFX)(TET,DOX)	
■ TSR2 H82017105 F 2017 ** 13.942.9 b/aTEM-f, aec(67)-45-cr 9 (AMP) (CFXCR>TO(AZM)(NAL_CLPLEV)(GBM.STR)(TET)(CH.)(SUL,SXT)(AM2) ST330 H82017044 F 2017 * 20.455.5 2 (AMP) (CFX) ST337 B82017051 F 2017 * 4.720.1 3 (CFX)(NAL)(TET,DCX) 00 ST326 H82017068 F 2017 * 6.242.5 b/aCTX-M9, qm/82 6 (AMP)(CTX,CFX,R0)(NAL)(TET,DCX) 00 ST326 H82017086 F 2017 * 6.242.5 b/aCTX-M9, qm/82 6 (AMP)(CTX,CFX,R0)(NAL)(TET,DCX)(CHL)(SUL,SXT) 01 ST376 H82017086 F 2017 ** 12.548.9 2 (AMP)(CFX) 01 ST375 H82017086 F 2017 ** 12.548.9 2 (AMP)(CFX) 01 ST375 H82017087 F 2017 ** 12.548.9 2 (AMP)(CFX) 01 ST375 H82017087 F 2017 ** 12.548.9 2 (AMP)(CFX) 02 </td <td></td> <td>ST363 HB2017082</td> <td>F</td> <td>2017</td> <td>**</td> <td>13.1±1.9</td> <td></td> <td>1</td> <td>(STR)</td> <td></td>		ST363 HB2017082	F	2017	**	13.1±1.9		1	(STR)	
Firsta H82017094 F 2017 ** 20.45.5 2 (AMP)(CFX) ST371 H82017091 F 2017 - 4.7±0.1 3 (CFX)(N4L)(TET,DOX) J_ST38 H82017091 F 2017 - 4.7±0.1 0 CFX 00 ST225 H82017096 F 2017 * 0.5±2.5 biaCTX-M9, gm/82 6 (AMP)(CTX,CFX,RO)(N4L)(TET,DOX)(CHL)(SUL,SXT) 100 ST225 H82017086 F 2017 */- 14.2±6.5 1 (CFX) 5 T375 H82017086 F 2017 */- 14.2±6.5 1 (CFX) 5 T375 H82017087 F 2017 */- 14.2±6.5 2 (AMP)(CTX,CFX,RO)(N4L)(TET,DOX)(CHL)(SUL,SXT) 5 T375 H82017087 F 2017 */- 14.2±6.5 0 5 T375 H82017087 F 2017 */- 14.2±6.1 5 (AMP)(CTX,CFX,STR)(CHL)(SUL,SXT) 5 T375 H82017087 F 2017 */- 4.2±4.1 5 (AMP)(CTX,CFX,STR)(CHL)(SUL,SXT) 5 T375 H82016032 E <td>d</td> <td>ST382 HB2017105</td> <td>F</td> <td>2017</td> <td>**</td> <td>13.9±2.9</td> <td>blaTEM-1, aac(6')-lb-cr</td> <td>9</td> <td>(AMP)(CTX,CFX,TIO)(AZM)(NAL,CLP,LEV)(GEM,STR)(TET)(CHL)(SUL,SXT)(AMZ)</td> <td></td>	d	ST382 HB2017105	F	2017	**	13.9±2.9	blaTEM-1, aac(6')-lb-cr	9	(AMP)(CTX,CFX,TIO)(AZM)(NAL,CLP,LEV)(GEM,STR)(TET)(CHL)(SUL,SXT)(AMZ)	
First He2017091 F 2017 - 4.720.1 3 (CFX)(NAL)(TET,DOX) 05 T252 He2017095 F 2009 * 0.550.1 0 05 T252 He2017095 F 2017 * 6.2±2.5 b/aCTX-MP4, gran2E 6 (AMP)(CTX,CFX,RD)(NAL)(TET,DOX)(CHL)(SUL,SXT) 05 T252 He2017086 F 2017 * 6.2±2.5 b/aCTX-MP4, gran2E 6 (AMP)(CTX,CFX,RD)(NAL)(TET,DOX)(CHL)(SUL,SXT) 05 T253 He2017086 F 2017 */ 14.2±6.5 1 (CFX) 15 T375 HE2017086 F 2017 */ 12.5±9 2 (AMP)(CTX,CFX,RD)(NAL)(TET,DOX)(CHL)(SUL,SXT) 15 T375 HE2017087 F 2017 *17.5±19 or 18.0±3.0 0 0 15 T375 HE2017097 F 2016 * 4.2±4.1 5 (AMP) (CTX,CFX,(STR)(CH_L)(SUL,SXT) 5 T375 HE2010037 HE201701 F 2017.216 *17.1±1.10111±2.2 aec(6)-10-Cr 6 (AMP) (CTX,CFX,(STR)(OR,L)(SUL,ST) (MMP)(CTX,CFX,(STR)(OR,L)(SUL	ľ	C ST330 HB2017044	F	2017	**	20.4±5.5		2	(AMP) (CFX)	
Image: ST358 AF2009006 F 2009 ** 0.5±0.1 0 00 ST326 H82017095 F 2017 ** 0.5±2.5 bia CTX-M49, qm/B2 6 (AMP)(CTX,CFX,RO)(NAL)(TET,DOX)(CH_J(SUL,SXT) - ST376 H82017088 F 2017 ** 1.2±8.5 1 (CFX) - ST375 H82017087 F 2017 ** 1.2±8.9 2 (AMP)(CFX) - ST375 H82017087 F 2017 ** 1.2±8.9 2 (AMP)(CFX) - ST375 H82017087 F2 2017 ** 1.2±8.9 2 (AMP)(CFX) - ST375 H82017087 F2 2017 ** 1.2±8.9 0 0 - ST375 H82017087 H82017110 F 2016 * 4.2±4.1 5 (AMP) (CTX,CFX)(STR)(CHL)(SUL,STT) - ST327 H82016035 H82017009 E 2017, 2016 +/17.111.107.111.152.2 a@c(6)-Ib-cr (ump) cTX,CFX,TON(unL,CUL9)(sIL,SUT) q/ump/cTX,CFX,TON(unL,CUL9)(sIL,SUT) q/ump/cTX,CFX,TON(unL,CUL9)(sIL,SUT) q/ump/cTX,CFX,TON(unL,CUL9)(sIL,SUT) <	1	- ST371 HB2017091	F	2017	-	4.7±0.1		3	(CFX)(NAL)(TET,DOX)	
D0 ST225 H82017095 F 2017 ** 6.222.5 blaCTX-M9, gr/82 6 (AMP/CTX,CPX,RO)(N4L)(TET,DOX)(CHL)(SUL,SXT) = ST376 H82017086 F 2017 +f- 14.246.5 1 (GFX) = ST375 H82017086 F 2017 *1 2.548.9 2 (AMP/CTX,CPX,RO)(N4L)(TET,DOX)(CHL)(SUL,SXT) 55 1375 H82017087 H82017110 F 2017 *17.541.9 or 18.0±3.0 0 51207 H82010632 E 2016 *14.2±4.1 5 (AMP)(CTX,CPX,RO)(N4L)(TET,DOX)(CHL)(SUL,SXT) 51207 H82010637 H8201070 E 2016 *14.2±4.1 5 (AMP)(CTX,CPX,STR)(CHL)(SUL,SXT) 51207 H82010637 H8201070 E 2016 *14.2±4.1 5 (AMP)(CTX,CPX,STR)(CHL)(SUL,SXT)		r ST58 AH2009006	F	2009	**	0.5±0.1		0		
ST376 HB2017098 F 2017 +/- 14 2±6.5 1 (CFX) ST375 HB2017087 F 2017 ** 12.5±8.9 2 (AMP)(CFX) ST375 HB2017087 HB2017087 HB2017087 F 2017 **17.5±1.9 or 18.0±3.0 0 ST375 HB2017087 HB2017087 HB2017087 E 2016 * 4.2±4.1 5 (AMP) (CTX,CFX)(STR)(CH.)(SUL,SXT) ST327 HB2016035 HB2017090 E 2017 +17.1±1.1 or 11.1±2.2 aac(6)-lb-cr 6 (MP) cTX,CFX)(STR)(CH.)(SUL,SXT) = // MPP)(CTX,CFX TO (MAL,CPLEV)(TET.DOX)(CH.)(SUL,SXT)	na	0 ST225 HB2017095	F	2017		6.2±2.5	bla CTX-M-9, qnrB2	6	(AMP)(CTX,CFX,RIO)(NAL)(TET,DOX)(CHL)(SUL,SXT)	
ST351 HB2017080 F 2017 ** 1.25±8.9 2 (АМР)(CFX) GT3753 HB2017087 H2017087 H2017101 F 2017 **1.75±1.9 or 18.0±3.0 0 GT375 HB2016032 E 2016 ** 4.2±4.1 5 (AMP) (CTX,CFX)(STR)(CHL)(SUL,SXT) ST327 HE0010053 H2017009 E 2016 *4.2±4.1 5 (AMP) (CTX,CFX)(STR)(CHL)(SUL,SXT)	ļ	- ST376 HB2017098	F	2017	+/-	14.2±6.5		1	(CFX)	
Il 51375 H82017087 H82017110 F 2017 **17.5±1.9 or 18.0±3.0 0 IS1375 H82017087 H82017087 E 2016 * 4.2±4.1 5 (AMP) (CTX.CFX)(STR)(CH_L)(SUL,SXT) S1287 H82010032 E 2017.2016 +17.1±1.1 or 11.1±2.2 aec(6)-10-cr 6 (имр. стх.гер.срх.торик.curlex/gettigeoorgieul.sxt) = // имл.ctx.rtx.p/mm.ctx.p/mm.ctx.rtx.p/mm.c	55	- ST351 HB2017068	F	2017		12.5±8.9		2	(AMP)(CEX)	
L ST295 HB2016032 E 2016 * 4.2±4.1 5 (AMP) (CTX,CFX)(STR)(CH.)(SUL,SXT) ST297 HB2016035 HB2017099 E 2017, 2016 +/-17.1±1.1 or 11.1±2.2 aac(6)-lb-cr 6 (MP) cTX,CFXT0(ML,CPLEY)(STR)(DA)(SUL,SXT) / (MP)(CTX,CFXT0(ML,CPLEY)(STR)(DA)(SUL,SXT) / (MP)(CTX,CFXT0(ML,CPLEY)(STR)(STR)(STR) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STR)(STR) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STR)(STR) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STR)(STX) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STR) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STR) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STR) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STR) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STX) / (MP)(STX,CFXT0(ML,CPLEY)(STR)(STX) / (MP)(STX,CFXT0(ML,CPLEX) / (MP)(STX,CFXT0(ML,CPLEX)(STX) / (MP)(ST	٦J,	- ST375 HB2017097 HB2017110	F	2017	** 17.5:	±1.9 or 18.0	±3.0	0		
L ST297 HB2016035 HB2017099 E 2017, 2016 +/-17.1±1.1 or 11.1±2.2 aac(6)-/b-cr 6 (MIP) CTXFEP.CFX.TO(NALCLPLEV/STR)[D0X/SULSXT) \(\alpha\) (MIP) (CTXFEX.TO(NALCLPLEV/STR)[D0X/SULSXT) \(\alpha\) (MIP) (TTXFEX.TO(NALCLPLEV/STR)[MIP) (MIP) (MIP) (TTXFEX.TO(NALCLPLEV/STR)[MIP) (MIP) (TTXFEX.TO(NALCLPLEV/STR)[MIP) (MIP) (MIP) (TTXFEX.TO(NALCL	JT	- ST295 HB2016032	Е	2016		4.2±4.1		5	(AMP) (CTX,CFX)(STR)(CHL)(SUL,SXT)	
	۱L	- ST297 HB2016035 HB2017099	Е	2017, 2016	+/-17.1:	E1.1 or 11.1	±2.2 aac(6')-lb-cr	6	(AMP) CTX,FEP,CFX,TIO)(NAL,CLP,LEV)(STR)(DOX)(SUL,SXT) or (AMP)(CTX,CFX,TIO)(NAL,CLP,LEV)(TET,DOX)(CHL)	(SU
ST355 HB2017072 D 2017 25.2±4.0 1 (CFX)		- ST355 HB2017072	D	2017		25.2±4.0		1	(CFX)	
		Salmonella LT2								

Figure 4. Phylogenetic relationships of the 53 *C. braakii* isolates (lineage III) from this study and our previous study [10]. The phylogenetic tree of the 53 *C. braakii* isolates was constructed using the concatenated sequences of the seven housekeeping genes by the neighbor-joining method. Bootstrap values of 50% or more from 1000 replicates were shown. The presence of ESBLs and *qnr* genes, source, year, NDR (number of drugs resistant to), adhesion, LDH and antibiotic resistance phenotype of an isolate is shown on the right. The tree was constructed using the neighbor joining algorithm. ST, D, F, H, and LDH indicate sequence types, isolates from diarrheal patients, foods and healthy individuals, and lactate dehydrogenase, respectively. Adhesion index: ***, >50; **, >1 and <50; *, <1; +/-, ambivalent or no adhesion; -, no adhesion.

2.2. Prevalence of Antimicrobial Resistance

Susceptibility to 22 antibiotics was tested on the 128 *Citrobacter* isolates using the broth microdilution method according to CLSI recommendations (Table 2). All isolates were sensitive to amikacin (AMI). For the 67 *C. freundii* isolates, most were resistant to one or more of the β -lactams, especially to penicillins (58.2%), cephalosporins (9.0–94.0%), monobactams (7.5%) and carbapenems (1.5–4.5%). Resistance to the three quinolones tested ranged from 7.5% to 23.9%. Resistance to other antibiotics was as follows: Aminoglycosides (0–20.9%), tetracyclines (16.4–32.8%), phenicols (25.4%), sulfonamides (22.4–25.4%) and macrolides (10.4%). For the 45 *C. braakii* isolates, resistance to different β -lactams was as follows: Penicillins (51.1%), cephalosporins (3.0–88.9%), monobactams (11.1%) and carbapenems (0–2.2%), and resistance to quinolones (6.7–44.4%), aminoglycosides (0–22.2%), tetracyclines (31.1–42.2%), phenicols (20.0%), sulfonamides (24.4–28.9%) and macrolides

.(SXT)

(6.7%). For 16 *C. youngae* isolates, varied resistance was found to penicillins (50.0%), cephalosporins (0–68.8%), monobactams (6.3%), quinolones (6.3–12.5%), aminoglycosides (0–12.5%), tetracyclines (12.5–18.8%), phenicols (12.5%) and sulfonamides (18.8%).

Antibiotic	C. freundii	(n = 67) Resi	stance (%)	C. youngae	(n = 16) Resi	stance (%)	C.braakii (n = 45) Resistance (%)			
	D (n = 30)	F (n = 30)	E (n = 7)	D (n = 12)	F (n = 4)	E (n = 0)	D (n = 8)	F (n = 33)	E (n = 4)	
PENICILLINS										
Ampicillin	15 (50.0)	23 (76.7)	1 (14.3)	5 (41.7)	3 (75.0)	0 (0)	3 (37.5)	16 (48.5)	4 (100.0)	
CEPHALOSPORINS										
Cefotaxime	6 (20.0)	12 (40.0)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	7 (21.2)	2 (50.0)	
Ceftazidime	3 (10.0)	3 (10.0)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	1 (3.0)	0 (0)	
Cefepime	0 (0)	6 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.0)	0 (0)	
Cefoxitin	28 (93.3)	29 (96.7)	6 (85.7)	8 (66.7)	3 (75.0)	0 (0)	7 (87.5)	29 (87.9)	4 (100.0)	
Ceftiofur Sodium	3 (10.0)	11 (36.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (21.2)	1 (25.0)	
MONOBACTAMS										
Aztreonam	2 (6.7)	3 (10.0)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	5 (15.2)	0 (0)	
CARBAPENEMS										
Imipenem	0 (0)	1 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.0)	0 (0)	
Meropenem	0 (0)	3 (10.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
QUINOLONES										
Nalidixicacid	4 (13.3)	12 (40.0)	0 (0)	2 (16.7)	0 (0)	0 (0)	3 (37.5)	15 (45.5)	2 (50.0)	
Ciprofloxacin	2 (6.7)	4 (13.3)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	3 (9.1)	0 (0)	
Levofloxacin	1 (3.3)	4 (13.3)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	3 (9.1)	0 (0)	
AMINOGLYCOSIDES										
Gentamicin	0 (0)	7 (23.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6.1)	0 (0)	
Amikacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Streptomycin	2 (6.7)	11 (36.7)	1 (14.3)	2 (16.7)	0 (0)	0 (0)	0 (0)	8 (24.2)	2 (50.0)	
Kanamycin	0 (0)	3 (10.0)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	1 (3.0)	0 (0)	
TETRACYCLINES										
Tetracycline	7 (23.3)	15 (50.0)	0 (0)	3 (25.0)	0 (0)	0 (0)	1 (12.5)	17 (51.5)	1 (25.0)	
Doxycycline	2 (6.7)	9 (30.0)	0 (0)	2 (16.7)	0 (0)	0 (0)	1 (12.5)	12 (36.4)	1 (25.0)	
PHENICOLS										
Chloramphenicol	6 (20.0)	11 (36.7)	0 (0)	2 (16.7)	0 (0)	0 (0)	0 (0)	7 (21.2)	2 (50.0)	
SULFONAMIDES										
Trimethoprim/Sulfamethoxazole	4 (13.3)	13 (43.3)	0 (0)	2 (16.7)	1 (25.0)	0 (0)	0 (0)	11 (33.3)	2 (50.0)	
Sulfafurazole	3 (10.0)	12 (40.0)	0 (0)	3 (25.0)	0 (0)	0 (0)	0 (0)	9 (37.3)	2 (50.0)	
MACROLIDES										
Azithromycin	1 (3.3)	6 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (9.1)	0 (0)	
MDR	9 (30.0)	17 (56.7)	1 (14.3)	3 (25.0)	0 (0)	0 (0)	1 (12.5)	17 (51.5)	3 (75.0)	

Table 2. Prevalence of resistance to different antibiotics by species and source.

D, diarrheal patients; F, foods; E, environment. MDR: With resistance to at least one antibiotic of three or more distinct classes (MDR \ge 3).

Resistance to at least one antibiotic of three or more distinct classes was defined as multidrug-resistant (MDR). We found 51 MDR isolates which were distributed in five lineages, and mainly in Lineages I, II, and III which included 14/29 (48.3%), 13/38 (34.2%) and 21/45 (46.7%) MDR isolates, respectively. Ten of the 51 MDR isolates were isolated from 2016, and the remaining 41 were from 2017; By source, 13 of the 51 MDR isolates (25.5%) were isolated from diarrheal patient, four (7.8%) from the environment and 34 (66.7%) from foods (Table 1).

There were 27 MDR *C. freundii* isolates, including 9/30 (30.0%) from diarrheal patient, 17/30 (56.7%) from foods and 1/7 (14.3%) from the environment (Table 2). A total of 58 MDR *C. freundii* isolates were isolated from this study and our previous study, including 28/94 (29.8%) from diarrheal patient, 22/41 (63.7%) from foods, 7/19 (36.8%) from healthy individuals and 1/7 (14.3%) from the environment (Supplementary Table S2).

For *C. youngae*, 3 MDR isolates were isolated from this study, including 3/12 (25.0%) from diarrheal patient (Table 2) and together with 9 MDR isolates from our previous study, 5/30 (16.7%) were from diarrheal patient, 6/24 (25.0%) from foods and 1/3 (33.3%) from healthy individuals (Supplementary Table S2).

For *C. braakii*, 21 MDR isolates were isolated from this study, including 1/8 (12.5%) from diarrheal patient, 17/33 (51.5%) from foods and 3/4 (75.0%) from the environment (Table 2). Five MDR isolates were isolated from our previous study. Taken together, the MDR rate was 1/8 (12.5%) from diarrheal patient, 22/40 (55.0%) from foods and 3/4 (75.0%) from the environment (Supplementary Table S2).

2.3. Prevalence of ESBLs and Fluoroquinolone Resistance

Among the 128 *Citrobacter* isolates, 12 ESBL isolates were detected, which harbored *bla*_{CTX-M-3}, *bla*_{CTX-M-9} or *bla*_{TEM-1}, 11 of which were MDR. No isolate carried *bla*_{SHV}, *bla*_{GES}, *bla*_{PER} or *bla*_{VEB} genes.

Thirty eight (29.7%) of the 128 *Citrobacter* isolates were resistant to fluoroquinolones, including 16 *C. freundii*, 2 *C. younga*e and 20 *C. braakii* isolates, all of which were resistant to NAL (MICs \geq 32 µg/mL); 12 resistant to CIP (MICs \geq 2 µg/mL); and 10 resistant to LEV (MICs \geq 8 µg/mL). By source, 27 (71.1%) of the 38 fluoroquinolones resistant isolates were from food, two from the environment and nine from diarrheal patients. These isolates were distributed among different phylogenetic lineages, 9/38 (23.7%) in Lineage I, 7/38 (1.8%) in Lineage II, 20/38 (52.6%) in Lineage III, one each in Lineage IV and V. Thirty three (86.8%) of the 38 fluoroquinolones resistant isolates were MDR (MDR \geq 3), and 9 (23.7%) were EMBLs carrying the *bla*CTX-M-3, *bla*CTX-M-9, or *bla*TEM-1 gene (Table 3).

Table 3. Quinolone resistant *Citrobacter* isolates and the presence of quinolone resistance genes and alterations in the *gyrA* gene.

Isolates	Species	Year	Source	ST	NDR	NAL	CIP	LEV	PMQR	gyrA position
HB2016008	C. freundii	2016	F	274	8	>128	4		aac(6')-Ib-cr,qnrB2	Thr59Ile
HB2016017	C. freundii	2016	F	282	1	>128				Thr59Ile
HB2017011	C. freundii	2017	D	163	7	>64	16	8	aac(6')-Ib-cr	Thr59Ile
HB2017018	C. freundii	2017	D	125	2	>128				Thr59Ile
HB2017027	C. freundii	2017	D	314	4	>64				No mutation
HB2017055	C. freundii	2017	F	161	6	>128				Thr59Ile
HB2017056	C. freundii	2017	F	340	4	>128				Thr59Ile
HB2016019	C. freundii	2016	F	284	9	>128		8	qnrB9	Thr59Ile
HB2016023	C. freundii	2016	F	288	9	>128	32	16	aac(6')-Ib-cr, qnrB9	Thr59Ile
HB2016024	C. freundii	2016	F	289	3	>128	4	8		Thr59Ile
HB2016034	C. freundii	2016	F	260	7	32			qnrS1	No mutation
HB2017026	C. freundii	2017	D	313	6	>128	4		aac(6')-Ib-cr	Thr59Ile
HB2017040	C. freundii	2017	F	326	8	>128				Thr59Ile
HB2017042	C. freundii	2017	F	328	9	>128	8	16		Thr59Ile
HB2017045	C. freundii	2017	F	331	7	64			qnrS1	No mutation
HB2017060	C. freundii	2017	F	344	7	>128				No mutation
HB2016015	C. braakii	2016	F	280	5	>128			qnrS1	Thr59Ile
HB2016033	C. braakii	2016	F	296	2	>128				Thr59Ile
HB2016035	C. braakii	2016	F	297	6	>128	8	8	aac(6')-Ib-cr, qnrB2	Thr59Ile
HB2017070	C. braakii	2017	D	353	2	>64				Thr59Ile
HB2017076	C. braakii	2017	D	358	4	>128				Thr59Ile
HB2017078	C. braakii	2017	D	359	1	>64	2			No mutation
HB2017079	C. braakii	2017	Е	360	3	>128				Thr59Ile
HB2017084	C. braakii	2017	F	365	3	>64				Thr59Ile
HB2017087	C. braakii	2017	F	367	8	>128			aac(6')-Ib-cr, qnrB2	Thr59Ile
HB2017090	C. braakii	2017	F	370	8	>128	8	>16		Thr59Ile
HB2017091	C. braakii	2017	F	371	3	>128				Thr59Ile
HB2017092	C. braakii	2017	F	372	4	64				No mutation
HB2017095	C. braakii	2017	F	225	6	>128			qnrB2	No mutation
HB2017099	C. braakii	2017	E	297	6	>128	8	8	aac(6')-Ib-cr	Thr59Ile
HB2017102	C. braakii	2017	F	379	3	32				No mutation
HB2017103	C. braakii	2017	F	380	4	>128				Thr59Ile
HB2017105	C. braakii	2017	F	382	9	>128	8	16	aac(6')-Ib-cr	No mutation
HB2017106	C. braakii	2017	F	383	4	32				No mutation
HB2017107	C. braakii	2017	F	384	7	>128				Thr59Ile
HB2017108	C. braakii	2017	F	385	6	>128			aac(6')-Ib-cr, qnrB2	Thr59Ile
HB2017007	C. youngae	2017	D	237	8	>64	8	8	aac(6')-Ib-cr	Thr59Ile
HB2017065	C. youngae	2017	D	348	7	>128				Thr59Ile, Gln111Arg, Ile134Val

NAL, nalidixicacid; CIP, ciprofloxacin; LEV, levofloxacin. NDR, number of drugs resistant to. PMQR, plasmid-mediated quinolone resistance (PMQR) genes.

Twenty eight (73.7%) of the 38 NAL-resistant isolates analyzed contained mutations in the QRDR of the *gyrA* gene, with 27 having one mutation in codon 59 (Thr59Ile) and one having three mutations in codons 59, 111and 134 (Thr59Ile, Gln111Arg and Ile134Val). No mutations were found in the QRDR region of the *parC* gene (Table 3).

Of the 28 NAL-resistant isolates with *gyrA* mutations, 12 belonged to *C. freundii*, 14 to *C. braakii*, 2 to *C. youngae*. Among the 12 *C. freundii* isolates with *gyrA* mutations, six were resistant to CIP (MIC of $\geq 4 \ \mu g/mL$), and five were resistant to LEV (MIC of $\geq 8 \ \mu g/mL$); of the 14 *C. braakii* with *gyrA* mutations, three were resistant to CIP (MIC of 8 $\ \mu g/mL$), and three to LEV (MIC of $\geq 8 \ \mu g/mL$); of the two *C. youngae* isolates with *gyrA* mutations, only one was resistant to CIP and LEV (MIC of 8 $\ \mu g/mL$) (Table 3).

2.4. Prevalence of qnrB Genes

Nineteen *Citrobacter* isolates, including 4 *C. braakii* and 15 *C. freundii* isolates, were found to harbor *qnrB* genes (including *qnrB2*, *qnrB9*, *qnrB17*, *qnrB76*, *qnrB13*, *qnrB93* and *qnrB94*) (Table 1). Four *C. braakii* and one *C. freundii* isolates harbored *qnrB2*, all of which were resistant to NAL, (MICs \geq 128 µg/mL), and were MDR. *QnrB9* was found in five *C. freundii* isolates with three (HB2017053, HB2016023 and HB2017031) isolated from food and MDR, and two (HB2016004 and HB2017031) isolated from diarrheal patients and none was MDR. Among the three *qnrB9*-carrying MDR isolates, two (HB2016023 and HB2017031) were resistant to NAL (MICs \geq 128 µg/mL). *QnrB17* was harbored in one *C. freundii* isolate (HB2017039) which was isolated from food and was not MDR. *qnrB76* was harbored by two *C. freundii* isolates with one from food and one from the environment. A variant of *qnrB76* (*qnrB76* contained a LexA binding site) was harbored by three *C. freundii* isolates, all of which were isolated from diarrheal patients. A variant of *qnrB13* (*qnrB13* contained a LexA binding site) was harbored by three *C. freundii* patients. A variant of *qnrB13* (*qnrB13* contained a LexA binding site) was harbored by two a diarrheal patients.

Two isolates (HB2017059 and HB2017038) were found to harbor a new *qnrB* gene, both of which were isolated from food (Table 1). Sequence analysis revealed that the new *qnrB* gene harbored by HB2017059 differed from the *qnrB13* gene (GenBank accession no. EU273756.1) by one nucleotide change (a G→A change at nt85 resulting in Asp→Asn), and this new *qnrB* allele was assigned *qnrB93* (GenBank accession no.MK047606). The new *qnrB* gene harbored by HB2017038 differed from the *qnrB11* gene (GenBank accession no. EU136183.1) by seven nucleotide change, including two non-synonymous changes, and A→G change at nt430 resulted in Thr→Ala and an A→C change at nt556 resulted in Ile→Leu, and five synonymous changes, a T→G change at nt246, A→C change at nt357, G→A change at nt399, C→T change at nt468 and G→C change at nt564. This new *qnrB* allele was assigned *qnrB94* (GenBank accession no.MK047607) [26].

qnrB genes were predominantly harboured by Lineage I (*C. freundii*) (Table 1). When all *C. freundii* data combined, 26/53 (49.1%) Lineage I isolates carried a *qnrB* allele (Figure 2).

2.5. Adherence and Cytotoxicity of Citrobacter Isolates

The 128 *Citrobacter* isolates were tested for adhesion and cytotoxicity to Hep-2 cells as done previously [10] (Table 1). Fourteen isolates (10.9%) had an adhesion index greater than 50 and were classified as high adhesion. Fifty-seven (44.5%) isolates had an adhesion index between 1 and 50 and were regarded as intermediate adhesive. Thirty seven (28.9%) isolates showed little adhesion (adhesion index of <1). The remaining 20 (15.6%) isolates showed ambivalent adhesion or no adhesion. By cytotoxicity, of the 128 isolates, 13 (10.2%) were highly cytotoxic, 40 (31.3%) intermediate cytotoxic and 75 (58.6%) non-cytotoxic.

Among the 14 highly adhesive isolates, four isolates released LDH more than 24%, and were considered highly cytotoxic (Figure 5); seven isolates released LDH from 19.4% to 22.9% and were considered intermediate cytotoxic; the remaining three isolates showed LDH release less than 16.5% and were likely to be non-cytotoxic (Table 1 and Supplementary Table S3).





Figure 5. Adhesion and cytotoxicity of *Citrobacter* isolates to the human epidermoid laryngocarcinoma (Hep-2, CCC0068) cell line. (**A**) Light micrographs of the adherence patterns exhibited by the 13 highly cytotoxic *Citrobacter* isolates, and control strains CF74 and CF72. Bar: 10μ m. (**B**) Cytotoxicity of the 13 highly cytotoxic *Citrobacter* isolates was measured by the amount of the LDH released after 8 h exposure by Hep-2 cells. CF72 and CF74 were control strains. CF72 was a non-cytotoxic and non- adhesive negative control, CF74 was a highly adherent and cytotoxic positive control.

Among the 57 intermediate adhesive isolates, seven isolates showed high cytotoxicity with more than 24% LDH released (Figure 5); 23 isolates released LDH from 18.0% to 23.5% and were intermediate cytotoxic, the remaining 27 isolates were considered to be non-cytotoxic (Table 1 and Supplementary Table S3).

Among the 37 less adhesive isolates, two showed high cytotoxicity with more than 24% LDH released (Figure 5); nine were considered intermediate cytotoxic which released LDH from 17.9% to 22.2%; the remaining 26 isolates showed LDH release less than 15.9% and are likely to be non-cytotoxic (Table 1 and Supplementary Table S3).

The 20 non adhesive isolates were also non-cytotoxic with all, except one showing intermediate cytotoxicity, releasing LDH from 0.6% to 17.1% (Table 1 and Supplementary Table S3).

We examined any differences in adhesion and cytotoxicity between lineages. We analyzed the difference using data in this study alone (Figure 6A,C) and also using combined data with our two previous studies (Figure 6B,D). Between Lineages I and II which exclusively contained *C. freundii* isolates, Lineage II showed higher proportion of high or intermediate adhesive and cytotoxic isolates than in Lineage I and the difference is statistically significant (p < 0.01) (Figure 6); Between Lineage IV and V which contained only *C. youngae* isolates, the percentage of highly adhesive isolates in Lineage V was higher than in Lineage IV (p < 0.01) (Figure 6A,C), and the percentage of the highly or intermediate cytotoxic isolates in Lineage V was also higher than in Lineage IV (p < 0.05) (Figure



6B,D). When the two virulence traits were considered together, Lineages II and V had higher adhesive and cytotoxic isolates than Lineages I, III, and IV (Figure 7).

Figure 6. The percentage of adhesive and cytotoxic isolates in different lineages. (**A**) and (**B**) The percentage of high, intermediate, little or no adhesive or cytotoxic isolates based on the 128 *Citrobacter* isolates from this study. (**C**) and (**D**) The percentage of high, intermediate, little or no adhesive or cytotoxic isolates in different lineages based on the128 *Citrobacter* isolates from this study and 95 *Citrobacter* isolates from our previous studies [10,16]. The statistical significance between Lineages I and II or Lineages IV and V was determined by Mann-Whitney U test. *, *p* < 0.05; **, *p* < 0.01.



Figure 7. The number of adhesive and cytotoxic isolates in different lineages. The data were based on 128 isolates from this study and 95 *Citrobacter* isolates from our previous studies [10,16].

3. Discussion

Citrobacter spp. are opportunistic pathogens that can cause diarrhea, septicemia, meningitis, and urinary tract infections, especially in immunocompromised patients [27]. Together with our previous studies [10,16], we found 25 highly cytotoxic *Citrobacter* isolates out of 271 (9.2%), 15 were isolated from diarrheal patients (11.4% of diarrheal isolates), seven from foods (6.7% food isolates), two from healthy individuals (8.7%) and 1 from the environment (9.1%). The 22 highly cytotoxic *Citrobacter* isolates were distributed among the five lineages identified. However, Lineage II (*C. freundii*) and Lineage V (*C. youngae*) disproportionally contained more adhesive and more cytotoxic isolates than Lineages I, III, and IV and are likely to pose a higher risk to human health.

3.1. High Genetic Diversity of Citrobacter spp. Across China and Internationally.

The 128 *Citrobacter* isolates were divided into 123 STs, displaying high genetic diversity. We compared our STs with 268 STs from the *Citrobacer* MLST database and found 22 STs in this study were present in the database with isolates from other countries or regions, or from different sources. Among these 22 STs, ST1 contained isolates from diarrheal patients, healthy individuals, animals, insects and environment in our previous study [9]; ST12 contained isolates from a rectal swab (Israel in 2009) and a diarrheal patient (China in 2015) [16]; ST17 contained isolates from a skin necrosis, urine (Poland, 2012), two rectal swabs (Latvia, 2008 and 2009), and two diarrheal patients (China in

2014) [16]; ST163 and ST169 contained isolates from water (Canada in 2015); ST161 contained isolates from water (Canada in 2015) and a diarrheal patient (China in 2015) [16]; ST85, ST183, ST214, ST216, ST217, ST225, and ST237 each contained isolates from diarrheal patient in our previous studies [10,18]; ST187 and ST219 each contained isolates from healthy individuals in our previous study [16]; ST258 and ST260 each contained isolates from the Netherlands. Our analysis by clonal complexes which allowed one to examine closely related STs, found 17 *C. freundii* CCs containing at least 2 STs per CC contained isolates from different countries with one CC present in 5 different countries. For *C. youngae* and *C. braakii*, fewer CCs were identified, and narrower geographic distribution was found. However, there was no information from other countries for comparison for these latter two species. Thus, some *Citrobacter* STs and CCs are likely to be widely present in fecal, food, and other reservoirs and spread in different countries or regions. However, there are very limited reports of *Citrobacter* spp. from different sources or geographical regions. A recent study in companion animals reported nosocomial dissemination of *C. freundii* strains resistant to extended-spectrum cephalosporins [28]. Our studies underscore the need for further study to understand the genetic diversity, virulence and antibiotic resistance and their risks to human health.

3.2. Association of C. freundii Lineage II and C. youngae Lineage V with Higher Adhesion and Cytotoxicity

The clustering of highly adhesive and cytotoxic isolates in specific lineages is most interesting as it suggests that different lineages have different levels of virulence and/or may cause different types of diseases. Combining this study with our previous studies [10,16], there were nine highly cytotoxic *C. freundii* isolates, of which six were isolated from diarrheal patients, two from foods, and one from the environment. Among these six highly cytotoxic *C. freundii* isolates from diarrheal patients, five isolates were clustered in Lineage II and one in Lineage I, suggesting that Lineage II is more likely to cause diarrheal disease. *C. freundii*, as the most common *Citrobacter* species, has caused gastroenteritis associated outbreaks [3]. However, despite reports of different virulence factors involved [9,10], little is known of its pathogenic mechanisms and there have been no means that can differentiate strains that can cause diarrheal disease from those of harmless colonizers. The identification of Lineage II as more likely to cause diarrheal more likely to cause other infections.

Similarly, combining with our previous studies [10,16], seven of the eight highly cytotoxic *C. youngae* strains fell into Lineage V, of which four were isolated from diarrheal patients, suggest that Lineage V is likely to cause diarrheal disease. However, *C. younage* had not been recognized as a diarrheal pathogen and Lineage V should be further investigated for their potential to cause diarrheal disease.

C. braakii has been isolated from foods, hospital infections and UTIs [14], and acute peritonitis patients [7,8]. In our previous study, we isolated *C. braakii* isolates from food source, but not human fecal samples [10]. In this study, we obtained 45 *C. braakii* isolates from food, environment and diarrheal patients with two highly adhesive and five highly cytotoxic isolates. Three of the five highly cytotoxic *C. braakii* isolates were isolated from diarrheal patients, and it remains to be determined whether *C. braakii* can cause diarrhea.

3.3. Higher Prevalence of Multidrug Resistance in C. braakii Isolates and Citrobacter Isolates from Food Sources

MDR has been reported in *Citrobacter* isolates, especially *C. freundii* [17]. Considering isolates from this study only, 58 of the 128 isolates were MDR, mainly distributed among Lineages I to III. When combined with our previous studies [10,16], 96 of the 271 *Citrobacter* isolates (35.4%) were MDR, including 58 *C. freundii* isolates (36.0% of *C. freundii* isolates, Lineages I and II), 12 *C. youngae* isolates (21.1% of *C. youngae* isolates, Lineages IV and V) and 26 *C. braakii* isolates (49.0% of *C. braakii* isolates, Lineage III). The difference in the prevalence of MDR between *C. youngae* and *C. braakii* is statistically significant (p < 0.01).

Among the 96 MDR isolates, 34 were isolated from diarrheal patients (25.8% of diarrheal isolates), 50 from foods (47.6% of the food isolates), four from the environment (36.4%) and eight from healthy individuals (34.8%). Interestingly MDR were more prevalent among food isolates. Since most of the food source was related to meat or meat products [10,16], the MDR may have been a result of extensive use of antibiotics in animals. Moreover, four highly cytotoxic strains (11.8%) were found in 34 MDR isolates from diarrheal patients, highlighting the combined increased risk of high cytotoxicity and MDR of *Citrobacter* to human health.

3.4. Carriage of ESBL Genes by Citrobacter spp. was Relatively Low

ESBL producing *Enterobacteriaceae* has been a major challenge in infection control [29,30]. Previous studies have reported that *Citrobacter* isolated from human clinical infections that carried ESBL genes varied from 5.6% to 67.5%, including *bla*_{TEM-1}, *bla*_{SHV-12}, *bla*_{CTX-M-15}and *bla*_{CTX-M}[3,28]. In our studies, including our previous studies [10,16], we found 2.6% of *Citrobacter* isolates carried the *bla*_{CTX-M-9} gene, 1.1% carried the *bla*_{CTX-M-3} gene, 3.7% carried the *bla*_{TEM-1} gene, and none carried the *bla*_{SHV} gene. The ESBL carrying *Citrobacter* isolates consisted of 12 *C. freundii* isolates (7.5% of *C. freundii* isolates) and four *C. braakii* isolates (7.5% of *C. braakii* isolates). The four ESBL carrying *C. braakii* isolates were isolated from foods. Three of the 12 ESBLs carrying *C. freundii* isolates were isolated from diarrheal patients (2.3% of diarrheal isolates), eight from foods (7.6%) and one from healthy individuals (4.3%). In comparison to other reports, ESBL carriage is lower in our isolates. The isolates of our previous two studies [10,16] were from the south of China (Anhui province), while isolates of this study were from northern China (Hebei Province), suggesting likely low prevalence of ESBLs in *Citrobacter* spp. In China. However, other regions of China would need to be sampled to obtain a more comprehensive picture.

3.5. Higher Prevalence of Quinolone Resistance in Lineages I and III of Citrobacter spp with Multiple Mechanism of Resistance Detected

The prevalence of quinolone resistance varied among the lineages. Lineage III (*C. braakii*) had the highest proportion of resistance isolates (52.6%), followed by Lineage I (*C. freundii*) with 23.7%. We analyzed the carriage of potential quinolone resistance genes or mutations which include both the QRDR of *gyrA* and *parC* associated resistance, and PMQR genes mediated resistance [22]. *Citrobacter* isolates with mutations in the QRDR of *gyrA*, *including* Thr83Ile and Asp87Asn have shown reduced susceptibility to fluoroquinolones [22,23]. In our previous study [18], four (66.7%) of the six ciprofloxacin-resistant *C. freundii* isolates were found to have mutations in codons 59, 111, and/or 134 in the QRDR region of the *gyrA* gene, namely, Thr59Ile, Gln111Arg, and Ile134Val. In this study, 28 ciprofloxacin-resistant isolates carried mutations in the QRDR of *gyrA* with 27 having one mutation, Thr59Ile and one having three mutations, Thr59Ile, Gln111Arg and Ile134Val. However, it should be noted that these three mutations have not been experimentally confirmed whether they affect fluoroquinolone resistance.

For PMQR gene-mediated resistance, 14.0% of our *Citrobacter* isolates carried a *qnr* gene, and 5.5% of *Citrobacter* isolates carried the *aac(6')-lb-cr* gene. Our results contrast previous reports of high prevalence of *qnr* and *aac(6')-lb-cr* genes from clinical infections in China at 72.8% and 68.9% in *C. freundii* isolates; and 42.9% and 42.9% in *C. braakii* isolates, respectively from the study of Zhang et al. [29], and 63.3% and 26.7% in *C. freundii* from the study of Yang *et al.* [31]. This difference could be due to different regions of China or the sources of samples.

3.6. Citrobacter spp. Carried Many Variants of the qnrB Gene with C. freundii Lineage I as the Main Reservoir

qnrB is known to be harbored by *Citrobacter* [26]. Surprisingly our *Citrobacter* isolates harboured many *qnrB* variants with 11 *qnrB* allelic variants found, including *qnrB2*, *qnrB9*, *qnrB13*, *qnrB16*, *qnrB17*, *qnrB63*, *qnrB76*, *qnrB77*, *qnrB92*, *qnrB93* and *qnrB94*. Interestingly, although only 17% (28 of 161) of the *C. freundii* isolates harbored *qnrB* genes, all except one *qnrB* positive isolate belonged to

Lineage I, suggesting that Lineage I is a main reservoir of *qnrB* genes. However, carriage of *qnrB* gene does not always confer high level of quinolone resistance [29]. Only two *qnrB9*-carrying *C*. *freundii* isolates in Lineage I were resistant to quinolones, while other *qnrB*-carrying *C*. *freundii* isolates in Lineage I were susceptible to NAL, CIP and LEV. The only *qnrB* carrying Lineage II isolate is quinolones resistant. Four *qnrB*-carrying *C*. *braakii* isolates were resistant to NAL (MICs \geq 128 µg/mL). These results suggest that *Citrobacter* isolates carrying different *qnrB* alleles may have different levels of quinolone resistance.

QnrB has a growing number of allelic variants [32]. In our previous study, we found a variant of *qnrB77* (a *qnrB77* contained a LexA binding site) and a new *qnrB* allele (*qnrB92*) [10,16]. In this study, we found a variant of *qnrB76* (a *qnrB76* contained a LexA binding site), a variant of *qnrB13* (a *qnrB13* contained a LexA binding site), and two new *qnrB* alleles, *qnrB93* and *qnrB94*.

4. Conclusion

We analyzed 128 *Citrobacter* isolates (67 *C. freundii*, 16 *C. youngae* and 45 *C. braakii* isolates) from human diarrheal patients, foods and environment in Shijiazhuang, Hebei Province in the north of China. This study expands the genetic diversity observed from our previous studies of human and food isolates from South China [10,16]. The isolates showed high diversity with 123 STs of which 101 were novel STs. Only 22 STs (17.9%) were found from different sources and/or geographic regions. Phylogenetic analysis divided the 128 isolates into five lineages. Lineages I and II contained *C. freundii* isolates, while Lineages IV and V contained *C. youngae* isolates and Lineage III contained all *C. braakii* isolates.

The 51 MDR isolates were mainly distributed in Lineage I (*C. freundii*) and Lineage III (*C. braakii*) with 48.3% and 46.7% of the isolates in these lineages being MDR, respectively. Food isolates were also more likely to be MDR. Combining data with our previous studies [10,16], we found that the prevalence of quinolone resistance was highest in Lineage I (*C. freundii*) and Lineage III (*C. braakii*). *Citrobacter spp.* carried many variants of the *qnrB* gene with the carriage by *C. freundii* Lineage I isolates being the highest. Surprisingly, the prevalence of ESBL genes in *Citrobacter spp.* is relatively low.

There were 22 highly cytotoxic *Citrobacter* isolates, which were preferentially distributed in *C. freundii* Lineage II and *C. youngae* Lineage V, suggesting that these two lineages are more virulent than others, and thus, more likely to cause disease. This study has shed more light on the genetic diversity, pathogenicity and antibiotic resistance of *Citrobacter*.

5. Methods

5.1. Citrobacter Isolates

128 *Citrobacter* spp. isolates were isolated from 50 diarrheal patients, 11 environment and 67 food samples in Shijiazhuang Hebei Province, China from 2016 to 2017. 50 diarrheal patient fecal samples included 30 *C. freundii*, eight *C. braakii* and 12 *C. youngae* isolates, and four of the 50 diarrheal patient fecal samples harbored *norovirus* (HB2016002 and HB2017022) or *Vibrio parahaernolyticus* (HB2016004 and HB2016036); the 11 environment isolates had seven *C. freundii* and four *C. braakii* which were isolated from food processing place; 67 food isolates included 30 *C. freundii*, 33 *C. braakii* and four *C. youngae* isolates which were isolates which were isolated from chicken, pork, duck and vegetables (Supplementary Table S4). The species identity of each isolate was confirmed using API 20E test strips (bioMérieux, La Balme les Grottes, France). Bacterial culture media and conditions were as previously described [10].

5.2. MLST and Phylogenetic Analysis

The *Citrobacter* MLST scheme (http://pubmlst.org/*cfreundii*/) was used. PCR amplification and sequencing were carried out using published primers as described previously [10]. SeqMan 7.0 software was used to analyze the sequences.

5.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out as previously described [10,33]. Minimum inhibitory concentration (MIC) was interpreted in 22 antibiotics, including ampicillin, cefotaxime, ceftazidime, cefepime, cefoxitin, ceftiofur Sodium, aztreonam, imipenem, meropenem, nalidixicacid, ciprofloxacin, levofloxacin, gentamicin, amikacin, streptomycin, kanamycin, tetracycline, doxycycline, chloramphenicol, trimethoprim/sulfamethoxazole, sulfafurazole and azithromycin (Table 2). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls for MICs.

5.4. PCR Amplification and Sequencing.

Detection of *blatem*, *blashv*, *blactx-m-1*, *blactx-m-2*, *blactx-m-8*, *blactx-m-9*, *blages*, *blaper* and *blaveb*, *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *aac*(6')-*lb-cr* and *qepA* genes were carried out as previously described [10,34–39].

5.5. In Vitro Adhesion and Cytotoxicity Assays.

The human epidermoid laryngocarcinoma (HEp-2, CCC0068) cell line was obtained from the cell resource center at Peking Union Medical College. HEp-2 cell line has been used as a human cell line to study bacteria-host interactions in many studies [40–42]. Note that Hep-2 cell line may have been a misidentified cell line, raising concerns of relevance to laryngeal cancer research [43], but the issue is not relevant here. *In vitro* adhesion to host cells was performed as previously described [10]. The mean number of bacteria per HEp-2 after examination of 10 visual fields was determined and was used as an adhesion index (<1; >1 and <50; >50) [10].

The lactate dehydrogenase (LDH) released by the HEp-2 cells was determined as previously described [10]. All experiments were performed three times in duplicate.

5.6. Statistical Analysis.

SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA) was used to conduct all statistical comparisons. A nonparametric test (Mann–Whitney U-test) and chi-square test were used to compare the different groups for statistical significance. *p*-value of ≤ 0.05 (2-tailed) was considered to be statistically significant.

5.7. Ethics Approval and Consent to Participate

This study was reviewed and approved by the ethics committee of the National Institute for Communicable Disease Control and Prevention, the Chinese CDC. Human fecal specimens were acquired with the written informed consent of the diarrheal patients with the approval of the ethics committee of the National Institute for Communicable Disease Control and Prevention, according to the medical research regulations of Ministry of Health (ICDC-2016004).

Supplementary Materials: The following are available online at www.mdpi.com/2076-0817/9/3/195/s1, Figure S1, Phylogenetic relationships as determined by MLST data for the 123 *C. freundii* isolates from this study and two previous studies; Table S1, List of clonal complexes of *Citrobacter* isolates from this study and the public MLST database[#]; Table S2, Prevalence of MDR isolates in different *Citrobacter* species; Table S3, Adherence and Cytotoxicity in 128 *Citrobacter* Isolates; Table S4, Source, drugs resistance, Genotypes and Antibiotic resistance phenotype of 128 *Citrobacter* Isolates.

Author Contributions: Formal Analysis, L.Q., S.H., X.L., X.C., C.Z. and H.S.; Resources, B.X., Y.G. and R.J.; Writing–Original Draft Preparation, L.L.; Writing–review and editing, L.L. and R.L.; Supervision, J.X. All authors have read and approved the final version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (No. 81301401, 81290345), and the National Key Research and Development Program of China (2016YFC1201903, 2016YFC1202700).

Conflicts of Interest: The authors declare no conflict of interest.

Reference

- 1. Park, Y.-J.; Yu, J.K.; Lee, S.; Oh, E.J.; Woo, G.-J. Prevalence and diversity of qnr alleles in AmpC-producing Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii and Serratia marcescens: A multicentre study from Korea. *J. Antimicrob. Chemother.* **2007**, *60*, 868–871.
- Guerrant, R.L.; Dickens, M.D.; Wenzel, R.P.; Kapikian, A.Z. Toxigenic bacterial diarrhea: Nursery outbreak involving multiple bacterial strains. J. Pediatr. 1976, 89, 885–891.
- Tschäpe, H.; Prager, R.; Streckel, W.; Fruth, A.; Tietze, E.; Böhme, G. Verotoxinogenic Citrobacter freundii associated with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: Green butter as the infection source. *Epidemiol. Infect.* 1995, *114*, 441–450.
- 4. Warner, R.D. A large nontypical outbreak of Norwalk virus. Gastroenteritis associated with exposing celery to nonpotable water and with Citrobacter freundii. *Arch. Intern. Med.* **1991**, *151*, 2419–2424.
- Doulgeraki, A.; Paramithiotis, S.; Nychas, G.-J.E. Characterization of the Enterobacteriaceae community that developed during storage of minced beef under aerobic or modified atmosphere packaging conditions. *Int. J. Food Microbiol.* 2011, 145, 77–83.
- Giammanco, G.M.; Aleo, A.; Guida, I.; Mammina, C. Molecular Epidemiological Survey of Citrobacter freundii Misidentified as Cronobacter spp. (Enterobacter sakazakii) and Enterobacter hormaechei Isolated from Powdered Infant Milk Formula. *Foodborne Pathog. Dis.* 2011, *8*, 517–525.
- Samonis, G.; Karageorgopoulos, D.; Kofteridis, D.P.; Matthaiou, D.; Sidiropoulou, V.; Maraki, S.; Falagas, M.E. Citrobacter infections in a general hospital: Characteristics and outcomes. *Eur. J. Clin. Microbiol. Infect. Dis.* 2008, 28, 61–68.
- 8. Mohanty, S.; Singhal, R.; Sood, S.; Dhawan, B.; Kapil, A.; Das, B.K. Citrobacter infections in a tertiary care hospital in Northern India. *J. Infect.* **2007**, *54*, 58–64.
- 9. Bai, L.; Xia, S.; Lan, R.; Liu, L.; Ye, C.; Wang, Y.; Jin, N.; Cui, Z.; Jing, H.; Xiong, Y.; et al. Isolation and Characterization of Cytotoxic, Aggregative Citrobacter freundii. *PLoS ONE* **2012**, *7*, e33054.
- 10. Liu, L.; Lan, R.; Liu, L.; Wang, Y.; Zhang, Y.; Wang, Y.; Xu, J. Antimicrobial Resistance and Cytotoxicity of Citrobacter spp. in Maanshan Anhui Province, China. *Front. Microbiol.* **2017**, *8*, 1357.
- 11. Chen, K.J.; Chen, T.H.; Sue, Y.M. Citrobacter Youngae and Pantoea Agglomerans Peritonitis in a Peritoneal Dialysis Patient. *Perit. Dial. Int.* **2013**, *33*, 336–337.
- 12. Basra, P.; Koziol, A.; Wong, A.; Carrillo, C. Complete Genome Sequences of Citrobacter braakii Strains GTA-CB01 and GTA-CB04, Isolated from Ground Beef. *Genome Announc.* **2015**, *3*, e01307-14.
- Kwak, H.L.; Han, S.K.; Park, S.; Park, S.H.; Shim, J.Y.; Oh, M; Ricke, S.C.; Kim, H.Y. Development of a rapid and accurate identification method for Citrobacter species isolated from pork products using a Matrix-Assisted Laser-Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOFMS). J. Microbiol. Biotechnol. 2015, 25, 1537–1541.
- 14. Arens, S.; Verhaegen, J.; Verbist, L. Differentiation and susceptibility of Citrobacter isolates from patients in a university hospital. *Clin. Microbiol. Infect.* **1997**, *3*, 53–57.
- 15. Chao, C.-T.; Lee, S.-Y.; Yang, W.-S.; Chen, H.-W.; Fang, C.-C.; Yen, C.-J.; Chiang, C.-K.; Hung, K.-Y.; Huang, J.-W. Citrobacter Peritoneal Dialysis Peritonitis: Rare Occurrence with Poor Outcomes. *Int. J. Med Sci.* **2013**, *10*, 1092–1098.
- Liu, L.; Chen, D.; Liu, L.; Lan, R.; Hao, S.; Jin, W.; Sun, H.; Wang, Y.; Liang, Y.; Xu, J. Genetic Diversity, Multidrug Resistance, and Virulence of Citrobacter freundii From Diarrheal Patients and Healthy Individuals. *Front. Microbiol.* 2018, *8*, 233.
- 17. Akya, A.; Jafari, S; Ahmadi, K.; Elahi, A. Frequency Of blaCTX-M, blaTEM and blaSHV Genes in Citrobacters Isolated from Imam Reza Hospital in Kermanshah. *J. Mazand. Univ. Med. Sci.* **2015**, *25*, 65–73.
- Oliveira, H.; Pinto, G.; Oliveira, A.; Oliveira, C.; Faustino, M.A.; Briers, Y.; Domingues, L.; Azeredo, J. Characterization and genome sequencing of a Citrobacter freundii phage CfP1 harboring a lysin active against multidrug-resistant isolates. *Appl. Microbiol. Biotechnol.* 2016, 100, 10543–10553.
- Moland, E.S.; Hanson, N.D.; Black, J.A.; Hossain, A.; Song, W.; Thomson, K.S. Prevalence of Newer β-Lactamases in Gram-Negative Clinical Isolates Collected in the United States from 2001 to 2002. *J. Clin. Microbiol.* 2006, 44, 3318–3324.
- Choi, S.-H.; Lee, J.E.; Park, S.J.; Kim, M.-N.; Choo, E.J.; Kwak, Y.G.; Jeong, J.-Y.; Woo, J.H.; Kim, N.J.; Kim, Y.S. Prevalence, microbiology, and clinical characteristics of extended-spectrum β-lactamase-producing

Enterobacter spp., Serratia marcescens, Citrobacter freundii, and Morganella morganii in Korea. *Eur. J. Clin. Microbiol. Infect. Dis.* **2007**, *26*, 557–561.

- 21. Shao, Y.; Xiong, Z.; Li, X.; Hu, L.; Shen, J.; Li, T.; Hu, F.; Chen, S. Prevalence of plasmid-mediated quinolone resistance determinants in Citrobacter freundii isolates from Anhui province, PR China. *J. Med Microbiol.* **2011**, *60*, 1801–1805.
- 22. Minarini, L.; Darini, A.L.C. Mutations in the quinolone resistance-determining regions of gyrA and parC in Enterobacteriaceae isolates from Brazil. *Braz. J. Microbiol.* 2012, *43*, 1309–1314.
- Weigel, L.M.; Steward, C.D.; Tenover, F.C. gyrA Mutations Associated with Fluoroquinolone Resistance in Eight Species of Enterobacteriaceae. *Antimicrob. Agents Chemother.* 1998, 42, 2661–2667.
- 24. Feil, E.J. Small change: Keeping pace with microevolution. Nat. Rev. Genet. 2004, 2, 483-495.
- Wirth, T.; Falush, D.; Lan, R.; Colles, F.; Mensa, P.; Wieler, L.H.; Karch, H.; Reeves, P.; Maiden, M.C.; Ochman, H.; et al. Sex and virulence in Escherichia coli: An evolutionary perspective. *Mol. Microbiol.* 2006, 60, 1136–1151.
- 26. Jacoby, G.; Cattoir, V.; Hooper, D.; Martínez-Martínez, L.; Nordmann, P.; Pascual, A.; Poirel, L.; Wang, M. qnr Gene Nomenclature v. *Antimicrob. Agents Chemother.* **2008**, *52*, 2297–2299.
- Liu, X.; Huang, Y.; Xu, X.; Zhao, Y.; Sun, Q.; Zhang, Z.; Zhang, X.; Wu, Y.; Wang, J.; Zhou, N.; et al. Complete Genome Sequence of Multidrug-Resistant Citrobacter freundii Strain P10159, Isolated from Urine Samples from a Patient with Esophageal Carcinoma. *Genome Announc.* 2016, 4, e01754-15.
- Harada, K.; Shimizu, T.; Ozaki, H.; Kimura, Y.; Miyamoto, T.; Tsuyuki, Y. Characterization of Antimicrobial Resistance in Serratia spp. and Citrobacter spp. Isolates from Companion Animals in Japan: Nosocomial Dissemination of Extended-Spectrum Cephalosporin-Resistant Citrobacter freundii. *Microorganisms* 2019, 7, 64.
- Liu, L.-H.; Wang, N.-Y.; Wu, A.Y.-J.; Lin, C.-C.; Lee, C.-M.; Liu, C.-P. Citrobacter freundii bacteremia: Risk factors of mortality and prevalence of resistance genes. J. Microbiol. Immunol. Infect. 2018, 51, 565–572.
- Zhang, R.; Ichijo, T.; Huang, Y.-L.; Cai, J.; Zhou, H.; Yamaguchi, N.; Nasu, M.; Chen, G.-X. High prevalence of qnr and aac(6')-Ib-cr genes in both water-borne environmental bacteria and clinical isolates of Citrobacter freundii in China. *Microbes Environ*. 2012, *27*, 158–163.
- Yang, H.; Chen, H.; Yang, Q.; Chen, M.; Wang, H. High Prevalence of Plasmid-Mediated Quinolone Resistance Genes qnr and aac(6')-Ib-cr in Clinical Isolates of Enterobacteriaceae from Nine Teaching Hospitals in China v. Antimicrob. Agents Chemother. 2008, 52, 4268–4273.
- 32. Jacoby, G.A.; Griffin, C.M.; Hooper, D.C. Citrobacter spp. as a Source of qnrB Alleles v. Antimicrob. Agents Chemother. 2011, 55, 4979–4984.
- Clinical and Laboratory Standards Institute. M100-S28 Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Eighth Informational Supplement; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018; p. 353.
- Poirel, L.; Walsh, T.; Cuvillier, V.; Nordmann, P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn. Microbiol. Infect. Dis.* 2011, 70, 119–123.
- Voets, G.M.; Fluit, A.C.; Scharringa, J.; Stuart, J.C.; Hall, M.A.L.-V. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. *Int. J. Antimicrob. Agents* 2011, *37*, 356–359.
- Liu, Y.-Y.; Wang, Y.; Walsh, T.; Yi, L.-X.; Zhang, R.; Spencer, J.; Doi, Y.; Tian, G.; Dong, B.; Huang, X.; et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *Lancet Infect. Dis.* 2016, 16, 161–168.
- Xavier, B.B.; Lammens, C.; Ruhal, R.; Kumar-Singh, S.; Butaye, P.; Goossens, H.; Malhotra-Kumar, S. Identification of a novel plasmid-mediated colistin-resistance gene,mcr-2, inEscherichia coli, Belgium, June 2016. *Eurosurveillance* 2016, *21*, 30280.
- Zhao, W.-H.; Hu, Z.-Q. IMP-type metallo-?-lactamases in Gram-negative bacilli: Distribution, phylogeny, and association with integrons. *Crit. Rev. Microbiol.* 2011, 37, 214–226.
- Mange, J.-P.; Stephan, R.; Borel, N.; Wild, P.; Kim, K.S.; Pospischil, A.; Lehner, A. Adhesive properties of Enterobacter sakazakii to human epithelial and brain microvascular endothelial cells. *BMC Microbiol.* 2006, 6, 58.

- Dallal, M.M.S.; Validi, M.; Douraghi, M.; Fallah-Mehrabadi, J.; Lormohammadi, L. Evaluation the cytotoxic effect of cytotoxin-producing Klebsiella oxytoca isolates on the HEp-2 cell line by MTT assay. *Microb. Pathog.* 2017, 113, 416–420.
- 42. Konkel, M.E.; Joens, L.A. Adhesion to and invasion of HEp-2 cells by Campylobacter spp. *Infect. Immun.* **1989**, *57*, 2984–2990.
- 43. Philippe, G. A comprehensive review of Hep-2 cell line in translational research for laryngeal cancer. *Am. J. Cancer Res.* **2019**, *9*, 644–649.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).