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Original Article

Clinical and molecular epidemiological characterization of extensively drug-resistant Acinetobacter baumannii in patients with liver disease

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Abstract: The global prevalence of extensively drug-resistant (XDR) Acinetobacter baumannii (A. baumannii) has become a healthcare-associated problem. We aimed to investigate the molecular characteristics of XDR A. baumannii in patients with liver disease. Fifty-nine non-duplicated XDR A. baumannii from our hospital patients with liver disease were collected in China between December 2009 and October 2012. All isolates was performed by antibiotic susceptibility test and multilocus sequence typing. Resistance genes were detected by PCR. All isolates were resistance to common antibiotics except polymyxin B. The OXA-23, OXA-51, GIM and OXA-58 genes were prevalent in the XDR A. baumannii. Three clones were widespread in 59 isolates: ST381 (11 isolates, 18.6%), ST425 (11 isolates, 18.6%) and ST492 (12 isolates, 20.3%). We explored the drug resistance and molecular typing of XDR A. baumannii isolates from our hospital. The major clones, ST381, ST425 and ST492, have disseminated in our hospital patients with liver disease.

Keywords: Acinetobacter baumannii, extensively drug-resistant, multilocus sequence typing (MLST)

Introduction

Acinetobacter baumannii (A. baumannii) is agram-negative, ubiquitous pathogen, causing serious nosocomial infections, mainly in intensive care unit (ICU) [1, 2]. This microorganism is commonly present high resistance to multiple antimicrobial agents, even including fluoroquinolones and aminoglycosides [3]. Therefore, extensively drug-resistant Acinetobacter baumannii (XDR A. baumannii) has risen globally as a health care-associated opportunistic pathogen [4], and the infection rate is increasing worldwide in recent years [5, 6]. Treatment of XDR A. baumannii is a challenging problem in the Hospital [7]. Colistin is a clinical potential choice for managing XDR A. baumannii.

Materials and methods

Patient information and bacterial isolates

Between December 2009 and October 2012, 59 non-repetitive XDR A. baumannii isolates were collected, which derived from various clinical samples (spit, blood, bile, urine, peritoneal fluid, intravenous catheter) of different wards in patients with liver disease. A. baumannii strains were identified by PHOENIX-100 automated Vitek Systems (BD, USA), and confirmed by gyrBgene. All strains were maintained at -80°C and inoculated into blood agar plates at 35°C.

Antibiotic susceptibility test and detection of resistance genes

Antibiotic susceptibility test was performed by PHOENIX-100 automated Vitek Systems. The minimal inhibitory concentrations of amikacin, gentamicin, ticarcillin-clavulanic acid, piperacillin, imipenem, meropenem, ciprofloxacin, ceftazidime, cefepime and polymyxin B were confirmed and interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2011). The quality strains were Pseudomonas aeruginosa ATCC27853 and Escherichia coli-ATCC25922. Resistance genes (VIM-1, VIM-2, SIM-1, IMP-R, GIM, SPMI, OXA-23, OXA-24, OXA-51, and OXA-58) were detected by PCR following previous method [8].

Table 1. General information in 59 XDRAB isolates

NO.	Isolates	Year	Wards	Specimen	Sex	Age
1	140558	2009	IICU	Blood	Female	49
2	140472	2009	IICU	Sputum	Male	51
3	140567	2009	IICU	Sputum	Female	52
4	141074	2009	IICU	Sputum	Female	49
5	140567	2009	IICU	Blood	Female	52
6	141182	2009	IICU	Sputum	Male	25
7	141027	2009	IICU	Sputum	Female	53
8	133401	2009	IICU	Sputum	Male	48
9	141285	2009	SICU	Sputum	Female	55
10	103560	2009	SICU	Blood	Female	45
11	48155	2009	SICU	Sputum	Female	64
12	141285	2009	General surgery	Bile	Female	55
13	48155	2010	General surgery	Blood	Female	64
14	232310	2010	Infection	Sputum	Female	49
15	145547	2010	IICU	Sputum	Female	64
16	144768	2010	IICU	Sputum	Male	80
17	134768	2010	Infection	Sputum	Female	70
18	138286	2010	Infection	Urine	Male	44
19	150081	2010	Infection	Sputum	Female	79
20	169407	2010	HICU	Sputum	Female	29
21	235780	2010	Hepatobiliary surgery	Bile	Female	63
22	40661	2010	Gastroenterology	Sputum	Female	68
23	188660	2010	HICU	Peritoneal fluid	Female	73
24	188660	2010	HICU	Peritoneal fluid	Female	61
25	193417	2010	Infection	Sputum	Female	65
26	193417	2010	Infection	Sputum	Female	65
27	18452	2011	IICU	Bile	Female	53
28	221255	2011	HICU	Blood	Female	60
29	232768	2011	IICU	Sputum	Female	52
30	240833	2011	General surgery	Blood	Female	54
31	248631	2011	IICU	Sputum	Female	50
32	248752	2011	IICU	Sputum	Female	19
33	248666	2011	IICU	Sputum	Female	10
34	248904	2011	Infection	Sputum	Female	10
35	242977	2011	Oncology	Sputum	Male	60
36	248631	2011	IICU	Sputum	Female	65
37	242889	2011	IICU	Peritoneal fluid	Female	10
38	229777	2011	General surgery	Peritoneal fluid	Female	64
39	272656	2011	IICU	Spit	Female	70
40	265082	2011	HICU	Peritoneal fluid	Male	57
41	273174	2011	HICU	Sputum	Male	32
42	132910	2012	IICU	Blood	Male	88
43	253584	2012	Infection	Sputum	Female	54
44	276604	2012	IICU	Sputum	Male	54
45	275753	2012	IICU	Sputum	Female	44
46	278758	2012	SICU	Sputum	Female	53
47	265014	2012	SICU	Blood	Female	52
48	283826	2012	IICU	Sputum	Female	78

49	283878	2012	Infection	Intravenous catheter	Female	62
50	248014	2012	IICU	Sputum	Male	73
51	252822	2012	IICU	Sputum	Female	47
52	279589	2012	IICU	Sputum	Female	66
53	296867	2012	Infection	Sputum	Male	12
54	247588	2012	HICU	Blood	Female	84
55	300522	2012	Urinary surgery	Urine	Female	62
56	313034	2012	Hepatobiliary surgery	Sputum	Male	64
57	324410	2012	HICU	Sputum	Male	71
58	326010	2012	IICU	Sputum	Female	76
59	283354	2012	IICU	Sputum	Female	55

Abbreviations: Infectious Intensive Care Unit (IICU), Hepatic Intensive Care Unit (HICU).

Table 2. The results of antibiotic susceptibility in 59 XDR A B isolates

No.	Strains	Antibiotics									
		AMK	GEN	TIM	PIP	IMP	MER	CIP	CAZ	CPM	PMB
1	140558	R	R	R	R	R	R	R	R	R	S
2	140472	R	R	R	R	R	R	R	R	R	S
3	140567	R	R	R	R	R	R	R	R	R	S
4	141074	R	R	R	R	R	R	R	R	R	S
5	140567	R	R	R	R	R	R	R	R	R	S
6	141182	R	R	R	R	R	R	R	R	R	S
7	141027	R	R	R	R	R	R	R	R	R	S
8	133401	R	R	R	R	R	R	R	R	R	S
9	141285	R	R	R	R	R	R	R	R	R	S
10	103560	R	R	R	R	R	R	R	R	R	S
11	48155	R	R	R	R	R	R	R	R	R	S
12	141285	R	R	R	R	R	R	R	R	R	S
13	48155	R	R	R	R	R	R	R	R	R	S
14	232310	R	R	R	R	R	R	R	R	R	S
15	145547	R	R	R	R	R	R	R	R	R	S
16	144768	R	R	R	R	R	R	R	R	R	S
17	134768	R	R	R	R	R	R	R	R	R	S
18	138286	R	R	R	R	R	R	R	R	R	S
19	150081	R	R	R	R	R	R	R	R	R	S
20	169407	R	R	R	R	R	R	R	R	R	S
21	235780	R	R	R	R	R	R	R	R	R	S
22	40661	R	R	R	R	R	R	R	R	R	S
23	188660	R	R	R	R	R	R	R	R	R	S
24	188660	R	R	R	R	R	R	R	R	R	S
25	193417	R	R	R	R	R	R	R	R	R	S
26	193417	R	R	R	R	R	R	R	R	R	S
27	18452	R	R	R	R	R	R	R	R	R	S
28	221255	R	R	R	R	R	R	R	R	R	S
29	232768	R	R	R	R	R	R	R	R	R	S
30	240833	R	R	R	R	R	R	R	R	R	S
31	248631	R	R	R	R	R	R	R	R	R	S
32	248752	R	R	R	R	R	R	R	R	R	S
33	248666	R	R	R	R	R	R	R	R	R	S

34	248904	R	R	R	R	R	R	R	R	R	S
35	242977	R	R	R	R	R	R	R	R	R	S
36	248631	R	R	R	R	R	R	R	R	R	S
37	242889	R	R	R	R	R	R	R	R	R	S
38	229777	R	R	R	R	R	R	R	R	R	S
39	272656	R	R	R	R	R	R	R	R	R	S
40	265082	R	R	R	R	R	R	R	R	R	S
41	273174	R	R	R	R	R	R	R	R	R	S
42	132910	R	R	R	R	R	R	R	R	R	S
43	253584	R	R	R	R	R	R	R	R	R	S
44	276604	R	R	R	R	R	R	R	R	R	S
45	275753	R	R	R	R	R	R	R	R	R	S
46	278758	R	R	R	R	R	R	R	R	R	S
47	265014	R	R	R	R	R	R	R	R	R	S
48	283826	R	R	R	R	R	R	R	R	R	S
49	283878	R	R	R	R	R	R	R	R	R	S
50	248014	R	R	R	R	R	R	R	R	R	S
51	252822	R	R	R	R	R	R	R	R	R	S
52	279589	R	R	R	R	R	R	R	R	R	S
53	296867	R	R	R	R	R	R	R	R	R	S
54	247588	R	R	R	R	R	R	R	R	R	S
55	300522	R	R	R	R	R	R	R	R	R	S
56	313034	R	R	R	R	R	R	R	R	R	S
57	324410	R	R	R	R	R	R	R	R	R	S
58	326010	R	R	R	R	R	R	R	R	R	S
59	283354	R	R	R	R	R	R	R	R	R	S

Abbreviations: AMK, amikacin; GEN, gentamicin; TIM, ticarcillin-clavulanic acid; PIP, piperacillin; IMP, imipenem; MEM, meropenem; CIP, ciprofloxacin; CAZ, ceftazidime; CPM, cefepime; PMB, polymyxin B; A. baumannii, Acinetobacter baumannii.

Table 3. Resistant genotyping in 59XDRAB isolates

NO.	Isolates	Resistant genes									
		OXA-23	OXA-24	OXA-51	OXA-58	VIM-1	VIM-2	SIM-1	IMP-R	GIM	SPMI
1	140558	+	-	+	+	-	-	-	-	+	-
2	140472	+	-	+	-	-	-	-	-	+	-
3	140567	+	-	+	+	-	-	-	-	+	-
4	141074	+	-	+	+	-	-	-	-	+	-
5	140567	+	-	+	+	-	-	-	-	+	-
6	141182	+	-	+	+	-	-	-	-	+	-
7	141027	+	-	+	-	-	-	-	-	+	-
8	133401	+	-	+	-	-	-	-	-	+	-
9	141285	+	-	+	+	-	-	-	-	+	-
10	103560	+	-	+	-	-	-	-	-	+	-
11	48155	+	-	+	+	+	+	-	-	+	-
12	141285	+	-	+	+	+	+	-	-	+	-
13	48155	+	-	+	+	+	+	-	-	+	-
14	232310	+	-	+	+	-	-	-	-	+	-
15	145547	+	-	+	-	-	-	-	-	+	-
16	144768	+	-	+	+	-	-	-	-	+	-
17	134768	+	-	+	+	-	+	-	-	+	-

18	138286	+	-	+	+	-	+	-	-	+	-
19	150081	+	-	+	+	-	+	-	-	+	-
20	169407	+	-	+	+	-	-	-	-	+	-
21	235780	+	-	+	-	-	-	-	-	+	-
22	40661	+	-	+	+	-	-	-	-	+	-
23	188660	+	-	+	+	-	-	-	-	+	-
24	188660	+	-	+	+	-	-	-	-	+	-
25	193417	+	-	+	-	-	-	-	-	+	-
26	193417	+	-	+	+	-	-	-	-	+	-
27	18452	+	-	+	+	-	-	-	-	+	-
28	221255	+	-	+	-	-	-	-	-	+	-
29	232768	+	-	+	+	-	-	-	-	+	-
30	240833	+	-	+	+	-	-	-	-	+	-
31	248631	+	-	+	+	-	-	-	-	+	-
32	248752	+	-	+	+	-	-	-	-	+	-
33	248666	+	-	+	+	-	+	-	+	+	-
34	248904	+	-	+	+	-	+	-	+	+	-
35	242977	+	-	+	+	-	+	-	+	+	-
36	248631	+	-	+	+	-	+	-	+	+	-
37	242889	+	-	+	+	-	+	-	+	+	-
38	229777	+	-	+	+	-	+	-	+	+	-
39	272656	+	-	+	+	-	+	-	+	+	-
40	265082	+	-	+	+	-	+	-	+	+	-
41	273174	+	-	+	+	+	+	-	+	+	+
42	132910	+	+	+	+	+	+	-	+	+	+
43	253584	+	+	+	+	+	+	-	+	+	+
44	276604	+	+	+	+	+	+	-	+	+	+
45	275753	+	+	+	+	+	+	-	+	+	+
46	278758	+	+	+	+	+	+	-	+	+	+
47	265014	+	+	+	+	+	+	-	+	+	+
48	283826	+	+	+	+	+	+	-	+	+	+
49	283878	+	+	+	+	-	-	-	+	+	-
50	248014	+	-	+	+	-	-	-	+	+	-
51	252822	+	-	+	+	-	-	-	+	+	-
52	279589	+	+	+	+	-	-	-	+	+	-
53	296867	+	+	+	+	-	-	-	+	+	-
54	247588	+	+	+	+	-	-	-	+	+	-
55	300522	+	-	+	+	-	-	-	+	+	-
56	313034	+	-	+	+	-	-	-	+	+	-
57	324410	+	-	+	+	-	-	-	-	+	-
58	326010	+	+	+	+	-	-	-	+	+	+
59	283354	+	-	+	+	-	-	-	-	+	-

Multilocus sequence typing (MLST)

MLST sequences of seven housekeeping genes (gltA, gyrB, gdhB, recA, cpn60, gpi, rpoD) was analyzed as described previously [9]. Sequence types (STs) were assigned according to the A. baumannii MLST database (http://pubmlst.org/abaumannii/). The new STs were submitted to the above databases.

Statistical analysis

Data were shown as range, median or a proportion of all patients. Fisher's exact test was

Table 4. Molecular typing in 59 XDRAB isolates

NO.	Isolates	MLST	Allelic profiles						
		ST	gltA	gyrB	gdhB	recA	cpn60	gpi	rpoD
1	140558	464	1	3	3	2	46	142	3
2	140472	425	1	3	3	2	2	100	3
3	140567	425	1	3	3	2	2	100	3
4	141074	425	1	3	3	2	2	100	3
5	140567	425	1	3	3	2	2	100	3
6	141182	425	1	3	3	2	2	100	3
7	141027	425	1	3	3	2	2	100	3
8	133401	425	1	3	3	2	2	100	3
9	141285	464	1	3	3	2	2	100	3
10	103560	464	1	3	3	2	46	142	3
11	48155	425	1	3	3	2	46	142	3
12	141285	425	1	3	3	2	2	100	3
13	48155	351	1	3	3	2	2	100	3
14	232310	STx7	1	17	3	39	2	143	7
15	145547	373	1	3	3	2	46	106	3
16	144768	218	1	12	12	11	4	103	3
17	134768	STx5	1	3	3	2	2	102	3
18	138286	STx2	1	3	3	2	46	102	3
19	150081	208	1	3	3	29	46	97	3
20	169407	425	1	3	3	29	2	97	3
21	235780	467	1	3	3	2	2	100	3
22	40661	STx6	1	3	107	2	46	97	3
23	188660	STx2	1	102	107	2	2	102	3
24	188660	191	1	3	3	29	46	97	3
25	193417	191	1	3	3	2	2	94	3
26	193417	STx4	1	3	3	2	2	94	3
27	18452	381	1	3	3	2	46	94	3
28	221255	381	1	81	3	2	2	16	3
29	232768	381	1	81	3	2	2	16	3
30	240833	381	1	81	3	2	2	16	3
31	248631	381	1	81	3	2	2	16	3
32	248752	381	1	81	3	2	2	16	3
33	248666	381	1	81	3	2	2	16	3
34	248904	381	1	81	3	2	2	16	3
35	242977	381	1	81	3	2	2	16	3
36	248631	381	1	81	3	2	2	16	3
37	242889	381	1	81	3	2	2	16	3
38	229777	STx8	1	81	3	2	2	16	3
39	272656	368	1	3	3	2	2	94	3
40	265082	STx1	1	3	3	2	2	140	3
41	273174	492	51	3	3	2	46	119	3
42	132910	492	1	3	3	2	2	117	3
43	253584	492	1	3	3	2	2	117	3
44	276604	492	1	3	3	2	2	117	3
45	275753	492	1	3	3	2	2	117	3
46	278758	492	1	3	3	2	2	117	3
47	265014	STx1	1	3	3	2	2	117	3

used. Data analyses were performed by using the SPSS 13.0 software (SPSS Inc., USA).

Results

Clinical characteristics of patients

From December 2009 and October 2012, all 59XDR A. baumannii isolates from patients with liver disease were selected. Among these isolates, Thirty-eight (64.4%) isolates was from specimen of Sputum, Nine (15.3%) was from blood, intravascular catheters, five (8.5%) was from peritoneal fluid, three (5.1%) was from bile, two (3.4%) was from urine, one (1.7%) was from intravenous catheter, one (1.7%) was from spit. 59 patients (43 males and 16 females) had a median age of 55 years (from 10 to 88 years). Thirty-eight (64.4%) were in the ICU, 15 (25.4%) were in medical wards, and 6 (10.2%) were in surgical wards (**Table 1**).

Antibiotic susceptibilities

In our study, Antibiotic susceptibilities of XDR A. baumannii were tested by VITEK-2 and PHOENIX-100 system. All 59XDR A. baumannii isolates displayed 100% resistance to amikacin, gentamicin, ticarcillin-clavulanic acid, piperacillin, imipenem, meropenem, ciprofloxacin, ceftazidime, cefepime. In addition, they displayeda high susceptibility to polymyxin B (100%) (**Table 2**).

Detection of resistance related genes

In this study, All 59 isolates from 2009 to 2012 carried the OXA-23, OXA-51 and GIM genes. 51 isolates (86.4%) had the OXA-58 gene, 35 isolates (59.3%) had the IMP-R gene and 22 isolates (37.3%) had the VIM-2 gene. OXA-24, VIM-1 and SPMI genes were positive in 20.3%, 18.6% and 15.3% of XDR A. baumannii, respectively. No SIM-1 gene was found (**Table 3**).

MLST genotypic results

The sequence typing of all 59 isolates was revealed in **Table 4**. The unas-

48	283826	STx1	51	3	3	2	46	119	3
49	283878	492	51	3	3	2	46	119	3
50	248014	492	1	3	3	2	2	117	3
51	252822	492	1	3	3	2	2	117	3
52	279589	492	1	3	3	2	2	117	3
53	296867	492	1	3	3	2	2	117	3
54	247588	492	1	3	3	2	2	117	3
55	300522	STx1	1	3	3	2	2	117	3
56	313034	STx1	51	3	3	2	46	119	3
57	324410	STx3	51	3	3	2	46	119	3
58	326010	STx1	51	3	3	2	2	119	3
59	283354	425	51	3	3	2	46	119	3

signed STs were given consecutive numbers (STx1, STx2, STx3, etc.). MLST analysis showed ST381 (11 isolates, 18.6%), ST425 (11 isolates, 18.6%) and ST492 (12 isolates, 20.3%) were the predominant clones. 45 isolates determinately belonged to ST191 (2/45), ST208 (1/45), ST218 (1/45), ST351 (1/45), ST368 (1/45), ST373 (1/45), ST381 (11/45), ST425 (11/45), ST464 (3/45), ST467 (1/45), and ST492 (12/45). Other 14 isolates genotypes were still unknown STs.

Discussion

A. baumannii firstly emerged in Brooklyn, New York in 1997 [10]. From then on, the incidence of XDR A. baumannii has gradually increased in worldwide. Its frequent emergence result in nosocomial outbreaks. Se-veral nosocomial infections caused by XDR A. baumannii clones have been reported in some hospitals [11]. In the present study, we inve-stigated the molecular characteristics of XDR A. Baumannii from patients with liver disease during a 3-year period in our hospital.

In this study, all 59 A. baumannii from different clinical specimens were resistant to amikacin, gentamicin, ticarcillin-clavulanic acid, piperacillin, imipenem, meropenem, ciprofloxacin, ceftazidime, cefepime, but susceptible to polymyxin B. Therefore, they were designated XDR A. baumannii [12].

Previous studies have shown that carbapenem resistant A. baumannii harbored OXA-58 and OXA-23 genes in European countries [13]. Our work Screened for some genes encoding metallo-beta-lactamases (MBL) (VIM-1, VIM-2, SIM-1, IMP-R, GIM and SPMI) and OXA-type genes (OXA-23, OXA-24, OXA-51, and OXA-58 genes). The presence of OXA-type enzymes was associated with carbapenem resistance in all study isolates. Meanwhile, MBL genes are probably responsible for Beta-lactam antibiotic resistance according to the previous findings [14].

In addition, we carried out MLST to examine molecular typing of XDRA. Baumannii isolates in patients with liver disease. MLST analysis displayed ST381, ST425 and ST492 were the prevalent clones of XDR A. baumannii from 2009 to 2012. In earlier studies, ST92 and ST69 isolates have been described as major clones in carbapenem-resistant A. baumannii [15, 16]. The above reports are inconsistent with our results, which was possibly due to all XDR A. baumannii in our study.

We explored the drug resistance and molecular typing of XDRA. baumannii isolates from patients with liver disease. The major clones, ST381, ST425 and ST492, have disseminated in patients with liver disease.

Disclosure of conflict of interest

None.

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