

Supplementary Materials and Methods

Patient data. Patients who suffered breakthrough bacteremia due to the combination of CRAb and a carbapenem-susceptible pathogen during carbapenem therapy were identified from a database of *A. baumannii* bacteremia collected in Taipei Veterans General Hospital from 2000 to 2008. Sources of bacteremia were determined according to recommendations by the U.S. CDC (1).

Supplementary Table 1 Clinical characteristics of patients with polymicrobial bacteremia caused by carbapenem-resistant *Acinetobacter baumannii* and other carbapenem-susceptible bacteria during carbapenem therapy

Patient No.	Age/sex	Underlying conditions	Source of bacteremia	<i>A. baumannii</i> /CHDL gene and upstream IS ^a /plasmid location/ IPM MIC (mg/L)	Concomitant bacteria/ IPM MIC (mg/L)	Antimicrobial therapy ^b	Patient outcome ^c
1	61/M	DM. Hepatitis C and alcohol-related liver cirrhosis	Pneumonia	Ab1969/ISAba3-IS1 008-	<i>Escherichia coli</i> Ec1003/ 0.125	Tigecycline and sulbactam	Died on day 5

		(Child C). Receipt liver transplantation.		<i>bla</i> _{OXA-58} /11-kb plasmid/64			
2	64/F	RA with methotrexate and etanercept therapy. Disseminated TB. Small intestinal perforation.	Pneumonia	Ab0509/IS <i>Aba1</i> - <i>bla</i> _{OXA-82} / 50-kb plasmid/16	<i>Klebsiella pneumoniae</i> KpTL425/0.25	IPM and amikacin	Died on day 8
3	78/M	Aortic dissection with 2terna wound infection. Hypertension. CAD. Chronic renal failure.	Surgical wound	Ab0505/IS <i>Aba1</i> - <i>bla</i> _{OXA-82} / 50-kb plasmid/32	<i>Pseudomonas aeruginosa</i> PaTL424/2	Nil	Died on day 2
4	72/M	ESRD, under regular haemodialysis. Hypertension. CAD. Old CVA.	Pneumonia	Ab1203/IS <i>Aba1</i> - <i>bla</i> _{OXA-172} /50 kb plasmid/32	<i>Proteus mirabilis</i> Pm1231/ 1; ESBL-producing <i>E. coli</i> Ec1231/1	Nil	Died on same day

CHDL, carbapenem-hydrolysing class D β -lactamase; IS, insertion sequence; IPM, imipenem; M, male; DM, diabetes mellitus; F, female; RA, rheumatoid arthritis; TB, tuberculosis; CAD, coronary arterial disease; ESRD, end-stage renal disease; CVA, cerebral vascular accident; ESBL, extended-spectrum β -lactamase.

^a The CHDL genes were amplified with LA Taq polymerase (Takara Shuzou, Otsu, Japan), TA cloned into the pCR-TOPOII vector (Invitrogen, Carlsbad, CA), and sequenced by Mission Biotech (Taipei, Taiwan). The localization of the CHDL gene on plasmid was performed as previously described (2).

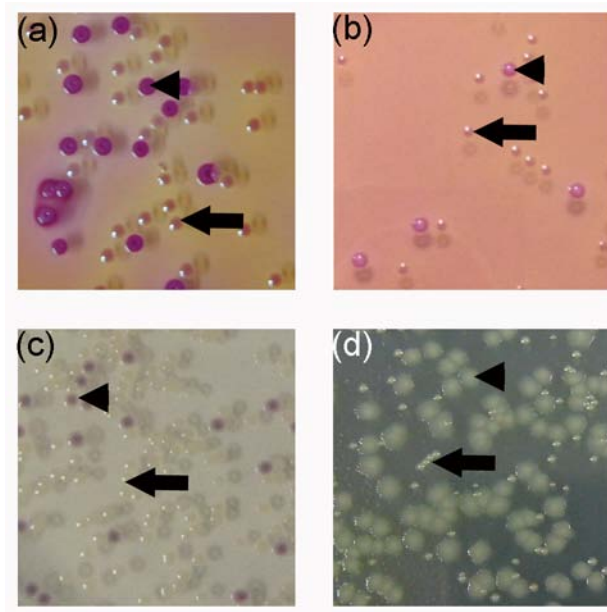
^b Antibiotics that were prescribed after culture was available

^c After bacteremia onset

Supplementary Table 2. Primers used in this study^a

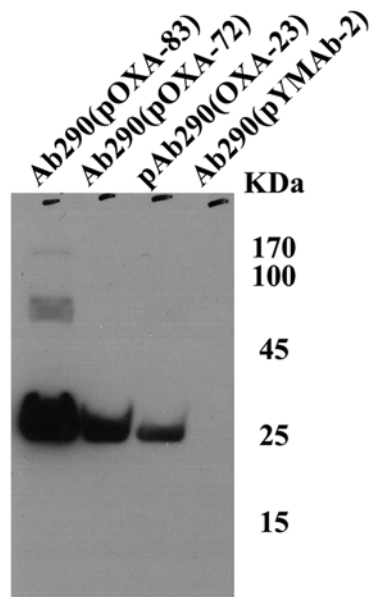
Primers	Sequences, 5' – 3'
IS1008(<i>Xba</i> I)F	tctagaTCTATTTGCAACAGTGCCATTTTTCTT
IS1008 (M- <i>Bam</i> HI)R	ggatccCATGATATACAACCTTTAAAATTTGG
OXA-58 (<i>Xho</i> I)R	ctcgagTTATAAATAATGAAAAACACC
IS1008-ΔP2-1(<i>Xba</i> I)F	tctagaTTGCAACAGTGCCATTTTTCTT
IS1008-ΔP2-2(<i>Xba</i> I)F	CTAGtctagaAACAGTGCCATTTTTCTTTATAC
IS1008-ΔP2-3(<i>Xba</i> I)F	CTAGtctagaACAGTGCCATTTTTCTTTATACTAT
ISAbal(<i>Xba</i> I)F	tctagaCACGAATGCAGAAGTTG
OXA-23-like(<i>Xho</i> I)R	ctcgagAATAATATTCAGCTGTTTTAATG
OXA-24(<i>Xba</i> I)F	tctagaCTCTAAGCCCCAAAATTCC
OXA-24-like(<i>Xho</i> I)R	ctcgagAATGATTCCAAGATTTTCTAGCG
OXA-51-like(<i>Xho</i> I)R	ctcgagTAAAATACCTAATTGTTCTAA
OXA-58-21(<i>Bam</i> HI)F	ggatccGAGCATAGTATGAGTCGA
OXA-58-21(<i>Xho</i> I)R	ctcgagTTATAAATAATGAAAAACACC
Gyrase-A(<i>Kpn</i> I)P1	ggtaccAAGCATTCATATTTAGATTAC
Gyrase-A(<i>Pst</i> I)P2	ctcgagCTCTTTTACTAACTCGGC

^a Lower-case letters indicate restriction sites.

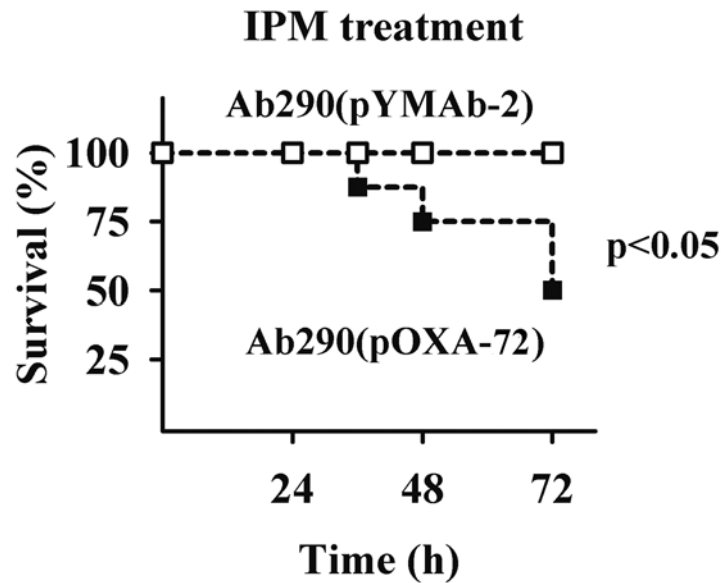


Supplementary FIG 1. Differentiation of cocultured bacteria in different agar plates.

Bacteria cocultured in broth containing imipenem were differentiated by their colony characteristics on appropriate agar plates. *Acinetobacter baumannii* strain Ab290 (pOXA-58-2) (arrow) was differentiated from *Escherichia coli* strain Ec1003 (A, arrowhead) and *Klebsiella pneumoniae* strain KpTL425 (B, arrowhead) on MacConkey agar (Becton Dickinson and Company, Franklin Lakes, NJ). Ab290 (pOXA-58-2) (arrow) was differentiated from *Enterobacter cloacae* strain EntC YT240 (C, arrowhead) on Harlequin *E. coli*/Coliform medium (LabM, Lancashire, UK) and from *Pseudomonas aeruginosa* strain PaTL424 (D, arrowhead) on Mueller-Hinton agar (Becton Dickinson and Company).



Supplementary FIG 2. Detection of other carbapenem-hydrolysing class D β -lactamases in extracellular fractions from *A. baumannii*. Extracellular localizations of His-tagged OXA-83, OXA-72, and OXA-23 were detected using an anti-His antibody. Extracellular fractions of transformants bearing the shuttle vector pYMAb-2 were used as negative controls.



Supplementary FIG 3. Co-infection with *Escherichia coli* and *Acinetobacter baumannii* producing OXA-72 enhances the pathogenesis of polymicrobial infection in the presence of carbapenem therapy. The 48-h mortality was significantly higher in mice co-infected with Ec1003 and Ab290(pOXA-72) than in those co-infected with Ec1003 and Ab290 carrying a shuttle vector (pYMAb-2).

References

1. **Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM.** 1988. CDC definitions for nosocomial infections, 1988. *Am J Infect Control* **16**:128-140.
2. **Chen TL, Chang WC, Kuo SC, Lee YT, Chen CP, Siu LK, Cho WL, Fung CP.** 2010. Contribution of a plasmid-borne *bla*_{OXA-58} gene with its hybrid promoter provided by IS1006 and an IS*Aba3*-like element to beta-lactam resistance in *Acinetobacter* genomic species 13TU. *Antimicrobial agents and chemotherapy* **54**:3107-3112.