

**Figure S1.** Determination of colony forming units (CFU) in a 0.4 to 0.6 McFarland suspension. Three bacterial strains (*E. coli* 1103, *B. melitensis* reference strain ATCC 23456 and *S. aureus*) were diluted to McFarland standards between 0.4 to 0.6 and afterwards, the corresponding OD<sub>600</sub> were determined. 10-fold serial dilutions in 0.9% NaCl were made and 100 µl of each dilution were plated on Colombia agar plates. Subsequently, colonies were counted and the number of CFU/ml in the original undiluted solution was calculated.

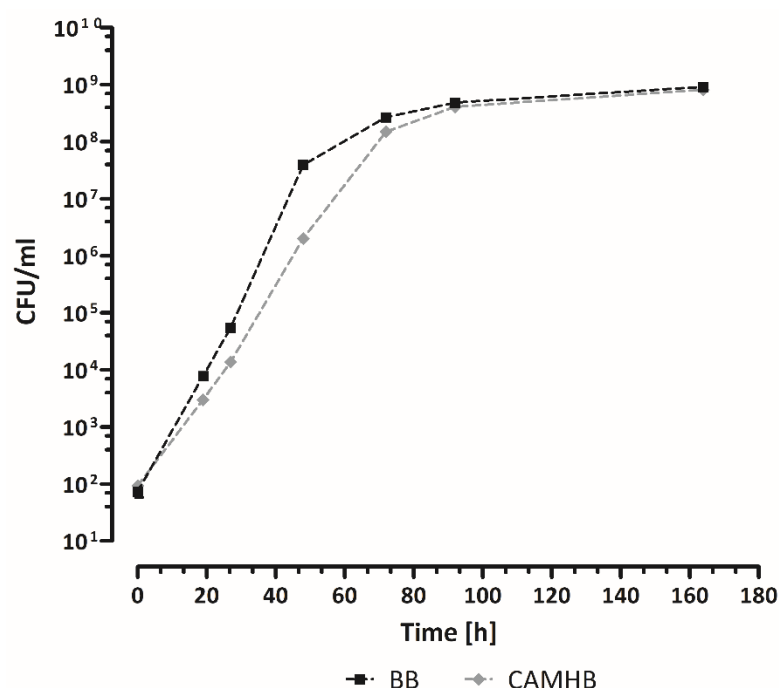
**Table S1.** Comparison of five different culture media recommended for fastidious organism (BB, CAMHB, CAMHB-F, CAMHB-X and H- medium). BMD was performed in triplicates using CAMHB, CAMHB-F, CAMHB-X and H-medium and repeated six times for BB. MIC values were determined after incubation in a 5% CO<sub>2</sub> atmosphere for 48 h. The numbers in the table represent the range of the tested MIC values (a) *B. melitensis* reference strain ATCC 23456. (b) *B. melitensis* clinical isolate Bm150048. CIP, ciprofloxacin; LEV, levofloxacin; DOX, doxycycline; GEN, gentamicin; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; RIF, rifampicin

(a)

Antimicrobial substance	BB (mg/L)	CAMHB (mg/L)	CAMHB-X (mg/L)	CAMHB-F (mg/L)	H-Medium (mg/L)
GEN	0.5-1	0.5	0.5	0.5	0.5
STR	2-4	2	2	2	1-2
RIF	1-2	1	0.5	1-4	2
DOX	0.06-0.125	0.06	0.06	0.125-0.5	0.25-0.5
SXT	0.5-1	0.02	0.125	0.25	2
LEV	0.5	0.5	0.5	0.5	0.5
CIP	0.5	0.5	0.5	0.5	0.5

(b)

Antimicrobial substance	BB (mg/L)	CAMHB (mg/L)	CAMHB-X (mg/L)	CAMHB-F (mg/L)	H-Medium (mg/L)
GEN	0.5-2	0.25	0.25-0.5	0.5	0.5
STR	2	1	0.5-1	2	1
RIF	4	4	2	4	4
DOX	0.125-0.25	0.125	0.25	0.25	0.25-0.5
SXT	1-4	0.06	0.125-0.25	0.25-0.5	4
LEV	0.5	0.5	0.5	0.5	0.5
CIP	0.5	0.5	0.5	0.5	0.5



**Figure S2.** Growth curve of Bm150048 in two different culture media (BB, CAMHB). Each dot represents the mean value of 3 replicates. BB, *Brucella* broth; CAMHB, cation adjusted Mueller-Hinton II broth;

**Table S2.** Comparison of BMD and AD using two different culture media (BB, CAMHB). *B. melitensis* reference strain ATCC 23456 and *B. melitensis* clinical isolate Bm150048 were used in triplicates to compare MIC values obtained with BMD and AD when using (a) BB or (b) CAMHB. MIC values were obtained after incubation in a 5% CO<sub>2</sub> atmosphere for 48 h. The numbers in the table represent the MIC or range of MICs of the repeated tests. DOX, doxycycline; GEN, gentamicin; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; RIF, rifampicin

(a)

Antimicrobial substance	ATCC 23456		Bm150048	
	BMD	AD	BMD	AD
GEN (mg/L)	0.5-1	1	0.5-1	0.5-1
STR (mg/L)	2-4	4	2	2-8
RIF (mg/L)	2-4	2	4	4
DOX (mg/L)	0.06-0.125	0.25-0.5	0.25	0.25-0.5
SXT (mg/L)	0.5-1	1	0.5-1	0.5-1

(b)

Antimicrobial substance	ATCC 23456		Bm150048	
	BMD	AD	BMD	AD
GEN (mg/L)	0.5	0.25-0.5	0.25	0.25-0.5
STR (mg/L)	2	2-4	1	2-4
RIF (mg/L)	1	1	4	4
DOX (mg/L)	0.06	0.25	0.25	0.25
SXT (mg/L)	0.02	0.03-0.06	0.06	0.06-0.125

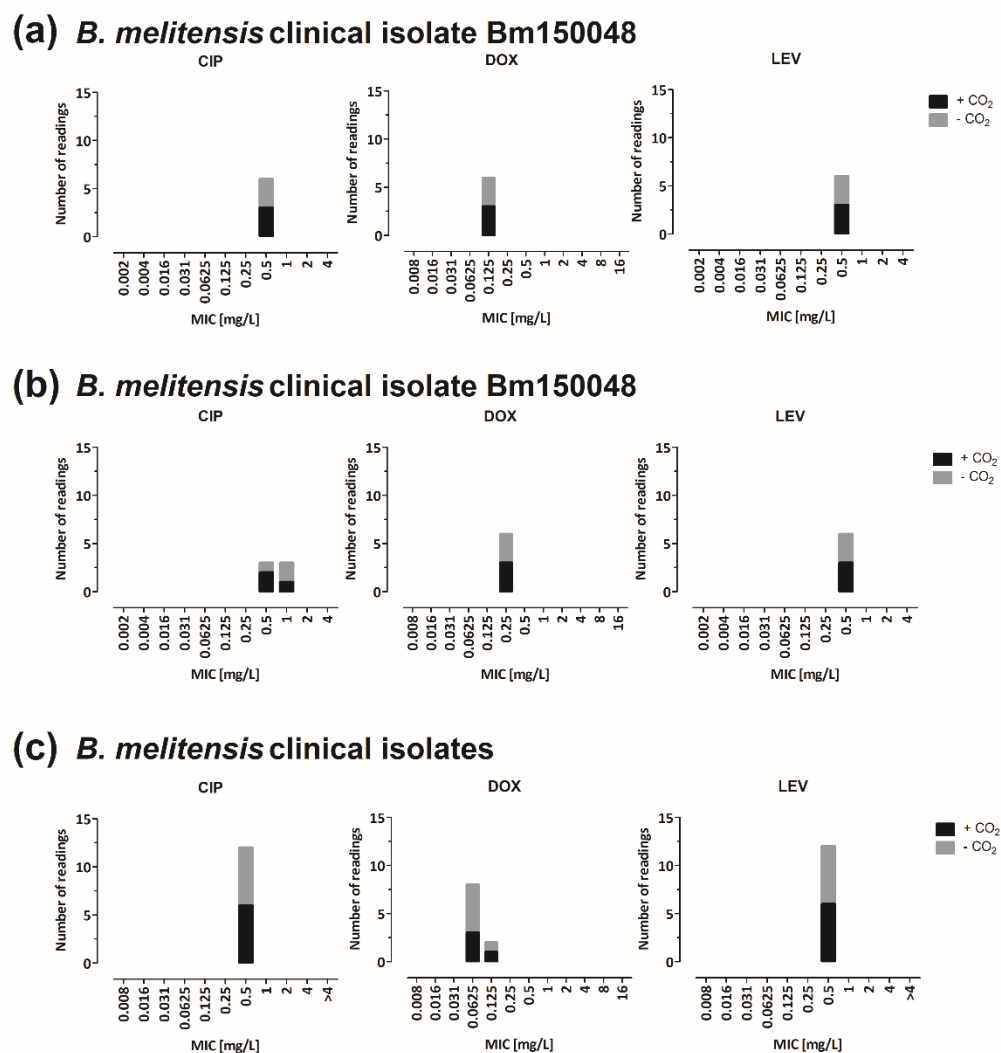
**Table S3.** Impact of incubation time on MIC values. BMD was performed in triplicates and MIC values were determined after incubation in 5% CO<sub>2</sub> for 18–64 h. The numbers in the table represent the MIC or range of MICs of the repeated tests. **(a)** CAMHB **(b)** BB. CIP, ciprofloxacin; LEV, levofloxacin; DOX, doxycycline; GEN, gentamicin; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; RIF, rifampicin

**(a)**

Antimicrobial substance	18 h	24 h	42 h	48 h	64 h
GEN (mg/L)	<0.016	<0.016	0.25	0.25	0.25
STR (mg/L)	<0.125	<0.125	0.5	1	1
RIF (mg/L)	<0.25	<0.25	2–4	4	4
DOX (mg/L)	<0.016	<0.016	0.125	0.125	0.125–0.25
SXT (mg/L)	<0.008	<0.008	0.031	0.031	0.062
LEV (mg/L)	<0.004	<0.004	0.5	0.5	0.5
CIP (mg/L)	<0.004	<0.004	0.5	0.5	0.5

**(b)**

Antimicrobial substance	18 h	24 h	42 h	48 h	64 h
GEN (mg/L)	<0.016	0.25	0.5	0.5	0.5
STR (mg/L)	<0.125	0.5–1	2	2	2
RIF (mg/L)	<0.25	2	2–4	4	4
DOX (mg/L)	<0.016	0.0625	0.125	0.125	0.25
SXT (mg/L)	<0.008	1	1–2	1–2	1–2
LEV (mg/L)	<0.004	0.25–0.5	0.5	0.5	0.5
CIP (mg/L)	<0.004	0.5	0.5	0.5	0.5



**Figure S3.** Impact of the cultivation atmosphere on MIC values. MIC values were determined in triplicates (a, b) or once (c) after incubation at  $36 \pm 1^\circ\text{C}$  with or without 5% CO<sub>2</sub> for 48 h. CAMHB was used as culture medium. (a) Reference strain ATCC 23456. (b) Clinical isolate Bm150048. (c) Six clinical isolates. CIP, ciprofloxacin; LEV, levofloxacin; DOX, doxycycline;