## Bacillus subtilis variant natto Bacteremia of Gastrointestinal Origin, Japan

## Appendix

Appendix Table. Minimum inhibitory concentrations of each antimicrobial agent and their interpretation against the Bacillus subtilis var. *natto* strains isolated from the patient\*

Antimicrobial agent	MIC (µg/mL)	Interpretation†
Penicillin G	≦0.12	S
Ampicillin	≦0.06	S
Imipenem	≦1	S
Gentamicin	≦1	S
Erythromycin	≦0.25	S
Clindamycin	≦0.25	S
Levofloxacin	≦0.5	S

\*MIC, minimum inhibitory concentration; S, susceptible; R, resistant. <sup>†</sup>Interpretation (susceptible, intermediate, or resistant) was based on the Clinical and Laboratory Standards Institute criteria (M45).

The isolate	1 GTGTTTATCCTGACATTGTGATCGGCACCTTAAGCAAAGCTGTTGGTACGGAAGGAGGTT 60	0
natto	1 GTGTTTATCCTGACATTGTGATCGGCACCTTAAGCAAAGCTGTTGGTACGGAAGGAGGTT 60	
The isolate	61 TTGCGGCTGGATCAGCGGTCTTCATCGACTTTTTGCTGAACCATGCCAGAACATTTATCT 120	6
natto	61 TTGCGGCTGGATCAGCGGTCTTCATCGACTTTTTGCTGAACCATGCCAGAACATTTATCT 120	9
The isolate	121 TTCAAACCGCTATTCCGCCAGCCAGCTGTGCGGCTGCTCACGAGGCTTTCAACATCAGAA 180	1
natto	121 TTCAAACCGCTATTCCGCCAGCCAGCTGTGCGGCTGCTCACGAGGCTTTCAACATCAGAA 180	Ģ
The isolate	181 CCAGTTTGAAGAATATGGGTTATGTGGTGAAAGGAGATCACACCGATTATTCCTGTAG 240	18
natto	181 CCAGTTTGAAGAATATGGGTTATGTGGTGAAAGGAGATCACACACCGATTATTCCTGTAG 240	6
The isolate	241 TCATTGGCGATGCCCATAAAACGGTCATATTTGCTGAAAAACTGCAGGGCAAGGGAATTT 300	
natto	241 TCATTGGCGATGCCCATAAAACGGTCATATTTGCTGAAAAACTGCAGGGCAAGGGAATTT 300	č
The isolate	301 ATGCTCCTGCCATTCGGCCGGCCGACCGTTGCGCCGGGTGAAAG 343	
natto	301 ATGCTCCTGCCATTCGGCCGGCCGACCGTTGCGCCGGGTGAAAG 343	

**Appendix Figure 1.** The isolate showed 100% homology to *Bacillus subtilis* var. *natto* standard strain in the *bioF* sequence. natto, *Bacillus subtilis* var. *natto* standard strain.

The isolate	1	GTGT	TAT		GACA	TTG	TGA	TCGG	CAC	CTT	AAG	CAA	AGC	TGT	TGGI	ACGO	GAAG	GAGGTT	60
subtilis	1	GTGT	ttgti		GACA	ttg	tga.		GCAC	ċĦ	ÅÅĠ	ĊĂĂ	GĞĊ	tgt	tggo	cccci	GAAG	GAGGTT	60
The isolate	61	TTGC	GGCTI	GGAT	CAG	CGG	TCT	TCAT	CGA	CTT	TTT	GCT	GAA	CCA	TGCC	AGA/	ACAT	TATCT	120
subtilis	61	ttgc	GGCA	GGAT	TCAG	CGG	tct	TCAT	ĊĠĂ	ĊŦŦ	+++	GCT	GAA	ĊĊĂ	tgco	AGA/	LLLL.	ttätct	120
The isolate	121	TTCA		GCTA	TTC	CGC	CAG		GCTG	TGC	GGC	TGC	TCA	CGA	GGC1	TTC	ACA'	TCA -	177
subtilis	121	ttc.		GCTA	ttc	cgc	CÁGI		sctg	tgc	GGC	tgc	tca	ĊĠĂ	GGCI	ttci	ACA-	tca -	177
The isolate	174	ATCA	GAAC	CAGI		AAG	AAT.	ATGO	GTT	ATG	GTGG	TGA	AAG	GAG	ATC/	ACAC.	ACCG.	ATTATT	233
The isolate subtilis		ATCA(      ATCA(		Ĩ	İİİ	ΠĨ	TTT	ATG(         ATG(	Ĩ	ÏĬĬ	II	ĨĨ	III	ĨIĬ	ΪĬĬ	IIII	IIII	i i i i i i	233 287
	228		GAAC	Ĩ		 AAG		ATG(      ATG( CAT/	GTT	III Atg	I I Stcg	    TGA	 AAG	 GAG	ATC/	IIII Acac.	ACCG.	i i i i i i	
subtilis	228 234	ATCA	Î I I Î Gaac Tagt	CAG CAG	I II Icto Iggo IIII	 GAAG	AAT.	CAT#	GTT GTT	ATC ATC	TCG TCG	TGA	III AAG TTG	III GAG CTG	ЦЦЦ АТС/ ААА/	ACAC	ACCG GCAGI		287 293
<i>subtilis</i> The isolate	228 234 288		TAGT GAAC TAGT TGGT	CAGI CAGI CATI LIL CATI	  CTC        GGC	 GAAG	GCC GCC GCC	CAT#	GTT GGTT	ATC ATC III CGC	TCG TCG TCA	TGA TGA 111 111 111	III AAG TTG III TTG	CTG CTG CTG CTG	ATC/ ATC/ AAA/ III AAA/	ACAC	ACCG GCAGI	GGCAAG IIIIII GGCAAG	287 293

**Appendix Figure 2.** The isolate showed a 50-nt deletion in the *bioF* sequence compared to *Bacillus subtilis* subsp. *subtilis* standard strain. subtilis, *Bacillus subtilis* subsp. *subtilis* standard strain.

The isolate	1	TGCGCTCGCTTCAAAGGTAAGCCGGCACCCGGCAGCCATTGCAGAATTATGCTGGTCGGA	60
natto	1	TGCGCTCGCTTCAAAGGTAAGCCGGCACCCGGCAGCCATTGCAGAATTATGCTGGTCGGA	60
The isolate	61	CGATCCGGATTACATAACAGGCTATGTTGCGGGTAAGAAAATGGGCTATCAGCGTATTAC 1	20
natto	61	CGATCCGGATTACATAACAGGCTATGTTGCGGGTAAGAAAATGGGCTATCAGCGTATTAC 1	20
The isolate	121		80
natto	121	AGCAATGAAAGAATACGGGACTGAAGAGGGCTGACGAGTCTTTTTTATTGATGGATCCAA 1	80
The isolate	181	TGATGTAAACACGTACATACATGATCTGGAGAAGCAGCCTATTTTAATAGAGTGGGAGG 2	239
natto	181	TGATGTAAACACGTACATACATGATCTGGAGAAGCAGCCTATTTTAATAGAGTGGGAGG 2	239

**Appendix Figure 3.** The isolate showed 100% homology to *Bacillus subtilis* var. *natto* standard strain in the *bioW* sequence. natto, *Bacillus subtilis* var. *natto* standard strain.

The isolate	1 TGCGCTCGCTTCAAAGGTAAGCCGGCACCCGGCAGCCATTGCAGAATTATGCTGGTCGGA	60
subtilis	1 TGCGCTCGCTTCAAAGGTAAGCCGGCACCCGGCAGTCGTTGCAGAATTATGCTGGTCGGA	60
The isolate	61CGATCCGGATTACATAACAGGCTATGTTGCGGGTAAGAAAATGGGCTATCAGCGTATTAC	120
subtilis	61 CGATCCGGATTACATAACAGGCTATGTTGCGGGTAAGAAAATGGGCTATCAGCGTATTAC	120
The isolate	121 AGCAATGAAAGAATACGGGACTGAAGAGGGCTGACGAGTCTTTTTTTT	180
subtilis	121 AGCAATGAAAGAATACGGGACTGAAGAGGGC <mark>tgd</mark> cgaGtCtttttttattgatggatccaa	180
The isolate	181 TGATGTAAACACGTACATACATGATCTGGAGAAGCAGCCTATTTTAATAGAGTGGGAGG	239
subtilis	181 TGATGTAAACACGTACATACATGACCTGGAGAAGCAGCCTATTTTAATAGAGTGGGAGG	239

**Appendix Figure 4.** The isolate showed a single nucleotide mutation that resulted in a termination codon and stopped amino acid synthesis compared to *B. subtilis* subsp. *subtilis* standard strain in the *bioW* sequence. subtilis, *Bacillus subtilis* subsp. *subtilis* standard strain.