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APPENDICES

APPENDIX I

REAGENTS AND INSTRUMENTS

A. REAGENT

Absolute ethanol	(Merck,U.S.A)
Methanol	(Merck, U.S.A)
Agarose (ultrapure)	(Biorad, U.S.A)
EDTA	(Amresco, U.S.A)
Ethidium bromide	(Amresco, U.S.A)
Glacial acetic acid	(Merck, Germany)
Sodium hydroxide	(Merck, Germany)
Mineral oil	(Sigma, U.S.A)
Mueller-Hinton agar	(Oxoid, England)
Na_2HPO_4	(Sigma, U.S.A)
KH_2PO_4	(Sigma, U.S.A)
Tris (ultrapure)	(Amresco, U.S.A)
Middlebrook 7H11 agar base	(BBL, U.S.A)
Middlebrook 7H9 broth	(Difco, U.S.A)

B. MATERIAL

X-ray film	(Kodak, Japan)

C. INSTRUMENTS

BACTEC MGIT 960 system	(Becton Dickinson, U.S.A)
Water bath	(Memmert, U.S.A)
Incubator	(Forma Scientific, U.S.A)
Microcentrifuge	(Eppendorf, U.S.A)

APPENDIX I (CONTINUED)

Perkin Elmer GeneAmp PCR system 9600	(Perkin Elmer, U.S.A)
ABI Prism^{TM} 310 Automate sequencer	(Perkin Elmer, U.S.A)

APPENDIX II

MEDIA, SOLUTION FOR CULTURE AND SUSCEPTIBILITY TESTING

1. Ogawa media

Mineral salt solution

Potassium dihydrogen phosphate anhydrous (KH_2PO_4)	3.0	g
Sodium glutamate	3.0	g
Distilled water	300	ml
Glycerine	18	ml
2%Malachite green solution	18	ml
Homogenised whole eggs	600ml (12-16 eggs)	

Autoclaving mineral salt solution at 121°C for 15 minutes to sterilise. Cool to room temperature. The following ingredients are aseptically pooled in a large, sterile flask and mixed well: glycerine, 2% malachite green solution, homogenised whole eggs. The medium is mixed well and distributed in 6-8 ml volumes in sterile 20x150 mm screw-capped test tubes. Place the bottles in a slanted position in the inspissator and coagulate the medium for 45 minutes at 80-85 °C. Cool and store at 4 °C until used.

2. Middlebrook 7H9 broth with 5% oleic acid–albumin-dextrose-catalase (OADC)

Middlebrook 7H9	4.7	g
Middlebrook OADC Enrichment	50	ml
Distilled water	950	ml

Suspend 4.7 g of the powder in 950 ml of distilled water. Autoclaving at 121°C for 15 minutes. Aseptically add 50 ml Middlebrook OADC Enrichment to the medium when cooled to 45 °C. Dispense to 4 ml per tube. Cool and store at 4 °C until used.

APPENDIX II (CONTINUED)

3. Mueller-Hinton agar with 10% OADC

Muller-Hinton agar	38	g
Middlebrook OADC Enrichment	100	ml
Distilled water	900	ml

Suspend 38 g in 900 ml of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121 °C for 15 minutes. Cool to about 50 °C and aseptically add 100 ml of Middlebrook OADC Enrichment. Dispense to 20 ml per petri dish. Cool and store at 4 °C until used.

4. Middlebrook 7H11 medium

Middlebrook 7H11 agar base	19	g
Glycerol	5	ml
Middlebrook OADC Enrichment	100	ml
Distilled water	900	ml

Suspend 4.7 g of the powder in 900 ml of distilled water containing 5 ml Glycerol. Swirl to obtain a smooth suspension without boiling, sterilize by autoclaving at 121°C for 15 minutes. Cool to about 50°C and aseptically add 100 ml of Middlebrook OADC Enrichment. Dispense to 20 ml per petri dish. Cool and store at 4 °C until used.

5. Phosphate buffer (pH 6.8)

KH_2PO_4	4.032	g
Na_2HPO4	10.14	g
Distilled water	1000	ml

The solution was sterilized by autoclaving at 121°C for 15 minutes. Cool and store at room temperature until used.

APPENDIX III

REAGENTS AND PREPARATIONS

1. 0.5 M Ethylene diamine tetraacetic acid (EDTA), pH 8.0

Dissodium ethylene diamine tetraacetate.$2H_2O$	186.1 g
DDW	800.0 ml
Adjust pH to 8.0	
Adjust volume to 1,000 ml	

2. 1 M Tris-HCl, pH 8.0

Tris (ultapure)	121.1 g
DDW	800.0 ml
Adjust to pH 8.0 by adding conc. HCl	42.0 ml
Sterililize by autoclaving	

3. 50 x Tris-acetate buffer (TAE)

Tris (ultrapure)	242.0 g
Glacial acetic acid	57.1 g
0.5 M EDTA	100.0 ml
Adjust the volume to 1,000 ml with DDW	
Sterilize by autoclaving	

4. 10 x TE buffer

Tris	12.11 g
0.5 M EDTA	20 ml

Adjust to pH 8.0 by adding conc. HCl, Adjust volume to 1,000 ml. Sterilize by autoclaving

APPENDIX III (CONTINUED)

REAGENT FOR AGAROSE GEL ELECTROPHIRESIS

1. 10 mg/ml Ethidium bromide

Ethidium bromide	1	g
DDW	100	g

Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer to a dark bottle and stored at 4°C

2. 1.5% Agarose gel

Agarose (ultrapure)	0.3	g
1 x TAE	20.0	ml
10 mg/ml Ethidium bromide	1.0	µl

3. 3 Kb DNA Ladder fragment

Prepare from stock 500 µl/ml

Add DDW 550 µl to final concentration 60 ng/ml

Use 5 µl per lane agarose gel

APPENDIX IV

MIC results determined by broth microdilution, BACTEC MGIT 960 and E test methods.

Strain	MIC (microgram/milliliter)		
	Broth microdilution	BACTEC MGIT 960	E test
MAC001	8	≤16	6
MAC002	8	≤16	8
MAC003	>256	>64	>256
MAC004	4	≤16	8
MAC005	8	≤16	8
MAC006	2	≤16	1
MAC007	8	≤16	4
MAC008	4	≤16	8
MAC009	4	≤16	8
MAC010	4	≤16	6
MAC011	2	≤16	4
MAC012	4	≤16	6
MAC013	4	≤16	8
MAC014	4	≤16	6
MAC015	1	≤16	2
MAC016	8	≤16	16
MAC017	4	≤16	8
MAC018	8	≤16	8
MAC019	4	≤16	8
MAC020	2	≤16	1
MAC021	8	≤16	4
MAC022	8	≤16	8
MAC023	8	≤16	16
MAC024	8	≤16	12
MAC025	8	≤16	8
MAC026	8	≤16	12
MAC027	4	≤16	8

APPENDIX IV (CONTINUED)

Strain	MIC (microgram/milliliter)		
	Broth microdilution	BACTEC MGIT 960	E test
MAC028	4	≤16	8
MAC029	>256	>64	>256
MAC030	0.5	≤16	0.5
MAC031	4	≤16	4
MAC032	8	≤16	16
MAC033	4	≤16	8
MAC034	1	≤16	1
MAC035	4	≤16	8
MAC036	4	≤16	6
MAC037	2	≤16	3
MAC038	4	≤16	8
MAC039	4	≤16	4
MAC040	4	≤16	6
MAC041	8	≤16	4
MAC042	1	≤16	2
MAC043	4	≤16	2
MAC044	8	≤16	16
MAC045	8	≤16	8
MAC046	0.5	≤16	4
MAC047	>256	>64	>256
MAC048	2	≤16	3
MAC049	4	≤16	8
MAC050	4	≤16	8
MAC051	8	≤16	6
MAC052	8	≤16	12
MAC053	4	≤16	8
MAC054	4	≤16	8
MAC055	4	≤16	4
MAC056	4	≤16	8

APPENDIX IV (CONTINUED)

Strain	MIC (microgram/milliliter)		
	Broth microdilution	BACTEC MGIT 960	E test
MAC057	2	≤16	6
MAC058	1	≤16	0.5
MAC059	1	≤16	1.5
MAC060	4	≤16	4
MAC061	4	≤16	6
MAC062	2	≤16	3
MAC063	4	≤16	8
MAC064	1	≤16	2
MAC065	4	≤16	8
MAC066	4	≤16	8
MAC067	2	≤16	8
MAC068	0.5	≤16	1
MAC069	2	≤16	4
MAC070	1	≤16	2
MAC071	2	≤16	4
MAC072	2	≤16	8
MAC073	4	≤16	8
MAC074	2	≤16	4
MAC075	2	≤16	4
MAC076	2	≤16	4
MAC077	4	≤16	6
MAC078	4	≤16	2
MAC079	4	≤16	8
MAC080	4	≤16	6
MAC081	4	≤16	8
MAC082	4	≤16	8
MAC083	4	≤16	3
MAC084	2	≤16	4
MAC085	2	≤16	4

APPENDIX IV (CONTINUED)

Strain	MIC (microgram/milliliter)		
	Broth microdilution	BACTEC MGIT 960	E test
MAC086	2	≤16	6
MAC087	4	≤16	2
MAC088	2	≤16	4
MAC089	2	≤16	4
MAC090	4	≤16	8
MAC091	4	≤16	6
MAC092	4	≤16	8
MAC093	4	≤16	6
MAC094	2	≤16	4
MAC095	4	≤16	4
MAC096	4	≤16	8
MAC097	2	≤16	4
MAC098	4	≤16	4
MAC099	4	≤16	4
MAC100	4	≤16	4

BIOGRAPHY

Miss Nifaridah Waba was born on January 11, 1981 in Pattani, Thailand. She graduated with bachelor degree of science in Microbiology from the Faculty of Science at Prince of Songkla University in 2002.