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APPENDICES

APPENDIX I

REAGENTS, MATERIALS AND INSTRUMENTS

A. REAGENTS

Absolute ethanol	(Merck, Germany)
Agarose	(Biorad, USA)
Brain heart infusion agar	(Oxoid, England)
EDTA	(Ameresco, USA)
Ethidium bromide	(Ameresco, USA)
Horse serum	(GibcoBRL, USA)
Trypticase soy agar	(Oxoid, England)
NaCL	(Merck, Germany)
Tris	(Ameresco, USA)

B. METATERIALS

C. INSTRUMENTS

Water bath	(Mettler, USA)
Perkin Elmer GeneAmp PCR system 9600	(Perkin Elmer, USA)
Camera Gel Doc TM MZL	(BIO-RAD, USA)
Incubator	(BIO-RAD, USA)
Microcentrifuge	(Eppendorf, USA)
Spectrophotometer	(BIO-RAD, USA)

APPENDIX II

MEDIA, SOLUTION AND IDENTIFICATION PRODURES

1. Trypticase soy agar with 5% sheep blood

Trypticase agar base	40	g/L
Sheep blood	50	ml
Distilled water	910	ml

The medium was sterilized by autoclaving at 121°C, 15 pounds/inch² pressure, for 15 minutes. The sterile medium was cooled to 50°C. Add blood into base medium. Dispense 20 ml per Petri dish. Cool and store at 4°C until used.

2. Antibiotic solution preparation

Erythromycin, final concentration 10 mg/L

Prepare a stock solution ; dissolve 0.028 g in 5.78 ml distilled water

Clarithromycin, final concentration 10 mg/L

Prepare a stock solution ; dissolve 0.028 g in 5.78 ml distilled water

Clindamycin, final concentration 10 mg/L

Prepare a stock solution ; dissolve 0.028 g in 5.78 ml distilled water

Penicillin, final concentration 10 mg/L

Prepare a stock solution ; dissolve 0.028 g in 5.78 ml distilled water

Vancomycin, final concentration 10 mg/L

Prepare a stock solution ; dissolve 0.028 g in 5.78 ml distilled water

Tetracycline, final concentration 10 mg/L

Prepare a stock solution ; dissolve 0.028 g in 5.78 ml distilled water

Ciprofloxacin, final concentration 10 mg/L

Prepare a stock solution ; dissolve 0.028 g in 5.78 ml distilled water

3. Brain heart infusion with 50% horse serum

Brain heart infusion	3.7	g.
Horse serum	100	ml.
Distilled water	100	ml.

Sterile by autoclaving at 121°C, 15 pounds/inch² pressure, for 15 minutes. Aliquot into sterile screw cap tubes (400 µl/tube). Store tubes in refrigerator at 4°C until used.

4. Sterile saline solution

Sodium chloride	8.5	g/L
Distilled water	1	L

Sterilize by autoclaving at 121°C, 15 pound/inch² pressure, for 15 minutes. Store at room temperature.

APPENDIX III

REAGENTS AND PREPARATION

1. 5X Tris-borate buffer (TBE)

Tris base	54	g/L
Boric acid	27.5	g/L
0.5 M EDTA	20	ml

Adjust volume to 1 liter with distilled water, The solution was mixed and sterilized by autoclaving at 121°C for 15 min.

2. 0.5 MEDTA (pH 8.0)

Disodium ethylene diamine tetra-acetate 2H ₂ O	186.1	g/L
Distilled water	1	L

Adjust pH to 8.0 and volume to 1 liter. Store at room temperature for no longer than 1 year.

3. 1.5% Agarose gel

Agarose	1.5	g
0.5X BE	100	ml

Dissolved by heating in microwave oven and occasional mix unit no granules of agarose are visible.

4. 6X Loading buffer 100 ml

Tris HCL	0.6	g
EDTA	1.68	g
SDS	0.5	g
Bromphenol Blue	0.1	g
Sucrose	40	g

Adjust volume to 100 ml with distilled water. Mix the solution, aliquot into 1.5 microtubes and store at 4°C.

5. Reagent for DNA Extraction

5.1 Protease K

Reconstituted of protease K (lyophilized) with 1.25 ml protease solvent, stored at -20°C

5.2 Buffer AL (Ready to used)

5.3 Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add the 25 ml of ethanol (96-100%) to buffer AW1 concentrate as indicated on the bottle.

5.4 Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the 30 ml of ethanol (96-100%) to buffer AW2 concentrate as indicated on the bottle.

5.5 Buffer AE (Ready to used)

6. Reagent for PCR product purification

Buffer PE

Buffer PE is supplied as a concentrate. Before using for the first time, add the 55 ml of ethanol (96-100%) to buffer PE concentrate as indicated on the bottle.

Buffer PB (Ready to used)



APPENDIX IV

THE RESULTS OF ALL TESTS IN THIS STUDY

**Results of antibiotic susceptibility and the presence of resistance genes of
S. pneumoniae.**

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
46-13	2.0	1.0	0.0625	<i>mef(E)/mel</i>
46-19	>512	512	256	<i>erm(B)</i>
46-20	1.0	2.0	0.0625	<i>mef(E)/mel</i>
46-29	2.0	2.0	0.0625	<i>mef(E)/mel</i>
46-31	16	8.0	0.0625	<i>mef(E)/mel</i>
46-33	0.125	0.0625	0.0625	-
46-34	>512	512	256	<i>erm(B)</i>
46-37	>512	512	512	<i>erm(B)</i>
46-42	>512	512	512	<i>erm(B)</i>
46-46	16.0	16	0.0625	<i>mef(E)/mel</i>
46-49	0.125	0.0625	0.125	-
46-50	0.0625	0.0625	0.0625	-
46-53	>512	512	512	<i>erm(B)</i>
46-55	8.0	4.0	0.125	<i>mef(E)/mel</i>
46-56	>512	512	512	<i>erm(B)</i>
46-57	2.0	4.0	0.0625	<i>mef(E)/mel</i>
46-58	8.0	8.0	0.0625	<i>mef(E)/mel</i>
46-60	8.0	8.0	0.0625	<i>mef(E)/mel</i>

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
46-61	0.0625	0.0625	0.0625	-
46-64	>512	512	512	<i>erm(B)</i>
46-66	8.0	4.0	0.125	<i>mef(E)/mel</i>
46-69	0.125	0.125	0.0625	-
46-71	0.125	0.0312	0.125	-
46-72	0.125	0.0625	0.0312	-
46-73	>512	512	512	<i>erm(B)</i>
46-75	8.0	4.0	0.125	<i>mef(E)/mel</i>
46-76	0.125	0.03125	0.0625	-
46-77	8.0	4.0	0.125	<i>mef(E)/mel</i>
46-78	8.0	4.0	0.125	<i>mef(E)/mel</i>
46-86	>512	512	512	<i>erm(B)</i>
46-90	0.125	0.0625	0.0625	-
46-93	1.0	2.0	0.03125	<i>mef(E)/mel</i>
46-96	0.0625	0.0625	0.0625	-
46-97	0.125	0.125	0.0625	-
46-98	4.0	4.0	0.0625	<i>mef(E)/mel</i>
46-99	2.0	2.0	0.03125	<i>mef(E)/mel</i>
46-100	>512	512	256	<i>erm(B)</i>
46-102	8.0	4.0	0.125	<i>mef(E)/mel</i>
46-104	0.125	0.0625	0.0625	-
46-105	>512	512	512	<i>erm(B)</i>
46-106	0.125	0.125	0.0625	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
46-108	>512	512	512	<i>erm(B)</i>
46-110	>512	512	512	<i>erm(B)</i>
46-111	>512	512	256	<i>erm(B)</i>
46-112	>512	512	512	<i>erm(B)</i>
46-113	4.0	8.0	0.125	<i>mef(E)/mel</i>
46-114	4.0	4.0	0.125	<i>mef(E)/mel</i>
46-115	0.0625	0.125	0.0625	-
46-116	8.0	8.0	0.125	<i>mef(E)/mel</i>
46-117	8.0	4.0	0.125	<i>mef(E)/mel</i>
46-118	0.125	0.0625	0.125	-
46-119	>512	512	128	<i>erm(B)</i>
46-120	0.0625	0.125	0.125	-
46-121	>512	512	512	<i>erm(B)</i>
46-122	2.0	2.0	0.0625	<i>mef(E)/mel</i>
46-123	2.0	2.0	0.0625	<i>mef(E)/mel</i>
46-124	0.125	0.0625	0.125	-
46-125	0.0625	0.03125	0.0625	-
46-126	8.0	8.0	0.125	<i>mef(E)/mel</i>
46-127	>512	512	256	<i>erm(B)</i>
46-129	>512	512	256	<i>erm(B)</i>
46-130	0.125	0.0625	0.125	-
46-131	0.0312	0.03125	0.0625	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
46-132	>512	512	512	<i>erm(B)</i>
46-133	>512	512	512	<i>erm(B)</i>
46-134	4.0	2.0	0.125	<i>mef(E)/mel</i>
46-135	>512	512	512	<i>erm(B)</i>
46-136	16.0	4.0	128	<i>erm(B)</i>
46-143	>512	512	512	<i>erm(B)</i>
46-144	0.125	0.0312	0.125	-
46-146	>512	512	512	<i>erm(B)</i>
46-147	0.125	0.0625	0.125	-
46-149	>512	512	512	<i>erm(B)</i>
46-150	>512	512	512	<i>erm(B)</i>
46-155	0.125	0.0625	0.0625	-
46-156	>512	512	128	<i>erm(B)</i>
46-158	0.125	0.0625	0.125	-
46-159	4.0	4.0	0.0625	<i>mef(E)/mel</i>
46-161	0.125	0.0625	0.125	-
46-163	0.125	0.0625	0.125	-
47-1	0.125	0.0625	0.125	-
47-13	>512	512	512	<i>erm(B)</i>
47-15	0.25	0.0312	0.0625	-
47-18	0.125	0.0625	0.125	-
47-19	8.0	2.0	0.125	<i>mef(E)/mel</i>
47-21	8.0	4.0	0.125	<i>mef(E)/mel</i>

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
47-22	16.0	8.0	0.125	<i>mef(E)/mel</i>
47-27	16.0	4.0	0.125	<i>mef(E)/mel</i>
47-33	0.125	0.0625	0.125	-
47-34	>512	512	512	<i>erm(B)</i>
47-36	0.125	0.125	0.125	-
47-39	0.125	0.0625	0.0312	-
47-40	>512	512	0.125	<i>erm(13)</i>
47-41	4.0	2.0	0.125	<i>mef(E)/mel</i>
47-42	>512	512	512	<i>erm(B)</i>
47-43	0.125	0.0625	0.0312	-
47-44	0.125	0.0625	0.0625	-
47-45	>512	512	512	<i>erm(B)</i>
47-50	>512	512	512	<i>erm(B)</i>
47-51	8.0	4.0	0.125	<i>mef(E)/mel</i>
47-53	8.0	4.0	0.125	<i>mef(E)/mel</i>
47-54	1.0	0.125	0.125	-
47-56	8.0	4.0	0.125	<i>mef(E)/mel</i>
47-57	0.125	0.125	0.125	-
47-60	>512	512	512	<i>erm(B)</i>
47-61	0.125	0.0625	0.125	-
47-62	0.125	0.0625	0.125	-
47-63	0.125	0.0625	0.125	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
47-64	0.125	0.0625	0.0312	-
47-65	8.0	4.0	0.125	<i>mef(E)/mel</i>
47-66	4.0	4.0	0.125	<i>mef(E)/mel</i>
47-67	4.0	4.0	0.125	<i>mef(E)/mel</i>
47-68	0.03125	0.0312	0.0625	-
47-69	8.0	4.0	0.125	<i>mef(E)/mel</i>
47-70	0.125	0.125	0.125	-
47-71	0.125	0.125	0.0625	-
47-72	>512	512	512	<i>erm(B)</i>
47-73	4.0	4.0	0.0625	<i>mef(E)/mel</i>
47-74	0.125	0.125	0.0625	-
47-75	0.125	0.125	0.125	-
47-76	0.125	0.0625	0.125	-
47-77	0.125	0.0625	0.125	-
47-78	0.125	0.0625	0.125	-
47-79	0.125	0.0625	0.125	-
47-80	>512	512	512	<i>erm(B)</i>
47-81	0.03125	0.0312	0.03125	<i>erm(B)</i>
47-82	0.125	0.125	0.0625	-
47-83	0.125	0.0625	0.125	-
47-84	>512	512	512	<i>erm(B)</i>
47-85	0.125	0.0625	0.125	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
47-86	0.125	0.0625	0.125	-
47-90	>512	512	512	<i>erm(B)</i>
47-91	>512	512	512	<i>erm(B)</i>
47-92	0.125	0.125	0.125	-
47-93	0.125	0.125	0.125	-
47-95	>512	512	512	<i>erm(B)</i>
47-96	>512	512	512	<i>erm(B)</i>
47-97	4.0	4.0	0.0625	<i>mef(E)/mel</i>
47-98	4.0	4.0	0.0625	<i>mef(E)/mel</i>
47-102	0.125	0.125	0.125	-
47-103	8.0	8.0	0.0625	<i>mef(E)/mel</i>
47-104	8.0	8.0	0.125	<i>mef(E)/mel</i>
47-105	>512	512	512	<i>erm(B)</i>
47-106	0.125	0.125	0.125	-
47-107	>512	512	512	<i>erm(B)</i>
47-108	>512	512	512	<i>erm(B)</i>
47-109	0.125	0.125	0.125	-
47-110	0.0625	0.0312	0.125	-
47-111	8.0	8.0	0.125	<i>mef(E)/mel</i>
47-112	0.125	0.125	0.0625	-
47-113	>512	512	512	<i>erm(B)</i>
47-114	>512	512	512	<i>erm(B)</i>

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
47-116	0.125	0.0625	0.0625	-
47-117	0.25	0.125	0.125	-
47-118	0.125	0.125	0.125	-
47-119	0.125	0.125	0.125	-
47-121	>512	512	512	<i>erm(B)</i>
47-122	>512	512	256	<i>erm(B)</i>
47-123	0.125	0.03125	0.125	-
47-126	>512	512	512	<i>erm(B)</i>
47-128	0.125	0.125	0.0312	-
47-129	0.125	0.125	0.0312	-
47-130	0.25	0.125	0.0625	-
47-136	>512	512	256	<i>erm(B)</i>
48-2	0.125	0.125	0.0625	-
48-3	0.125	0.0625	0.0625	-
48-4	0.125	0.125	0.125	-
48-5	0.125	0.125	0.0625	-
48-7	8.0	4.0	0.125	<i>mef(E)/mel</i>
48-9	0.03125	0.0312	0.03125	-
48-10	0.125	0.0625	0.0625	-
48-11	8.0	4.0	0.125	<i>mef(E)/mel</i>
48-13	0.125	0.125	0.0625	-
48-14	0.125	0.125	0.125	-
48-15	0.125	0.125	0.0625	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
48-16	8.0	8.0	0.0625	<i>mef(E)/mel</i>
48-17	>512	256	256	<i>erm(B)</i>
48-18	>512	256	512	<i>erm(B)</i>
48-19	0.125	0.125	0.0625	-
48-20	0.125	0.125	0.125	-
48-21	0.125	0.125	0.0625	-
48-22	2.0	1.0	0.0625	<i>mef(E)/mel</i>
48-23	8.0	8.0	0.125	<i>mef(E)/mel</i>
48-24	>512	512	512	<i>erm(B)</i>
48-25	0.125	0.0625	0.125	-
48-26	0.125	0.0625	0.125	-
48-27	8.0	8.0	0.125	<i>mef(E)/mel</i>
48-29	1.0	1.0	0.0625	<i>mef(E)/mel</i>
48-30	>512	512	32	<i>erm(B)</i>
48-35	2.0	4.0	0.0625	<i>mef(E)/mel</i>
48-37	0.25	0.25	0.0625	-
48-38	0.125	0.0625	0.125	-
48-39	0.125	0.125	0.125	-
48-41	0.125	0.0625	0.125	-
48-42	0.125	0.0625	0.0625	-
48-44	>512	512	256	<i>erm(B)</i>
48-45	0.125	0.125	0.125	-
48-46	0.125	0.0625	0.0625	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
48-48	8.0	8.0	0.03125	<i>mef(E)/mel</i>
48-49	1.0	1.0	0.0625	<i>mef(E)/mel</i>
48-50	0.125	0.0625	0.0625	-
48-52	>512	256	512	<i>erm(B)</i>
48-58	4.0	4.0	0.125	<i>mef(E)/mel</i>
48-59	>512	512	512	<i>erm(B)</i>
48-60	0.0625	0.125	0.0625	-
48-63	0.0625	0.0625	0.0625	-
48-67	0.125	0.125	0.0625	-
48-71	>512	512	512	<i>erm(B)</i>
48-72	2.0	4.0	0.0625	<i>mef(E)/mel</i>
48-75	0.0625	0.125	0.0625	-
48-76	1.0	4.0	0.0625	<i>mef(E)/mel</i>
48-79	0.0625	0.0625	0.0625	-
48-81	8.0	8.0	0.125	<i>mef(E)/mel</i>
48-84	>512	32	128	<i>erm(B)</i>
48-86	4.0	4.0	0.125	<i>mef(E)/mel</i>
48-87	>512	256	512	<i>erm(B)</i>
48-91	0.125	0.0625	0.0625	-
48-97	8.0	8.0	0.125	<i>mef(E)/mel</i>
48-100	4.0	8.0	0.125	<i>mef(E)/mel</i>
48-101	>512	512	512	<i>mef(E)+ erm(B)</i>

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
48-105	0.0625	0.125	0.125	-
48-107	0.0625	0.125	0.125	-
48-109	0.0625	0.0625	0.125	-
48-110	>512	512	512	<i>erm(B)</i>
48-111	0.0625	0.0625	0.125	-
48-112	0.125	0.0625	0.125	-
48-114	0.125	0.125	0.03125	-
48-115	0.125	0.125	0.125	-
48-116	8.0	4.0	0.125	<i>mef(E)/mel</i>
48-121	0.0625	0.0625	0.125	-
48-122	8.0	4.0	0.125	<i>mef(E)/mel</i>
48-123	4.0	2.0	0.125	<i>mef(E)/mel</i>
48-124	8.0	4.0	0.125	<i>mef(E)/mel</i>
48-125	0.125	256	128	-
48-126	>512	512	512	<i>erm(B)</i>
48-130	0.125	0.125	0.125	-
48-131	0.125	0.125	0.125	-
48-134	>512	512	512	<i>erm(B)</i>
48-135	>512	512	512	<i>erm(B)</i>
48-136	0.0625	0.0625	0.125	-
49-2	8.0	4.0	0.0625	<i>mef(E)/mel</i>
49-3	>512	>512	512	<i>erm(B)</i>
49-4	0.125	0.03125	0.125	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
49-5	0.125	0.03125	0.125	-
49-6	0.125	0.03125	0.125	-
49-7	4.0	2.0	0.125	<i>mef(E)/mel</i>
49-8	0.125	0.03125	0.125	-
49-9	0.125	0.03125	0.125	-
49-10	8.0	4.0	0.125	<i>mef(E)/mel</i>
49-11	8.0	2.0	0.0625	<i>mef(E)/mel</i>
49-12	4.0	4.0	0.0625	<i>mef(E)/mel</i>
49-13	8.0	4.0	0.125	<i>mef(E)/mel</i>
49-14	>512	512	512	<i>erm(B)</i>
49-15	0.125	0.03125	0.125	-
49-16	0.0625	0.0625	0.125	-
49-17	8.0	2.0	0.125	<i>mef(E)/mel</i>
49-18	>512	>512	512	<i>erm(B)</i>
49-19	4.0	2.0	0.0625	<i>mef(E)/mel</i>
49-20	0.125	0.03125	0.125	-
49-21	0.0625	0.03125	0.0625	-
49-22	0.125	0.0312	0.031	-
49-23	0.0625	0.0625	0.125	-
49-24	0.03125	0.03125	0.0625	-
49-25	2.0	2.0	0.125	<i>mef(E)/mel</i>
49-26	0.125	0.03125	0.0625	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
49-27	0.125	0.0312	0.125	-
49-28	0.0625	0.03125	0.0625	-
49-29	>512	>512	512	<i>erm(B)</i>
49-30	>512	>512	512	<i>erm(B)</i>
49-31	>512	>512	512	<i>erm(B)</i>
49-32	8.0	4.0	0.0625	<i>mef(E)/mel</i>
49-33	>512	>512	512	<i>erm(B)</i>
49-34	>512	>512	512	<i>erm(B)</i>
49-35	>512	>512	512	<i>erm(B)</i>
49-36	>512	>512	512	<i>erm(B)</i>
49-38	>512	>512	512	<i>erm(B)</i>
49-39	0.125	0.03125	0.125	-
49-41	0.125	0.03125	0.125	-
49-42	0.125	0.03125	0.125	-
49-43	0.125	0.0625	0.125	-
49-44	4.0	2.0	0.0625	<i>mef(E)/mel</i>
49-45	4.0	2.0	0.125	<i>mef(E)/mel</i>
49-46	0.125	0.0625	0.125	-
49-47	4.0	2.0	0.125	<i>mef(E)/mel</i>
49-48	>512	512	512	<i>erm(B)</i>
49-49	>512	>512	512	<i>erm(B)</i>
49-50	>512	>512	512	<i>erm(B)</i>
49-51	0.125	0.03125	0.125	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
49-52	4.0	8.0	0.125	<i>mef(E)/mel</i>
49-53	0.125	0.125	0.125	-
49-54	2.0	2.0	0.125	<i>mef(E)/mel</i>
49-55	0.125	0.03125	0.125	-
49-56	0.0625	0.03125	0.125	-
49-57	4.0	2.0	0.125	<i>mef(E)/mel</i>
49-58	4.0	2.0	0.125	<i>mef(E)/mel</i>
49-59	0.0312	0.0312	0.125	-
49-60	0.0312	0.0625	0.25	-
49-61	0.0625	0.0625	0.125	-
49-62	>512	512	512	<i>erm(B)</i>
49-63	4.0	8.0	0.125	<i>mef(E)/mel</i>
49-64	>512	512	256	<i>erm(B)</i>
49-65	>512	512	512	<i>erm(B)</i>
49-66	2.0	4.0	0.0312	<i>mef(E)/mel</i>
49-68	2.0	4.0	0.0625	<i>mef(E)/mel</i>
49-69	4.0	2.0	0.125	<i>mef(E)/mel</i>
49-70	0.125	0.0312	0.125	-
49-71	0.125	0.0625	512	-
49-72	>512	512	512	<i>erm(B)</i>
50-1	8.0	4.0	0.125	<i>mef(E)/mel</i>
50-2	0.125	0.0625	0.125	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
50-3	0.0625	0.03125	0.125	-
50-4	0.0625	0.0625	0.125	-
50-5	0.125	0.0625	0.0625	-
50-6	0.03125	0.03125	0.0625	-
50-7	0.03125	0.03125	0.125	-
50-8	2.0	2.0	0.125	<i>mef(E)/mel</i>
50-9	0.125	0.125	0.125	-
50-10	0.125	0.125	0.125	-
50-11	0.125	0.0625	0.125	-
50-12	>512	512	>512	<i>erm(B)</i>
50-13	4.0	4.0	0.125	<i>mef(E)/mel</i>
50-14	0.25	0.125	0.125	-
50-15	0.25	0.03125	0.0625	-
50-16	0.25	0.25	0.0625	-
50-17	0.125	0.125	0.0625	-
50-18	>512	512	512	<i>erm(B)</i>
50-19	4.0	4.0	0.125	<i>mef(E)/mel</i>
50-21	8.0	8.0	0.125	<i>mef(E)/mel</i>
50-22	0.125	0.125	0.125	-
50-23	0.06125	0.125	0.0625	-
50-24	1.0	4.0	0.0625	<i>mef(E)/mel</i>
50-25	4.0	2.0	0.0625	<i>mef(E)/mel</i>
50-26	0.25	0.125	0.125	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
50-27	0.25	0.125	0.125	-
50-28	4.0	4.0	0.125	<i>mef(E)/mel</i>
50-29	0.06125	0.125	0.125	-
50-30	2.0	2.0	0.125	<i>mef(E)/mel</i>
50-31	2.0	8.0	0.125	<i>mef(E)/mel</i>
50-32	4.0	2.0	0.125	<i>mef(E)/mel</i>
50-33	4.0	4.0	0.125	<i>mef(E)/mel</i>
50-34	0.06125	0.125	0.125	-
50-35	0.125	0.125	0.125	-
50-36	0.125	0.125	0.125	-
50-37	0.06125	0.125	0.125	-
50-39	0.06125	0.125	0.125	-
50-40	0.125	0.125	0.125	-
50-41	8.0	2.0	0.125	<i>mef(E)/mel</i>
50-42	0.125	0.125	0.125	-
50-43	0.125	0.125	0.125	-
50-44	0.125	0.125	0.0625	-
50-46	0.125	0.125	0.125	-
50-47	8.0	4.0	0.0625	<i>mef(E)/mel</i>
50-48	8.0	4.0	0.125	<i>mef(E)/mel</i>
50-49	4.0	4.0	0.125	<i>mef(E)/mel</i>
50-50	4.0	2.0	0.0625	<i>mef(E)/mel</i>

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
50-51	8.0	4.0	0.125	<i>mef(E)/mel</i>
50-52	8.0	8.0	0.0625	<i>mef(E)/mel</i>
50-53	0.125	0.125	0.125	-
50-54	8.0	2.0	0.125	<i>mef(E)/mel</i>
50-55	8.0	4.0	0.125	<i>mef(E)/mel</i>
50-56	>512	>512	512	<i>erm(B)</i>
50-57	>512	>512	512	<i>erm(B)</i>
50-58	>512	64	32	<i>erm(B)</i>
50-59	0.25	0.0625	0.125	-
50-60	>512	>512	512	<i>erm(B)</i>
50-61	>512	512	512	<i>erm(B)</i>
50-63	0.125	0.03125	0.125	-
50-64	>512	>512	512	<i>erm(B)</i>
50-65	0.125	0.125	0.125	-
50-66	>512	512	128	<i>erm(B)</i>
50-67	0.03125	0.0625	0.0625	-
50-68	2.0	2.0	0.125	<i>mef(E)/mel</i>
50-69	0.125	0.125	0.125	-
50-70	2.0	2.0	0.0625	<i>mef(E)/mel</i>
50-71	8.0	8.0	0.125	<i>mef(E)/mel</i>
50-72	0.125	0.125	0.0625	-
50-73	0.0625	0.125	0.0625	-
50-74	0.0625	0.03125	0.0625	-

No.	MICs Erythromycin µg/ml	MICs Clarithromycin µg/ml	MICs Clindamycin µg/ml	Resistance gene
50-75	4.0	2.0	0.125	<i>mef(E)/mel</i>
50-77	0.125	0.125	0.125	-
50-78	8.0	0.125	0.0625	<i>mef(E)/mel</i>
50-79	0.0625	0.03125	0.0625	-
50-80	0.0625	0.03125	0.125	-
50-81	>512	512	256	<i>erm (B)</i>
50-82	>512	512	512	<i>erm (B)</i>
ATCC 49619	0.125	0.0625	0.25	-
<i>mef</i>	4.0	8.0	0.125	<i>mef(E)/mel</i>
<i>erm(B)</i>	>512	512	512	<i>erm(B)</i>

APPENDIX V

Result of MICs with CCCP inhibited macrolide efflux pump of 112 *mef*-positive
S. pneumoniae

No.	MICs Ery ($\mu\text{g/ml}$) Without CCCP	MICs Ery ($\mu\text{g/ml}$) with CCCP 10 $\mu\text{g/ml}$	No. of fold decreased
46-013	2.0	0.015	7
46-020	2.0	0.015	7
46-029	2.0	0.015	7
46-031	16.0	0.03125	9
46-046	16.0	0.03125	9
46-055	8.0	0.015	9
46-057	2.0	0.015	7
46-058	8.0	0.03125	8
46-060	8.0	0.015	9
46-066	8.0	0.015	9
46-075	8.0	0.015	9
46-077	8.0	0.03125	8
46-078	8.0	0.03125	8
46-093	1.0	0.015	6
46-098	2.0	0.015	7
46-099	2.0	0.015	7
46-102	8.0	0.015	9
46-113	4.0	0.03125	7
46-114	4.0	0.03125	7
46-116	8.0	0.015	9
46-117	8.0	0.015	9
46-122	2.0	0.015	7
46-123	4.0	0.015	8
46-126	4.0	0.015	8
46-134	4.0	0.03125	7
46-159	4.0	0.03125	7
47-19	8.0	0.015	9
47-21	8.0	0.015	9
47-22	16.0	0.03125	9

No.	MICs Ery ($\mu\text{g/ml}$) Without CCCP	MICs Ery ($\mu\text{g/ml}$) with CCCP 10 $\mu\text{g/ml}$	No. of fold decreased
47-27	16.0	0.03125	9
47-41	4.0	0.03125	7
47-51	8.0	0.03125	8
47-53	8.0	0.03125	8
47-56	8.0	0.03125	8
47-65	8.0	0.03125	8
47-66	4.0	0.015	8
47-67	4.0	0.0625	6
47-69	8.0	0.0625	7
47-73	4.0	0.015	8
47-97	4.0	0.015	8
47-98	4.0	0.03125	7
47-103	8.0	0.03125	8
47-104	8.0	0.03125	8
47-111	8.0	0.03125	8
48-07	8.0	0.03125	8
48-11	8.0	0.015	9
48-16	8.0	0.015	9
48-22	2.0	0.015	7
48-23	8.0	0.015	9
48-27	8.0	0.03125	8
48-29	1.0	0.015	6
48-35	2.0	0.03125	6
48-048	8.0	0.015	9
48-049	1.0	0.015	6
48-58	4.0	0.015	8
48-072	2.0	0.015	7
48-076	2.0	0.015	7
48-081	8.0	0.03125	8
48-086	4.0	0.015	8
48-097	8.0	0.03125	8
48-100	4.0	0.015	8
48-116	8.0	0.0625	7
48-122	8.0	0.03125	8
48-123	4.0	0.03125	7
48-124	8.0	0.015	9

No.	MICs Ery ($\mu\text{g/ml}$) Without CCCP	MICs Ery ($\mu\text{g/ml}$) with CCCP 10 $\mu\text{g/ml}$	No. of fold decreased
49-2	8.0	0.015	9
49-7	4.0	0.015	8
49-10	8.0	0.015	9
49-11	8.0	0.015	9
49-12	4.0	0.015	8
49-13	8.0	0.015	9
49-17	8.0	0.015	9
49-19	4.0	0.015	8
49-25	2.0	0.015	7
49-32	8.0	0.03125	8
49-44	4.0	0.03125	7
49-45	4.0	0.0625	6
49-47	4.0	0.0625	6
49-52	4.0	0.03125	7
49-54	2.0	0.03125	6
49-57	4.0	0.0625	6
49-58	4.0	0.03125	7
49-63	4.0	0.03125	7
49-66	2.0	0.015	7
49-68	2.0	0.015	7
49-69	2.0	0.015	7
50-1	8.0	0.03125	8
50-8	2.0	0.015	9
50-13	4.0	0.015	8
50-19	4.0	0.015	8
50-21	8.0	0.015	9
50-24	1.0	0.015	6
50-25	4.0	0.015	8
50-28	4.0	0.03125	7
50-30	2.0	0.015	7
50-31	2.0	0.015	7
50-32	4.0	0.015	8
50-33	4.0	0.015	8
50-41	8.0	0.015	9
50-47	8.0	0.015	9
50-48	8.0	0.015	9

No.	MICs Ery ($\mu\text{g/ml}$) Without CCCP	MICs Ery ($\mu\text{g/ml}$) with CCCP 10 $\mu\text{g/ml}$	No. of fold decreased
50-49	4.0	0.015	8
50-50	4.0	0.015	8
50-51	8.0	0.015	9
50-52	4.0	0.015	8
50-54	8.0	0.015	9
50-55	8.0	0.015	9
50-68	2.0	0.015	7
50-70	2.0	0.015	7
50-71	8.0	0.015	9
50-75	2.0	0.015	7
50-78	8.0	0.015	9
48-101 <i>erm(B)+mef(E)</i>	>512	>512	-
<i>mefE</i> positive control	4.0	0.015	8
<i>erm (B)</i> positive control	>512	>512	-
ATCC49619 Sensitive strain	0.125	0.125	-

	771		840
SP.AF274302	TTCTATTACG	GAAATTTCTT	TTGCATTGG
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	841		910
SP.AF274302	TTCGAAAAGC	ATGTATTACT	AATAACAAGT
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	911		980
SP.AF274302	TACTTCTCC	AAATGGATT	GTAATATTCG
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	981		1050
SP.AF274302	TATCGGTGTG	CAAACAGCTC	TTTTTCAGGA
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	1051		1120
SP.AF274302	ATCGGAAGTA	TCATGTCACT	TGCTATGCCA
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	1121		1190
SP.AF274302	GTGTAATCA	TGGTTTTTA	CTATCAGGTA
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus

	1191	1218
SP.AF274302	TGAGGTTAGA	AAATTAGATT TAAAATAA
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus

APPENDIX VII

Multiple nucleotide sequence alignment of entire *mef* gene from *S. pneumoniae* (SP.AF274302) and those of *S. pyogenes* (SY.AF445042, SY.AY657002 and SY.AB227521), *S. pneumoniae* (SP.AF376746), *S. aureus* (SA.AY064721), *S. salivarius* (SS.AJ318993), *S. intermedius* (SI.AY064722), *S. agalactiae* (SAG.DQ445273), viridans streptococcus (SV.EF042094), and *N. gonorrhoeae* (NG.AY319932).

```

1                                     70
SP.AF274302 ATGGAATAAT ACAACAATTG GAAAGAAA TTTTATGAA TATGGGCAGG GCAAGCAGTA TCATTAATCA
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AF445042 .....TT..G...A...
SY.AF657002 .....TT..G...A...
SY.AF227521 .....TT..G...A...
Consensus .....tt..g...a...

71                                     140
SP.AF274302 TAGTGGTAT CCTGCAATG GATTATTT TTTACCTTAC AGAAAAAATA GATCTGGA TGGCTTGGC
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AF445042 .....T.....T.....C.....
SY.AF657002 .....T.....T.....C.....
SY.AF227521 .....T.....T.....C.....
Consensus .....t.....t.....c.....

141                                     210
SP.AF274302 TAGGTTTA TTAGTAGGTT TTTACCCTA TGTAATG GACCTGCA TTGGTGTGCT AGTGGATCGT
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AF445042 .....C..T.....G..C..T.....A.....
SY.AF657002 .....C..T.....G..C..T.....A.....
SY.AF227521 .....C..T.....G..C..T.....A.....
Consensus .....c..t.....g..c..t.....a.....

211                                     280
SP.AF274302 TAGATAGA AGAAGATAAT GATTGGTGC GATTTAATTA TCGCAGAA TGGTGAATG CTTGATTG
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AF445042 .....T.....T..G...A.....
SY.AF657002 .....T.....T..G...A.....
SY.AF227521 .....T.....T..G...A.....
Consensus .....t.....t..g...a.....

```

```

281
SP.AF274302 TTGCATTCTG TATGGAGCTA CCTGTCTGGA TGATTATGAT AGTATTGTTT ATCCGCTAGCA TTGGAACAGC
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
S.AGDQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 .....A.....G.....
SY.AY657002 .....A.....G.....
SY.AF227521 .....A.....G.....
Consensus .....a.....g.....

```

```

351
SP.AF274302 TTTTATATAC CCAAGACTEA ATGCGGTTAC AACTACTTTTA GTACCAGAAG AACAGCTAAC GAAATGCGCA
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 .....C...G..T...G.....T.....T...
SY.AY657002 .....C...G..T...G.....T.....T...
SY.AF227521 .....C...G..T...G.....T.....T...
Consensus .....c...g..t...g.....t.....t...

```

```

421
SP.AF274302 TTTTATATAC AGTCTTTTGA GTCTATAAGC TATATTGTTA GTCCGGCAAT TGCAGCACTC TTATACTCCG
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 .....G.....
SY.AY657002 .....G.....
SY.AF227521 .....G.....
Consensus .....g.....

```

```

491
SP.AF274302 TTGGGGATTT AAATGCTATT ATTGCCATCG ACGTATTGGG TGCTGTGATT GCATCTATTA CGGTAGCAAT
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 .....AC.....T.....
SY.AY657002 .....AC.....T.....
SY.AF227521 .....AC.....T.....
Consensus .....ac.....t.....

```

```

561
SP.AF274302 TGTACGTATA CCTAAGCTGG GTATCAAAAT GCAAAGTTTA GAACCAAATT TCATAAGGGA GATGAAGAAA
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 .....T.....G...GC...G...C.....A...A...C...
SY.AY657002 .....T.....G...GC...G...C.....A...A...C...
SY.AF227521 .....T.....G...GC...G...C.....A...A...C...
Consensus .....t.....g...gc...g...c.....a...a...c...

```

```

631
SP.AF274302 TGTAGT TGG TCTGAA A A AACAAAGGA TTGTTTACT TATTACTCT AAGAACACTA TATACTTTTG
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 .....A.G.CT...A...AC.G...T.....A.....T.....G.T.....T.....TG...
SY.AY657002 .....A.G.CT...A...AC.G...T.....A.....T.....G.T.....T.....TG...
SY.AF227521 .....A.G.CT...A...AC.G...T.....A.....T.....G.T.....T.....TG...
Consensus .....a.g.ct...a...ac.g...t.....a.....t.....g.t.....t.....tg...

```

701 770
 SP.AF274302 TTTATATGCC AATCAATGCA CTATTTCCTT TAATAAATCAT GGAACACTTT AATGGAACGC CTGTGCATAT
 SP.AF376746
 SA.AY064721
 SS.AJ318993
 SV.EF042094
 SAG.DQ445273
 SI.AY064722
 NG.AY319932
 SY.AY445042T.....C.....T.....TT.....A.....
 SY.AY657002T.....C.....T.....TT.....A.....
 SY.AF227521T.....C.....T.....TT.....A.....
 Consensust.....c.....t.....tt.....a.....

771 840
 SP.AF274302 TTCTATTACG GAAATTTTCT TTGCATTTGG GATGCTAGCA GGAAGCTTAT TATTAGGAAG ATTAGGGGGC
 SP.AF376746
 SA.AY064721
 SS.AJ318993
 SV.EF042094
 SAG.DQ445273
 SI.AY064722
 NG.AY319932
 SY.AY445042C...A...T.GAT...G...TC...GTT...T...AAT
 SY.AY657002C...A...T.GAT...G...TC...GTT...T...AAT
 SY.AF227521C...A...T.GAT...G...TC...GTT...T...AAT
 Consensusc...a...t.gat...g...tc...gtt...t...aat

841 910
 SP.AF274302 TTTGAAAAT ATGATTACT AATAAAAGT TATTITTTTA TAATGGGGAC CATTTAGGC GTTCCGAAA
 SP.AF376746
 SA.AY064721
 SS.AJ318993
 SV.EF042094
 SAG.DQ445273
 SI.AY064722
 NG.AY319932
 SY.AY445042 .A.C.....GAA.C...T.....GGCA...CA......G.....T A.C...A...A...A...T
 SY.AY657002 .A.C.....GAA.C...T.....GGCA...CA......G.....T A.C...A...A...A...T
 SY.AF227521 .A.C.....GAA.C...T.....GGCA...CA......G.....T A.C...A...A...A...T
 Consensusa.c...t.....gaa.c...t.....ggca...ca......g.....t a.c...a...a...a...t

911 980
 SP.AF274302 TACTTCTTC AATGGGATT GAAATATTCC TAGTTTGGTG TGCAATAATG GGGCTTTCGG TGTATTTTA
 SP.AF376746
 SA.AY064721
 SS.AJ318993
 SV.EF042094
 SAG.DQ445273
 SI.AY064722
 NG.AY319932
 SY.AY445042C.A...G.....T.C...T..T...C.....T...T.G.....
 SY.AY657002C.A...G.....T.C...T..T...C.....T...T.G.....
 SY.AF227521C.A...G.....T.C...T..T...C.....T...T.G.....
 Consensusc.a...g.....t.c...t..t...c.....t...t.g.....

981 1050
 SP.AF274302 TAGGGGTGTG CAAACAGCTC TTTTTCAGSA GAAAATTAAG CCTGAATATT TAGGACGTGT ATTTTCTTTG
 SP.AF376746
 SA.AY064721
 SS.AJ318993
 SV.EF042094
 SAG.DQ445273
 SI.AY064722
 NG.AY319932
 SY.AY445042 C.....C.....A.....
 SY.AY657002 C.....C.....A.....
 SY.AF227521 C.....C.....A.....
 Consensus C.....c.....a.....

1051 1120
 SP.AF274302 ATCGAAGTA TCATGTCA T TGCTATGCCA ATTGAGTTAA TTCTTTTTEG ATTTTGTGCT GATAAATCG
 SP.AF376746
 SA.AY064721
 SS.AJ318993
 SV.EF042094
 SAG.DQ445273
 SI.AY064722
 NG.AY319932T T.CT.....
 SY.AY445042 .CT.....T.....A.....C...C.....G.....
 SY.AY657002 .CT.....T.....A.....C...C.....G.....
 SY.AF227521 .CT.....T.....A.....C...C.....G.....
 Consensus .ct.....t.....a.....c...c.....g.....

```

1121                                     1190
SP.AF274302 GTGTAATCA TGGGTTTTTA CTATCAGGTA TTTTAATTAT TGGCATTGCT ATAGTTTGCC AAATGATAAC
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 ..... C.....T.....A.....C.....A
SY.AY657002 ..... C.....T.....A.....C.....A
SY.AF227521 ..... C.....T.....A.....C.....A
Consensus .....C.....T.....A.....C.....A

```

```

1191                                     1218
SP.AF274302 TGAGGTTAGA AAATTAGATT TAAAATAA
SP.AF376746 .....
SA.AY064721 .....
SS.AJ318993 .....
SV.EF042094 .....
SAG.DQ445273 .....
SI.AY064722 .....
NG.AY319932 .....
SY.AY445042 ...A.....
SY.AY657002 ...A.....
SY.AF227521 ...A.....
Consensus ...A.....

```


APPENDIX VIII

Multiple nucleotide sequence alignment of entire *mel* gene from 10 *mel*-positive *S. pneumoniae* isolates with those of *S. pneumoniae* (SP.AF274302) in GenBank.

```

1                               70
SP.AF274302 ATGGGAATTAA TATTA AAAAGACATT CGTGTGGAAT TCAAAGGACG CGATGTTTTA GATATAAATG
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

71                               140
SP.AF274302 AATTAGAAGT ATATGATTAT GACCGTATTG GTTTAGTAGG AGCAAATGGT GCTGGAAAAA GCACTTTACT
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

141                               210
SP.AF274302 TAACTTACTT TTAGGAGAAT TAACTCCCCC AGGATGTAAA ATGAATCGTC TGGGTGAACT TGCTTATAIT
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

211                               280
SP.AF274302 GGGCAGTTGG ACGAAGTAAC TCTGCAGGAG GAAAAAGATT TTGCACITGT AGGCAAGCTA GGTGTTGAGC
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

281                               350
SP.AF274302 AATTA AATAT ACAGACTATG AGCGGTGGTG AAGAAACAAG GCTTAAAAATA GCACAGGCCT TATCGGCACA
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

351                                     420
SP.AF274302 GGTTCATGGT ATTTTAGCGG ATGAACCTAC GAGCCATTTA GACCGTGAAG GAATTGATT TCTAATAGGA
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

421                                     490
SP.AF274302 GAGCTAAAAT ATTTTACAGG TGCACGTGTA GTTATTAGCC ATGACCGCTA TTTTCTTGAT GAAATAGTAG
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

491                                     560
SP.AF274302 ATAAAAATATG GGAACGTAAA GATGGCAAAA TCACTGAGTA TTGGGGAAAC TATTCTGATT ATCTTCGTCA
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

561                                     630
SP.AF274302 GAAAAGAGGAA GAACGTAAAG GCCAAGCTGC AGAATACGAA CAATTTATTG CGGAACGTGC CCGATTGGAA
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

631                                     700
SP.AF274302 AGGGCTGCGG AGGAAAAAGG AAAACAGGCT CGTAAAAATG AACAGAAGGC AAAAGGTTCT TCAAAGAAAA
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

701                                     770
SP.AF274302 AAAGTACTGA AGACGGAGAG CGTTTAGCTC ATCAAAAATC AATAGGAAGT AAGGAAAAAA AGATGTATAA
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

	771		840
SP.AF274302	TGCTGCTAAA	ACCCTAGAGC	ACAGGATTGC
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	841		910
SP.AF274302	ATTGTTTCA	GGCAAAGTAA	AGCATTGGAG
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	911		980
SP.AF274302	AAGTATTGG	GGATAAGGCT	CTGTTTGAAA
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	981		1050
SP.AF274302	AACTGGTGGT	AATGGAATCG	GAAAAACAAC
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	1051		1120
SP.AF274302	ATTTCGCTA	AGGCAAAAAT	AGGTTACTTT
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus
	1121		1190
SP.AF274302	TTGATTTTAT	GCAGAAGGAT	TGTGACTACA
SP47-22
SP50-30
SP50-24
SP50-19
SP49-68
SP49-54
SP49-25
SP49-2
SP48-58
SP47-27
Consensus

```

1191                                     1260
SP.AF274302 CAAACAGAAC GATATTGGAA AAAGTTTATC TGTTTTAAGC GGTGGAGAAA TTATAAAATT GTTGCTTGCT
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

1261                                     1330
SP.AF274302 AAAATBTTA TGGGTAGATA TAACATCCTA ATAATGSATG AACCCAGTAA CTCCTTGAC ATACCAAGTT
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

1331                                     1400
SP.AF274302 TAGAGCTTT GGAATACTA ATGAAGGAGT ACACCGGAAC TATCGTGGT ATCACCCACG ATAAACGATT
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

```

1401                                     1464
SP.AF274302 ACTCGAAAAT GTAGCAGATG TAGTTTATGA AATTAGAGAT AAGAAAAATA ATCTGAAACA TTAA
SP47-22 .....
SP50-30 .....
SP50-24 .....
SP50-19 .....
SP49-68 .....
SP49-54 .....
SP49-25 .....
SP49-2 .....
SP48-58 .....
SP47-27 .....
Consensus .....

```

APPENDIX IX

Multiple nucleotide sequence alignment of entire *mel* gene from *S. pneumoniae* (SP.AF274302) and those of *S. pyogenes* (SY.AF445042, SY.AY657002 and SY.AB227521), *S. pneumoniae* (SP.AF376746), viridans streptococcus (SV.EF042094) and *S. salivarius* (SS.AJ318993).

```

1                               70
SP.AF274302 ATGGAATTAA TATTAAAAGC AAAAGACATT CGTGTGGAAT TCAAAGGACG CGATGTTTTA GATATAAATG
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ..... A ..... A .....
SY.AY657002 ..... A ..... A .....
SY.AF227521 ..... A ..... A .....
Consensus ..... C ..... g .....

71                               140
SP.AF274302 AATTAGAAGT ATATGATTAT JACCGTATTG GTTTAGTAGG AGCAAATGGT G'TGGAAAAA GCACCTTACT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ..... A ..... T .....
SY.AY657002 ..... A ..... T .....
SY.AF227521 ..... A ..... T .....
Consensus ..... t ..... C .....

141                               210
SP.AF274302 CAGGATCTCT TTAGGAGAAT TAACTCCCCC AGGATGTAAA ATGAATCGTC TGGGTGAACT TGCCTATATT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ..A..... T..... A.....
SY.AY657002 ..A..... T..... A.....
SY.AF227522 ..A..... T..... A.....
Consensus ..g..... C..... g.....

211                               280
SP.AF274302 TTTAGTTGG ACGAAGTAAC TCTGCAGGAG GAAAAAGATT TTGCACTTGT AGGCAAGCTA GGTGTTGAGC
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ..... G.....
SY.AY657002 ..... G.....
SY.AF227521 ..... G.....
Consensus ..... G.....

281                               350
SP.AF274302 AATTAAATAT ACAGATATAT AGCGGTGGTG AAGAAACAAG GCTTAAAATA GCACAGGCCT TATCGGCACA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ..... C.....
SY.AY657002 ..... C.....
SY.AF227521 ..... C.....
Consensus ..... t.....

351                               420
SP.AF274302 CGTTGATGCT ATTTTAGCGG ATGAACCTAC GAGCCATTTA GACCGTGAAG GAATTGATT TCTAATAGGA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....
SY.AY657002 .....
SY.AF227521 .....
Consensus .....

421                               490
SP.AF274302 CAGTAAAAAT ATTTTACAGG TGCACTGTTA GTTATTAGCC ATGACCGCTA TTTTCTTGAT GAAATAGTAG
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....
SY.AY657002 .....
SY.AF227521 .....
Consensus .....

```

```

491                                     560
SP.AF274302 ATAAATATG GGAAGTAAA GATGGCAAAA TCACTGAGTA TTGGGAAAC TATTCTGATT ATCTTCGTCA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....
SY.AY657002 .....
SY.AF227521 .....
Consensus .....

561                                     630
SP.AF274302 GAAAGAGGAA GAACGTAAGA GCCAAGCTGC AGAATACGAA CAATTTATTG CGGAACGTGC CCGATTGGAA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....A.....T.....
SY.AY657002 .....A.....T.....
SY.AF227521 .....A.....T.....
Consensus .....C.....C.....

631                                     700
SP.AF274302 AGGCTTCGGG AGGAAAAAGG AAAACAGGCT CGTAAATAG AACAGAAGGC AAAAGGTTCT TCAAAGAAAA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....
SY.AY657002 .....
SY.AF227521 .....
Consensus .....

701                                     770
SP.AF274302 AAAGTACTGA AGACGGAGGG CGTTTAGCTC ATCAAAAATC AATAGGAAGT AAGGAAAAAA AGATGTATAA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....G.....C.....
SY.AY657002 .....G.....C.....
SY.AF227521 .....G.....C.....
Consensus .....a.....t.....

771                                     840
SP.AF274302 TGTGATATAA ACCCTAAGGC AAGGATTGTC GGCCTTAGGA AAAGTAGAAG CTCGGGAGG CATTCCGAGA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ...C.....T.....A.....A.....
SY.AY657002 ...C.....T.....A.....A.....
SY.AF227521 ...C.....T.....A.....A.....
Consensus .....t.....a.....C.....C.....

841                                     910
SP.AF274302 ATTCGTTTCA GGCAAAATAA AGCATTGGAG CTCATAATC CATACCCAT AGTCGGTGCA GAAATTAATA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....G.....
SY.AY657002 .....G.....
SY.AF227521 .....G.....
Consensus .....a.....

911                                     980
SP.AF274302 AAGTATTGGG GGATAAGGCT CTGTTTGAAA ATGCATCTTT TCAAAATCCG TTAGGAGCAA AAGTGGCGTT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....A.....C.....A.....
SY.AY657002 .....A.....C.....A.....
SY.AF227521 .....A.....C.....A.....
Consensus .....t.....t.....t.....

981                                     1050
SP.AF274302 AACTAGTGGT AATGGAATCG GAAAAACAAC TTTAATCCAA ATGATCTTAA ACCATGAAGA AGGAATTTCT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ...G.....C.....
SY.AY657002 ...G.....C.....
SY.AF227521 ...G.....C.....
Consensus .....t.....t.....

1051                                    1120
SP.AF274302 ATTATGNTA AGGCAAAAAT AGGTTACTTT GCACAGAATG GTTACAAGTA CAACAGTAAT CAGAATGTTA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 ...A.....
SY.AY657002 ...A.....
SY.AF227521 ...A.....
Consensus .....g.....

```

```

1121                                     1190
SP.AF274302 TGGAGTTTAT GCAGAAGGAT TGTGACTACA ATATATCAGA AATTCGTTCA GTGCTAGCAT CTATGGGGTT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....T.....T.....
SY.AY657002 .....T.....T.....
SY.AF227521 .....T.....T.....
Consensus .....C.....a.....

1191                                     1260
SP.AF274302 TAAACGSAAC GATATTGGAA AAAGTTTATC TGTTTTAAGC GGTGGAGAAA TTATAAAATT GTTGCTTGCT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....C.....
SY.AY657002 .....C.....
SY.AF227521 .....C.....
Consensus .....t.....

1261                                     1330
SP.AF274302 AAAATGCTCA TGGGTAGATA TAACATCCTA ATAATGGATG AACCCAGTAA CTTCTTGAC ATACCAAGTT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....
SY.AY657002 .....
SY.AF227521 .....
Consensus .....

1331                                     1400
SP.AF274302 TAAAGTCTTT GGAAATACTA ATGAAGGAGT ACACCGGAAC TATCGTGTIT ATCACCCACG ATAAACGATT
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....
SY.AY657002 .....
SY.AF227521 .....
Consensus .....

1401                                     1464
SP.AF274302 ACTCGAAAAA GTAGCAATG TAGTTTATGA AATTAGAGAT AAGAAAAATA ATCTGAAACA TTAA
SP.AF376746 .....
SV.EF042094 .....
SS.AJ318993 .....
SY.AY445042 .....T.....T.....G.....
SY.AY657002 .....T.....T.....G.....
SY.AF227521 .....T.....T.....G.....
Consensus .....a.....a.....t.....

```

APPENDIX X**64 CODON ON DNA**

One- and Three-Letter symbols for the amino acids

A	Ala	Alanine
B	Asx	Asparagine or aspartic acid
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
Z	Glx	Gln or Glu

The standard genetic code

First position (5'end)	Second position				Third position (3' end)
	U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met ^a	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

^aAUG forms part of the initiation signal as well as coding for internal Met residues.

BIOGRAPHY

Miss Paopa-nga Monthanapisut was born on October 12, 1978 in Phitsanuloke province, Thailand. She graduated with a Bachelor degree of Science in Microbiology Major from Faculty of Science, Naresuan University in 2001. Now, She works at Research Laboratory of Dentistry Faculty, Thammasart University.

