

MALDI-TOF mass spectrometry-based optochin susceptibility testing for differentiation of *Streptococcus pneumoniae* from other *Streptococcus mitis* group streptococci

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Supplementary Materials

Table S1.

Results of molecular/biochemical species identification and bile solubility/optochin disk diffusion for the development set of isolates ($n=13$).

Table S2.

Results of molecular/biochemical species identification and bile solubility/optochin disk diffusion for the test set of isolates ($n=24$).

Table S3.

Results of optochin minimum inhibitory concentration (MIC) for the development set ($n=13$) and the test set ($n=24$) isolates.

Table S1. Results of molecular/biochemical species identification and bile solubility/optochin disk diffusion for the development set of isolates ($n=13$).

	#	Molecular identification				Biochemical identification (VITEK 2)			Bile solubility					Optochin disk diffusion			
									Tube test McF 0.5		Tube test McF 4.0		Plate test	Incubation at 35 ± 1 °C in ambient air		Incubation at 35 ± 1 °C with 5% CO ₂	
		<i>sodA</i> sequencing	<i>rpoB</i> sequencing	<i>lytA</i>	<i>ply</i>	Species	Qualifying message (% probability)	Time to result [h]	30 min	3 hours	30 min	3 hours		Inhibition zone [mm]	Result	Inhibition zone [mm]	Result
Development set	2P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Excellent (97)	5.00	+	+	+	+	+	28	S	20	S
	5P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Very good (94)	5.98	+	+	+	+	+	32	S	22	S
	6P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Good (91)	6.05	+	+	+	+	+	26	S	24	S
	8P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Good (92)	6.77	+	+	+	+	+	28	S	21	S
	1M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus mitis/oralis</i>	Good (91)	7.02	-	-	-	-	-	-	R	-	R
	3M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus mitis/oralis</i>	Very good (93)	5.80	~	~	~	+	-	-	R	-	R
	4M	<i>S. oralis</i>	ND	-	-	<i>Streptococcus mitis/oralis</i>	Excellent (99)	5.07	-	-	-	-	-	-	R	-	R
	5M	<i>S. oralis</i>	ND	-	-	<i>Streptococcus mitis/oralis</i>	Excellent (98)	5.07	-	-	-	-	-	-	R	-	R
	<i>S. pneumoniae</i> DSM20566	ND	ND	ND	ND	<i>Streptococcus pneumoniae</i>	Very good (93)	4.98	-	-	+	+	-	28	S	26	S
	<i>S. mitis</i> DSM12643	ND	ND	ND	ND	<i>Streptococcus mitis/oralis</i>	Good (91)	5.97	-	-	-	-	-	-	R	-	R
	<i>S. pseudopneumoniae</i> DSM18670	ND	ND	ND	ND	Low discrimination (repeated test: unidentified)	Low discrimination (50) (repeated test: unidentified)	7.90 (repeated test: 2.83)	-	-	-	-	?	32	S	20	S
	<i>S. oralis</i> DSM20627	ND	ND	ND	ND	<i>Streptococcus mitis/oralis</i>	Excellent (97)	5.97	-	-	-	-	-	-	R	-	R
	<i>S. pneumoniae</i> ATCC49619	ND	ND	ND	ND	<i>Streptococcus pneumoniae</i>	Excellent (97)	2.57	+	+	+	+	+	22	S	22	S

+, positive; -, negative; ~, almost transparent (ambiguous result); ND, not done; ?, not readable/unclear result; R, resistant; S, susceptible

Table S2. Results of molecular/biochemical species identification and bile solubility/optochin disk diffusion for the test set of isolates (n=24).

	#	Molecular identification				Biochemical identification (VITEK 2)			Bile solubility					Optochin disk diffusion			
									Tube test McF 0.5		Tube test McF 4.0		Plate test	Incubation at 35 ± 1 °C in ambient air		Incubation at 35 ± 1 °C with 5% CO ₂	
		<i>sodA</i> sequencing	<i>rpoB</i> sequencing	<i>lytA</i>	<i>ply</i>	Species	Qualifying message (% probability)	Time to result [h]	30 min	3 hours	30 min	3 hours		Inhibition zone [mm]	Result	Inhibition zone [mm]	Result
Test set	R2P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Very good (93)	5.78	+	+	+	+	+	24	S	24	S
	R3P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Excellent (97)	4.83	+	+	+	+	+	27	S	25	S
	R5P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Excellent (99)	4.78	+	+	+	+	+	23	S	18	S
	R6P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Excellent (99)	4.82	+	+	?	~	+	24	S	14	R (?)
	R8P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Excellent (97)	5.03	+	+	+	+	+	23	S	23	S
	R10P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Very good (94)	5.03	+	+	+	+	+	23	S	21	S
	R11P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Excellent (97)	3.32	?	+	-	~	+	28	S	22	S
	R13P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Good (91)	5.77	+	+	+	+	+	29	S	25	S
	R14P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Very good (93)	5.98	+	+	+	+	+	32	S	22	S
	R15P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Very good (94)	5.98	?	+	+	+	+	25	S	25	S
	R16P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Very good (95)	6	?	+	+	+	+	30	S	22	S
	R17P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S. pneumoniae</i>	+	+	<i>Streptococcus pneumoniae</i>	Very good (95)	4.78	?	+	+	+	?	28	S	25	S

+, positive; -, negative; ~, almost transparent (ambiguous result); ND, not done; ?, not readable/unclear result; R, resistant; S, susceptible

Table S2 (continuation). Results of molecular/biochemical species identification and bile solubility/optochin disk diffusion for the test set of isolates ($n=24$).

	#	Molecular identification				Biochemical identification (VITEK 2)			Bile solubility					Optochin disk diffusion			
									Tube test McF 0.5		Tube test McF 4.0		Plate test	Incubation at 35 ± 1 °C in ambient air		Incubation at 35 ± 1 °C with 5% CO ₂	
		<i>sodA</i> sequencing	<i>rpoB</i> sequencing	<i>lytA</i>	<i>ply</i>	Species	Qualifying message (% probability)	Time to result [h]	30 min	3 hours	30 min	3 hours		Inhibition zone [mm]	Result	Inhibition zone [mm]	Result
Test set	R1P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S.</i> <i>pseudopneumoniae</i>	-	+	Low discrimination (repeated test: unidentified)	Low discrimination (50) (repeated test: unidentified)	7.90 (repeated test: 3.32)	?	?	-	+	?	24	S	13	R (?)
	R12P	<i>S. pneumoniae</i> / <i>pseudopneumoniae</i>	<i>S.</i> <i>pseudopneumoniae</i>	-	+	<i>Streptococcus</i> <i>pneumoniae</i>	Very good (93)	6.98	+	+	~	~	?	25	S	22	S
	R2M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Excellent (98)	5.78	-	-	-	-	-	-	R	-	R
	R5M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Very good (95)	6.97	-	-	-	-	-	-	R	-	R
	R6M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Excellent (99)	4.8	-	-	-	-	-	-	R	-	R
	R7M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Very good (94)	5.03	-	-	-	-	-	-	R	-	R
	R9M	<i>S. oralis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Excellent (98)	5.02	-	-	-	-	-	-	R	-	R
	R11M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Excellent (99)	4.82	-	-	-	-	-	-	R	-	R
	R12M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Very good (95)	5.78	-	-	-	-	-	-	R	-	R
	R15M	<i>S. parasanguinis</i> / <i>cristatus</i>	ND	-	-	Low discrimination (repeated test: <i>S. thoraltensis</i>)	Low discrimination (50) (repeated test: 95)	7.93 (repeated test: 2.58)	-	-	-	-	-	-	R	-	R
	R16M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Excellent (99)	5.05	-	-	-	-	-	-	R	-	R
	R18M	<i>S. mitis</i>	ND	-	-	<i>Streptococcus</i> <i>mitis/oralis</i>	Excellent (99)	5.03	-	-	-	-	-	-	R	-	R

+, positive; -, negative; ~, almost transparent (ambiguous result); ND, not done; ?, not readable/unclear result; R, resistant; S, susceptible

Table S3. Results of optochin minimum inhibitory concentration (MIC) for the development set ($n=13$) and the test set ($n=24$) isolates.

	#	Species	MIC ($\mu\text{g/ml}$)
Development set	2P	<i>S. pneumoniae</i>	0.5
	5P	<i>S. pneumoniae</i>	0.25
	6P	<i>S. pneumoniae</i>	0.25
	8P	<i>S. pneumoniae</i>	0.25
	1M	<i>S. mitis</i>	32
	3M	<i>S. mitis</i>	32
	4M	<i>S. oralis</i>	64
	5M	<i>S. oralis</i>	128
	DSM20566	<i>S. pneumoniae</i>	0.5
	DSM12643	<i>S. mitis</i>	8
	DSM18670	<i>S. pseudopneumoniae</i>	0.5
	DSM20627	<i>S. oralis</i>	32
	ATCC49619	<i>S. pneumoniae</i>	0.5
Test set	R2P	<i>S. pneumoniae</i>	1
	R3P	<i>S. pneumoniae</i>	0.25
	R5P	<i>S. pneumoniae</i>	1
	R6P	<i>S. pneumoniae</i>	1
	R8P	<i>S. pneumoniae</i>	0.5
	R10P	<i>S. pneumoniae</i>	1
	R11P	<i>S. pneumoniae</i>	0.25
	R13P	<i>S. pneumoniae</i>	0.5
	R14P	<i>S. pneumoniae</i>	0.5
	R15P	<i>S. pneumoniae</i>	4
	R16P	<i>S. pneumoniae</i>	0.25
	R17P	<i>S. pneumoniae</i>	0.25
	R1P	<i>S. pseudopneumoniae</i>	1
	R12P	<i>S. pseudopneumoniae</i>	0.5
	R2M	<i>S. mitis</i>	64
	R5M	<i>S. mitis</i>	32
	R6M	<i>S. mitis</i>	64
	R7M	<i>S. mitis</i>	256
	R9M	<i>S. oralis</i>	256
	R11M	<i>S. mitis</i>	32
	R12M	<i>S. mitis</i>	64
	R15M	<i>S. parasanguinis/ cristatus</i>	128
	R16M	<i>S. mitis</i>	32
	R18M	<i>S. mitis</i>	4