

## Supplementary Material

### Utilizing Metagenomic Next-Generation Sequencing (mNGS) for Rapid Pathogen Identification and to Inform Clinical Decision-Making: Results From a Large Real-World Cohort

#### Running title: Application of mNGS in clinical practice

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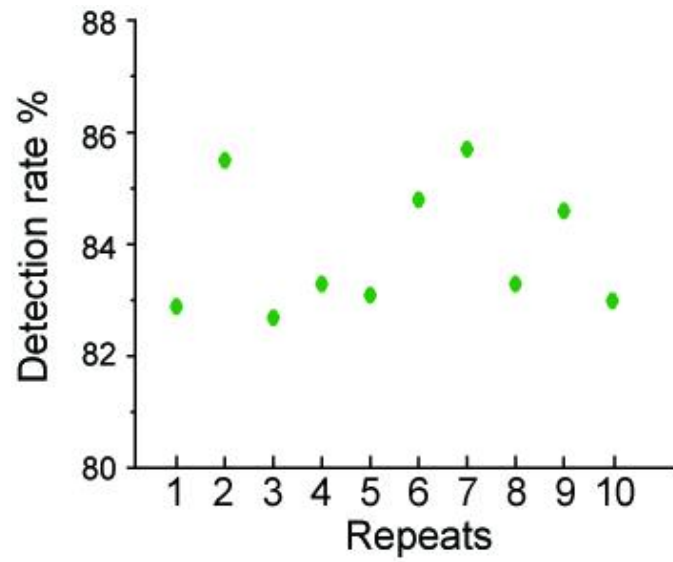
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**Figure S1.** The performance of mNGS assay using simulated data.

Simulation was repeated 10 times using randomly selected microbes (N = 1000).

**Table S1.** The comparison of mNGS and CT results of 188 patients (201 samples) with various infectious diseases.

SID	PID	Sex	Age	Sample Type	Infection Site	CMT	real-time PCR	CT (CMT + real-time PCR)	mNGS	mNGS/CT comparison	Micro Match Level	Concordant Pathogen Type
1	P1	M	80	Blood	LRI	negative	n.a.	negative	Pneumocystis jirovecii + Human betaherpesvirus 5	mNGS-positive	-	-
2	P2	F	42	Blood	Unknown	Corynebacterium tuberculostearicum	n.a.	Corynebacterium tuberculostearicum	Corynebacterium tuberculostearicum	double-positive (concordant)	species	G+
3	P3	F	64	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
4	P4	M	87	sputum	LRI	Staphylococcus aureus + Pseudomonas aeruginosa	n.a.	Staphylococcus aureus + Pseudomonas aeruginosa	Stenotrophomonas maltophilia + Pseudomonas aeruginosa	double-positive (overlapping)	species	-
5	P5	M	29	sputum	Unknown	negative	n.a.	negative	Human herpesvirus 1	mNGS-positive	-	-
6	P1	M	80	sputum	LRI	Acinetobacter baumannii	n.a.	Acinetobacter baumannii	Acinetobacter baumannii	double-positive (concordant)	species	G-
7	P6	M	33	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus oralis	double-positive (concordant)	species	G+
8	P7	M	51	sputum	LRI	negative	n.a.	negative	Staphylococcus haemolyticus + Enterococcus faecium + Mycobacterium tuberculosis Complex	mNGS-positive	-	-
9	P8	M	24	BALF	LRI	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-
10	P9	M	75	BALF	LRI	Candida albicans	n.a.	Candida albicans	Candida albicans	double-positive (concordant)	species	Fungi
11	P10	M	15	BALF	Unknown	negative	n.a.	negative	Staphylococcus capitis + Human herpesvirus 6	mNGS-positive	-	-
12	P3	F	64	BALF	Unknown	Aspergillus	n.a.	Aspergillus	Klebsiella aerogenes	double-positive (discordant)	-	-
13	P11	F	23	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
14	P12	M	56	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus agalactiae + Human betaherpesvirus 7	double-positive (overlapping)	genus	-
15	P13	M	73	sputum	Unknown	Candida + Staphylococcus lugdunensis + Corynebacterium tuberculostearicum	n.a.	Candida + Staphylococcus lugdunensis + Corynebacterium tuberculostearicum	Staphylococcus lugdunensis + Corynebacterium tuberculostearicum + Candida albicans	double-positive (concordant)	genus	Polymicrobial (bacteria and fungi)
16	P14	M	29	BALF	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis Complex	double-positive (concordant)	genus	MTB

17	P15	M	86	BALF	LRI	Candida albicans + Viridans Streptococci	n.a.	Candida albicans + Viridans Streptococci	Human herpesvirus 1	double-positive (discordant)	-	-
18	P16	M	62	BALF	LRI	Candida	n.a.	Candida	Enterococcus faecium	double-positive (discordant)	-	-
19	P17	M	38	BALF	LRI	negative	n.a.	negative	negative	double-negative	-	-
20	P18	M	62	BALF	Unknown	negative	n.a.	negative	negative	double-negative	-	-
21	P19	M	54	BALF	LRI	negative	Legionella pneumophila (-)	negative	Legionella pneumophila	mNGS-positive	-	-
22	P20	M	56	BALF	LRI	Viridans Streptococci	n.a.	Viridans Streptococci	Human betaherpesvirus 5	double-positive (discordant)	-	-
23	P21	F	52	BALF	LRI	Neisseria	n.a.	Neisseria	Neisseria subflava	double-positive (concordant)	genus	G-
24	P22	M	46	BALF	LRI	negative	Pneumocystis jirovecii (+)	Pneumocystis jirovecii	Pneumocystis jirovecii	double-positive (concordant)	species	Fungi
25	P23	M	18	BALF	LRI	Viridans Streptococci	n.a.	Viridans Streptococci	Haemophilus parainfluenzae	double-positive (discordant)	-	-
26	P24	M	65	BALF	LRI	negative	n.a.	negative	Mycoplasma hominis	mNGS-positive	-	-
27	P25	F	19	BALF	LRI	negative	Human betaherpesvirus 5 (+)	Human betaherpesvirus 5	Human betaherpesvirus 5	double-positive (concordant)	species	Viruses
28	P26	M	16	BALF	LRI	Viridans Streptococci + Klebsiella pneumoniae	n.a.	Viridans Streptococci + Klebsiella pneumoniae	Klebsiella pneumoniae + Streptococcus oralis	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
29	P27	M	62	BALF	LRI	Proteus mirabilis	n.a.	Proteus mirabilis	Pseudomonas aeruginosa + Klebsiella pneumoniae + Acinetobacter baumannii + Proteus mirabilis	double-positive (overlapping)	species	-
30	P28	M	57	BALF	LRI	negative	Pneumocystis jirovecii (-), Human betaherpesvirus 5 (+)	Human betaherpesvirus 5	Human betaherpesvirus 5 + Pneumocystis jirovecii	double-positive (overlapping)	species	-
31	P29	F	71	Blood	BSI	negative	Escherichia coli (-)	negative	Escherichia coli	mNGS-positive	-	-
32	P27	M	62	Blood	LRI	negative	Pseudomonas aeruginosa (+)	Pseudomonas aeruginosa	Pseudomonas aeruginosa	double-positive (concordant)	species	G-
33	P11	F	23	Blood	Unknown	negative	Pneumocystis jirovecii (-)	negative	Pneumocystis jirovecii	mNGS-positive	-	-
34	P30	M	54	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
35	P31	M	24	Blood	Typhoid fever	negative	n.a.	negative	negative	double-negative	-	-
36	P32	F	38	Blood	Unknown	negative	Pneumocystis jirovecii (+), Human betaherpesvirus 5 (+)	Pneumocystis jirovecii + Human betaherpesvirus 5	Pneumocystis jirovecii + Human betaherpesvirus 5	double-positive (concordant)	species	Polymicrobial infection (fungi and viruses)
37	P33	M	69	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
38	P34	M	60	Blood	LRI	negative	n.a.	negative	negative	double-negative	-	-

39	P35	F	68	Blood	Unknown	Escherichia coli	n.a.	Escherichia coli	Escherichia coli	double-positive (concordant)	species	G-
40	P36	F	19	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
41	P37	M	47	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
42	P38	M	83	Blood	LRI	negative	n.a.	negative	Cryptococcus neoformans + Enterococcus faecalis	mNGS-positive	-	-
43	P39	M	20	Blood	BSI	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-
44	P40	F	27	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
45	P41	M	76	Blood	CNSI	negative	n.a.	negative	Human herpesvirus 1 + Acinetobacter baumannii + Epstein-Barr virus	mNGS-positive	-	-
46	P7	M	51	Blood	LRI	negative	n.a.	negative	Epstein-Barr virus	mNGS-positive	-	-
47	P42	M	67	Blood	LRI	negative	n.a.	negative	Pneumocystis jirovecii + Human herpesvirus 1	mNGS-positive	-	-
48	P43	F	68	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
49	P44	M	81	Blood	LRI	negative	n.a.	negative	Pneumocystis jirovecii	mNGS-positive	-	-
50	P45	F	43	Blood	BSI	Escherichia coli	n.a.	Escherichia coli	Escherichia coli	double-positive (concordant)	species	G-
51	P46	M	56	Blood	Unknown	negative	n.a.	negative	Torque teno virus 29	mNGS-positive	-	-
52	P47	F	37	Blood	BSI	Enterococcus faecium	n.a.	Enterococcus faecium	Enterococcus faecium	double-positive (concordant)	species	G+
53	P48	M	67	Blood	Unknown	Escherichia coli	n.a.	Escherichia coli	Enterobacter cloacae + Stenotrophomonas maltophilia	double-positive (discordant)	-	-
54	P49	F	52	Blood	Unknown	negative	n.a.	negative	negative	double-negative	-	-
55	P50	F	28	Blood	BSI+LRI	negative	Acinetobacter baumannii (-)	negative	Acinetobacter baumannii	mNGS-positive	-	-
56	P51	M	69	sputum	Unknown	Viridans Streptococci + Neisseria	n.a.	Viridans Streptococci + Neisseria	Neisseria subflava + Streptococcus parasanguinis	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
57	P52	M	67	sputum	Unknown	Pseudomonas aeruginosa	n.a.	Pseudomonas aeruginosa	Pseudomonas aeruginosa	double-positive (concordant)	species	G-
58	P53	M	31	sputum	LRI	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus salivarius	double-positive (concordant)	genus	G+
59	P54	F	89	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Stenotrophomonas maltophilia + Candida glabrata	double-positive (discordant)	-	-
60	P55	F	31	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Stenotrophomonas maltophilia + Haemophilus parainfluenzae	double-positive (discordant)	-	-
61	P56	M	65	sputum	Unknown	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-

62	P57	F	17	sputum	Unknown	<i>Pseudomonas aeruginosa</i>	n.a.	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	double-positive (concordant)	species	G-
63	P58	F	75	sputum	LRI	<i>Acinetobacter baumannii</i>	n.a.	<i>Acinetobacter baumannii</i>	<i>Acinetobacter baumannii</i>	double-positive (concordant)	species	G-
64	P59	M	88	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	<i>Stenotrophomonas maltophilia</i>	double-positive (discordant)	-	-
65	P60	F	81	sputum	Unknown	<i>Acinetobacter baumannii</i>	n.a.	<i>Acinetobacter baumannii</i>	<i>Acinetobacter baumannii</i>	double-positive (concordant)	species	G-
66	P61	M	24	sputum	LRI	<i>Mycobacterium</i>	n.a.	<i>Mycobacterium</i>	<i>Mycobacterium tuberculosis</i>	double-positive (concordant)	genus	MTB
67	P62	M	19	sputum	Unknown	negative	n.a.	negative	<i>Prevotella jejuni</i>	mNGS-positive	-	-
68	P63	M	63	sputum	Unknown	Viridans Streptococci + <i>Rothia mucilaginosa</i>	n.a.	Viridans Streptococci + <i>Rothia mucilaginosa</i>	<i>Streptococcus oralis</i> + <i>Rothia mucilaginosa</i>	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
69	P64	F	71	sputum	Unknown	<i>Stenotrophomonas maltophilia</i>	n.a.	<i>Stenotrophomonas maltophilia</i>	<i>Stenotrophomonas maltophilia</i>	double-positive (concordant)	species	G-
70	P65	M	68	sputum	Unknown	<i>Neisseria</i>	n.a.	<i>Neisseria</i>	<i>Neisseria subflava</i>	double-positive (concordant)	genus	G-
71	P66	F	64	sputum	Unknown	<i>Staphylococcus epidermidis</i>	n.a.	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus epidermidis</i>	double-positive (concordant)	species	G+
72	P67	M	63	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	<i>Staphylococcus haemolyticus</i> + <i>Gordonia bronchialis</i>	double-positive (discordant)	-	-
73	P68	M	50	sputum	Unknown	<i>Klebsiella pneumoniae</i>	n.a.	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	double-positive (concordant)	species	G-
74	P69	M	60	sputum	Unknown	Viridans Streptococci + <i>Serratia marcescens</i> + <i>Aggregatibacter segnis</i>	n.a.	Viridans Streptococci + <i>Serratia marcescens</i> + <i>Aggregatibacter segnis</i>	<i>Streptococcus anginosus</i> + <i>Serratia marcescens</i> + <i>Aggregatibacter segnis</i>	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
75	P70	M	60	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	<i>Haemophilus parainfluenzae</i>	double-positive (discordant)	-	-
76	P71	M	74	sputum	Unknown	<i>Acinetobacter baumannii</i>	n.a.	<i>Acinetobacter baumannii</i>	<i>Acinetobacter baumannii</i>	double-positive (concordant)	species	G-
77	P72	F	86	sputum	LRI	<i>Staphylococcus aureus</i> + <i>Enterobacter cloacae</i>	n.a.	<i>Staphylococcus aureus</i> + <i>Enterobacter cloacae</i>	<i>Staphylococcus aureus</i>	double-positive (overlapping)	species	-
78	P73	M	71	sputum	Unknown	<i>Staphylococcus aureus</i>	n.a.	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	double-positive (concordant)	species	G+
79	P74	M	84	sputum	Unknown	<i>Candida albicans</i>	n.a.	<i>Candida albicans</i>	<i>Klebsiella pneumoniae</i>	double-positive (discordant)	-	-

80	P75	F	69	sputum	Unknown	Neisseria	n.a.	Neisseria	Stenotrophomonas maltophilia	double-positive (discordant)	-	-
81	P76	M	49	sputum	LRI	Neisseria + Enterobacter cloacae	n.a.	Neisseria + Enterobacter cloacae	Neisseria subflava + Enterobacter cloacae	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
82	P77	F	38	sputum	Unknown	Viridans Streptococci + Neisseria	n.a.	Viridans Streptococci + Neisseria	Streptococcus salivarius + Neisseria elongata	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
83	P78	M	38	sputum	Unknown	negative	n.a.	negative	Stenotrophomonas maltophilia	mNGS-positive	-	-
84	P79	M	63	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Enterococcus faecalis	double-positive (discordant)	-	-
85	P80	F	41	sputum	LRI	Candida albicans	n.a.	Candida albicans	Candida glabrata + Klebsiella pneumoniae	double-positive (discordant)	-	-
86	P81	F	/	sputum	Unknown	Aspergillus fumigatus	n.a.	Aspergillus fumigatus	Candida parapsilosis	double-positive (discordant)	-	-
87	P82	F	86	sputum	Unknown	Candida glabrata + Candida albicans	n.a.	Candida glabrata + Candida albicans	Candida glabrata	double-positive (overlapping)	species	-
88	P83	M	53	sputum	LRI	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-
89	P84	M	53	sputum	LRI	Rothia mucilaginosa	n.a.	Rothia mucilaginosa	Rothia mucilaginosa	double-positive (concordant)	species	G+
90	P85	M	72	sputum	Unknown	Rothia mucilaginosa	n.a.	Rothia mucilaginosa	Rothia mucilaginosa	double-positive (concordant)	species	G+
91	P86	M	52	sputum	Unknown	negative	n.a.	negative	Klebsiella pneumoniae + Pseudomonas aeruginosa	mNGS-positive	-	-
92	P87	F	38	sputum	Unknown	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-
93	P88	M	56	sputum	LRI	Klebsiella pneumoniae + Acinetobacter baumannii + Corynebacterium striatum	n.a.	Klebsiella pneumoniae + Acinetobacter baumannii + Corynebacterium striatum	Klebsiella pneumoniae + Acinetobacter baumannii + Corynebacterium striatum	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
94	P89	F	45	sputum	Unknown	negative	n.a.	negative	Aspergillus fumigatus	mNGS-positive	-	-
95	P90	M	47	sputum	LRI	Acinetobacter baumannii	n.a.	Acinetobacter baumannii	Acinetobacter baumannii	double-positive (concordant)	species	G-
96	P91	M	81	sputum	Unknown	Viridans Streptococci + Candida	n.a.	Viridans Streptococci + Candida	Candida albicans	double-positive (overlapping)	genus	-
97	P92	M	55	sputum	LRI	Neisseria	n.a.	Neisseria	Neisseria subflava	double-positive (concordant)	genus	G-
98	P93	M	50	sputum	Unknown	Corynebacterium + Klebsiella pneumoniae	n.a.	Corynebacterium + Klebsiella pneumoniae	Corynebacterium striatum + Klebsiella pneumoniae	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)



99	P94	M	61	sputum	LRI	<i>Pseudomonas aeruginosa</i>	n.a.	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	double-positive (concordant)	species	G-
100	P95	M	92	sputum	LRI	<i>Pseudomonas aeruginosa</i>	n.a.	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	double-positive (concordant)	species	G-
101	P96	M	78	sputum	LRI	<i>Candida albicans</i>	n.a.	<i>Candida albicans</i>	<i>Streptococcus salivarius</i>	double-positive (discordant)	-	-
102	P97	M	49	sputum	LRI	<i>Acinetobacter baumannii</i>	n.a.	<i>Acinetobacter baumannii</i>	<i>Acinetobacter baumannii</i>	double-positive (concordant)	species	G-
103	P98	F	54	sputum	Unknown	<i>Klebsiella pneumoniae</i> + <i>Viridans Streptococci</i>	n.a.	<i>Klebsiella pneumoniae</i> + <i>Viridans Streptococci</i>	<i>Streptococcus mitis</i>	double-positive (overlapping)	species	-
104	P99	F	82	sputum	Unknown	<i>Viridans Streptococci</i>	n.a.	<i>Viridans Streptococci</i>	<i>Streptococcus mitis</i>	double-positive (concordant)	species	G+
105	P100	F	43	sputum	Unknown	<i>Candida</i>	n.a.	<i>Candida</i>	negative	CT-positive	-	-
106	P101	F	67	sputum	Unknown	<i>Candida</i> + <i>Corynebacterium striatum</i>	n.a.	<i>Candida</i> + <i>Corynebacterium striatum</i>	<i>Corynebacterium striatum</i>	double-positive (overlapping)	species	-
107	P102	M	87	sputum	LRI	<i>Staphylococcus aureus</i> + <i>Acinetobacter baumannii</i> + <i>Pseudomonas aeruginosa</i>	n.a.	<i>Staphylococcus aureus</i> + <i>Acinetobacter baumannii</i> + <i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i> + <i>Acinetobacter baumannii</i> + <i>Pseudomonas aeruginosa</i>	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
108	P103	M	65	sputum	LRI	<i>Rothia mucilaginosa</i>	n.a.	<i>Rothia mucilaginosa</i>	<i>Rothia mucilaginosa</i>	double-positive (concordant)	species	G+
109	P64	F	71	sputum	Unknown	<i>Penicillium</i>	n.a.	<i>Penicillium</i>	<i>Stenotrophomonas maltophilia</i> + <i>Burkholderia stabilis</i> + Human herpesvirus 1	double-positive (discordant)	-	-
110	P104	F	80	sputum	Unknown	<i>Pseudomonas aeruginosa</i>	n.a.	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	double-positive (concordant)	species	G-
111	P105	M	81	sputum	LRI	<i>Pseudomonas aeruginosa</i>	n.a.	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	double-positive (concordant)	species	G-
112	P106	M	78	sputum	LRI	<i>Aspergillus fumigatus</i> + <i>Aspergillus flavus</i>	n.a.	<i>Aspergillus fumigatus</i> + <i>Aspergillus flavus</i>	Human herpesvirus 1	double-positive (discordant)	-	-
113	P107	M	60	sputum	LRI	<i>Viridans Streptococci</i> + <i>Neisseria</i>	n.a.	<i>Viridans Streptococci</i> + <i>Neisseria</i>	<i>Neisseria elongata</i>	double-positive (overlapping)	genus	-
114	P108	M	67	sputum	LRI	<i>Proteus mirabilis</i>	n.a.	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i>	double-positive (concordant)	species	G-
115	P109	M	78	sputum	LRI	<i>Staphylococcus aureus</i>	n.a.	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	double-positive (concordant)	species	G+

116	P110	M	66	sputum	LRI	Corynebacterium striatum + Burkholderia cepacia	n.a.	Corynebacterium striatum + Burkholderia cepacia	Corynebacterium striatum + Burkholderia cepacia	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
117	P111	F	86	sputum	Unknown	Enterococcus faecium + Stenotrophomonas maltophilia + Acinetobacter baumannii + Staphylococcus haemolyticus	n.a.	Enterococcus faecium + Stenotrophomonas maltophilia + Acinetobacter baumannii + Staphylococcus haemolyticus	Enterococcus faecium + Stenotrophomonas maltophilia + Acinetobacter baumannii + Staphylococcus haemolyticus	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
118	P70	M	60	sputum	Unknown	Candida albicans	n.a.	Candida albicans	Candida albicans	double-positive (concordant)	species	Fungi
119	P112	M	67	sputum	Unknown	Candida	n.a.	Candida	Streptococcus anginosus	double-positive (discordant)	-	-
120	P13	M	73	sputum	Unknown	Candida + Staphylococcus lugdunensis	n.a.	Candida + Staphylococcus lugdunensis	Staphylococcus lugdunensis + Candida albicans	double-positive (concordant)	genus	Polymicrobial (bacteria and fungi)
121	P113	M	30	sputum	LRI	Acinetobacter baumannii	n.a.	Acinetobacter baumannii	Acinetobacter baumannii	double-positive (concordant)	species	G-
122	P114	F	58	sputum	LRI	Pseudomonas aeruginosa	n.a.	Pseudomonas aeruginosa	Pseudomonas aeruginosa	double-positive (concordant)	species	G-
123	P115	M	63	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Staphylococcus haemolyticus	double-positive (discordant)	-	-
124	P116	F	68	sputum	Unknown	Proteus mirabilis + Neisseria	n.a.	Proteus mirabilis + Neisseria	Proteus mirabilis + Neisseria subflava	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
125	P117	M	60	sputum	Unknown	Acinetobacter baumannii + Burkholderia cepacia + Corynebacterium striatum	n.a.	Acinetobacter baumannii + Burkholderia cepacia + Corynebacterium striatum	Acinetobacter baumannii + Burkholderia cepacia + Corynebacterium striatum	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
126	P118	F	75	sputum	LRI	Acinetobacter baumannii	n.a.	Acinetobacter baumannii	Acinetobacter baumannii	double-positive (concordant)	species	G-
127	P40	F	27	sputum	Unknown	Neisseria	n.a.	Neisseria	Neisseria flavescens	double-positive (concordant)	genus	G-
128	P119	M	87	sputum	LRI	Acinetobacter baumannii	n.a.	Acinetobacter baumannii	Acinetobacter baumannii	double-positive (concordant)	species	G-
129	P120	F	35	sputum	Unknown	Staphylococcus epidermidis	n.a.	Staphylococcus epidermidis	Staphylococcus epidermidis	double-positive (concordant)	species	G+
130	P121	F	40	sputum	Unknown	Enterobacter cloacae	n.a.	Enterobacter cloacae	Enterobacter cloacae	double-positive (concordant)	species	G-

131	P122	M	33	sputum	LRI	Aspergillus fumigatus	n.a.	Aspergillus fumigatus	Aspergillus fumigatus	double-positive (concordant)	species	Fungi
132	P123	F	67	sputum	Unknown	Candida + Burkholderia stabilis	n.a.	Candida + Burkholderia stabilis	Burkholderia stabilis + Candida albicans	double-positive (concordant)	genus	Polymicrobial (bacteria and fungi)
133	P124	M	42	sputum	LRI	Staphylococcus aureus + Viridans Streptococci + Neisseria + Candida	n.a.	Staphylococcus aureus + Viridans Streptococci + Neisseria + Candida	Staphylococcus aureus + Streptococcus mitis + Neisseria subflava	double-positive (overlapping)	genus	-
134	P125	M	50	sputum	Unknown	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-
135	P126	M	25	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus anginosus	double-positive (concordant)	species	G+
136	P127	M	75	sputum	LRI	Pseudomonas aeruginosa	n.a.	Pseudomonas aeruginosa	Pseudomonas aeruginosa	double-positive (concordant)	species	G-
137	P124	M	42	sputum	LRI	Viridans Streptococci + Neisseria + Candida + Staphylococcus aureus + Haemophilus parainfluenzae	n.a.	Viridans Streptococci + Neisseria + Candida + Staphylococcus aureus + Haemophilus parainfluenzae	Streptococcus sanguinis + Neisseria subflava + Staphylococcus aureus + Haemophilus parainfluenzae	double-positive (overlapping)	genus	-
138	P128	M	84	sputum	Unknown	Enterococcus faecium + Stenotrophomonas maltophilia	n.a.	Enterococcus faecium + Stenotrophomonas maltophilia	Enterococcus faecium	double-positive (overlapping)	species	-
139	P129	F	61	sputum	Unknown	Viridans Streptococci + Neisseria + Haemophilus parainfluenzae	n.a.	Viridans Streptococci + Neisseria + Haemophilus parainfluenzae	Streptococcus anginosus + Neisseria subflava + Haemophilus parainfluenzae	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
140	P130	F	40	sputum	LRI	Staphylococcus aureus + Viridans Streptococci + Neisseria	n.a.	Staphylococcus aureus + Viridans Streptococci + Neisseria	Streptococcus sanguinis	double-positive (overlapping)	genus	-
141	P131	M	56	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus salivarius	double-positive (concordant)	genus	G+
142	P132	M	80	sputum	Unknown	Candida albicans + Lactobacillus fermentum + Viridans Streptococci + Rothia mucilaginosa	n.a.	Candida albicans + Lactobacillus fermentum + Viridans Streptococci + Rothia mucilaginosa	Lactobacillus fermentum + Rothia mucilaginosa + Streptococcus sanguinis	double-positive (overlapping)	genus	-
143	P133	M	84	sputum	LRI	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-
144	P134	M	51	sputum	LRI	Viridans Streptococci + Neisseria	n.a.	Viridans Streptococci + Neisseria	Streptococcus sanguinis + Neisseria elongata	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)

145	P135	M	69	sputum	LRI	Acinetobacter baumannii	n.a.	Acinetobacter baumannii	Acinetobacter baumannii	double-positive (concordant)	species	G-
146	P136	F	71	sputum	Unknown	Proteus mirabilis	n.a.	Proteus mirabilis	Proteus mirabilis	double-positive (concordant)	species	G-
147	P137	M	50	sputum	Unknown	Corynebacterium striatum	n.a.	Corynebacterium striatum	Corynebacterium striatum	double-positive (concordant)	species	G+
148	P138	F	45	sputum	LRI	Stenotrophomonas maltophilia + Staphylococcus aureus + Corynebacterium striatum	n.a.	Stenotrophomonas maltophilia + Staphylococcus aureus + Corynebacterium striatum	Stenotrophomonas maltophilia + Staphylococcus aureus + Corynebacterium striatum	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
149	P139	M	62	sputum	LRI	Pseudomonas aeruginosa + Staphylococcus aureus	n.a.	Pseudomonas aeruginosa + Staphylococcus aureus	Pseudomonas aeruginosa + Staphylococcus aureus	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
150	P140	M	56	sputum	Unknown	Klebsiella pneumoniae + Acinetobacter baumannii + Corynebacterium striatum	n.a.	Klebsiella pneumoniae + Acinetobacter baumannii + Corynebacterium striatum	Corynebacterium striatum + Acinetobacter baumannii	double-positive (overlapping)	species	-
151	P141	M	65	sputum	LRI	Viridans Streptococci + Neisseria + Staphylococcus epidermidis + Staphylococcus aureus + Pseudomonas aeruginosa	n.a.	Viridans Streptococci + Neisseria + Staphylococcus epidermidis + Staphylococcus aureus + Pseudomonas aeruginosa	Staphylococcus epidermidis + Staphylococcus aureus + Streptococcus anginosus + Neisseria subflava + Pseudomonas aeruginosa	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
152	P94	M	61	sputum	LRI	Pseudomonas aeruginosa	n.a.	Pseudomonas aeruginosa	Pseudomonas aeruginosa	double-positive (concordant)	species	G-
153	P142	M	53	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus sanguinis	double-positive (concordant)	genus	G+
154	P143	F	81	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus anginosus	double-positive (concordant)	species	G+
155	P144	M	44	sputum	LRI	Acinetobacter baumannii + Staphylococcus aureus	n.a.	Acinetobacter baumannii + Staphylococcus aureus	Acinetobacter baumannii + Staphylococcus aureus	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
156	P145	M	53	sputum	Unknown	Streptococcus dysgalactiae	n.a.	Streptococcus dysgalactiae	Streptococcus dysgalactiae	double-positive (concordant)	species	G+
157	P146	M	49	sputum	LRI	Aspergillus fumigatus	n.a.	Aspergillus fumigatus	Aspergillus fumigatus	double-positive (concordant)	species	Fungi
158	P147	F	58	sputum	LRI	Serratia marcescens	n.a.	Serratia marcescens	Serratia marcescens	double-positive (concordant)	species	G-

159	P148	M	45	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus oralis	double-positive (concordant)	species	G+
160	P149	M	92	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus pseudopneumoniae	double-positive (concordant)	species	G+
161	P150	M	70	sputum	Unknown	Viridans Streptococci + Neisseria + Rothia mucilaginosa + Streptococcus parasanguinis + Haemophilus parainfluenzae	n.a.	Viridans Streptococci + Neisseria + Rothia mucilaginosa + Streptococcus parasanguinis + Haemophilus parainfluenzae	Streptococcus parasanguinis + Neisseria subflava + Rothia mucilaginosa + Haemophilus parainfluenzae	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
162	P151	M	79	sputum	LRI	Acinetobacter baumannii + Pseudomonas aeruginosa + Corynebacterium striatum + Stenotrophomonas maltophilia	n.a.	Acinetobacter baumannii + Pseudomonas aeruginosa + Corynebacterium striatum + Stenotrophomonas maltophilia	Acinetobacter baumannii + Pseudomonas aeruginosa + Corynebacterium striatum + Stenotrophomonas maltophilia	double-positive (concordant)	species	Polymicrobial (multiple bacteria)
163	P152	M	17	sputum	Unknown	negative	Klebsiella pneumoniae (+)	Klebsiella pneumoniae	Corynebacterium resistens + Corynebacterium striatum + Klebsiella pneumoniae	double-positive (overlapping)	species	-
164	P14	M	29	sputum	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB
165	P62	M	19	sputum	Unknown	Haemophilus + Neisseria	n.a.	Haemophilus + Neisseria	Neisseria elongata	double-positive (overlapping)	genus	-
166	P153	M	30	sputum	Unknown	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB
167	P154	F	28	sputum	LRI	Acinetobacter baumannii	n.a.	Acinetobacter baumannii	Acinetobacter baumannii	double-positive (concordant)	species	G-
168	P155	F	34	sputum	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB
169	P156	M	72	sputum	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB
170	P157	M	64	sputum	LRI	Escherichia coli	n.a.	Escherichia coli	Escherichia coli	double-positive (concordant)	species	G-
171	P158	M	32	sputum	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus oralis	double-positive (concordant)	species	G+
172	P159	M	78	sputum	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB
173	P160	F	16	sputum	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB

174	P161	M	53	sputum	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB
175	P162	F	29	sputum	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis	double-positive (concordant)	genus	MTB
176	P163	M	59	sputum	Unknown	Rothia mucilaginosa	n.a.	Rothia mucilaginosa	Legionella pneumophila + Rothia mucilaginosa	double-positive (overlapping)	species	-
177	P164	M	58	sputum	LRI	Neisseria + Mycobacterium	n.a.	Neisseria + Mycobacterium	Neisseria subflava + Mycobacterium tuberculosis	double-positive (concordant)	genus	Polymicrobial (multiple bacteria)
178	P165	F	65	sputum	LRI	Klebsiella pneumoniae + Candida albicans	n.a.	Klebsiella pneumoniae + Candida albicans	Klebsiella pneumoniae + Candida albicans	double-positive (concordant)	species	Polymicrobial (bacteria and fungi)
179	P166	M	70	sputum	LRI	Viridans Streptococci	n.a.	Viridans Streptococci	Epstein-Barr virus	double-positive (discordant)	-	-
180	P167	M	63	BALF	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis + Pseudomonas aeruginosa	double-positive (overlapping)	genus	-
181	P168	F	34	BALF	LRI	Viridans Streptococci	n.a.	Viridans Streptococci	Pneumocystis jirovecii	double-positive (discordant)	-	-
182	P169	M	31	BALF	Unknown	negative	Pneumocystis jirovecii (-)	negative	Pneumocystis jirovecii	mNGS-positive	-	-
183	P170	M	56	BALF	Unknown	negative	Epstein-Barr virus (+)	Epstein-Barr virus	Klebsiella aerogenes + Epstein-Barr virus	double-positive (overlapping)	species	-
184	P171	M	66	BALF	Unknown	negative	n.a.	negative	Haemophilus parainfluenzae	mNGS-positive	-	-
185	P172	M	26	BALF	Unknown	negative	n.a.	negative	Pseudomonas putida	mNGS-positive	-	-
186	P173	F	17	BALF	LRI	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Pneumocystis jirovecii + Klebsiella pneumoniae	double-positive (overlapping)	species	-
187	P174	M	71	BALF	Unknown	Neisseria	n.a.	Neisseria	Elizabethkingia meningoseptica + Pseudomonas aeruginosa	double-positive (discordant)	-	-
188	P175	M	38	BALF	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Human betaherpesvirus 5 + Proteus mirabilis + Chryseobacterium indologenes + Streptococcus mitis	double-positive (overlapping)	species	-
189	P176	M	49	BALF	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Enterococcus faecium + Candida glabrata	double-positive (discordant)	-	-
190	P177	F	43	BALF	LRI	Mycobacterium	n.a.	Mycobacterium	Mycobacterium tuberculosis + Epstein-Barr virus	double-positive (overlapping)	genus	-
191	P178	M	14	BALF	Unknown	negative	n.a.	negative	negative	double-negative	-	-
192	P179	F	58	BALF	LRI	Klebsiella pneumoniae	n.a.	Klebsiella pneumoniae	Klebsiella pneumoniae	double-positive (concordant)	species	G-
193	P180	M	13	BALF	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	Streptococcus sanguinis	double-positive (concordant)	genus	G+

194	P181	F	22	BALF	Unknown	negative	n.a.	negative	<i>Stenotrophomonas maltophilia</i>	mNGS-positive	-	-
195	P182	F	54	BALF	Unknown	Neisseria	n.a.	Neisseria	Pneumocystis jirovecii + Human betaherpesvirus 5 + <i>Neisseria subflava</i>	double-positive (overlapping)	genus	-
196	P183	F	42	BALF	Unknown	Human betaherpesvirus 5	n.a.	Human betaherpesvirus 5	Human betaherpesvirus 5	double-positive (concordant)	species	Viruses
197	P184	M	26	BALF	Unknown	Viridans Streptococci	n.a.	Viridans Streptococci	negative	CT-positive	-	-
198	P185	F	43	BALF	Unknown	negative	n.a.	negative	<i>Chryseobacterium indologenes</i>	mNGS-positive	-	-
199	P186	M	69	BALF	Unknown	<i>Klebsiella pneumoniae</i>	n.a.	<i>Klebsiella pneumoniae</i>	Human betaherpesvirus 5	double-positive (discordant)	-	-
200	P187	M	59	BALF	Unknown	negative	n.a.	negative	negative	double-negative	-	-
201	P188	M	70	BALF	Unknown	<i>Mycobacterium</i>	n.a.	<i>Mycobacterium</i>	<i>Mycobacterium tuberculosis</i>	double-positive (concordant)	genus	MTB

Abbreviations: SID, sample ID; PID, patient ID; F, female; M, male; BALF, bronchoalveolar lavage fluid; LRI, lower respiratory infection; BSI, bloodstream infection; CNS, central nervous system infection; CMT, clinical microbiological testing; CT, convention test; mNGS, metagenomic next-generation sequencing; n.a., not available; MTB, *Mycobacterium tuberculosis*.

**Table S2.** The medical information of 24 cases whose mNGS results assisted treatment modification.

PID	SID	CT	mNGS	mNGS/CT comparison	Treatment	Outcome
P1	1	Negative	<i>Pneumocystis jirovecii</i> + <i>Human betaherpesvirus 5</i>	mNGS+	Ganciclovir, Tigecycline, Meropenem and Caspofungin combined with Sulfamethoxazole Tablet	Improve
P7	8	Negative	<i>Staphylococcus haemolyticus</i> + <i>Enterococcus faecium</i> + <i>Mycobacterium tuberculosis Complex</i>	mNGS+	Piperacillin tazobactam combined with teicoplanin	Improve
P19	21	Negative	<i>Legionella pneumophila</i>	mNGS+	Moxifloxacin Hydrochloride	Improve
P24	26	Negative	<i>Mycoplasma hominis</i>	mNGS+	Moxifloxacin	Improve
P29	31	Negative	<i>Escherichia coli</i>	mNGS+	Levofloxacin	Improve
P38	42	Negative	<i>Cryptococcus neoformans</i> + <i>Enterococcus faecalis</i>	mNGS+	Levofloxacin combined with Voriconazole	Improve
P41	45	Negative	<i>Human herpesvirus 1</i> + <i>Acinetobacter baumannii</i> + <i>Epstein-Barr virus</i>	mNGS+	Cefoperazone Sodium and Sulbactam Sodium	Lost to follow up
P42	47	Negative	<i>Pneumocystis jirovecii</i> + <i>Human herpesvirus 1</i>	mNGS+	sulfamethoxazole combined with oseltamivir	Death
P50	55	Negative	<i>Acinetobacter baumannii</i>	mNGS+	Tigecycline	Lost to follow up
P86	91	Negative	<i>Klebsiella pneumoniae</i> + <i>Pseudomonas aeruginosa</i>	mNGS+	Levofloxacin combined with Ceftizoxime Sodium	Improve
P12	14	<i>Viridans Streptococci</i>	<i>Streptococcus agalactiae</i> + <i>Human betaherpesvirus 7</i>	overlapping	Cefoperazone Sodium and Sulbactam Sodium	Improve
P27	29	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i> + <i>Klebsiella pneumoniae</i> + <i>Acinetobacter baumannii</i> + <i>Proteus mirabilis</i>	overlapping	Cefoperazone Sodium and Sulbactam Sodium	Lost to follow up
P73	77	<i>Staphylococcus aureus</i> + <i>Enterobacter cloacae</i>	<i>Staphylococcus aureus</i>	overlapping	Cefoperazone Sodium and Sulbactam Sodium	Lost to follow up
P82	87	<i>Candida glabrata</i> + <i>Candida albicans</i>	<i>Candida glabrata</i>	overlapping	Caspofungin	Improve
P107	113	<i>Viridans Streptococci</i> + <i>Neisseria</i>	<i>Neisseria elongata</i>	overlapping	Moxifloxacin	Improve
P132	142	<i>Candida albicans</i> + <i>Lactobacillus fermentum</i> + <i>Viridans Streptococci</i> + <i>Rothia mucilaginosa</i>	<i>Lactobacillus fermentum</i> + <i>Rothia mucilaginosa</i> + <i>Streptococcus sanguinis</i>	overlapping	Cefodizime Sodium	Improve
P170	183	<i>Epstein-Barr virus</i>	<i>Klebsiella aerogenes</i> + <i>Epstein-Barr virus</i>	overlapping	Cefoperazone Sodium and Sulbactam Sodium combined with Ganciclovir	Improve
P54	59	<i>Viridans Streptococci</i>	<i>Stenotrophomonas maltophilia</i> + <i>Candida glabrata</i>	discordant	SMZ combined with Voriconazole	Improve
P55	60	<i>Viridans Streptococci</i>	<i>Stenotrophomonas maltophilia</i> + <i>Haemophilus parainfluenzae</i>	discordant	Moxifloxacin Hydrochloride	Improve
P67	72	<i>Viridans Streptococci</i>	<i>Staphylococcus haemolyticus</i> + <i>Gordonia bronchialis</i>	discordant	Daptomycin	Improve
P74	79	<i>Candida albicans</i>	<i>Klebsiella pneumoniae</i>	discordant	Tigecycline	Improve
P96	101	<i>Candida albicans</i>	<i>Streptococcus salivarius</i>	discordant	Piperacillin	Improve
P166	179	<i>Viridans Streptococci</i>	<i>Epstein-Barr virus</i>	discordant	Ganciclovir	Lost to follow up
P186	199	<i>Klebsiella pneumoniae</i>	<i>Human betaherpesvirus 5</i>	discordant	DAA	Lost to follow up