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Profiling of Microbe Co-existence in Respiratory Tract Infections

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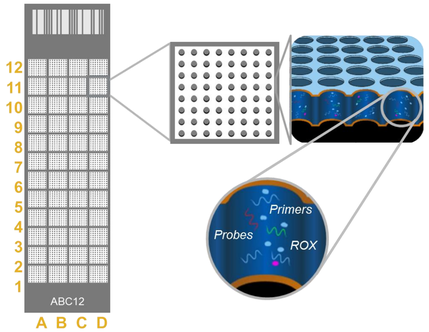
INTRODUCTION

Upper and lower respiratory tract infections can be caused by viral, bacterial or fungal agents. Although respiratory infection is usually diagnosed and treated with a single pathogen, coinfections with multiple agents have been reported in literature and could be common events. Characterizing microbial co-existence profiles will provide useful information for better understanding respiratory infections.

MATERIALS AND METHODS

A retrospective clinical research study was conducted. We tested about four hundred clinical repository specimens previously collected and tested by various laboratories. Nucleic acids from repository samples were extracted, pre-amplified by RT-PCR and tested via TaqMan qPCR method with a Respiratory Tract Microbiota (RTM) Panel on OpenArray (Thermo Fisher Scientific, Figure 1a and 1b). The RTM panel is designed to detect 42 viral, bacterial and fungal microbe targets.

Figure 1a. OpenArray™ system for high throughput RTM profiling



OpenArray™ technology utilizes a microscope slide–sized plate with 3,072 through-holes. Each plate contains 48 subarrays with 64 through-holes. Assays for different RTM targets are pre-spotted in individual through-holes. 1-4 subarrays can form a RTM panel depending on customer specification of the number of targets and replicates. 12 – 48 samples may be processed simultaneously on one OpenArray plate for RTM studies.

Figure 1b. RTM study workflow

DNA and RNA from various types of samples of respiratory tract samples may be extracted with KingFisher™ Flex purification system, followed by reverse transcription and pre-amplification. The reaction is then diluted and loaded into RTM OpenArray™ plates with the AccuFill™ system. Real time PCR of RTM assays is performed in QuantStudio™ 12K Flex instrument and results of positive or negative and Ct values are analyzed with QuantStudio™ 12 Flex software.

RESULTS

1. Multiple targets are detected in selected cohorts

This study presents data from 375 respiratory samples. Among them, 30 target microbes were detected at various levels of prevalence (Figure 1). The target microbes fall into two categories regarding their pathogenicity: those that are know to be pathogenic and some others that are commonly known as opportunistic pathogenic or even commensals. More opportunistic pathogenic targets in total number were detected than those of pathogenic targets.

Table 1. List of microbe targets detected in 375 respiratory samples

Pathogenic Target	Counts
Rhinovirus (RV)	80
Respiratory syncytial virus B (RSV B)	70
Respiratory syncytial virus A (RSV A)	51
Flu A H3	47
Human parainfluenza virus type 3 (hPIV3)	43
Flu B	31
Adenoviruses (AdV)	24
Flu A H1	20
Human parainfluenza virus type 1 (hPIV1)	18
Human metapneumovirus (hMPV)	18
Human bocavirus (HBoV)	18
human coronaviruses OC43 (CoV OC43)	13
human coronaviruses HKU1 (CoV HKU1)	10
Enterovirus (EV)	9
Human parainfluenza virus type 2 (hPIV2)	9
human coronaviruses 229E (CoV 229E)	7
human coronaviruses NL63 (CoV NL63)	7
Human parechovirus (HPeV)	6
Measles	3
Human parainfluenza virus type (hPIV4)	2
Enterovirus D68 (EV D68)	1
Mumps	1
Bordetella	1
Sum	489

Opportunistic Pathogenic Target	Counts
Staphylococcus aureus	100
HHV4 (Epstein–Barr virus, EBV)	95
Haemophilus influenzae	90
Streptococcus pneumoniae	89
Klebsiella pneumoniae	88
HHV6 (Human herpesvirus 6)	73
Moraxella catarrhalis	62
HHV5 (Cytomegalovirus)	32
Pneumocystis jirovecii	18
Sum	647

2. Target abundance distribution differs in two populations

In this study, Ct values reflect the relative abundance of targets. Lower Ct values represent more abundant targets. For pathogenic targets, sample counts distribute relatively evenly across the range of abundance levels. For opportunistic pathogenic targets, more samples bearing lower abundance level of targets were observed (Figure 2a and 2b). This is particularly true for *Klebsiella pneumoniae, Staphylococcus aureus*, HHV4 and HHV5 (Figure 2c).

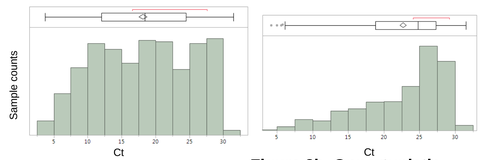


Figure 2a. Pathogenic **Figure 2b. Opportunistic pathogenic**

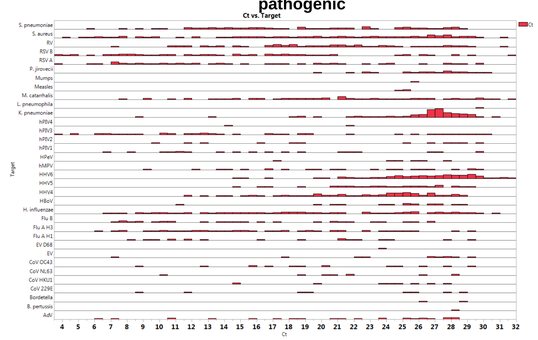


Figure 2c. All targets. Bar height represents sample counts

3. Microbe targets detected per sample

About 20% of 375 selected samples are positive for a single microbe, and 80% have two or more microbes, when all targets are considered at any level abundance (Ct under 28). However, this equilibrium changes when pathogenicity and abundance of targets are taken into consideration. For example, when only targets with abundance level of Ct 25 or earlier are considered, roughly 40% of samples have single targets and 60% have two or more targets (Figure 3, left charts). The proportions of samples with two or more microbes co-existing in one sample also changes when only known pathogenic targets are calculated (Figure 3, right charts).

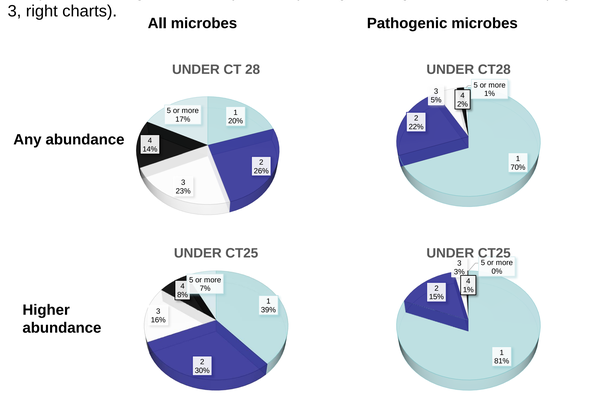
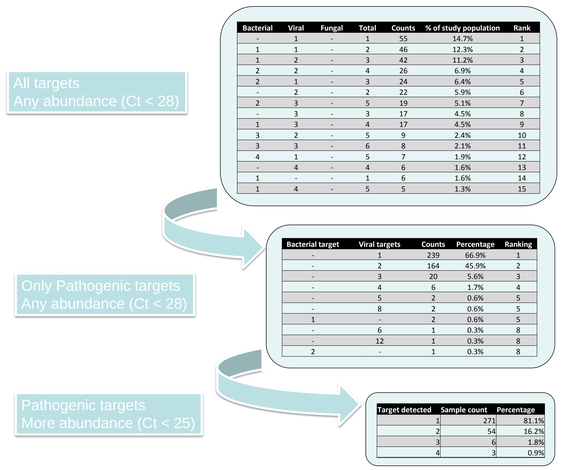


Figure 3. Frequency of target counts per sample in 375 respiratory samples

To further dissect the profiling of microbes existing in selected samples, the number and composition of pathogen types – bacterial, viral and fungal were profiled (Figure 4). In the tested samples, only viruses were observed as co-infection agents if pathogenic. Fewer than 20% samples have two or more viruses co-existing at significant abundance level (Figure 4).



Bacterial	Viral	Fungal	Total	Counts	% of study population	Rank
-	1	-	1	55	14.7%	1
1	1	-	2	46	12.3%	2
1	2	-	3	42	11.2%	3
2	2	-	4	26	6.9%	4
2	1	-	3	24	6.4%	5
-	2	-	2	22	5.9%	6
2	3	-	5	19	5.1%	7
-	3	-	3	17	4.5%	8
1	3	-	4	17	4.5%	9
3	2	-	5	9	2.4%	10
3	3	-	6	8	2.1%	11
4	1	-	5	7	1.9%	12
-	4	-	4	6	1.6%	13
1	-	-	1	6	1.6%	14
1	4	-	5	5	1.3%	15

Bacterial target	Viral targets	Counts	Percentage	Ranking
-	1	239	66.9%	1
-	2	164	45.9%	2
-	3	20	5.6%	3
-	4	6	1.7%	4
-	5	2	0.6%	5
-	8	2	0.6%	5
1	-	2	0.6%	5
-	6	1	0.3%	8
-	12	1	0.3%	8
2	-	1	0.3%	8

Target detected	Sample count	Percentage
1	271	81.1%
2	54	16.2%
3	6	1.8%
4	3	0.9%

Figure 4. Profiling of microbes in samples taking into consideration of target pathogenicity and abundance

4. Profiling of co-existence of viral pathogens in 63 clinical samples

All viral pathogens and their abundance levels were shown in Table 2. Among 63 samples with multiple targets detected, about 85% have with two pathogens, 10% have three, and 5% have as many as four pathogens. Table 3 summarizes the frequency of co-infections by pathogen targets. Interestingly, in contrast with the 20%-40% of co-infection rate in majority of the targets, influenza B virus is the only agent that serves as the sole pathogen in over 95% of influenza B positive samples tested.

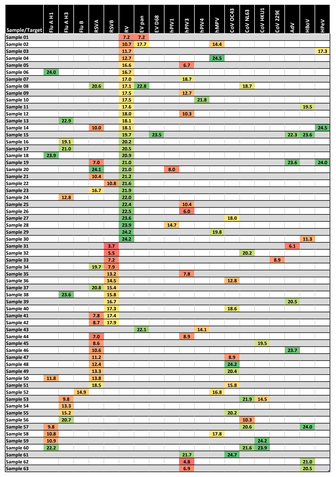


Table 2. Co-existence of pathogenic viral targets in 63 respiratory samples Ct values are shown for each target detected and are color coded. Red color represents high abundant and green color shows that target is less abundant.

Table 3. Prevalence of co-existence of pathogenic viral targets

Pathogen	Total Detected	With Other Pathogen	% Co-infection
RV	72	30	41.7%
RSVA	48	18	37.5%
Flu A H1	19	7	36.8%
AdV	14	5	35.7%
hMPV	15	5	33.3%
hPIV3	39	11	28.2%
Flu A H3	44	9	20.5%
RSVB	64	13	20.3%
hPIV1	16	2	12.5%
Flu B	27	1	3.7%
CoV_NL63	6	6	100.0%
HBoV	6	6	100.0%
EV D68	4	4	100.0%
CoV_OC43	11	9	81.8%
CoV_HKU1	7	5	71.4%
CoV_229E	4	1	25.0%
hPIV2	6	0	0.0%
HPeV	3	3	100.0%
hPIV4	2	2	100.0%
EV pan	1	1	100.0%

CONCLUSIONS

Multiple microbial types were found to co-exist in respiratory samples at different levels of abundance. While commensal, normally non-pathogenic microbes are more frequently detected in samples, often at lower levels, pathogenic microbes, mostly viruses in this study, are found in about 20% of samples examined.

The human respiratory tract microbiota panel (RTM) provides a useful tool to detect many microorganisms simultaneously for studying co-infection. Microbe profiling may help dissect and better understand viral-viral, viral-bacterial and microbe-human immunity interactions and thus could lead to more precise treatment for respiratory infectious diseases in the future.

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