The spectrum of simultaneously detected pathogens identified in infections by the FilmArray panels

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Summary

Aim of the study: It has been previously observed that the FilmArray Panel assays result in the simultaneous detection of multiple pathogens. However, there is limited data on the frequency of such simultaneous detections, the prevalence of certain pathogens in such co-detections and the possible existence of specific patterns.
Materials and Methodology: In order to investigate the spectrum of multiple isolations in our patient series, we have retrospectively looked at the results of all the samples which had been processed with the FilmArray panel assays, during the last one year-period in our hospital.
Results: Among 1041 samples, 8.3% revealed multiple pathogens (>1), 8.7% of the respiratory samples and 8.8% of the gastrointestinal samples. Human Rhinovirus/Enterovirus (HRV) and enteropathogenic *E.coli* (EPEC) were the most frequent pathogens detected on multiple respiratory

and gastrointestinal co-detections, respectively, while the combinations of HRV/Adenovirus and EPEC/*Campylobacter spp* were the commonest.

Conclusions: Our patient series revealed that the application of the FilmArray panel assays had a low, although significant, possibility of simultaneous detection of multiple pathogens, with HRV and EPEC predominating in such samples. Further studies are needed to explore the clinical significance of such pathogen-specific co-detections, arbitrarily named co-infections.

Key words

FilmArray; Multiple pathogens; multiplex PCR; co-infection; respiratory panel; gastrointestinal panel

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Introduction

Upper respiratory tract infections can be a serious burden to the healthcare system.[1] Although most of such infections are viral in origin, it is difficult to differentiate bacterial and viral infections, but also co-infections, due to the frequent overlapping of the clinical presentation and the variable sensitivity and lengthy turn-around time of the viral culture[1]. At the same time, gastrointestinal infections remain a major public health problem worldwide and their diagnosis is one of the main challenges. It has been suggested that enteric pathogen co-infections play an important role in gastroenteritis, but most efforts have only focused on a small range of species belonging to few pathogen groups.[2]

In the FilmArray Panel assays, an unprocessed clinical sample is subjected to nucleic acid purification, reverse transcription, a high-order nested multiplex polymerase chain reaction and amplicon melt curve analysis.[3] Biochemical reactions are enclosed in a disposable pouch, minimizing the PCR contamination risk.[3] The rapid turnaround time, the multiplex nature of the test (allowing simultaneous detection of an array of viruses, bacteria and parasites) and the superior sensitivity of FilmArray Panel assays seem to improve the evaluation and management of patients suspected of having respiratory[4] or gastrointestinal infections. The rapid and sensitive detection of respiratory viruses is essential for the early diagnosis and administration of appropriate antiviral therapy, as well as for the effective implementation of infection control measures.[5] Also, the use of gastrointestinal panels may improve decisions regarding patient isolation and reduce nosocomial transmission.[6] The additional significant value of the FilmArray Panel assays can be underlined by the fact that among pediatric patients who required intensive care unit (ICU) admission, 35.8% had a co-infection with multiple viruses.[7]

As the increasing use of the FilmArray Panel assays gradually reveals the significant incidence of co-infections, we present our first year of experience on the profile of co-detection of pathogens, which resulted by the use of FilmArray Panel assays in the routine clinical practice.

Material and methods

In order to investigate the prevalence of multiple isolations in the routine application of FilmArray Panel assays, but also the most frequently isolated pathogens or patterns of combination of pathogens, we retrospectively studied the results of all the samples which had been processed during one year-period (November 2015 to November 2016) with the FilmArray Panels, in the Central Laboratories of Hygeia & Mitera General Hospitals of Athens. Among 1041 samples processed during this one-year period, 656/1041(63%) were respiratory samples (**Rs**), 318/1041(31%) were gastroin-

testinal samples (**GIs**), 56/1041(5.4%) cerebrospinal fluids (**CSFs**) and 11/1041(1.1%) blood samples (**Bs**) derived from positive blood cultures. The median age of the 1041 patients was 21 years old (1 month – 92 years), while all patients were symptomatic.

BioFire FilmArray Panel assays. The FilmArray multiplex PCR panels consist of the respiratory panel (FA-RP), the gastrointestinal panel (FA-GP), the meningitis/encephalitis panel (FA-ME) and the blood culture identification panel (FA-BC).

FA-RP. Nasopharyngeal swab specimens (Rs) were collected from patients with symptomatic respiratory tract infections and processed with FA-RP. BioFire FA-RP assay version 1.7 (bioMérieux, Marcy l'Etoile, France) is U.S. FDA-cleared and CE-IVD certified.[8] The assay detects nucleic acids of 17 respiratory viruses and 3 bacteria, including adenovirus (Adv), HCoV or Coronavirus (OC43, NL63, 229E and HKU1), human respiratory syncytial virus (RSV), human metapneumovirus (hMPV), influenza A virus (H1/2009, H1 and H3), influenza B virus, parainfluenza viruses (PIV type 1 [PIV1] to PIV4), human rhinovirus/enterovirus (HRV), *Bordetella pertussis, Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae* in a multiplex PCR.[8]

FA-GP. FA-GP performs simultaneous detection of 22 different enteric pathogens directly from stool specimens: *Campylobacter* spp., *Clostridium difficile* (toxin A/B), *Plesiomonas shigelloides, Salmonella* spp., *Vibrio* spp., *Vibrio cholerae, Yersinia enterocolitica*, enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC) (stx1 and stx2) (including specific detection of *E. coli* O157), *Shigella* spp./enteroinvasive *E. coli* (EIEC), *Cryptosporidium* spp., *Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia*, adenovirus F 40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus.[9]

FA-ME. The FA-ME is a multiplex in vitro diagnostic test for the simultaneous, rapid detection of 14 pathogens directly from CSF specimens: *Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae*, cytomegalovirus, enterovirus, herpes simplex virus 1 and 2, human herpesvirus 6, human parechovirus, varicella-zoster virus, and *Cryptococcus neoformans/Cryptococcus gattii*.[10]

FA-BC. The FA-BC tests for a comprehensive list of 24 pathogens and 3 antibiotic resistance genes associated with bloodstream infections.

In all panels, the extraction, amplification, and detection steps take place in separate chambers of a self-contained, single-use pouch. The procedures were performed according to the manufacturer's instructions.

Results

Multiple co-detections. 86 out of 1041 samples (8.3%) revealed multiple pathogens (>1): 57/656(8.7%) of Rs and 28/318(8.8%) of GIs. Based on positive samples only, we had multiple pathogens in 57 out of 356 positive Rs (16%) and 28 out of 158 positive GIs (17.7%), respectively. Our target population, which is the group of patients with multiple isolations, consisted of 42/86(48.8%) males and 44/86(51.2%) females with a median age of 4 years old (1 month – 82 years). However, we have to underline that the median age of the 318 patiens with GIs was 38 years (0.1-89), while the median age of the 656 patients with Rs was 7 years old (0.1-92).

FA-RP. Among the Rs, HRV participated in most multiple isolations, being the most frequent isolated pathogen (37/57, 64.9%) (Figure 1). The second more frequently observed pathogens were PIV (1, 2 and 3), identified in 24 out of 57 samples (42.1%), and Adv identified in 23 out of 57 samples (40.4%), followed by RSV (15/57, 26.3%) (Figure 1). It is remarkable that the coexistence HRV/Adv was the most commonly observed, with 10 cases out of 57(17.5%), followed by HRV/RSV(6/57, 10.5%) (Table 1).

FA-GP. In GIs, Enteropathogenic E.coli (EPEC) was the most frequently isolated pathogen (18/28, 64.3%), followed by *Clostridium difficile* toxin A/B and *Campylobacter* sp (7/28, 25%, each) and *Salmonella* sp (6/28, 21.4%) (Figure 2). Moreover, the most common patterns of combination were the combinations of EPEC with *Campylobacter* sp, *Clostridium difficile* toxin A/B, *Salmonella* sp and Astrovirus (5/28, 3/28, 2/28 and 2/28, respectively) (Table 2).

FA-ME and FA-BC. The application of these panels did not reveal samples with simultaneous multiple detections.

Discussion

An increasing concern has been recently observed in the literature about the role of co-infections of the respiratory or gastrointestinal tract on the patient outcome. There are several reports on co-infections either of the respiratory or the gastrointestinal tract, discussing the prevalence of each pathogen on multiple detections that predominate. In the present study, we have discussed the basic profile of multiple co-detections, which have been obtained by routine clinical specimens in our hospital, trying to investigate a possible clinical significance.

Respiratory infections are a common cause of pediatric morbidity.[11] The available reference data on the

Figure 1

HRV was the most frequent isolated pathogen among the respiratory samples with simultaneously detected multiple pathogens. In the groups of Parainfluenza, Coronavirus and Influenza, all types of each separate group have been included.

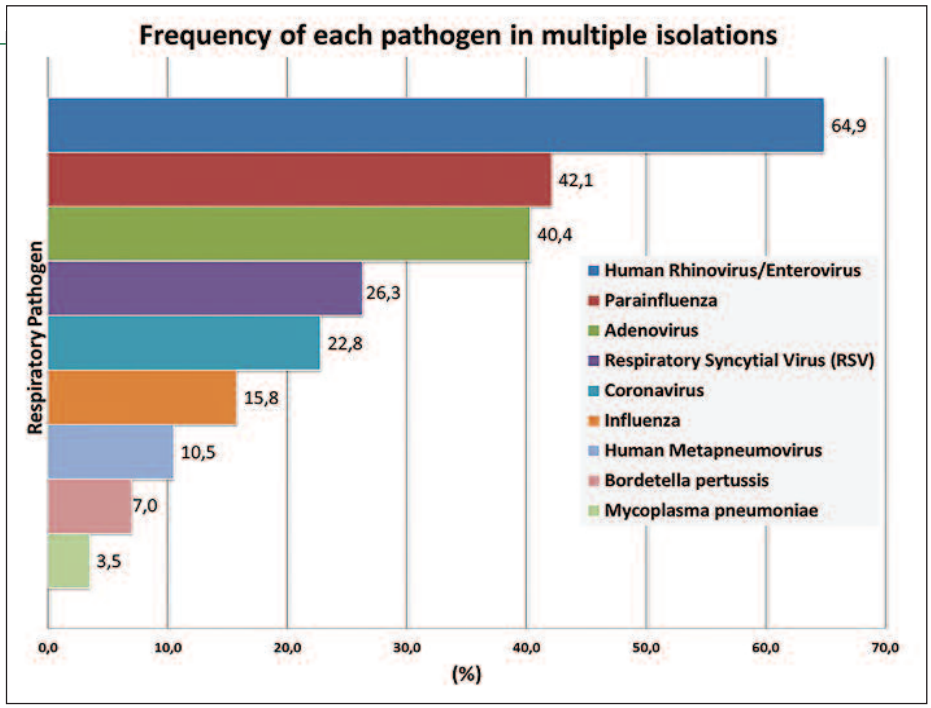

Table 1

The frequency of each respiratory combination pattern.

RESPIRATORY COMBINATION PATTERNS	N	(%)
HRV+Adv	10	17,5
HRV+RSV	6	10,5
HRV+Coronavirus	5	8,8
Adv+hMPV	4	7,0
HRV+PIV	4	7,0
HRV+*Bordetella*	3	5,3
RSV+INFLA	3	5,3
Coronavirus+Adv	3	5,3
Adv+Coronavirus+HRV	3	5,3
RSV+Coronavirus	2	3,5
HRV+hMPV	2	3,5
HRV+Adv+PIV	1	1,8
HRV+INFLA	1	1,8
RSV+Adv	1	1,8
Coronavirus+INFLA	1	1,8
Coronavirus+PIV	1	1,8
Coronavirus.1+Coronavirus.2+RSV	1	1,8
HRV+*Mycoplasma*	1	1,8
Adv+INFLB	1	1,8
HRV+INFLB	1	1,8
Mycoplasma+INFLB	1	1,8
Bordetella+INFLB	1	1,8
HRV+Coronavirus+PIV	1	1,8
	57	100

Coronavirus.1 and Coronavirus.2 refer to two different types of Coronavirus, which were simultaneously detected.
INFLA: influenza A, INFLB: influenza B.

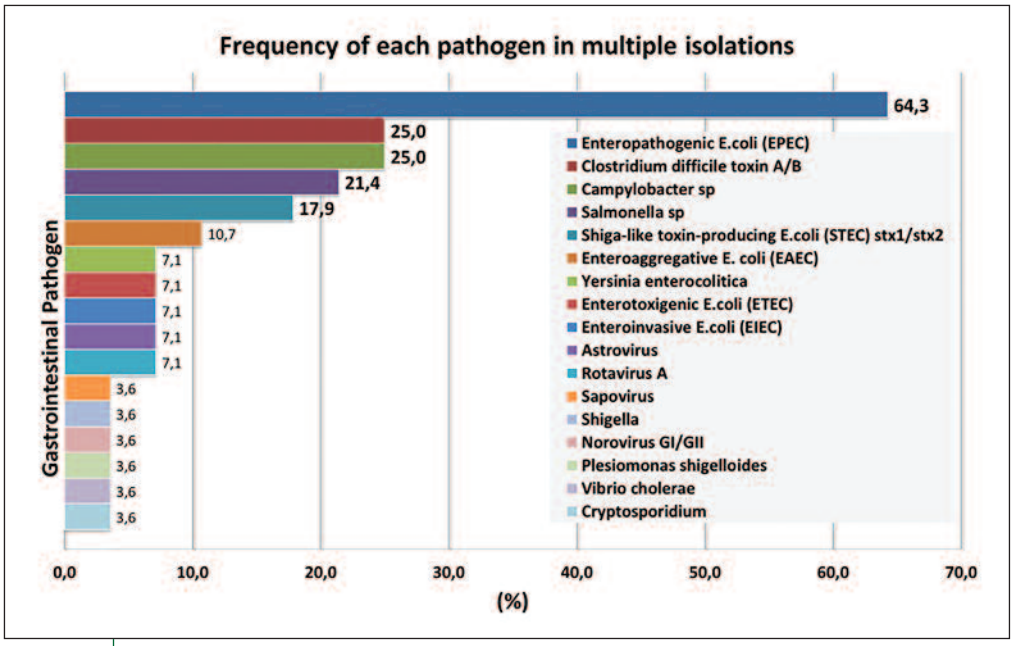

Figure 2 EPEC was the most frequent isolated pathogen among the gastrointestinal samples with simultaneous multiple detections, followed by *Clostridium difficile* toxin A/B, *Campylobacter* sp and *Salmonella* sp.

Table 2

The frequency of each gastrointestinal combination pattern.

GASTROINTESTINAL COMBINATION PATTERNS	N	(%)
EPEC+*Campylobacter*	5	17,9
EPEC+CDtoxA/B	3	10,7
EPEC+*Salmonella*	2	7,1
EPEC+Astrovirus	2	7,1
EPEC+*Salmonella*+Rotavirus	1	3,6
EPEC+*Salmonella* +CDtoxA/B	1	3,6
EPEC+ETEC	1	3,6
EPEC+Norovirus	1	3,6
EPEC+EAEC+*Yersinia*	1	3,6
Campylobacter+Sapovirus	1	3,6
Campylobacter+Yersinia	1	3,6
Salmonella+CDtoxA/B	1	3,6
Shigella+EIEC	1	3,6
CDtoxA/B+EAEC	1	3,6
CDtoxA/B+STEC	1	3,6
STEC+Adenovirus	1	3,6
Salmonella+STEC+Rotavirus	1	3,6
EPEC+STEC+*Cryptosporidium*	1	3,6
Plesiomonas+Vibrio	1	3,6
STEC+EIEC+ETEC+EAEC	1	3,6
	28	100

CDtoxA/B: Clostridium *difficile* (toxin A/B)

frequency of respiratory co-infections vary, although most reported percentages are higher than our results. In a study of 315 respiratory samples from children under 6 years of age, the FA-RP identified multiple co-infections (39%) with 2, 3, 4 and up to 5 different viruses.[12] In the literature co-infection has also been found in 31%,[13] 36%,[14] 37%,[15] 42%[16] and 51.8%[17] of positive respiratory samples. In our study, 9% of all our respiratory patients had multiple pathogens and 16% of all positive respiratory patients (almost one of six positive samples appeared with co-detections). There is no obvious explanation about the significant, although lower frequency of co-detection in our series of patients. However, the predominance of HRV in our single (data not shown) and multiple detections is in accordance with previously published data.[18,19] In a previous study, rhinoviruses have been more frequently detected and their co-infection rates have been also higher.[13] Human rhinovirus infections are highly prevalent, genetically diverse, and associated with both mild upper respiratory tract and more severe lower tract illnesses.[20] Rhinovirus has been found as the most common pathogen detected among symptomatic young children in a pediatric emergency department, while higher rhinovirus viral load and co-infection has increased the severity of the disease.[21] Moreover, HRV/Adv and HRV/RSV combinations have been previously mentioned as the most frequent co-infection patterns.[16,22] Interestingly, it has been suggested that the presence of RSV reduces the probability of rhinovirus infection, but that, if a co-infection occurs, both viruses cause clinical symptoms.[23]

At the same time, the etiology of pediatric diarrhea is rarely established because of overlapping signs and symptoms and expensive and inefficient testing methods.[24] Multiple pathogens have been detected in 7.8%,[25] 15%,[24] 16.4%,[26] 21.1%,[27] 22.2%,[6] 48.1%[28] and 49.3%[29] of positive stool samples. In our study, the application of FA-GP revealed that 17.7% of positive samples had multiple co-detections, a percentage closer to the obtained percentage (16.4%) by the European, multicentre, quarterly point-prevalence study of community-acquired diarrhoea (EUCODI).[26] This specific study, as well as another study which compared two commercially available multiplex panels (FilmArray and Luminex gastrointestinal panels), suggested significantly lower levels of sensitivity on the detection of mixed infections by routine methods, compared to the application of the FilmArray panel (5.5% vs 16.4% and 8.3% vs 21.1%, respectively).[30,31]

According to our knowledge, the prevailance of EPEC on the mixed infections has not been clearly reported elsewhere until now. Nevertheless, it has been usually reported as one of the most prevalent pathogens causing infectious gastroenteritis, detected either alone or included in the term "diarrheagenic *Escherichia coli*".[31-35] Combination patterns that have been previously described as prevalent are rotavirus and toxin-producing *C. difficile*,[36] rotavirus and *Shigella spp*[37] and rotavirus and norovirus GII,[2] while a positive association has been suggested for two pairs, rotavirus and adenovirus, and norovirus GII and *Salmonella*.[38] It is also important that the prevalence of co-infections with two enteric pathogens in diarrhea cases has been found higher than in asymptomatic children,[2] while children with co-infection had a more severe clinical presentation and had a higher probability to be severely dehydrated, independently of age and living community type.[36] Unfortunately, our patient series cannot be clinically assessed as they have been retrospectively analyzed and the clinical information is irrelevant, because of many different medical teams implicated. However, the predominance of EPEC either as the main pathogen or as colonization which accompanies the main pathogen, seem to need further investigation.

In conclusion, our first year experience on the routine application of FilmArray panels indicated a moderate, although remarkable, frequency of multiple co-detections on respiratory and stool specimens. In our patient series, HRV and EPEC were the predominant pathogens, which participated in co-infections, while HRV/Adv and EPEC/*Campylobacter* sp were the most frequent combinations observed. However, many are the remaining questions. What is the main reason that we have a wide variety on co-infections' frequency? It is an age-effect, as it has been previously proposed,[14,17] a time-effect,[39,40] or a combination of these, with factors such as the host phenotypic traits and the pathogen-pathogen interactions?[41] Finally, do we always have co-infections or single infections with accompanying colonization, and is it clearly established that "co-infections" are more severe than single infections? Future studies are considered imperative for the elucidation of all these questions.

Conflict of interest
The authors report no conflict of interests.

Transparency declaration
None to declare.

Περίληψη

Ταυτόχρονα ανιχνευθέντα παθογόνα που ταυτοποιήθηκαν σε λοιμώξεις με την τεχνολογία FilmArray

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Σκοπός της μελέτης: Προηγούμενες παρατηρήσεις έχουν δείξει ότι η εφαρμογή της τεχνολογίας του FilmArray μπορεί να οδηγήσει σε ταυτόχρονη ανίχνευση πολλαπλών παθογόνων. Ωστόσο, υπάρχουν περιορισμένα δεδομένα σχετικά με τη συχνότητα αυτών των ταυτόχρονων ανιχνεύσεων, τη συχνότητα εμφάνισης συγκεκριμένων παθογόνων σε τέτοιες συν-ανιχνεύσεις και την πιθανή ύπαρξη συγκεκριμένων συνδυασμών.
Υλικά και Μεθοδολογία: Για να μελετήσουμε την ταυτόχρονη ανίχνευση των πολλαπλών παθογόνων στη σειρά των ασθενών μας, αναλύσαμε αναδρομικά τα αποτελέσματα όλων των δειγμάτων που είχαν υποβληθεί στη μέθοδο του FilmArray, κατά τη διάρκεια του τελευταίου ενός έτους, στο Νοσοκομείο μας.
Αποτελέσματα: Μεταξύ 1041 δειγμάτων που υποβλήθηκαν στη μέθοδο, το 8,3% αποκάλυψε πολλαπλά παθογόνα (>1). Συγκεκριμένα, το 8.7% όλων των αναπνευστικών δειγμάτων και το 8.8% όλων των δειγμάτων γαστρεντερικού, αντίστοιχα. Ο ανθρώπινος ρινοϊός/εντεροϊός (Human Rhinovirus/Enterovirus ή HRV) και η εντεροπαθογόνος *E.coli* (enteropathogenic *E.coli* ή EPEC) ήταν τα συχνότερα ανιχνευθέντα παθογόνα στις πολλαπλές αναπνευστικές και γαστρεντερικές συν-ανιχνεύσεις, αντίστοιχα. Οι συνδυασμοί HRV/αδενοϊού και EPEC/*Campylobacter* sp ήταν οι συνηθέστεροι.
Συμπεράσματα: Η δική μας σειρά ασθενών έδειξε ότι η εφαρμογή των πάνελ του FilmArray είχε χαμηλή, αν και σημαντική, πιθανότητα ταυτόχρονης ανίχνευσης πολλαπλών παθογόνων, με τον HRV και την EPEC να υπερέχουν σε τέτοια δείγματα. Απαιτούνται περαιτέρω μελέτες για να διερευνηθεί η κλινική σημασία τέτοιων συν-ανιχνεύσεων, που αυθαιρέτως αποκαλούνται συλλοιμώξεις.

Λέξεις κλειδιά
FilmArray; πολλαπλά παθογόνα; πολυπλεκτική PCR; συλλοίμωξη; αναπνευστική λοίμωξη; γαστρεντερική λοίμωξη; μικροσυστοιχίες

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