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# Is there any specific association between respiratory viruses and bacteria in acute otitis media of young children?

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**Summary Background.** Respiratory viral infections are usually preceding or coinciding with acute otitis media (AOM) in children. It is not known if a given viral infection would facilitate invasion of bacterial pathogens into the middle ear in a species-specific way. We reanalysed the microbiological results of the two prospective Finnish Otitis Media (FinOM) studies for this purpose.

**Methods.** The children had been followed from 2 months to 2 years of age in specific study clinics and all referred AOM events were analysed. Combined results of virus detection tests from middle ear fluid and nasopharyngeal aspirate and those of bacterial culture from middle ear fluid were cross-tabulated for 529 AOM events in the FinOM Cohort Study and for 364 events in the FinOM Vaccine Trial.

**Results.** In both studies the main bacterial pathogens were *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* while the main viruses detected were rhinoviruses and respiratory syncytial virus (plus enteroviruses in the Vaccine Trial). No distinct species-specific associations were observed between the viral and bacterial findings.

**Conclusion.** We did not find support to the theory that respiratory infection caused by a given viral species would favour growth of a certain bacterial pathogen in the MEF more than another.

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Acute otitis media (AOM) is among the most common infectious illnesses in children all over the world. *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* are the most important bacterial pathogens in association

with acute otitis media, as reported e.g. by Kilpi et al.<sup>1</sup> while the previously important *Streptococcus pyogenes* has become less common. Viral upper respiratory infections (URI), usually preceding the clinical ear symptoms by a few days,<sup>2,3</sup> are increasingly acknowledged as important factors in the development of AOM in children, either together with bacterial pathogens or on their own.<sup>4-7</sup> Support for a synergistic effect of distinct viruses and bacteria in the pathogenesis of AOM has been obtained in an experimental animal model in chinchilla<sup>8,9</sup> while the matter has been examined in only a few human studies. Chonmaintree and co-workers found no association between specific bacterial and viral findings in 84 AOM events,<sup>10</sup> whereas Heikkinen and co-workers reported, from another small study, that *S. pneumoniae* bacteria were cultured from influenza virus containing MEF specimens more frequently than from those containing other viruses.<sup>11</sup>

Apart from spreading to the middle ear itself, a virus infection in the nasopharynx may contribute to the pathogenesis of AOM by causing inflammation of the mucosa, blocking the Eustachian tube and impairing the host's immune functions<sup>12-14</sup> thus aiding in the invasion by bacteria. Infections caused by respiratory syncytial virus especially have been suggested to result in AOM more often than those by other viruses.<sup>11</sup> Other viruses or virus groups often associated with AOM include influenza virus A, adenoviruses and parainfluenza virus types 1 and 3. Lately, with the use of PCR as a diagnostic method, rhinovirus and enterovirus groups have emerged among the most common viruses in this context.<sup>15-17</sup>

The Finnish Otitis Media Studies (FinOM) comprise two large prospective studies in young children carried out in 1990s, with the FinOM Cohort Study examining the epidemiology and different risk factors of AOM<sup>1</sup> and the FinOM Vaccine Trial evaluating the efficacy and safety of two pneumococcal conjugate vaccines.<sup>18,19</sup> Main bacteriological and virological observations in these studies have been published separately in the previous reports.<sup>1,5,6,18,19</sup> In the present study, we reanalysed microbiological data collected in the studies to assess specific viral-bacterial associations in AOM.

## Materials and methods

### Children, specimens and definitions

The children of the present study belong to two groups of children, the FinOM Cohort Study

comprising 329 healthy children and a random sample of 198 children from the control group (i.e. those who had received hepatitis B vaccine instead of either of the two pneumococcal conjugate vaccines) of the FinOM Vaccine Trial.<sup>6</sup> The Cohort Study was conducted from April 1994 through July 1997 and the FinOM Vaccine Trial from December 1995 through March 1999 in Tampere district in Southern Finland.<sup>18</sup>

The parents of the children were advised to take their child to the study clinic every time when symptoms of an acute respiratory infection occurred and especially if acute otitis media was suspected.<sup>1</sup> Middle ear fluid specimens for viral and bacteriological testing were collected from all children with AOM in both study groups. Nasopharyngeal aspirates collected at the same AOM visit were subjected to virological testing only. An informed written consent was obtained from the parents or guardian of the study children. The consent forms and the study protocols were all accepted by the Ethics Committee of the National Public Health Institute and by those of the Tampere Health Centre and Tampere University Hospital.

An evaluable event of AOM in the present study is an AOM event diagnosed by the study doctor according to defined clinical criteria<sup>20</sup> and with both NPA, and MEF from one or both ears, available for viral analysis and the MEF(s) for bacterial culture. Only the first AOM event of a 30-day AOM episode as defined by Vesa et al.<sup>5</sup> was included, and the events with ongoing anti-microbial medication were excluded.

An event was considered virus positive if any virus was detected in the NPA and/or one or both of the MEF specimens from the two ears. For a positive bacteriological result, it was required that at least one of the four main pathogens *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* or *S. pyogenes* was cultured from the MEF. All the other bacteria classified as 'other' were included in the same group as negative cultures.

### Laboratory methods

The virological methods included antigen detection from the MEFs and the NPAs in all cases applying time-resolved fluoroimmunoassay<sup>21</sup> for adenovirus group, influenza viruses A and B, parainfluenza viruses 1,2 and 3 and RSV. In addition, the MEFs and NPAs were analysed for rhinoviruses by combined isolation-reverse transcriptase-PCR method in the Cohort Study<sup>22</sup> and by direct RT-PCR for the samples in the Vaccine Trial.<sup>6</sup> Enteroviruses and parechoviruses were searched for by RT-PCR and

lanthanide-labelled probes<sup>6</sup> in the Vaccine Trial only. The bacterial culture was performed from the freshly collected MEF samples as described by Kilpi et al.<sup>1</sup>

## Results

### Microbiological observations

The number of evaluable AOM events analysed both virologically and bacteriologically was 529 in the Cohort Study, originating from 201 children, and 364 in the Vaccine Trial, derived from 152 children. At least one of the main bacterial pathogens and/or the viruses searched for was detected in a similar proportion (85–86%) in both studies (Table 1). While the percentage of events with a bacterial pathogen isolated was likewise similar in the two studies, the proportion of AOM events associated with a viral infection was relatively larger in the Vaccine Trial, possibly due to the addition of the test for enteroviruses (Table 1). Out of the Cohort Study events with a single bacterial isolate, *S. pneumoniae* occurred in 116 (22%), *H. influenzae* in 95 (18%) and *M. catarrhalis* in 102 events (19%) while *S. pyogenes* was detected only once (Table 2). The corresponding figures in the Vaccine Trial were 94 (26%), 46 (13%), 61 (17%) and 0, respectively (Table 2).

Of the viruses detected in the Cohort Study as single viral findings, rhinovirus group occurred most frequently, detected in 207 (39%) events. The next most common was RSV in 6% while adenoviruses, influenza A and parainfluenza virus 3, when combined, constituted 4.5% of the events (Table 2). In the Vaccine Trial, the rhinoviruses were again the most frequently found viruses, occurring as single viral findings in 81 (22%) events, followed by

enteroviruses in 63 events (17%) and RSV in 35 events (10%; Table 3). Influenza A occurred in eight events, adenoviruses in four while influenza B and parechoviruses each occurred only once (Table 3).

### Specific associations

Specific associations between distinct viral and bacterial species were searched for in the above cross-tabulations in two ways: (i) by first categorizing the AOM events on the basis of viral findings and then asking in which proportions in each category a given bacterial pathogen had been cultured from MEF (Table 4), and (ii) the other way around, categorizing by bacterial findings and asking for occurrence of viral infections (Table 5). A detected respiratory viral infection appeared to have little effect on the overall results of the bacterial culture as the proportion of events with any bacterial pathogen detected in the MEF was similar in virus negative and virus positive events (Table 4). The proportion of virus infection associated events was similar whether a bacterial pathogen had been cultured from the MEF or not (Table 5).

As for the virus group specific associations, rhinovirus infection associated events revealed the presence of the main bacterial pathogens in similar proportions as all events, with the exception of *M. catarrhalis*, which appeared to coincide in somewhat smaller numbers in the Vaccine Trial (Table 4). Respiratory syncytial virus infection associated events, in contrast, showed lower figures for all major bacterial pathogens, with the exception of *M. catarrhalis* in the Vaccine Trial (Table 4). Indeed, RSV was detected relatively more frequently in events remaining negative for bacterial pathogens in both studies (Table 5). Enterovirus infection positive events in the Vaccine Trial appeared to segregate in the opposite way: both

**Table 1** Viral and bacterial findings in acute otitis media (AOM) events of children in the Finnish Otitis Media (FinOM) Cohort Study and in the FinOM Vaccine Trial

Virus/bact. positive events	FinOM Cohort Study (n=529), No. (%)	FinOM Vaccine Trial (n=364), No. (%)
Virus total	284 (54)	233 (64)
Bact. total	353 (67)	235 (65)
Bact. and virus	185 (35)	154 (42)
Bact. and/or virus	451 (85)	314 (86)
Bact. only	167 (32)	81 (22)
Virus only	99 (19)	79 (22)
No pathogen	78 (15)	50 (14)

Bacterium positive events include those with one or two of the four main pathogenic bacteria (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. pyogenes*) and the bacteriological results concern middle ear fluids (MEFs) only. Virus positivity means a viral finding in MEF and/or nasopharyngeal aspirate from the same AOM event whose MEF was also bacteriologically investigated. The group 'no pathogen' includes the events with no virus(es) and none of the four main pathogenic bacteria.

**Table 2** Number of acute otitis media events (out of a total of 529) with indicated viruses and bacteria detected in middle ear fluid and/or nasopharyngeal aspirate (viruses only) in 201 young children belonging to the Finnish Otitis Media Cohort Study

	<i>S. pn.</i>	<i>H. infl.</i>	<i>M. cat.</i>	<i>S. pyog.</i>	Mixture	Other or negative	Sum
Adenovirus	1	1	0	0	1	1	4
Influenza A	2	1	3	0	1	3	10
Parainfl. 3	2	0	4	0	1	3	10
RSV	5	4	3	1	4	15	32
Rhinovirus	42	41	41	0	15	68	207
Mixture	8	2	3	0	0	8	21
Negative	56	46	48	0	17	78	245
Sum	116	95	102	1	39	176	529

Virus positivity is defined as a viral finding in a nasopharyngeal aspirate and/or middle ear fluid (MEF) specimen(s) from the same AOM event whose MEF was also bacteriologically investigated. Virus negative event: no virus(es) detected. Two or more viruses from the same event are classified as viral mixtures. Bacterium positivity is defined in an analogous manner but concerning MEF only and exclusively the four main bacterial pathogens: *Streptococcus pneumoniae* (*S. pn.*), *Haemophilus influenzae* (*H. infl.*), *Moraxella catarrhalis* (*M. cat.*), *Streptococcus pyogenes* (*S. pyog.*). In the category 'other or negative' other bacteria beyond the four main pathogenic species may occur in the MEF or the fluid may be sterile.

*S. pneumoniae* and *H. influenzae* showed higher figures in these events than in general (Table 4) and enterovirus alone was found in a smaller proportion of events than together with one or more of the bacterial pathogens (Table 5). Other viruses occurred in much smaller numbers and their association with different bacterial pathogens was not evaluated beyond Table 3.

## Discussion

In this paper we have reanalysed the microbiological findings of two prospective AOM studies in young children in order to reveal possible specific associations between the species of the bacterial pathogen cultured from the MEF and the viral species or group causing the concomitant respiratory viral

**Table 3** Number of acute otitis media events (out of a total of 364) with indicated viruses and bacteria detected in middle ear fluid and/or nasopharyngeal aspirate (viruses only) in 152 young children belonging to the Finnish Otitis Media Vaccine Trial

	<i>S. pn.</i>	<i>H. infl.</i>	<i>M. cat.</i>	Mixture	Other or negative	Sum
Adenovirus	0	1	2	1	0	4
Influenza A	1	1	2	1	3	8
Influenza B	0	1	0	0	0	1
Parainfl. 3	3	0	2	0	4	9
RSV	7	2	6	6	14	35
Enteroviruses	22	10	10	6	15	63
Rhinoviruses	22	12	9	10	28	81
Parechovirus	0	0	1	0	0	1
Mixture	4	2	9	1	15	31
Negative	35	17	20	9	50	131
Sum	94	46	61	34	129	364

Virus positivity is defined as a viral finding in a nasopharyngeal aspirate and/or middle ear fluid (MEF) from the same AOM event whose MEF was also bacteriologically investigated. Two or more viruses detected from the same event are classified as viral mixtures. Virus negative event: no virus(es) detected. Bacterium positivity is defined in an analogous manner but concerning MEFs only and exclusively the four main bacterial pathogens: *Streptococcus pneumoniae* (*S. pn.*), *Haemophilus influenzae* (*H. infl.*), *Moraxella catarrhalis* (*M. cat.*), and *Streptococcus pyogenes*. One AOM event with a *S. pyogenes* finding in Table 5 is included in the category 'bacterial mixture' together with a rhinovirus. In the category 'other or negative', other bacteria beyond the four main bacterial pathogens may occur in the MEF or the fluid may be sterile.

**Table 4** Bacterium positivity related to virus positivity in events of acute otitis media (AOM) of children in the Finnish Otitis Media (FinOM) Cohort Study (529 AOM events) and in the FinOM Vaccine Trial (364 events)

	FinOM Cohort Study						FinOM Vaccine Trial				
	Total (N)	Any bact (%)	<i>S. pn.</i> (%)	<i>H. infl.</i> (%)	<i>M. cat.</i> (%)	<i>S. pyog.</i> (%)	Total (N)	Any bact (%)	<i>S. pn.</i> (%)	<i>H. infl.</i> (%)	<i>M. cat.</i> (%)
All events	529	67	22	18	19	0.2	364	65	26	13	17
Virus pos.	284	65	21	17	19	0.4	233	66	26	12	18
Rhinovirus pos.	207	67	20	20	20	0	81	65	28	15	11
RSV pos.	32	50	16	13	9	3	35	60	20	6	17
Enterovirus pos.							63	76	35	16	16
Virusmixt. pos.	21	62	38	10	14	0	31	52	13	6	29
Virus neg.	245	68	23	19	20	0	131	62	27	13	15

Data indicate percentage of bacterium positive AOM events out of the number of virologically defined events. Group 'any bact' includes the events with at least one of the main pathogenic bacteria: *Streptococcus pneumoniae* (*S. pn.*), *Haemophilus influenzae* (*H. infl.*), *Moraxella catarrhalis* (*M. cat.*) or *Streptococcus pyogenes* (*S. pyog.*) in the middle ear fluid (MEF). Virus positive AOM event is defined as a viral finding in a nasopharyngeal aspirate and/or MEF specimen(s) from the same acute otitis media event whose MEF was also bacteriologically investigated.

infection. Both studies had involved large groups of children visiting special study clinics, with all AOM events clinically well characterised and the microbiological aetiology thoroughly examined. In a large proportion of the studied events in both studies, a verified viral infection was coinciding with a positive MEF culture for one of the major bacteriological pathogens, but no species-specific associations were seen. This is in agreement with a previous report by Chonmaitree and co-workers.<sup>10</sup>

Viral respiratory infections are commonly known to accompany or precede acute otitis media and the significance of viral-bacterial interactions in the development of clinical AOM is generally accepted. Different pathogenetic mechanisms for these interactions have been suggested and they supposedly act together in a complicated manner depending on the immune status and genetic background of the host in addition to the properties of the microbes involved. We hypothesized that a given virus group or species might facilitate growth of a certain bacterial species in the middle ear, for instance, by inducing a cytokine response causing a specific pattern of inflammation in the nasopharynx, which would then favour one bacterial species more than another. This situation could be presented as a specific association between the indicated virus group and the benefited bacterial species. Our failure to detect any such association should not be taken as evidence for that such a situation could not exist, because several features in our set up might have prevented us from seeing it. Firstly, our microbiological data were limited to a single sampling time determined by the child's presentation at the study clinic after a varying length of

AOM symptoms, while the associated viral infection had persisted most likely for several days already. As a consequence, we may have missed some relevant viruses. Secondly, for a bacterial species to be detected in the MEF during AOM, it is required that the patient is carrying the bacterium in the nasopharynx before the onset of AOM. No relevant bacterial carriage data were available for these patients but it is obvious that every child was not carrying every bacterial pathogen constantly. This might be one reason for the observed variation in the distribution of the bacterial species in different datasets representing a single study. For instance, the sample of AOM events of the Vaccine Trial analysed in this study showed relatively more *S. pneumoniae* than larger materials of the same study analysed before.<sup>18,19</sup> Thirdly, in spite of using sensitive techniques to detect the different viruses in the samples, both biological and technical factors may have resulted in missing some infections caused by the virus groups tested for. The quantities and the lengths of excretion are likely to vary depending on both the virus species and the host individual. Likewise, the detection sensitivities of the tests—even if exploiting the same principle—usually vary from virus to virus.

Two groups of known respiratory viral pathogens were not tested for at all, human coronaviruses and human metapneumovirus. Testing for them might have increased the proportion of AOM events with documented coinciding defined virus infection, but we do not believe that it would have changed the main results of this study because the tested viruses covered two thirds of all AOM events, with or without MEF cultures positive for bacterial

**Table 5** Virus positivity related to bacterium positivity in events of acute otitis media (AOM) of children in the Finnish Otitis Media (FinOM) Cohort Study (529 AOM events) and in the FinOM Vaccine Trial (364 events)

	FinOM Cohort Study				FinOM Vaccine Trial				
	Total (N)	Any virus (%)	Rhino-virus (%)	RSV (%)	Total (N)	Any virus (%)	Rhino-virus (%)	RSV (%)	Enterovirus (%)
All events	529	54	39	6	364	64	22	10	17
Bact. pos.	353	53	39	4.5	235	66	23	9	20
<i>S. pn.</i> pos.	116	52	36	4	94	63	24	7	23
<i>H. infl.</i> pos.	95	52	43	4	46	63	26	4	22
<i>M. cat.</i> pos.	102	53	40	3	61	67	15	10	16
<i>S. pyog.</i> pos.	1	100	0	100					
Bact. mixt. pos.	39	56	38	10	34	73	29	18	18
Other or negative	176	56	38	9	129	61	22	11	12

Data indicate percentage of virus positive events out of the number of bacteriologically defined events. Bacterium positive AOM events are events with at least one of the main bacterial pathogens in the middle ear fluid (MEF), i.e. *Streptococcus pneumoniae* (*S. pn.*), *Haemophilus influenzae* (*H. infl.*), *Moraxella catarrhalis* (*M. cat.*) or *Streptococcus pyogenes* (*S. pyog.*). Bact. mixt. positive events are events with at least two of the main pathogenic bacteria concomitantly in the middle ear fluid (MEF). In the category 'other or negative' events other bacteria beyond the four main pathogenic bacteria may occur in the MEF or the fluid may be culturally negative. Virus positive AOM event is defined as a viral finding in a nasopharyngeal aspirate and/or MEF specimen(s) from the same acute otitis media event whose MEF was also bacteriologically investigated.

pathogens. Under these conditions, it is obvious that only very strong interactions would become apparent and our negative results for specific virus-bacterial associations in AOM should be valued accordingly.

The distribution of the specific viruses in the cross-tabulations seemed mostly quite haphazard, and apparent trends in one of the studies were not usually confirmed in the other. Only the occurrences of rhinoviruses, enteroviruses and RSV were analysed because the numbers of all other detected viruses were so small that random effects caused confusing, and most likely false segregations. This is exemplified in Table 4 where in the Cohort Study, pneumococci appear to associate with virus mixture much more often than with any single virus, whereas in the Vaccine Trial the opposite is true and here instead *M. catarrhalis* is specifically associated with virus mixture. Epidemiological studies indicate that occurrence of AOM is strictly associated with viral upper respiratory tract infections. In the prospective Cohort Study, about 40% of URI in young children were found to result in AOM.<sup>5</sup> In this study, we decided to compare the aetiology of URI, whether based on virus detection in NPA or MEF, with the bacterial MEF culture result, rather than relying on the MEF results only for both microbial classes. In these two studies, the overall rate of virus detection in the MEF was about two thirds of that in the NPA and the virus found in the MEF was also detected in the NPA

specimen in about 60 and 80%, respectively, of the MEF positive events.<sup>6</sup> We also made the cross-tabulations of the MEF results only (not shown) and found that, possibly due to smaller numbers of events, random variation appeared to cause some trends in one study but again, they were not seen in the other, and, therefore, not likely to be true.

RSV was detected in AOM slightly more often without bacteria than concomitantly with them. This trend is consistent with the previous concept<sup>11</sup> that, as compared with other viral infections, RSV infection is associated with an increased risk for AOM, even without bacteria. Similarly, RSV positive URI episodes were more often associated with AOM than URI due to other viruses<sup>5</sup> and, compared with other viruses, a relatively larger proportion of RSV positive NPA specimens was coinciding with RSV positive MEF specimens.<sup>6</sup> Enteroviruses, analysed in the Vaccine Trial only, appeared to occur more often with the main bacterial pathogens than without them. This might mean that enteroviruses are not very potential pathogens in the ear by themselves. Alternatively, the result could also suggest that enterovirus infection is relatively efficient in facilitating bacterial invasion into the middle ear.

In conclusion, the present study does not lend support to the hypothesis that some viral-bacterial combinations would be more apt to cause acute otitis media than the others. Still, this idea is worth pursuing further in other clinical materials, especially during influenza outbreaks.

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