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1 **Title**

2 Does respiratory virus co-infection increase the clinical severity of acute respiratory  
3 infection among children infected with respiratory syncytial virus?  
4

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28

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34

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36 Acute respiratory infections; viral co-infection; respiratory virus; RSV; RT-PCR

37

38 **Abbreviated title**

39 RSV/non-RSV respiratory virus co-infection and severity

40

41 **Running head title**

42 RSV and other respiratory virus co-infection

43

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47

## Abstract

48

49 **Background:** Respiratory syncytial virus (RSV) is a leading cause of acute  
50 lower respiratory infection in children less than 5 years of age. The impact of non-RSV  
51 respiratory virus co-infection on the severity of RSV disease is unknown.

52 **Methods:** This hospital-based prospective study was conducted in Nagasaki, Japan, on  
53 all children less than 5 years of age with acute respiratory infection (ARI) who had  
54 undergone a rapid RSV diagnostic test between April 2009 and March 2010. Thirteen  
55 respiratory viruses were identified from nasopharyngeal swab samples using a multiplex  
56 polymerase chain reaction (PCR); PCR positive samples were considered as confirmed  
57 respiratory virus infections. The cases were classified into three categories (pneumonia;  
58 moderate-to-severe non-pneumonic ARI; and mild ARI) according to the findings of the  
59 chest radiograph and the hospitalization records.

60 **Results:** Among 385 cases enrolled, 372 were eligible for analysis, of whom 86 (22%)  
61 were classified as pneumonia cases; 137 (36%), moderate-to-severe non-pneumonic  
62 ARI cases; and 162 (42%), mild ARI cases. RSV was detected in 172 cases (61.6%),  
63 and 31 cases (18.0%) had double or triple infections with other respiratory viruses. RSV  
64 infection was more frequently observed in pneumonia cases (OR, 2.2; 95% CI,  
65 1.27–3.8) and moderate-to-severe non-pneumonic ARI cases (OR, 2.95; 95% CI,

66 1.82–4.78) than in mild ARI cases. The association with moderate-to-severe  
67 non-pneumonic ARI cases was stronger with RSV/non-RSV respiratory virus  
68 co-infection (adjusted OR, 4.91; 95% CI, 1.9–12.7) than with RSV single infection  
69 (adjusted OR, 2.77; 95% CI, 1.64–4.7).

70 **Conclusions:** Non-RSV respiratory virus co-infection is not uncommon in  
71 RSV-infected children and may increase the severity of RSV disease.

72

73

74

## Background

75 Acute respiratory infection (ARI) is the leading cause of morbidity and  
76 mortality among infants and young children less than 5 years of age.[1] According to  
77 estimates from the World Health Organization and UNICEF, 2 million childhood deaths  
78 in the world are attributable to ARI annually.[2] Respiratory syncytial virus (RSV) is the  
79 most important viral pathogen, causing bronchiolitis, pneumonia, and severe forms of  
80 ARI in young children.[3] In addition to RSV, a wide range of respiratory viruses,  
81 including influenza virus, human metapneumovirus (HMPV), and human rhinovirus  
82 (HRV), are known to cause childhood ARI.[4]

83

84 Recently, multiplex polymerase chain reaction (PCR) has been used to detect  
85 concurrent infection with multiple respiratory viruses and has demonstrated that  
86 co-infection with more than one respiratory virus is common in children with and  
87 without ARI.[5-13] However, the clinical implications of multiple viral infections  
88 remain controversial. Several studies have shown that respiratory virus co-infection may  
89 increase the clinical severity of RSV disease,[5,7-9] while other studies have reported  
90 opposite findings.[10-12] These differences may be attributable to variations in clinical  
91 settings, study populations, and the seasonality of circulating viruses. No similar studies

92 have been formally conducted in East Asian countries, including Japan.

93

94 The aim of this study was to determine the frequency of respiratory virus

95 co-infection with RSV and to investigate the impact of RSV/non-RSV respiratory virus

96 co-infection on the severity of ARI among children less than 5 years of age.

97



98

## Materials and Methods

### 99 Participant and clinical data collection

100 This hospital-based prospective study was conducted in Nagasaki, Japan. The total  
101 population in the city in 2010 was 443,000, with 16,700 (3.8%) individuals less than 5  
102 years of age. Nagasaki City Municipal Hospital (NCMH) is one of two referral hospitals  
103 for sick children in Nagasaki. NCMH has 414 beds, including 38 beds in the pediatric  
104 ward. The hospital also cares for patients who walk in with mild symptoms. About a  
105 half of outpatients are cases without referral letters. We expect that the characteristics of  
106 these children do not differ from those patients in neighboring clinics.

107

108 We approached all children less than 5 years of age who showed signs of ARI  
109 and who were tested using the rapid RSV diagnostic kit in either the outpatient  
110 department (OPD) or the pediatric ward of NCMH. The children were evaluated by  
111 hospital pediatricians regardless of their participation in the study and the rapid  
112 diagnostic tests were ordered by them based on their clinical judgment; no standardized  
113 testing/admission criteria were used in the study. All children who had been  
114 administered the rapid RSV diagnostic tests were enrolled in the study regardless of  
115 their test findings." A commercial RSV diagnostic kit (a rapid

116 immunochromatographic assay; Check RSV, Alfresa Farmer Co., Ltd., Japan)[14] was  
117 routinely used whenever RSV infection was clinically suspected. The sensitivity and  
118 specificity of the RSV diagnostic kits which were commercially available in Japan were  
119 32-79% and 100% when compared with RT-PCR.[15]

120

121           The study period was from April 1st 2009 through March 31st 2010 including  
122 the four seasons: spring (March-May), summer (June-August), fall (September-November),  
123 and winter (December-February). Information from clinical findings, chest radiographs,  
124 and blood examinations were retrospectively obtained from the hospital records. The  
125 information on some risk factors for severe diseases such as prematurity and  
126 breastfeeding status were not always available in the hospital records, and the blood  
127 examination was not usually done for the majority of outpatients. Thus these data were  
128 not collected in our study.

129

### 130 **Sample collection and storage**

131           Residual nasopharyngeal swabs were temporarily stored at  $-20^{\circ}\text{C}$  in the  
132 laboratory department of NCMH after being used for the rapid RSV diagnostic test.  
133 Within a week, the samples were transported to the Institute of Tropical Medicine at

134 Nagasaki University for storage in a deep freezer (−80 °C) until they were used for  
135 further molecular tests.

136

### 137 **Multiplex PCR analysis**

138           The viral nucleic acid was extracted using a QIA viral RNA minikit (QIAGEN  
139 Inc., Valencia, CA). The following four multiplex PCR assays were performed for each  
140 sample to detect 13 respiratory viruses in the RNA: (1) RSV, influenza A/B (FLUA,  
141 FLUB), and HMPV; (2) human parainfluenza virus (HPIV) types 1–4; (3) HRV and  
142 human coronavirus OC43/229E (HCoV); and (4) human adenovirus (HAdV) and  
143 human bocavirus (HBoV). The details of the multiplex PCR assays are published  
144 elsewhere.[13,16] Briefly, reverse transcription-PCR (RT-PCR) assays were performed  
145 using the one-step RT-PCR kit from QIAGEN. Confirmatory-PCR was performed using  
146 a hemi-nested PCR assay on samples that were positive in the initial PCR test. Samples  
147 positive for both PCR assays were defined as positive, and PCR positive samples were  
148 considered as confirmed respiratory virus infections. The results of rapid RSV  
149 diagnostic test kit were not used to define our cases.

150

### 151 **Data analysis**

152           The patients were classified into one of the following three case categories: (1)  
153 a pneumonia case, if the hospitalized child had consolidation on the chest radiograph;  
154 (2) a moderate-to-severe non-pneumonic ARI case, if the hospitalized child did not have  
155 consolidation on the chest radiograph; and (3) a mild ARI case, if the child was not  
156 hospitalized and did not have a chest radiograph. Most of the moderate-to-severe  
157 non-pneumonic ARI cases had bronchiolitis or severe bronchitis. Children who were  
158 hospitalized but did not have a chest radiograph were excluded from the analysis.

159

160           Patient characteristics were compared using the chi-square test for categorical  
161 variables and the Kruskal-Wallis test for continuous variables. Odds ratios were  
162 calculated to demonstrate the associations between the viral infection status and the  
163 disease severity (i.e. mild ARI vs. moderate-to-severe non-pneumonic ARI and mild  
164 ARI vs. pneumonia). The effect of RSV infection with or without other respiratory viral  
165 co-infection on the disease severity were investigated using logistic regression models.  
166 Sex and age group (<12 months, 12-23 months, and 24-59 months) were included in the  
167 final model as potential confounders. The Hosmer-Lemeshow test was used to  
168 determine the goodness-of-fit of the model. All data management was performed using  
169 Microsoft Excel 2007, and statistical analyses were performed using STATA 11.2

170 (STATA Corp., USA). The significant level was taken to be 5% for two-tailed tests.

171

172 **Ethics approval**

173 The study was approved by the Institutional Review Board (IRB) of NCMH

174 and the IRB of the Institute of Tropical Medicine, Nagasaki University, Nagasaki,

175 Japan.

176

## Results

177

178           During the study period, a total of 385 children were tested for RSV, and all  
179 were recruited for this study. In all, 86 (22%) children were classified as pneumonia  
180 cases, 137 (36%) were classified as moderate-to-severe non-pneumonic ARI cases, and  
181 149 (39%) were classified as mild ARI cases. Thirteen (3%) children were hospitalized  
182 but did not have a chest radiograph; these children were excluded from the analysis.  
183 Among the 372 children, 227 (61%) were male, and the median age (inter-quartile  
184 range) was 12 (5.5) months, ranging from 0 to 96 months. When the characteristics of  
185 the three groups were compared, there were no significant differences in sex and age.  
186 However, pneumonia cases had significantly higher white blood cell (WBC) counts and  
187 C-reactive protein (CRP) levels than the moderate-to-severe non-pneumonic ARI cases  
188 (Table 1).

189

190           At least one virus was detected in 279 (75%) of the cases by the multiplex PCR  
191 assay. Of these, two or more viruses were concurrently detected in 44 (15.8%) cases; 39  
192 (14.0%) cases had dual virus infections, and 5 (1.8%) cases had triple infections. RSV  
193 was by far the most common pathogen and was identified in 172 (61.7%) children,  
194 followed by HRV (n = 63, 22.6%), HMPV (n = 23, 8.2%), HPIV-1 (n = 22, 7.8%), and

195 HAdV (n = 21, 7.5%) (Table 2). Among the 172 children with confirmed RSV infection,  
196 31 (18.0%) of RSV positive children had double or triple infections with other  
197 respiratory viruses; HRV was the most common co-infecting virus, followed by HAdV  
198 (Table 2). Among the 200 RSV-negative children, 107 (53.5%) were positive for at least  
199 one non-RSV respiratory virus, and 13 were double or triple infections. HRV/HAdV (n  
200 = 3) was the leading combination followed by HRV/HBoV (n = 2) and HRV/PIV-1 (n =  
201 2).

202

203 We then analyzed associations between infection with each virus and the  
204 disease categories. RSV infection had significant associations with both pneumonia  
205 cases and moderate-to-severe non-pneumonic ARI cases in comparison with mild ARI  
206 cases; the level of association was stronger with moderate-to-severe non-pneumonic  
207 ARI cases (OR, 2.95; 95% CI, 1.82–4.78;  $P < 0.001$ ) than with pneumonia cases (OR,  
208 2.2; 95% CI, 1.27–3.8;  $P = 0.004$ ). In addition, HPIV-3 infection was strongly  
209 associated with pneumonia cases (OR, 7.54; 95% CI, 1.56–36.4;  $P = 0.012$ ) but was not  
210 significantly associated with moderate-to-severe non-pneumonic ARI cases (OR, 2.21;  
211 95% CI, 0.4–12.3;  $P = 0.4$ ) (Table 3).

212

213           Because we found the most significant association between moderate-to-severe  
214 non-pneumonic ARI cases and RSV infection, we further analyzed the possible risk  
215 factors for moderate-to-severe non-pneumonic ARI. An age of older than 24 months  
216 was protective against moderate-to-severe non-pneumonic ARI. Interestingly, when  
217 RSV-infected children were stratified into those that were or were not co-infected with  
218 other viruses, the association with moderate-to-severe non-pneumonic ARI was stronger  
219 among RSV-infected children with non-RSV virus co-infection than in children with  
220 RSV single infection. This association was even clearer in the multivariate analysis,  
221 which adjusted for sex and age groups (Table 4). However, non-RSV respiratory virus  
222 co-infection did not exhibit a significant effect on the degree of association with  
223 pneumonia cases (data not shown). We also analyzed the association between  
224 moderate-to-severe non-pneumonic ARI and non-RSV respiratory virus infection  
225 among RSV-negative children. The association was stronger with multiple non-RSV  
226 respiratory virus infections than single non-RSV respiratory virus infection, but the  
227 confidence intervals were wide because of the small sample size (data not shown).

228

229           Results from the PCR and the RSV rapid test were almost compatible. The rate  
230 of overall agreement between both assays was 86% (n = 332/385, Cohen's kappa



231 coefficient=0.72,  $p=0.0001$ ); 30 samples were positive for PCR but negative for the  
232 rapid test, and 23 samples were negative for PCR but positive for the rapid test.

233

## Discussion

234

235           In our study population, we showed that respiratory virus co-infection with  
236 RSV infection had a prevalence of 18% and was associated with moderate-to-severe  
237 non-pneumonic ARI. The rates of respiratory virus co-infection in RSV-infected cases  
238 have varied substantially in past studies, ranging from 11% to 53%.[17,18] The  
239 co-infection rate may be affected by the composition of the study population; more  
240 hospitalized children may result in a higher rate of co-infection. In our case, OPD  
241 children constituted 40% of the total population, and their low co-infection rate (14.6%)  
242 may have reduced the overall rate. Seasonal variations in circulating respiratory viruses  
243 also determine the co-infection rate. In our one-year study, the co-infection rate during  
244 the cold season (from October to March, 19.7%) was higher than the rate during the hot  
245 season (from April to September, 12.5%). Thus, the comparison of co-infection rates  
246 between studies requires careful interpretation.

247

248           Our findings indicate that respiratory virus co-infections with RSV infection  
249 increases the risk of developing more severe ARI with respiratory distress, such as  
250 bronchiolitis, compared to RSV single infection. The effect of multiple viral infections  
251 on the clinical picture of ARI has been controversial. Previous studies have reported that

252 co-infection with non-RSV respiratory viruses, particularly HMPV, increased the risk of  
253 hospitalization, pneumonia, and the receipt of mechanical ventilation among  
254 RSV-infected children,[5,7-9] whereas other studies noted no association between  
255 respiratory virus co-infection and the severity of RSV disease.[10-12,19] Inconsistent  
256 findings have also been reported for associations between ARI severity and respiratory  
257 virus co-infections with non-RSV respiratory virus infections.[12,20] These variations  
258 may be explained by the different combinations of virus co-infections. Franz et al.  
259 reported that RSV/non-RSV co-infection was more strongly associated with pneumonia  
260 than RSV single infection, and the primary co-infecting virus in their study was HBoV  
261 (8.8% of all RSV infections, n = 14/160).[9] However, Martin et al. found that multiple  
262 viral infections did not increase the severity of RSV single infection; the leading  
263 combinations in their study were RSV/HAdV (25% of dual viral infections, n = 24/96)  
264 and RSV/HCoV (17.7% of dual infections, n = 17/96).[12] In our study, the most  
265 common virus that was present in an RSV co-infection was HRV (11%, n = 19/172).  
266 Therefore, our findings may not be directly comparable with those from previous  
267 reports.

268

269 Several hypotheses have been proposed to explain the association between

270 multiple respiratory virus co-infections and ARI severity; these hypotheses include  
271 modification of immune responses after the initial infection,[17,21] host susceptibility  
272 to multiple viruses,[8] and associations of specific respiratory viruses with colonized  
273 bacteria.[9,16] However, our study does not give a causal explanation for the observed  
274 phenomenon due to limited biological data. In our study, the evidence for an increased  
275 risk of developing pneumonia when co-infected with non-RSV respiratory viruses was  
276 not significant, which is probably due to the small sample size or because bacterial  
277 infection played a major role in the pathogenesis of pneumonia. Our results involving  
278 associations between clinical severity and higher WBC counts and CRP levels may  
279 reflect the latter explanation.

280

281           Despite the small sample size, we again demonstrate a strong and significant  
282 association of HPIV-3 infection with severe ARI in the Japanese population. We have  
283 previously shown a compatible association with children with ARI in Vietnam.[13] Both  
284 parainfluenza viruses and RSV are enveloped RNA viruses that are in the family  
285 Paramyxoviridae. Parainfluenza viruses have many epidemiologic and clinical  
286 similarities to RSV and thus require medical attention as the second most important  
287 viral cause of hospitalization for ARI in young children.[22]

288

289           On the other hand, the positivity of influenza was conspicuously low (1.3%) in  
290 our children. This was because in our setting, a commercial rapid influenza diagnostic  
291 test (RIDT) was routinely performed for any outpatient with influenza-like illnesses  
292 prior to the RSV diagnostic test and children with positive RIDT results were usually  
293 not tested for RSV. Consequently influenza cases were screened out from our study  
294 population. Further investigations are needed to evaluate the association between  
295 influenza infection and the disease severity.

296

297           In our study population, 25% of the children less than 5 years of age were  
298 negative for any virus. This result was compatible with previous reports that found that  
299 virus is not detected in a considerable proportion (10–30%) of patients with respiratory  
300 diseases, despite intensive investigations.[6,7,9,12,20,23] Several reasons have been  
301 proposed to explain this virus-negative population. First, conventional virus detection  
302 tests, such as immunochromatography, are not sensitive. In our study, the sensitivity of  
303 the RSV rapid test was 83.2%, referring to the PCR results. However, the sensitivity of  
304 this rapid test is 100% if compared with other immunochromatography-based tests.[14]  
305 Second, infection with other respiratory viruses, such as HCoV-NL63,[24]

306 HCoV-HKU1,[25] and human enteroviruses[26] that are not included as target viruses  
307 in our current multiplex PCR system may have been missed. Third, targeted viruses  
308 might have been cleared by the time of sampling because it was often several days or  
309 over a week after the onset of disease when the sampling was performed. Such delayed  
310 sampling may underestimate the true viral infection rate.[9,27,28] Fourth, some viral  
311 RNA may have been degraded during the temporary storage at  $-20^{\circ}\text{C}$  in the hospital.  
312 However, the rate of viral detection was not low in our study as compared with previous  
313 reports. Finally, there is always the possibility that an unknown virus might have caused  
314 the disease.

315

316 In addition to the examples mentioned above, this study has additional  
317 limitations. First is the nature of cross-sectional studies. We expect that sequential  
318 sample collections will uncover the natural courses of respiratory virus co-infections  
319 and their causal effects on the clinical outcomes. Second, our study lacked data  
320 regarding the timing of infection and disease onset. Third, our specimens were not  
321 tested for viral load by quantitative PCR. The viral load of RSV may have been  
322 associated with the co-infection status and the disease severity.[12] Fourth, we did not  
323 have information regarding bacterial infection because non-invasive collection of

324 appropriate sputum samples is not feasible among young children.

325

326 In summary, RSV is the leading viral cause of lower respiratory infections and

327 causes substantial hospitalization burden during the winter season in Japan.[29] Our

328 findings indicate that co-infection with RSV and other respiratory viruses is not

329 uncommon and that co-infections may increase the severity of RSV disease in children.

330 Further investigations are warranted to investigate the determinants of RSV disease

331 severity.

332

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336



337

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1 **Table 1**  
 2 Characteristics of the study patients by their case categories.  
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	Mild ARI N = 149	Moderate-to-severe non-pneumonic ARI N = 137	Pneumonia N = 86	Total N = 372	<i>P</i> value <sup>a</sup>
Sex, N (%)					
Male	94 (63.1)	83 (60.6)	50 (58.1)	227 (61)	n.s.
Female	55 (36.9)	54 (39.4)	36 (41.9)	145 (39)	
Age average (months), median (IQR)	12 (7)	12 (7)	12 (13)	12 (5.5)	n.s.
Age group (months), N (%)					
<12	62 (41.6)	63 (46)	26 (30.2)	151 (40.6)	n.s.
12–23	52 (34.9)	57 (41.6)	37 (43)	146 (39.2)	
>24	35 (23.5)	17 (12.4)	23 (26.8)	75 (20.2)	
WBC (per mm <sup>3</sup> ), median (IQR)	n.a.	10,200 (5,900)	11,250 (7,200)	10,700 (6,400) <sup>b</sup>	0.04
CRP (mg/dl), median (IQR)	n.a.	0.9 (2.1)	3.1 (4.7)	1.6 (3.4) <sup>b</sup>	0.0001

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 5 NOTE. n.s., not significant; n.a., data not available; SD, standard deviation; IQR, inter-quartile range.  
 6 <sup>a</sup>P value calculated from univariate analysis that the chi-square test was used for categorical variables whereas the Kruskal-Wallis test was  
 7 applied for continuous variables.  
 8 <sup>b</sup>Data was available for 223 patients.

1 **Table 2**  
 2 Virus distribution in the total samples and in the RSV-positive samples.

	No. of positives in total samples, N=372			No. of positives in RSV-positive samples, N=172	
	N	%		N	%
RSV	172	46.2	HRV	19	11.1
HRV	63	16.9	HAdV	6	3.5
HMPV	23	6.2	HBoV	3	1.7
HPIV-1	22	5.9	HMPV	2	1.2
HAdV	21	5.7	HPIV-1	2	1.2
HPIV-3	14	3.8	FluA	2	1.2
HBoV	7	1.9			
FluA	5	1.3			
HPIV-4	1	0.3			

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 4 NOTE. RSV, respiratory syncytial virus; HRV, human rhinovirus; HMPV, human metapneumovirus; HPIV, human parainfluenza virus;  
 5 HAdV, human adenovirus; HBoV, human bocavirus; FluA, influenza A virus.

1 **Table 3**  
 2 Comparison of respiratory viral infections and clinical case categories.  
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		Mild ARI	Moderate-to-severe non-pneumonic ARI	Pneumonia	Mild vs. moderate-to-severe non-pneumonic ARI			Mild vs. pneumonia		
		N = 149 (%)	N = 137 (%)	N = 86 (%)	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
RSV	Pos	48 (32.2)	80 (58.4)	44 (51.2)	2.95	1.82–4.78	<0.001	2.2	1.27–3.80	0.004
	Neg	101 (67.8)	57 (41.6)	42 (48.8)	Reference			Reference		
HRV	Pos	25 (16.8)	30 (21.9)	8 (9.3)	1.39	0.77–2.51	0.3	0.5	0.22–1.18	0.1
	Neg	124 (83.2)	107 (78.1)	78 (90.7)	Reference			Reference		
HMPV	Pos	9 (6)	9 (6.6)	5 (5.8)	1.09	0.42–2.84	0.9	0.96	0.31–2.96	0.9
	Neg	140 (94)	128 (93.4)	81 (94.2)	Reference			Reference		
HAdV	Pos	10 (6.7)	6 (4.4)	5 (5.8)	0.64	0.23–1.80	0.4	0.86	0.28–2.60	0.8
	Neg	139 (93.3)	131 (95.6)	81 (94.2)	Reference			Reference		
HPIV-1	Pos	11 (7.4)	9 (6.6)	2 (2.3)	0.88	0.35–2.20	0.8	0.3	0.06–1.38	0.1
	Neg	138 (92.6)	128 (93.4)	84 (97.7)	Reference			Reference		
HPIV-3	Pos	2 (1.3)	4 (2.9)	8 (9.3)	2.21	0.40–12.3	0.4	7.54	1.56–36.4	0.012
	Neg	147 (98.7)	133 (97.1)	78 (90.7)	Reference			Reference		

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 5 NOTE. OR, odds ratio; CI, confidence interval.

1 **Table 4**  
 2 Associations between RSV single/multiple infections and moderate-to-severe non-pneumonic ARI cases.  
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		Unadjusted OR	95% CI	<i>P</i> value	Adjusted OR	95% CI	<i>P</i> value
Sex	Male	Reference			Reference		
	Female	1.11	0.69–1.79	0.7	1.06	0.64–1.77	0.8
Age group (months)	<12	Reference			Reference		
	12–23	1.07	0.65–1.80	0.8	1.03	0.6–1.77	0.9
	>24	0.48	0.24–0.94	0.033	0.42	0.2–0.85	0.016
RSV	Negative	Reference			Reference		
	Single infection	2.68	1.61–4.47	<0.001	2.77	1.64–4.70	<0.001
	Multiple infection	4.56	1.80–11.57	0.001	4.91	1.90–12.7	0.001

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 5 NOTE. OR, odds ratio; CI, confidence interval.  
 6 Logistic regression analysis was done for mild ARI cases vs. moderate-to-severe non-pneumonic ARI cases. The multiple logistic  
 7 regression model included the explanatory variables listed in the table. The result of the Hosmer-Lemeshow test indicated high  
 8 goodness-of-fit for the final model (P=0.62).