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Epidemiology of human metapneumovirus among children with severe or very severe pneumonia in high pneumonia burden settings: the Pneumonia Etiology Research for Child Health (PERCH) study experience

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Author contributions

R.M. and M.D.K. participated in study conception and design (for subanalysis). W.A.B., H.C.B., D.R.F., L.L.H., S.R.C.H., K.L.K., O.S.L., S.A.M., D.R.M., K.L.O., J.A.G.S., D.M.T., R.A.K., P.S., S.O.S., M.D.T., and M.D.K. participated in study conception and design (for the parent PERCH study). W.A.B., C.P., H.C.B., D.R.F., L.L.H., S.R.C.H., K.L.K., O.S.L., S.A.M., D.R.M., K.L.O., J.A.G.S., D.M.T., M.A., J.O.A., C.B., A.J.D., B.E., N.S.F., M.M.H., R.A.K., D.P.M., S.C.M., J.M.M., D.E.P., M.Z.R., M.R., R.A.S., P. Sawatwong, P. Seidenberg, S.O.S., M.D.T., and M.D.K. participated in data collection. C.P. and M.D.K. participated in data curation for this subanalysis. R.M., H.Z., and M.D.K. participated in analysis of methods and materials and generation of figures. R.M., H.Z., W.A.B., and M.D.K. participated in interpretation of results and draft manuscript preparation. M.D.K. participated in funding acquisition for this subanalysis. All authors reviewed the results and approved the final version of the manuscript. R.M. and H.Z. contributed equally as co-first authors. M.D.K. contributed as the senior author.

Potential conflict of interest

The authors declare that they have no conflicts of interest.

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Abstract

Objectives: After respiratory syncytial virus (RSV), human metapneumovirus (hMPV) was the second-ranked pathogen attributed to severe pneumonia in the PERCH study. We sought to characterize hMPV-positive cases in high-burden settings, which have limited data, by comparing with RSV-positive and other cases.

Methods: Children aged 1–59 months hospitalized with suspected severe pneumonia and age/season-matched community controls in seven African and Asian countries had nasopharyngeal/oropharyngeal swabs tested by multiplex PCR for 32 respiratory pathogens, among other clinical and lab assessments at admission. Odds ratios adjusted for age and site (adjusted OR [aOR]) were calculated using logistic regression. Aetiologic probability was estimated using Bayesian nested partial latent class analysis. Latent class analysis identified syndromic constellations of clinical characteristics.

Results: hMPV was detected more frequently among cases (267/3887, 6.9%) than controls (115/4976, 2.3%), among cases with pneumonia chest X-ray findings (8.5%) than without (5.5%), and among controls with respiratory tract illness (3.8%) than without (1.8%; all $p \leq 0.001$). HMPV-positive cases were negatively associated with the detection of other viruses (aOR, 0.18), especially RSV (aOR, 0.11; all $p < 0.0001$), and positively associated with the detection of bacteria (aORs, 1.77; p 0.03). No single clinical syndrome distinguished hMPV-positive from other cases. Among hMPV-positive cases, 65.2% were aged <1 year and 27.5% had pneumonia danger signs; positive predictive value for hMPV aetiology was 74.5%; mortality was 3.9%, similar to RSV-positive (2.4%) and lower than that among other cases (9.6%).

Discussion: HMPV-associated severe paediatric pneumonia in high-burden settings was predominantly in young infants and clinically indistinguishable from RSV. HMPV-positives had low case fatality, similar to that in RSV-positives.

Keywords

Africa; Asia; Human metapneumovirus (hMPV); Paediatric; Severe pneumonia

Introduction

Lower respiratory infections are the most common cause of hospitalization and death in children aged 1–59 months [1,2]. In contrast to respiratory syncytial virus (RSV), whose role in paediatric respiratory infections has long been recognized, human metapneumovirus (hMPV) was only identified in 2001 [3]. It is a member of the *Pneumoviridae* family along with RSV and consists of two genotypes (A and B) based on surface glycoprotein sequence

variation in its attachment (G) and fusion (F) proteins, with further subdivisions in these genotypes [4].

HMPV has been shown to cause respiratory tract infections in all ages globally [1]. The clinical spectrum of hMPV infection ranges from mild upper respiratory symptoms, otitis media, bronchiolitis, bronchitis and pneumonia, and exacerbations of chronic lower airway diseases of asthma and chronic obstructive pulmonary disease (COPD) [1,5]. Treatment for hMPV-associated disease is currently supportive care and although no vaccines are available, several are under development [6], as are antiviral medications [1,7].

Although hMPV can infect all ages, infants <1 year have highest risk of severe hMPV-associated illness [8]. The droplet-spread virus infects most children (90–100%) by age 5–10 years and reinfection can occur [1,9]. In North America, the incidence of hMPV-associated severe hospitalized disease is similar to that of influenza and of parainfluenza 1, 2, and 3 combined [10]. In 2018, an estimated 14.2 million cases of hMPV acute lower respiratory infection occurred in children <5 years worldwide, including 643 000 hospitalizations and 7700 in-hospital deaths [8]. Children aged <12 months accounted for 71% of deaths and 64% of deaths were amongst children <6 months, of which 96% occurred in settings with high child mortality.

The Pneumonia Etiology Research for Child Health (PERCH) case-control study estimated the aetiology of pneumonia in children 1–59 months of age in five African and two Asian countries from 2011 to 2014. PERCH previously estimated that hMPV caused 6% of severe hospitalized radiographically confirmed pneumonia, the second most frequent viral pathogen after RSV [11]. Because of the high proportion of pneumonia cases attributed to hMPV, we further explored data from the PERCH study to evaluate the clinical and epidemiologic characteristics and outcomes of children infected with hMPV compared with other cases.

Methods

Participants

Between August 2011 and January 2014, children aged 1–59 months were enrolled at nine study sites in seven countries: Dhaka and Matlab, Bangladesh; Basse, The Gambia; Kilifi, Kenya; Bamako, Mali; Soweto, South Africa; Sa Kaeo and Nakhon Phanom, Thailand; and Lusaka, Zambia. Sites represented a mix of urban and rural populations and different prevalences of HIV, malaria, and infant mortality rates [11]. Each site enrolled subjects for 24 consecutive months.

Cases, enrolled at hospital admission, had WHO-defined severe or very severe pneumonia (pre-2013 definition): cough or difficulty breathing and either lower chest wall indrawing or a pneumonia danger sign (indicating very severe), including central cyanosis, difficulty feeding, vomiting everything, convulsions, lethargy, reduced consciousness, or head nodding [11]. Cases hospitalized within 14 days preceding enrolment, discharged as a PERCH case in the past 30 days, residing outside the study catchment area, or whose lower chest wall indrawing resolved after bronchodilator therapy were excluded. Controls were selected randomly from communities within the catchment area of the cases and were frequency-

matched to cases by enrolment date and age stratum (28 days–5 months, 6–11 months, 12–23 months, and 24–59 months). Controls meeting case-defining criteria were excluded but could have respiratory illness.

Data collection

Standardized assessment was performed for cases and controls at enrolment, including demographic information, medical history, clinical data, comorbidities, and risk factors, described in detail previously [11]. Mortality status of discharged cases was assessed 30–90 days after admission. Respiratory tract illness (RTI) in controls was defined as having any of cough, rhinorrhoea, fever, or sore throat, plus one or more of the following: ear discharge, wheezing, or difficulty breathing. Leucocytosis was defined as white blood cell count $>15 \times 10^9$ cells/L for children aged 1–11 months and $>13 \times 10^9$ cells/L for children aged 12–59 months. Chest X-rays (CXRs) taken within 72 hours of admission were read by two trained readers blinded to the clinical laboratory findings using WHO-standardized procedures [12]. Discordant readings were adjudicated by two other expert readers. Findings were classified as normal, other infiltrates only, consolidation with or without other infiltrates or uninterpretable. CXR-positive cases had either consolidation or other infiltrates identified.

Specimen collection and testing

Flocked nasopharyngeal (NP) and rayon oropharyngeal (OP) swabs collected from all participants were tested by multiplex PCR for 33 pathogens [13]. High density *Streptococcus pneumoniae* and *Haemophilus influenzae* were defined using thresholds associated with case status: $>6.9 \log_{10}$ copies/mL and $>5.9 \log_{10}$ copies/mL, respectively [14].

Blood from cases and controls was tested by *lytA* PCR; blood cultures were obtained from cases [15]. Cases had induced sputum and gastric aspirates tested for *Mycobacterium tuberculosis* by PCR and culture, respectively. Lung aspirates and pleural fluid were tested by multiplex PCR and cultured.

Statistical analysis

Cases and controls without HIV infection and with NP/OP test results were included in analyses. Odds ratios and 95% CIs were calculated using logistic regression adjusted for site and age in months. Cases with hMPV detected in NP/OP ('hMPV-positive'), RSV-positive and cases negative for hMPV and RSV ('hMPV-negative/RSV-negative').

Aetiologic probability of hMPV as the cause of the pneumonia was estimated using the PERCH Integrated Analysis (PIA) adjusted for age and site, described elsewhere [11,16,17]. The PIA estimates the probability for each case; the mean across cases estimates the proportion of all cases attributed to hMPV. Briefly, PIA is a Bayesian nested partially latent class analysis that integrates laboratory evidence from multiple sources from both cases and controls to estimate both individual and population-level aetiology probabilities. Pathogens detected in invasive specimens and *M. tuberculosis* results from induced sputum were considered 100% specific and attributed 100% aetiology. PIA is a single-cause model; when evidence for multiple pathogens exists, aetiologic probability is divided among them.

Positive predictive value was defined as the median (or mean) aetiologic probability among hMPV-positive cases.

Latent class analysis (LCA) was used to identify constellations of clinical characteristics among hMPV-positive and RSV-positive cases. We evaluated 3- to 6-class LCA models of cough, fever, CXR consolidation, C-reactive protein (CRP) ≥ 40 mg/L, leucocytosis, and wheeze. Sensitivity analyses restricted to CXR-positive cases, cases with high aetiologic probability and controls with RTI were conducted.

Two-sided $p < 0.05$ was considered statistically significant. Analyses were performed using Stata version 15.1 (StataCorp LLC, College Station, TX), and R Statistical Software 4.3.1 (The R Development Core Team, Vienna, Austria). PIA was performed using Bayesian inference software JAGS 4.2.0 (<http://mcmc-jags.sourceforge.net/>) and the publicly available R package Bayesian Analysis Kit for Etiology Research (website: <https://github.com/zhenkewu/baker>).

Ethical considerations

The study protocol was approved by the institutional review board or ethical review committee overseeing each site and at the Johns Hopkins Bloomberg School of Public Health. Written informed consent was obtained from parents or legal guardians of participants.

Results

We analysed 3887 of 4232 (91.9%) cases and 4976 of 5325 (93.4%) controls who were not infected with HIV and had hMPV results. HMPV was detected in NP/OP of 267 (6.9%) cases and 115 (2.3%) controls (adjusted OR [aOR], 3.0; 95% CI, 2.42–3.80; $p < 0.0001$; Fig. 1 and Table 1) [18]. hMPV positivity was higher among CXR-positive cases (8.5% vs. 5.5% CXR-negative; aOR, 1.62; $p = 0.001$) and among controls with RTI (3.8% vs. 1.8% in controls without RTI; aOR, 2.81; $p < 0.0001$). Although cases aged 6–11 months (8.8%) and 12–23 months (7.4%) had the highest proportion of hMPV-positive cases (vs. 6.1% in younger and 5.2% in older age groups, $p = 0.03$; Table S1), most (65.2%) hMPV-positive cases were < 1 year of age and 16.5% were aged 1–2 months. HMPV was detected in one of 37 lung aspirate specimens, which was also positive for *H. influenzae* (non-type b) and *Moraxella catarrhalis*, in a 6-month-old child.

NP/OP hMPV positivity ranged from 3.6% in Thailand to 8.9% in Zambia for cases and 1.2% in Mali to 3.3% in South Africa for controls (Table 1 and S1). HMPV seasonal and annual patterns and co-circulation with RSV also differed within and across sites (Fig. 2). Detection of other viruses was negatively associated with hMPV positivity (cases: aOR, 0.18; 95% CI, 0.14–0.23; controls: aOR, 0.47; 95% CI, 0.32–0.69), particularly for RSV (cases: aOR, 0.11; 95% CI, 0.06–0.20) (Table 1). Conversely, bacteria were detected in NP/OP more frequently among hMPV-positive than hMPV-negative cases (aOR, 1.77; $p = 0.03$) and controls (aOR, 1.93; $p = 0.16$). Results for CXR-positive cases were similar (Table S2).

PIA analyses estimated that 6.0% (range, 2.1–7.6% across sites) of all pneumonia cases (6.4% among severe, 5.4% among very severe) and 7.5% (range, 2.5–11.6%) of CXR-positive cases were caused by hMPV (Fig. S1), which decreased in older age groups (Fig. S1B). Among all hMPV-positive cases, 39.0% ($n = 104$) had high ($\geq 80\%$) probability that hMPV was the cause of the pneumonia and 70.0% ($n = 187$) had $\geq 50\%$ probability (Fig. 3). The positive predictive value (median probability among hMPV-positive cases) of hMPV as the sole cause was 74.5% (mean, 65.6%).

Among all cases, 27.5% of hMPV-positive cases were very severe, similar to RSV-positive cases (26.7%) but lower than hMPV-negative/RSV-negative cases (34.7%, aOR, 0.70; $p = 0.02$; Table 2). Results were similar when restricted to high ($\geq 80\%$) probability hMPV-positive and RSV-positive cases (Table S3); however, among CXR-positive cases, the proportion very severe was similar between hMPV-positive (30.5%) and hMPV-negative/RSV-negative cases (31.1%, Table S4). Ten hMPV-positive cases died (Table S5). Case fatality among hMPV-positive cases (3.9%) was similar to that of RSV-positive cases (2.4%; $p = 0.58$) but lower than hMPV-negative/RSV-negative cases (9.6%, aOR, 0.36; $p = 0.003$; Table 2).

Compared with RSV-positive cases, fewer hMPV-positive cases had wheeze (29.8% vs. 38.6%) and rhinorrhoea (41.6% vs. 47.1%), and more had vomiting (4.3% vs. 1.6%), consolidation on CXR (26.3% vs. 20.4%) and CRP ≥ 40 mg/L (31.8% vs. 17.4%; all $p \leq 0.05$, Table 2). Compared with hMPV-negative/RSV-negative cases, more hMPV-positive cases had cough, fever, chest wall retraction, hypoxemia, abnormal CXR (both consolidation and other infiltrates), and CRP ≥ 40 mg/L (all $p < 0.05$, Table 2). Among hMPV-positive cases, characteristics were generally similar regardless of aetiologic probability (Table S6). Latent class analyses assessing cough, fever, CXR consolidation, CRP ≥ 40 mg/L, leucocytosis, and wheeze and other stratified evaluations showed hMPV-positive cases exhibited no predominant constellations of clinical features (Tables S7A and S8).

Among controls, RTI (39.1% vs. 23.4%, $p < 0.0001$) and diarrhoea (4.4% vs. 1.9%, $p = 0.03$) was more common among hMPV-positive than hMPV-negative (Table S9), and hMPV prevalence was similar across age groups (range, 2.0–2.7%; $p = 0.76$; Table S1).

Discussion

This study characterized children ages 28 days to <5 years hospitalized with hMPV-associated severe pneumonia in seven African and Asian countries before the SARS-CoV-2 pandemic. Six percent of cases were estimated to be caused by hMPV in our single-cause aetiologic probability model and 70% of hMPV-positive cases had hMPV attributed as the most likely cause. Although less frequently detected than RSV, hMPV-positive cases had comparable severity and mortality with RSV-positive cases. Consolidation on CXR, leucocytosis, and high CRP were common among hMPV-positive cases, but, as observed previously [1], no single constellation of clinical findings at admission characterized them. The role hMPV plays in both upper and lower respiratory infections at our high pneumonia burden sites was supported by finding significantly greater hMPV positivity amongst cases

than controls and amongst controls with RTI vs. those without, in addition to finding hMPV in a lung aspirate specimen.

An estimated 96% of in-hospital hMPV-associated mortality in children <6 months of age occurs in high child mortality settings [8]. Only a few previous studies assessed the role of hMPV in pneumonia in high-burden African and Asian countries. Sites in the GABRIEL study (Cambodia, China, Haiti, India, Madagascar, Mali, Mongolia, and Paraguay) that conducted surveillance for 1–3 years found similar prevalences among hospitalized pneumonia cases with consolidation on CXR (8.6% vs. 8.5% in PERCH CXR-positive) and among controls who were restricted to children without RTI (1.1% vs. 1.8% in PERCH), estimating the population attributable fraction to be 7.8% [19]. The South African Drakenstein study, a 12-month longitudinal nested case-control study, observed 10% positivity among predominantly non-severe pneumonia cases; when restricted to severe cases as in PERCH, positivity (5%) was similar to PERCH South African cases (6.6%) [20]. Although this study found higher positivity in controls than in PERCH South African controls (11% vs. 3.3%), which was also higher than positivity in their cases resulting in no increased risk with case status, viral load among hMPV-positives was higher in Drakenstein cases than controls, as was similarly observed in a previous PERCH analysis of viral load [21]. However, another South African study, a pneumococcal conjugate vaccine trial, found that 11.1% of children hospitalized with clinical pneumonia or bronchiolitis in the placebo group were hMPV-positive [22]. Some variability in hMPV prevalence between studies may be expected due to annual and geographic variability, as was seen in PERCH, but the high positivity in Drakenstein asymptomatic controls was unusual.

As with all pneumonia cases, the absolute number of hMPV-positive cases was largest for younger children and decreased with age. Although cases aged 6–11 months had the highest proportion hMPV-positive, the proportion among children <6 months was restricted by the higher burden of RSV infection in that age group relative to other ages. Children aged 1–2 months represented 16.5% of hMPV-positive cases, and 4 of 10 hMPV-associated deaths were in children <6 months of age, data that could inform discussions on the role of maternal immunization. HMPV vaccines are currently under development, including combination vaccines with RSV and parainfluenza virus type 3 [6,23], as are neutralizing monoclonal antibodies for hMPV similar to those for RSV [1,7].

The negative association between detection of hMPV and other viruses, especially RSV, has been reported elsewhere [24]. Previous studies suggested different seasonality and responses to relative humidity [25,26], but three PERCH sites had hMPV and RSV co-circulation (South Africa, Kenya, and Bangladesh). In this context, immune responses such as interferon production may interfere with subsequent secondary infection [27]. Epidemiological surveillance of respiratory virus infection to understand dynamics between hMPV, RSV, SARS-CoV-2, and other viruses will continue to be important.

The diversity of clinical constellations among hMPV-positive cases may imply diverse aetiology among hMPV-associated illnesses, with some cases having hMPV as the sole aetiology, whereas others may be mixed infection with bacteria and still others may be due to another pathogen altogether because hMPV was detected in asymptomatic controls.

Clinical characteristics traditionally considered consistent with bacterial pneumonia, including consolidation on CXR, leucocytosis, and high CRP, were common among many hMPV-positive cases (although finding all three was rare, 5.5%). Along with association with bacterial co-carriage and evidence from other studies [28–30], these findings support that some hMPV-positive cases are likely hMPV-bacterial co-infections that may increase risk of progression to severe disease.

Our study has several limitations. Importantly, hMPV detection does not confirm aetiology because hMPV was detected among asymptomatic community controls. However, analyses accounted for specificity, sensitivity, and other pathogens, and most hMPV-positive cases had $\geq 75\%$ probability of hMPV being the aetiological agent. Poor sensitivity and specificity of bacterial microbiologic data limited ability to identify children with bacterial disease, likely underestimating bacterial-viral co-infection. Clinical characteristics were assessed at admission, likely underestimating very severe and CXR-positive cases whose disease progressed during hospitalization. Additionally, the study added physicians and provided oxygen, CXRs and microbiological testing that likely reduced mortality to all aetiologies compared with non-study conditions, but relative mortality between pathogens was likely unaffected.

Conclusions

HMPV-associated severe pneumonia among children aged <5 years in high-burden African and Asian settings was predominantly in young infants but occurred in all ages, and likely included some cases with bacterial co-infection. HMPV-positive case fatality was low, similar to that of RSV. HMPV-positive cases were characterized by a range of clinical constellations and were indistinguishable from RSV-positive cases. Moderately high NP/OP hMPV PCR test positive predictive value implies testing can distinguish most hMPV cases for evaluating disease burden and interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

The Pneumonia Etiology Research for Child Health dataset is publicly available at the ClinEpiDB website (University of Pennsylvania, Philadelphia, USA; <https://clinepidb.org>).

Appendix A.: Supplementary data

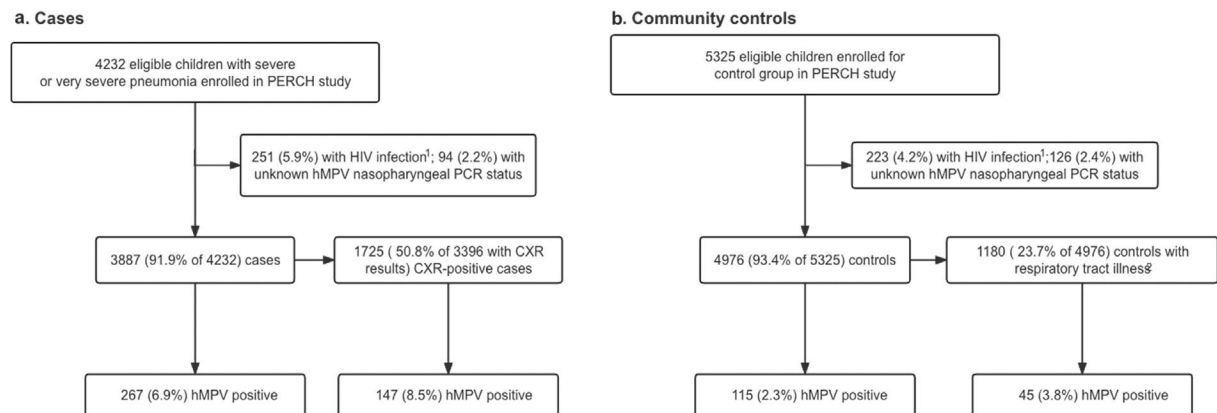
Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2024.10.023>.

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**Fig. 1.**

Case and control enrolment and eligibility for analysis and hMPV positivity by site and age. CXR, chest X-ray; hMPV, human metapneumovirus; PERCH, Pneumonia Etiology Research for Child Health. ^aHIV-infection was determined using either positive HIV PCR at any age, or positive HIV serology for those >18 months of age. HIV antibody testing was performed for cases from all sites except Bangladesh and for controls from Kenya, Mali, and Zambia. ^bRespiratory tract illness defined as having any of the following: (a) cough, (b) rhinorrhoea, or (c) fever or sore throat and one or more of following: the ear discharge, wheeze, or difficulty breathing.

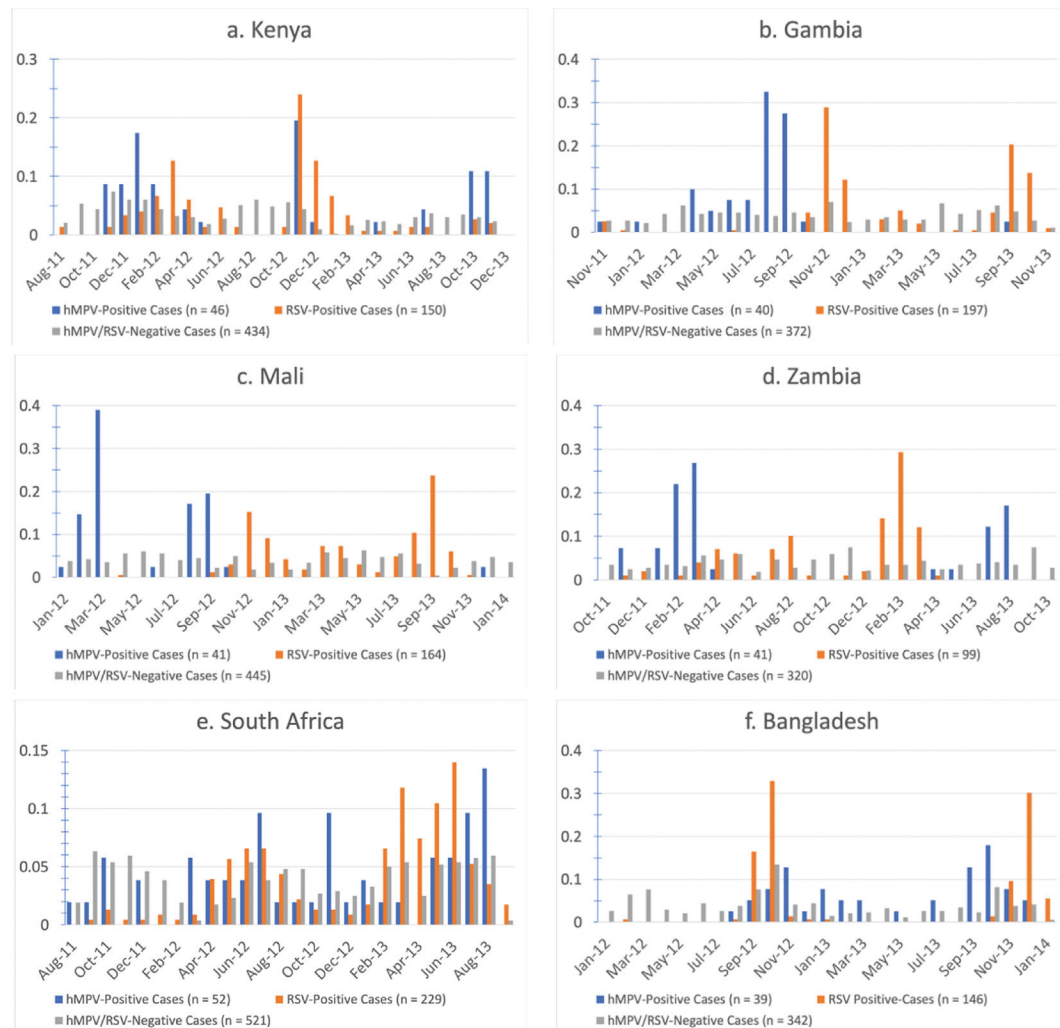


Fig. 2. Distribution of hMPV-positive, RSV-positive, and hMPV/RSV-negative cases across the enrolment period, by site. The proportion for the blue bars is determined using the denominator of all hMPV + cases, and for orange bars the denominator is all RSV + cases. The 12 cases positive for both hMPV and RSV (eight South Africa, two Kenya, two Bangladesh) are shown twice, included in both the hMPV + case distribution and the RSV + case distribution. Thailand is not shown due to few ($n = 8$) hMPV + cases. hMPV, human metapneumovirus; RSV, respiratory syncytial virus.

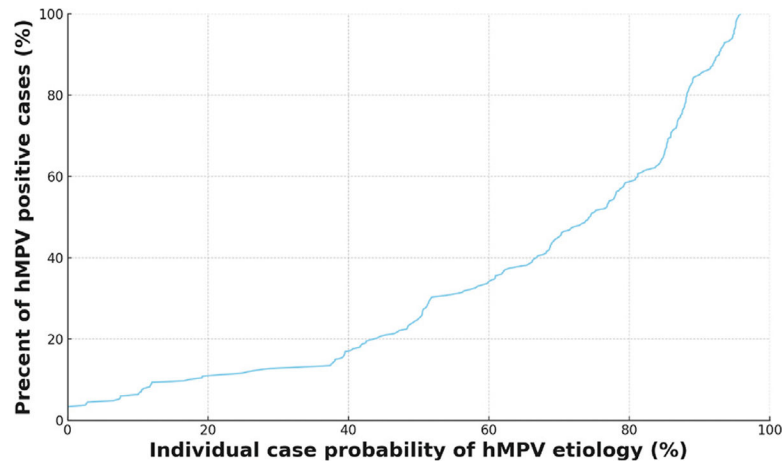


Fig. 3.

Cumulative probability that pneumonia aetiology was hMPV, estimated using the PERCH Integrated Analysis (PIA) among all NP/OP hMPV-positive cases. These are all cases that tested positive for hMPV in NP/OP, including the 12 that were positive for both hMPV and RSV. The PIA used all available data (e.g. blood cultures, NP/OP testing for other pathogens, etc.) and accounted for measurement error (sensitivity and specificity for each test/pathogen) to inform aetiology. The PIA assumed that a single pathogen was the cause of the pneumonia. Pathogens detected in normally sterile specimens (e.g. blood tested by culture, lung aspirates tested by PCR or culture and pleural fluid tested by PCR or culture) are assumed to be the cause and ignore data from other specimens (e.g. NP/OP, blood PCR, and gastric aspirate tested for *Mycobacterium tuberculosis* (Mtb)). Low aetiology probability for a particular pathogen can mean either that that pathogen is not likely the cause, or that it is a co-cause with one or more other pathogen(s) because this single-cause model splits the probability in mixed-cause pneumonia between the pathogens (e.g. pneumonia confirmed to be both *H. influenzae* and hMPV will be attributed at 50% to *H. influenzae* and 50% to hMPV). A case with a bacterial pathogen detected in blood by culture and hMPV detected in NP/OP will have 0% hMPV probability of aetiology in this single-cause model. Most cases that tested negative for hMPV in NP/OP have low (<10%) but non-zero aetiological probability (not shown). hMPV, human metapneumovirus; NP/OP, nasopharyngeal/oropharyngeal swab specimen; PERCH, Pneumonia Etiology Research for Child Health; RSV, respiratory syncytial virus.

Table 1
Characteristics associated with hMPV detection in NP/OP among severe pneumonia cases and community controls

Characteristics, n (%)	All cases		Community controls			
	hMPV A/B positive (n = 267)	hMPV A/B negative (n = 3620)	Adjusted OR (95% CI) ^a	p ^a	hMPV A/B positive (n = 115)	hMPV A/B negative (n = 4861)
Demographic						
Site				0.31		
Kenya	46 (17.2)	582 (16.1)	Reference		22 (19.1)	833 (17.1)
Gambia	40 (15.0)	569 (15.7)	0.88 (0.57–1.37)		13 (11.3)	611 (12.6)
Mali	41 (15.4)	609 (16.8)	0.84 (0.54–1.30)		9 (7.8)	715 (14.7)
Zambia	41 (15.4)	419 (11.6)	1.21 (0.78–1.89)		10 (8.7)	519 (10.7)
South Africa	52 (19.5)	742 (20.5)	0.87 (0.57–1.32)		27 (23.5)	796 (16.4)
Thailand	8 (3.0)	213 (5.9)	0.48 (0.22–1.04)		10 (8.7)	640 (13.1)
Bangladesh	39 (14.6)	486 (13.4)	1.02 (0.65–1.58)		24 (20.9)	747 (15.4)
Age group				0.03		
28 d–2 mo	44 (16.5)	760 (21.0)	Reference		4 (3.5)	421 (8.7)
3–5 mo	52 (19.5)	724 (20.0)	1.28 (0.84–1.96)		31 (26.9)	1096 (22.5)
6–11 mo	78 (29.2)	806 (22.3)	1.85 (1.17–2.93)		24 (20.9)	1158 (23.8)
12–23 mo	65 (24.3)	818 (22.6)	1.70 (0.86–3.38)		33 (28.7)	1199 (24.7)
24–59 mo	28 (10.5)	512 (14.1)	1.48 (0.40–5.55)		23 (20.0)	987 (20.3)
Female	118 (44.2)	1522 (42.0)	1.09 (0.85–1.40)	0.49	60 (52.2)	2410 (49.6)
Pneumonia risk factors						
Underweight ^b	84 (31.5)	1280 (35.4)	0.83 (0.63–1.09)	0.17	14 (12.2)	707 (14.5)
Day care attendance	37 (14.0)	606 (16.9)	0.85 (0.53–1.36)	0.50	16 (14.0)	886 (18.3)
Smoker in household	82 (31.1)	1229 (34.1)	0.93 (0.69–1.24)	0.61	36 (31.3)	1873 (38.7)
Never breastfed	21 (7.9)	340 (9.4)	0.88 (0.53–1.45)	0.61	13 (11.3)	328 (6.8)
Premature birth ^c	33 (12.7)	382 (10.8)	1.27 (0.85–1.88)	0.24	10 (8.9)	497 (10.4)
Low birth weight ^d	56 (21.4)	718 (20.0)	1.07 (0.78–1.45)	0.69	17 (14.8)	587 (12.1)
Premature ^c or low birth weight ^d	66 (25.2)	853 (23.7)	1.07 (0.80–1.44)	0.65	22 (19.1)	874 (18.0)
HIV-exposed, uninfected ^e	33 (36.3)	381 (34.2)	1.07 (0.85–1.34)	0.56	10 (27.8)	364 (28.2)

Characteristics, n (%)	All cases		Community controls					
	hMPV A/B positive (n = 267)	hMPV A/B negative (n = 3620)	Adjusted OR (95% CI) ^a	p ^a	hMPV A/B positive (n = 115)	hMPV A/B negative (n = 4861)	Adjusted OR (95% CI) ^a	p ^a
Prior antibiotics	131 (49.1)	1599 (44.2)	0.95 (0.76–1.20)	0.68	2 (1.7)	81 (1.7)	0.88 (0.46–1.66)	0.69
DTP vaccine (≥1 dose)	225 (84.3)	2861 (79.0)	1.69 (1.13–2.52)	0.01	105 (91.3)	4435 (91.2)	1.18 (0.53–2.62)	0.68
PCV vaccine (≥1 dose)	142 (53.2)	1854 (51.2)	1.23 (0.84–1.81)	0.29	56 (48.7)	2442 (50.2)	0.69 (0.36–1.32)	0.27
Microbiologic findings								
Microbiologically confirmed infection in sterile specimen hMPV (in lung aspirates) ^f	1 (0.4)	0 (0.0)	N/A	N/A	N/A	N/A	N/A	N/A
Any viruses (in lung aspirates, other than hMPV) ^g	3 (1.1)	0 (0.0)	N/A	N/A	N/A	N/A	N/A	N/A
Non contaminant bacteria								
Blood culture positivity	7 (2.7)	110 (3.1)	0.85 (0.39–1.86)	0.69	N/A	N/A	N/A	N/A
Any invasive specimen positivity ^h	9 (3.4)	121 (3.3)	1.01 (0.50–2.02)	0.98	N/A	N/A	N/A	N/A
<i>S. pneumoniae</i> ^h	2 (0.8)	42 (1.2)	0.67 (0.16–2.78)	0.58	N/A	N/A	N/A	N/A
<i>H. influenzae</i> ^h	2 (0.8)	19 (0.5)	1.41 (0.32–6.10)	0.65	N/A	N/A	N/A	N/A
<i>S. pneumoniae</i> PCR positive in whole blood								
Any positivity	14 (5.2)	234 (6.5)	0.82 (0.47–1.43)	0.49	8 (7.0)	245 (5.0)	1.31 (0.62–2.76)	0.48
Positivity >2.2 log ₁₀ copies/mL	12 (4.5)	160 (4.4)	1.05 (0.57–1.92)	0.88	4 (3.5)	139 (2.9)	1.18 (0.43–3.29)	0.75
Detection in NP/OP								
Any organism positive (other than hMPV)	258 (96.6)	3496 (96.6)	1.01 (0.51–2.02)	0.98	111 (96.5)	4624 (95.1)	1.31 (0.47–3.60)	0.61
Restricted to high density for <i>S. pneumoniae</i> and <i>H. influenzae</i> ⁱ	182 (68.2)	3084 (85.2)	0.37 (0.28–0.48)	<0.0001	67 (58.3)	3349 (68.9)	0.59 (0.40–0.87)	0.008
Viral detection in NP/OP								
Any NP viral positive (other than hMPV)	93 (34.8)	2687 (74.2)	0.18 (0.14–0.23)	<0.0001	40 (34.8)	2538 (52.2)	0.47 (0.32–0.69)	<0.0001
Respiratory syncytial virus	12 (4.5)	1024 (28.3)	0.11 (0.06–0.20)	<0.0001	2 (1.7)	159 (3.3)	0.54 (0.13–2.19)	0.39
Adenovirus	18 (6.7)	387 (10.7)	0.63 (0.38–1.02)	0.06	9 (7.8)	606 (12.5)	0.60 (0.30–1.19)	0.14
Human bocavirus	24 (9.0)	489 (13.5)	0.64 (0.42–0.99)	0.045	14 (12.2)	643 (13.2)	0.92 (0.52–1.63)	0.79
Human rhinovirus	27 (10.1)	874 (24.2)	0.34 (0.23–0.52)	<0.0001	19 (16.5)	1017 (20.9)	0.72 (0.43–1.18)	0.19
Parainfluenza virus	11 (4.1)	429 (11.9)	0.32 (0.17–0.58)	<0.0001	5 (4.4)	290 (6.0)	0.69 (0.28–1.70)	0.42

Characteristics, n (%)	All cases		Community controls					
	hMPV A/B positive (n = 267)	hMPV A/B negative (n = 3620)	Adjusted OR (95% CI) ^a	p ^a	hMPV A/B positive (n = 115)	hMPV A/B negative (n = 4861)	Adjusted OR (95% CI) ^a	p ^a
Human coronavirus	10 (3.8)	280 (7.7)	0.47 (0.24–0.89)	0.02	9 (7.8)	492 (10.1)	0.73 (0.37–1.45)	0.37
Influenza virus	8 (3.0)	171 (4.7)	0.62 (0.30–1.28)	0.19	1 (0.9)	113 (2.3)	0.35 (0.05–2.50)	0.29
Bacterial detection in NP/OP								
Any bacteria positive	250 (93.6)	3225 (89.1)	1.77 (1.07–2.93)	0.03	110 (95.7)	4453 (91.6)	1.93 (0.77–4.79)	0.16
Restricted to high density for <i>S. pneumoniae</i> and <i>H. influenzae</i> ^f	138 (51.7)	1708 (47.2)	1.22 (0.94–1.57)	0.14	46 (40.0)	1879 (38.7)	1.04 (0.70–1.53)	0.86
<i>S. pneumoniae</i>	220 (82.4)	2738 (75.6)	1.49 (1.07–2.08)	0.02	100 (87.0)	3976 (81.8)	1.47 (0.84–2.57)	0.18
<i>S. pneumoniae</i> >6.9 log ₁₀ copies/mL	36 (13.5)	456 (12.6)	1.10 (0.76–1.60)	0.60	5 (4.4)	374 (7.7)	0.54 (0.22–1.34)	0.18
<i>H. influenzae</i>	166 (62.2)	1922 (53.1)	1.48 (1.14–1.92)	0.003	65 (56.5)	2500 (51.4)	1.24 (0.84–1.83)	0.28
<i>H. influenzae</i> >5.9 log ₁₀ copies/mL	87 (32.6)	965 (26.7)	1.38 (1.05–1.82)	0.02	19 (16.5)	1058 (21.8)	0.70 (0.42–1.16)	0.16
<i>S. aureus</i>	48 (18.0)	577 (15.9)	1.16 (0.83–1.61)	0.40	20 (17.4)	656 (13.5)	1.28 (0.78–2.12)	0.33
<i>B. pertussis</i>	1 (0.4)	31 (0.9)	0.42 (0.06–3.12)	0.40	0 (0.0)	14 (0.3)	N/A	
<i>C. pneumoniae</i>	1 (0.4)	34 (0.9)	0.40 (0.05–2.91)	0.36	3 (2.6)	63 (1.3)	1.89 (0.58–6.17)	0.29
<i>M. pneumoniae</i>	4 (1.5)	40 (1.1)	1.57 (0.55–4.47)	0.39	6 (5.2)	46 (1.0)	6.23 (2.55–15.20)	<0.0001
<i>Salmonella</i> species	3 (1.1)	25 (0.7)	1.61 (0.48–5.42)	0.44	0 (0.0)	25 (0.5)	N/A	
Fungi detection in NP/OP								
<i>P. jirovecii</i>	16 (6.0)	293 (8.1)	0.69 (0.40–1.16)	0.16	5 (4.4)	376 (7.7)	0.54 (0.22–1.35)	0.19
<i>P. jirovecii</i> >4 log ₁₀ copies/mL	9 (3.4)	130 (3.6)	0.89 (0.44–1.78)	0.73	1 (0.9)	105 (2.2)	0.39 (0.05–2.87)	0.36

hMPV, human metapneumovirus; NP/OP, nasopharyngeal/oropharyngeal swab specimen; PCV, pneumococcal conjugate vaccine; DTP, Diphtheria-Tetanus-Pertussis; N/A, Not Applicable.

^a Adjusted for age in months and study site.

^b Underweight defined as weight for age less than −2 standard deviations of the median age-sex specific WHO reference [18].

^c Premature birth defined as gestational age <37 weeks.

^d Birth weight <2.5 kg.

^e HIV-exposed but uninfected defined as having had a negative HIV PCR test but either HIV seropositive for those <12 months of age or seronegative with maternal history of HIV infection. For HIV seronegative infants <7 months of age, perinatal exposure was defined as positive maternal serology. Positive maternal status based on self-report was accepted, except for seronegative children <7 months of age where documented positive maternal status was required. Analyses were limited to Zambia and South Africa, where were high HIV prevalence sites.

^f One case had hMPV detected in the lung aspirate (aged 6 months) with two other bacteria co-detected, *Haemophilus influenzae* and *Moraxella catarrhalis*. Human cytomegalovirus, *H. influenzae* and hMPV were detected in NP/OP specimen but not *Moraxella catarrhalis*. Blood culture was negative.

^gOne case (aged 9 months) had adenovirus detected and other two cases (aged 4 and 7 months) had human cytomegalovirus detected in the lung aspirates.

^hDetected by blood culture, by PCR or culture of lung aspirate, or by PCR, culture, or pneumococcal antigen detection (BinaxNOW) of pleural fluid.

ⁱRestricted to high density for *S. pneumoniae* (>6.9 log₁₀ copies/mL) and *H. influenzae* (>5.9 log₁₀ copies/mL).

Table 2
Comparison of clinical and laboratory findings by hMPV and RSV status in NP/OP swabs among all cases

Characteristics, n (%)	hMPV-positive ^a [A] (n = 255)	RSV-positive ^d [B] (n = 1024)	Negative for hMPV and RSV [C] (n = 2596)	A vs. B Adjusted OR (95% CI) ^b	p ^b	A vs. C Adjusted OR (95% CI) ^b	p ^b
Clinical							
Tachypnea ^c	216 (84.7)	863 (84.3)	2090 (80.5)	0.92 (0.61 – 1.38)	0.68	1.38 (0.95–2.00)	0.09
Rhinorrhoea	106 (41.6)	482 (47.1)	1112 (42.8)	0.69 (0.51–0.95)	0.02	1.01 (0.76–1.34)	0.95
Cough	188 (73.7)	777 (75.9)	1730 (66.6)	0.90 (0.64–1.26)	0.54	1.51 (1.11 – 2.06)	0.008
Fever ^d	100 (39.2)	297 (29.0)	777 (29.9)	1.29 (0.94–1.77)	0.11	1.60 (1.20–2.13)	0.001
Diarrhoea	30 (11.8)	137 (13.4)	405 (15.6)	0.89 (0.57–1.37)	0.59	0.70 (0.46–1.04)	0.08
Wheeze	76 (29.8)	395 (38.6)	845 (32.6)	0.62 (0.42–0.91)	0.02	0.86 (0.60–1.23)	0.42
Stridor	4 (1.6)	8 (0.8)	84 (3.2)	1.77 (0.50–6.22)	0.38	0.51 (0.18–1.41)	0.19
Nasal flaring	148 (58.0)	613 (59.9)	1494 (57.6)	0.98 (0.70–1.36)	0.89	1.08 (0.80–1.46)	0.62
Grunting	48 (18.8)	178 (17.4)	458 (17.6)	1.05 (0.68–1.62)	0.83	1.09 (0.73–1.62)	0.68
Chest wall retraction	245 (96.1)	1002 (97.9)	2310 (89.0)	0.59 (0.26–1.31)	0.19	2.88 (1.48–5.60)	0.002
Tachycardia ^e	128 (50.2)	525 (51.3)	1304 (50.2)	0.94 (0.70–1.25)	0.66	0.98 (0.75–1.28)	0.88
Very severe pneumonia	70 (27.5)	273 (26.7)	902 (34.7)	1.01 (0.73–1.42)	0.93	0.70 (0.52–0.95)	0.02
Vomiting	11 (4.3)	16 (1.6)	89 (3.4)	2.52 (1.12–5.70)	0.03	1.40 (0.72–2.72)	0.32
Inability to feed	19 (7.5)	72 (7.0)	209 (8.1)	1.11 (0.64–1.94)	0.71	0.88 (0.54–1.46)	0.63
Lethargy	24 (9.4)	51 (5.0)	325 (12.5)	1.64 (0.96–2.80)	0.07	0.73 (0.47–1.14)	0.17
Multiple convulsions	12 (4.7)	21 (2.1)	211 (8.1)	1.74 (0.80–3.76)	0.16	0.62 (0.33–1.14)	0.13
Nodding	38 (14.9)	204 (19.9)	397 (15.3)	0.77 (0.52–1.16)	0.21	0.98 (0.67–1.42)	0.90
Cyanosis	5 (2.0)	10 (1.0)	64 (2.5)	2.53 (0.82–7.82)	0.11	0.80 (0.32–2.04)	0.64
Hypoxemia ^f	101 (39.6)	380 (37.1)	900 (34.7)	1.28 (0.91 – 1.81)	0.16	1.40 (1.03–1.89)	0.03
Supplemental oxygen	110 (43.1)	426 (41.6)	1064 (41.0)	1.33 (0.92–1.94)	0.13	1.26 (0.90–1.76)	0.19
Radiographic findings ^g							
Abnormal ^h	141 (55.3)	500 (48.8)	1078 (41.5)	1.29 (0.97–1.71)	0.08	1.76 (1.36–2.28)	<0.0001
Consolidation	67 (26.3)	209 (20.4)	538 (20.7)	1.41 (1.01 – 1.97)	0.04	1.38 (1.02–1.86)	0.04
Other infiltrates	74 (29.0)	291 (28.4)	540 (20.8)	1.01 (0.74–1.38)	0.94	1.58 (1.18–2.12)	0.002
Laboratory findings							

Characteristics, n (%)	hMPV-positive ^d [A] (n = 255)	RSV-positive ^d [B] (n = 1024)	Negative for hMPV and RSV [C] (n = 2596)	A vs. B Adjusted OR (95% CI) ^b	p ^b	A vs. C Adjusted OR (95% CI) ^b	p ^b
Leucocytosis ⁱ	90 (35.3)	285 (27.8)	1206 (46.5)	1.27 (0.93–1.73)	0.13	0.66 (0.50–0.87)	0.003
Leukopenia	6 (2.4)	30 (2.9)	63 (2.4)	0.79 (0.31–2.01)	0.62	1.00 (0.42–2.36)	1.00
CRP (mg/mL)							
<5	62 (24.3)	281 (27.4)	629 (24.2)	Reference	0.02	Reference	0.22
5–<10	24 (9.4)	112 (10.9)	286 (11.0)	0.98 (0.58–1.67)		0.90 (0.55–1.48)	
10–<20	29 (11.4)	121 (11.8)	319 (12.3)	1.01 (0.61–1.67)		1.00 (0.63–1.60)	
20–<40	30 (11.8)	135 (13.2)	351 (13.5)	0.98 (0.59–1.61)		0.96 (0.60–1.52)	
40–<60	19 (7.5)	61 (6.0)	168 (6.5)	1.38 (0.76–2.51)		1.27 (0.73–2.19)	
≥60	62 (24.3)	117 (11.4)	468 (18.0)	2.01 (1.29–3.13)		1.49 (1.01–2.20)	
CRP ≥40 mg/mL	81 (31.8)	178 (17.4)	636 (24.5)	1.80 (1.29–2.52)	0.001	1.47 (1.09–1.98)	0.01
Prior antibiotics	123 (48.2)	458 (44.7)	1141 (44.0)	1.07 (0.82–1.38)	0.63	0.97 (0.76–1.23)	0.77
Microbiologic findings in NP/OP							
Any organism positive (other than hMPV and RSV)	247 (96.9)	957 (93.5)	2509 (96.7)	2.06 (0.97–4.40)	0.06	1.08 (0.52–2.27)	0.84
Any viral positive (other than hMPV and RSV)	81 (31.8)	362 (35.4)	1663 (64.1)	0.81 (0.60–1.10)	0.18	0.26 (0.19–0.34)	<0.0001
Any bacteria positive ^j	133 (52.2)	502 (49.0)	1206 (46.5)	1.29 (0.97–1.73)	0.08	1.24 (0.95–1.62)	0.11
<i>S. pneumoniae</i> >6.9 log ₁₀ copies/mL	36 (14.1)	139 (13.6)	317 (12.2)	1.12 (0.74–1.70)	0.59	1.18 (0.81–1.73)	0.39
<i>H. influenzae</i> >5.9 log ₁₀ copies/mL	84 (32.9)	275 (26.9)	690 (26.6)	1.47 (1.07–2.01)	0.02	1.36 (1.03–1.82)	0.03
Microbiologically confirmed infection in sterile specimen							
Any pathogen	10 (3.9)	10 (1.0)	124 (4.8)	3.65 (1.46–9.13)	0.006	0.80 (0.41–1.55)	0.51
Any bacteria	9 (3.5)	10 (1.0)	111 (4.3)	3.18 (1.24–8.16)	0.02	0.80 (0.40–1.60)	0.52
Outcomes							
Hospital stay >3 d ^k	167 (65.5)	572 (55.9)	1441 (55.5)	1.76 (1.28–2.41)	0.001	1.65 (1.24–2.20)	0.001
Hospital duration (median, IQR)	4.0 (3.0–7.0)	4.0 (3.0–6.0)	4.0 (3.0–7.0)	1.05 (1.02–1.08)	0.001	1.00 (0.98–1.02)	0.65
Mortality within 30 d of admission	10 (3.9) ^l	25 (2.4)	248 (9.6)	1.25 (0.57–2.71)	0.58	0.36 (0.19–0.71)	0.003

CRP, C-reactive protein; CXR, chest X-ray; hMPV, human metapneumovirus; IQR, interquartile range; NP/OP, nasopharyngeal/oropharyngeal swab specimen; RSV, respiratory syncytial virus.

^aExcludes 12 cases that were positive for both hMPV and RSV.

^bAdjusted for study sites and age in months.

- c_j Tachypnea defined as respiratory rate ≥ 60 breaths/min if aged < 2 months, respiratory rate ≥ 50 breaths/min if aged 2–12 months, respiration rate ≥ 40 breaths/min if aged > 12 months.
- d_j Fever defined as measured temperature $\geq 38^\circ\text{C}$ or reported history of fever.
- e_j Tachycardia defined as heart rate > 160 beats per minute (bpm) if aged < 11 months, heart rate > 150 bpm if aged 12–35 months, heart rate > 140 bpm if aged 36–59 months.
- f_j Children were considered to have hypoxemia if: (a) a room air pulse-oximetry reading indicated oxygen saturation $< 90\%$ at the two sites at elevation (Zambia and South Africa) or $< 92\%$ at all other sites, or (b) supplemental oxygen used if a room air oxygen saturation reading was not available.
- g_j Excluding missing or uninterpretable CXRs.
- h_j Defined as findings of consolidation or other infiltrates on chest radiographs.
- i_j Leucocytosis defined as white blood cell count $> 15\,000$ cells/ μL if age < 12 months, or $> 13\,000$ cells/ μL if age > 12 months.
- j_j Restricting to high density for *S. pneumoniae* (> 6.9 log₁₀ copies/mL) and *H. influenzae* (> 5.9 log₁₀ copies/mL).
- k_j Excluding cases with inpatient mortality.
- l_j See Table S10 for characteristics of the 10 hMPV cases that died.