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Pathogen Clearance and New Respiratory Tract Infections Among Febrile Children in Zanzibar Investigated With Multitargeting Real-Time Polymerase Chain Reaction on Paired Nasopharyngeal Swab Samples

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Background: New molecular methods have revealed frequent and often polymicrobial respiratory infections in children in low-income settings. It is not known whether presence of multiple pathogens is due to prolonged infections or to frequent exposure. The aim of this study was to analyze short-term pathogen clearance from nasopharynx and the rate of new respiratory tract infections in febrile preschool children.

Methods: Children (n = 207) with uncomplicated acute febrile illness 2–59 months of age presenting to a health center in Zanzibar, Tanzania, April–July 2011, were included. Paired nasopharyngeal swab samples, collected at enrolment and after 14 days, were analyzed by multiple real-time polymerase chain reaction for Adenovirus, bocavirus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, *Coronaviruses*, *Enterovirus*, influenza A and B virus, metapneumovirus, measles virus, *Mycoplasma pneumoniae*, parainfluenza virus, *Parechovirus*, respiratory syncytial virus and *Rhinovirus*. An age-matched and geographically matched healthy control group (n = 166) underwent nasopharyngeal sampling on 1 occasion.

Results: At baseline, 157/207 (76%) patients had at least 1 pathogen detected, in total 199 infections. At follow-up (day 14), 162/199 (81%) of these infections were not detected, including >95% of the previously detected infections with *Enterovirus*, influenza A virus, influenza B virus, metapneumovirus or parainfluenza virus. Still 115 (56%) children were positive for at least 1 pathogen at follow-up, of which 95/115 (83%) were not found at baseline. Detection of influenza B on day 14 was significantly associated with fever during follow-up.

Conclusion: The results suggest that children with acute febrile illness in Zanzibar rapidly clear respiratory tract infections but frequently acquire new infections within 14 days.

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Recent studies using sensitive molecular methods have generated new knowledge on the etiology of respiratory tract infections especially in children living in low-income countries.[1–3] When applying techniques that simultaneously target multiple pathogens, there has not only been an overall increased microbial detection but also higher rates of coinfections.[4] For many pathogens, high detection frequencies have been observed also among healthy controls, often similar to frequencies in symptomatic respiratory infections.[1,5] Moreover, new viruses have been discovered like the metapneumovirus, bocavirus and new types of *Coronavirus*, *Enterovirus* and *Rhinovirus*.[6] Hence, a more complex picture of the etiology of respiratory infections has emerged, often with a difficulty to determine which pathogen causes symptoms, if >1 pathogen is detected.

It is not well known if the high infection rates and frequent finding of multiple pathogens are due to persistence of some infections or a result of frequent exposure to new pathogens or new strains of the same pathogen, due to a lack of studies on this type of dynamics, especially studies using multitargeting molecular methods.

The aim of this study was to analyze pathogen clearance and frequencies of new infections in febrile children by obtaining paired sampling (day 0 and day 14) from 207 children for analysis with a real-time polymerase chain reaction (PCR) assay targeting a wide range of pathogens.

MATERIALS AND METHODS

Enrolment and Follow-Up of Patients

This is a substudy to a prospective short-term longitudinal cohort study that included children with acute uncomplicated febrile illness presenting to Kivunge primary health care center in Zanzibar, Tanzania, between April and July 2011. It is based on analysis of nasopharyngeal swab samples that were collected from 25% of the patients and also includes all the healthy controls in the parent study.

The aim of the parent study was to investigate causes of fever, and these results were recently published.[1] Inclusion criteria for enrolment in the parent study were as follows: (1) age 2–59 months old; (2) acute uncomplicated fever defined as history of fever in the preceding 24 hours (information from caretaker) and/or verified fever (axillary temperature of ≥37.5°C by electronic thermometer) and (3) written informed proxy consent from an accompanying

caretaker. Exclusion criteria were as follows: (1) signs of severe disease according to Integrated Management of Childhood Illness guidelines (http://www.who.int/child_adolescent_health/documents/IMCI_chartbooklet/en/index.html); (2) previous study enrolment in the past 28 days and (3) reported inability to return for follow-up.

Patients had a first scheduled follow-up on day 4 (±2 days) and a second on day 14 (±2 days) for evaluation of fever outcome (defined as degree of fever resolution; Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C932). Caretakers were instructed to return with their children in case any signs of severe disease occurred. If a child did not return for a scheduled follow-up, a study nurse visited the home to obtain follow-up information.

On the day of inclusion (day 0, baseline), all included patients in the parent study had a nasopharyngeal swab sampled. Of the 645 patients who came for day 14 follow-up in the parent study, 207 were randomly selected for nasopharyngeal paired sampling on day 14 (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C932). This randomization of 25% of the patients was performed by blind-picking color-coded candy (1 red for every 3 white) from a bag (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C932). Every child who got a red candy was subjected to nasopharyngeal swab sampling. Gender, age, fever characteristics and PCR positivity rates for pathogens on enrolment were similar in the subgroup of patients who were sampled on day 14 (n = 207) in comparison with all sampled patients (n = 677).

Enrolment of Healthy Controls

Healthy controls defined as children 2–59 months of age with no history of cough, runny nose or fever (by history and/or electronic axillary temperature <37.5°C) in the preceding 10 days with a written informed proxy consent from a caretaker were recruited during the same study time period from 8 villages in the catchment area. Recruitment aimed at a representative distribution of age, sex and geography. Each study week, one of the villages was visited and asymptomatic children were identified through house-to-house screening. Eligible children maximum 1 per household provided nasopharyngeal swabs for PCR analyses. Healthy controls were subjected to nasopharyngeal swab sampling on 1 occasion.

Nasopharyngeal Swab Sampling and Real-Time PCR Analysis in Patients and Healthy Controls

The swabs (Copan Regular Flocked Swab 502CS01; Copan Italia Spa, Brescia, Italy) were sampled in a standardized manner from the nasopharynx of the child. Directly after sampling, the swab was placed into a sterile vial containing 1-mL sterile NaCl 0.9%. Within 2 hours after sampling, the swabs and the saline were vortexed, after which the liquid content was transferred to a microtube using a disposable transfer pipette and stored in a controlled temperature of –70°C. After completion of the field trial, all samples were transported to Sweden on dry ice for molecular analyses.

The real-time PCR used for detection[7,8] as well as *Enterovirus*/*Rhinovirus* sequencing procedures[9] have been described before. Table (Supplemental Digital Content 2, http://links.lww.com/INF/C933) shows primers and probes for the real-time PCR. The result for each agent was recorded as the threshold cycle (Ct) value, which is inversely related to the pathogen load in each specimen. When a positive reaction was obtained for both *Enterovirus* and *Rhinovirus* and there was a >10 cycles difference in Ct value, the pathogen with the lowest Ct value was recorded as detected. When the Ct value differed <10 cycles, sequencing was performed to distinguish *Rhinovirus* and *Enterovirus*. Sequencing was also performed if *Rhinovirus*/*Enterovirus* was detected on both day 0 and day 14, with a Ct value >4 cycles lower on day 14 than on day 0 (indicating a probable new infection).

Ethical Considerations

The study was registered at clinicaltrials.org (NCT01094431) and approved by the Zanzibar Medical Research Ethics Committee in Tanzania (Reference number: ZAMREC/0001/April/010), and by the Regional Ethical Review Board in Gothenburg, Sweden (Reference number: 266-10). Written informed proxy consent was obtained from a caretaker of all enrolled patients and healthy controls before enrolment. No financial incentives were given.

Sample Size Calculation, Study Endpoints, Data Management and Statistical Analysis

The parent study was an exploratory study, which precluded a sample size calculation. However, it was estimated that a sample of 650 patients would be sufficient to obtain a representative classification of fever causes according to Integrated Management of Childhood Illness, and that at least 150 controls would be required to make comparisons of microbiologic findings obtained by PCR analysis of nasopharyngeal and rectal specimens. For this substudy, we estimated that to resample 25% of the 650 patients on day 14 would be sufficient to allow comparisons of pathogen detection frequencies on day 0 and day 14.

Data were double entered in CSPro (Census and Survey Processing System, United States Census Bureau) or Microsoft Excel for Mac, 2016, Microsoft, validated and exported to STATA 14 (StataCorp 2015, Stata Statistical Software: Release 14; StataCorp LP, College Station, TX) where all statistical analyses were performed. *P* values <0.05 were considered statistically significant. For independent samples, Fisher exact test was used for binary data and proportions. For paired samples, exact sign tests were used for binary data comparing positivity rates on day 0 and day 14 and paired *t* test were used for comparison of means.

TABLE 1. Characteristics of Patients (on Day 0, Baseline) and Healthy Controls

Characteristics	Patients (n = 207)	Healthy Controls (n = 166)
Female patients, n (%)	102 (49)	81 (49)
Median age (mo) (IQR)	15 (10–26)	24 (12–36)
2–11 mo, n (%)	67 (32)	32 (19)
12–23 mo, n (%)	68 (33)	42 (25)
24–35 mo, n (%)	37 (18)	41 (25)
36–59 mo, n (%)	35 (17)	52 (31)
Median reported fever duration on admission (d) (IQR)	3 (2–4)	—
Median body temperature (IQR)	37.3 (36.7–38.0)	36.5 (36.4–36.5)
Caretaker level of education, n (%)		
No school education	71 (34)	NA
N ≤ 6 yr education	26 (13)	NA
N > 6 yr education	91 (44)	NA
Fully vaccinated‡ >11 mo, n (%)	132 (94)§	NA
Underweight (weight/age) below –2 SD, n (%)	53 (26)	NA
Respiratory symptoms, n (%)		
N cough	180 (87)	0 (0)
N runny nose	156 (76)	0 (0)

‡Bacillus-Calmette-Guerin- vaccine, 1 dose (BCG); Oral polio vaccine, 3 doses (OPV3); Diphteria-Pertussis-Tetanus vaccine, 3 doses (DPT3), measles.

§Denominator patients >11 months: n = 140.

Denominators vary due to missing data (n ≤ 10 if not indicated).

IQR indicates interquartile range; NA, information not obtained from the healthy controls.

RESULTS

Pathogen Clearance, New Infections and Comparison With Healthy Controls

Table 1 shows participant characteristics of the 207 patients and for the 166 healthy controls. Tables 2 and 3 show nasopharyngeal PCR results from baseline and follow-up in patients, including analysis of pathogen clearance and new infections at follow-up. In coherence with the findings of the parent study,[1] the patients in this subset were more likely to be infected with influenza A and B virus, *Enterovirus* or respiratory syncytial virus (RSV), and less likely to be infected with *Rhinovirus* on day 0, as compared with healthy controls (Table 2).

On baseline sampling day 0, 157/207 (76%) patients had at least 1 pathogen detected. More than 1 pathogen was detected in 36 patients (range, 2–4 pathogens). In total, 199 infections were found on day 0. In healthy controls, 94/166 (56%) had 1 or more pathogens detected, of which 15 (9%) had 2 pathogens. On day 14, 139/158 (88%) patients had cleared a total of 162 infections. More than 95% of infections at baseline with *Enterovirus*, influenza A virus, influenza B virus or parainfluenza virus had become cleared (were PCR negative) on day 14. Despite this, 115/207 (56%) patients were positive for any pathogen at follow-up, with multiple pathogens detected in 32 patients (range, 2–4 pathogens). Of these 115 patients, 31 (28%) still had the same 1–2 pathogens (totally 36) detected at follow-up. In total, 46% of all patients (95/207) had 1–3 new pathogens detected at follow-up (totally 115 new infections). A large proportion of these 115 new infections were *Rhinovirus* (n = 48, 42%), influenza B virus (n = 10, 9%) or RSV (n = 23, 20%) (Table 3).

Of the 50 patients with no pathogen detected on day 0, 24 patients (48%) had 33 new infections detected on day 14. In total, 173 (84%) of patients had a change in PCR detection status for at least 1 pathogen (positive to negative; or negative to positive) from day 0 to day 14.

Pathogen Load in Patients on Inclusion and Follow-Up

Figure 1 shows the dynamics of the detected infections. The infections are represented by their real-time PCR Ct values on day 0 and day 14. This is the cycle during PCR when the fluorescence from the probe that binds to amplified fragments has reached detectable levels. Accordingly, a low pathogen load in the sample results in a high Ct value. Therefore, the Ct values on the Y axis are in reverse order, and a negative PCR (undetected pathogen) is represented by a Ct value of 40 in the plots.

Most of the pathogens that were detected at baseline were no longer detected on day 14. Pathogens detected at both baseline and follow-up in the same individual, in general, showed a higher Ct value on day 14, indicating a decrease in pathogen load. For influenza B and RSV, the mean Ct value increase was 8.4 ($P = 0.01$) and 7.7 ($P = 0.003$) cycles, respectively. A few pathogens detected at both time points showed lower Ct values on day 14. Those that were *Rhinovirus*/*Enterovirus* were subjected to sequencing to distinguish between new and persisting infections. In 3 cases (bocavirus, n = 1; RSV, n = 1; *Coronavirus* NL63, n = 1), the Ct value had declined >4 cycles, indicating a higher pathogen concentration at follow-up, suggesting a combination of clearance and new infection.

TABLE 2. Detection Frequencies at Baseline and Follow-Up (Day 14) in Patients and on 1 Occasion in Healthy Controls*

	BL (n = 207), n (%)	FU (n = 207), n (%)	Healthy Controls (n = 166), n (%)	P† BL vs. Controls	P† FU vs. Controls
Influenza A virus	17 (8)	1 (0)	2 (2)	0.002	0.59
Influenza B virus	31 (15)	12 (6)	4 (2)	<0.0005	0.13
Parainfluenza virus	6 (3)	0 (0)	1 (1)	0.137	0.44
RSV	53 (26)	37 (18)	26 (16)	0.022	0.58
Bocavirus	6 (3)	6 (3)	4 (2)	1	1
Enterovirus	23 (11)	5 (2)	1 (1)	<0.0005	0.23
Rhinovirus	32 (15)	60 (29)	48 (29)	0.002	1
Adenovirus	4 (2)	3 (1)	1 (1)	0.39	0.63
Coronavirus‡	17 (8)	16 (8)	23 (14)	0.093	0.06
Measles virus	3 (1)	6 (3)	0 (0)	0.257	0.036
Parechovirus	3 (1)	3 (1)	0 (0)	0.257	0.257

*Metapneumovirus, *Bordetella pertussis*, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* were also detected, but the detection was n ≤ 2 at any occasion.

†By Fisher exact test.

‡OC43, 225E, NL63, HKU1.

BL indicates baseline; FU, follow-up.

TABLE 3. Changes in Pathogen Detection Between BL and FU (Paired Data)

	Positive at BL	Cleared at FU	Proportion Cleared, %	New at FU	Fraction With New Infection,* %	P†
Influenza A virus	17	17	100	1	1	0.0034
Influenza B virus	31	29	94	10	6	0.0001
Parainfluenza virus	6	6	100	0	0	0.0313
RSV	53	39	74	23	15	0.056
Enterovirus	23	22	96	5	3	0.0015
Rhinovirus	32	20	63	48	27	0.0004

*Fraction of patients who were uninfected with this pathogen at baseline who had a new infection at FU.

†By sign test (2-sided).

BL indicates baseline; FU, follow-up.

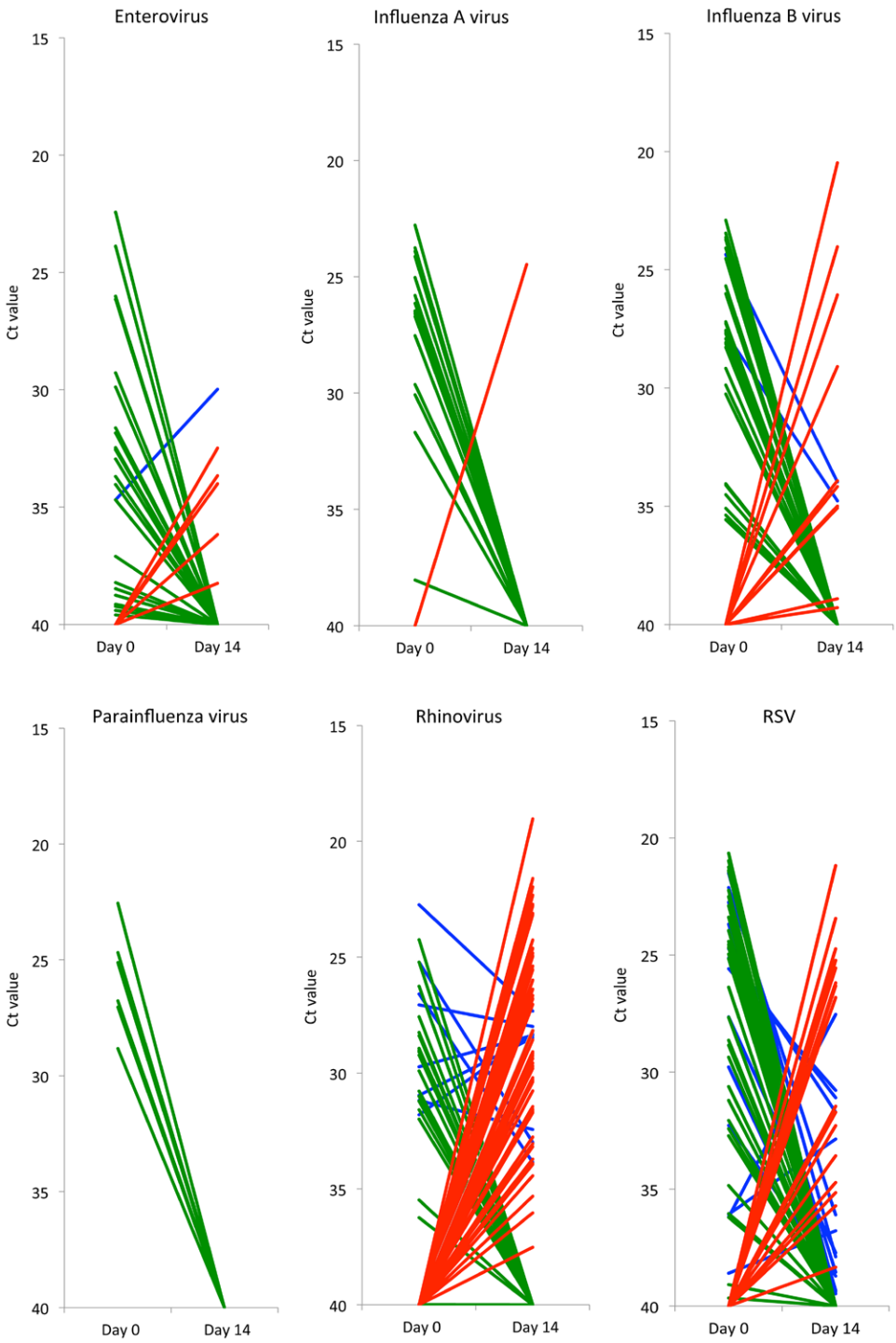

FIGURE 1. Real-time PCR Ct values on baseline and follow-up for selected pathogens. Each line represents 1 patient. Green lines for cleared infections and red lines for new infections on follow-up. Blue lines for agents detected at both baseline and follow-up. Ct values >40 were regarded as negative.

Fever Resolution

On follow-up day 14, 12/207 patients had a detected fever (≥37.5°C). Overall detection of a new pathogen at follow-up was not associated with measured fever on day 4 and/or day 14 when compared with early fever resolution, which was measured fever on day 0 only [18/37 (49%) vs. 77/170 (45%); $P = 0.76$]. Influenza B was the only pathogen significantly associated with the presence of fever after day 0; 4% (7/170) without versus 14% (5/32) with a measured fever on any follow-up had influenza B detected by PCR on day 14 ($P = 0.043$).

DISCUSSION

This is the first study that shows the short-term dynamics of a wide range of respiratory infections in children, as determined by real-time PCR conducted in a low-income setting. Most children cleared their infections within 2 weeks, but during this short

time span, almost half of the patients acquired new respiratory infections.

In comparison, previous studies of the duration of respiratory infections have included fewer patients or have focused on 1 or a few pathogens, and report lower frequencies of pathogens detected[10] than the present study. Also, respiratory tract coinfections[11–13] and pathogen clearance[14] are rarely studied at the same time.

The relatively high rates of new influenza B and RSV infections on day 14 might reflect ongoing epidemics with these viruses in Zanzibar at the time of the study. The observed high pathogen clearance rates for both RSV and influenza are in agreement with previous reports using both molecular and other methods that typically show an average shedding duration of <2 weeks.[14–19]

In the parent study,[1] *Enterovirus* and influenza A were important contributors to febrile disease with significantly more frequent detection in patients (at day 0) than in controls. On follow-up, these pathogens were cleared to a large extent, as also reported by others,[16,17,20,21] whereas new infections were rare (1 and 5 cases, respectively; Table 3). Asymptomatic infection with influenza virus A or B is reported to be uncommon.[1,16] This agrees with the observation that influenza virus B detected on day 14 that was associated with presence of measured fever after baseline sampling.

The high frequency of *Rhinovirus* both on inclusion and follow-up in this study is likely explained by many different *Rhinovirus* types circulating simultaneously, resulting in frequent exposure. New infections with *Rhinovirus* (48/207; 23%) were also more common than persisting infections (12/207; 5.7%), results consistent with previous longitudinal studies reporting that infections with new *Rhinovirus* strains are more common than prolonged (>30 days) carriage.[20,22,23] When the same pathogen is detected at follow-up as on baseline, it is important to know whether it actually is the same pathogen or a new infection with another strain. This is particularly necessary when many types of the same pathogen cocirculate, as is the case for rhinoviruses and enteroviruses, which also may be difficult to distinguish from another due to frequent cross reactivity in PCR between *Rhinovirus* and *Enterovirus*.[22] In our study, sequencing was applied when there was doubt about species, and we were hereby able to identify that *Enterovirus* was cleared in essentially all cases. In contrast, *Rhinovirus* infections were more frequent at baseline in patients than at follow-up as well as than in controls. One reason to this could be that *Rhinovirus* infections often do not cause fever which was 1 inclusion criteria for the patients in the parent study. For the other microbes, either the pathogen was not detected at follow-up or the Ct value was higher indicating lower concentration which suggests at least partial clearance. In such cases, distinction between new and prolonged infections was made without performing sequencing.

Quantification by standard curves is not appropriate for nasopharyngeal swab samples. Instead, we used Ct values to compare target concentrations on inclusion and follow-up, as shown in Figure 1 and Table 3. The lack of a standardized quantification is a limitation, but Ct values are acceptable estimates of target concentration and have been used in several previous studies.[24,25] For influenza B and RSV, Ct values increased by 7–8 cycles from day 0 to day 14, suggesting >99% reduction of pathogen concentration (because 6.6 cycles difference in theory represents a 100-fold change when PCR efficiency is perfect).

Another limitation of this study is that healthy controls were sampled only once; therefore, we do not know if children without symptoms clear their infections, or become infected with other agents, in a similar manner as those with history of fever. Because patients were not sampled beyond 2 weeks after baseline, the duration of prolonged infections could not be established.

The major strength of this study is that samples taken at 2 sampling occasions were analyzed for multiple pathogens using an assay that is highly sensitive and allows estimation of pathogen quantity. Using this assay, we could identify reduced microbial load in addition to clearance of pathogens when comparing the first with the second sampling. The results of this study are important for both future and ongoing studies that use real-time PCR techniques to determine etiology of fever and respiratory tract infections.[26] Our study shows that longitudinal sampling is of critical importance to understand the epidemiology and course of respiratory tract infection in children, which is particularly important in low-income countries.

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REFERENCES

1. Elfving K, Shakely D, Andersson M, et al. Acute uncomplicated febrile illness in children aged 2-59 months in Zanzibar—aetiologies, antibiotic treatment and outcome. *PLoS One*. 2016;11:e0146054.
2. D'Acremont V, Kilowoko M, Kyungu E, et al. Beyond malaria–causes of fever in outpatient Tanzanian children. *N Engl J Med*. 2014;370:809–817.
3. Waitumbi JN, Kuypers J, Anyona SB, et al. Outpatient upper respiratory tract viral infections in children with malaria symptoms in Western Kenya. *Am J Trop Med Hyg*. 2010;83:1010–1013.
4. El Kholy AA, Mostafa NA, Ali AA, et al. The use of multiplex PCR for the diagnosis of viral severe acute respiratory infection in children: a high rate of co-detection during the winter season. *Eur J Clin Microbiol Infect Dis*. 2016;35:1607–1613.
5. Feikin DR, Njenga MK, Bigogo G, et al. Viral and bacterial causes of severe acute respiratory illness among children aged less than 5 years in a high malaria prevalence area of western Kenya, 2007-2010. *Pediatr Infect Dis J*. 2013;32:e14–e19.
6. Debiaggi M, Canducci F, Ceresola ER, et al. The role of infections and coinfections with newly identified and emerging respiratory viruses in children. *Virol J*. 2012;9:247.
7. Andersson ME, Olofsson S, Lindh M. Comparison of the FilmArray assay and in-house real-time PCR for detection of respiratory infection. *Scand J Infect Dis*. 2014;46:897–901.
8. Brittain-Long R, Nord S, Olofsson S, et al. Multiplex real-time PCR for detection of respiratory tract infections. *J Clin Virol*. 2008;41:53–56.
9. Sansone M, Andersson M, Brittain-Long R, et al. Rhinovirus infections in western Sweden: a four-year molecular epidemiology study comparing local and globally appearing types. *Eur J Clin Microbiol Infect Dis*. 2013;32:947–954.
10. Regamey N, Kaiser L, Roiha HL, et al; Swiss Paediatric Respiratory Research Group. Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. *Pediatr Infect Dis J*. 2008;27:100–105.
11. Canducci F, Debiaggi M, Sampaolo M, et al. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. *J Med Virol*. 2008;80:716–723.
12. Kaida A, Kubo H, Takakura K, et al. Associations between co-detected respiratory viruses in children with acute respiratory infections. *Jpn J Infect Dis*. 2014;67:469–475.
13. Scotta MC, Chakr VC, de Moura A, et al. Respiratory viral coinfection and disease severity in children: a systematic review and meta-analysis. *J Clin Virol*. 2016;80:45–56.
14. Munywoki PK, Koech DC, Agoti CN, et al. Influence of age, severity of infection, and co-infection on the duration of respiratory syncytial virus (RSV) shedding. *Epidemiol Infect*. 2015;143:804–812.
15. Okiro EA, White LJ, Ngama M, et al. Duration of shedding of respiratory syncytial virus in a community study of Kenyan children. *BMC Infect Dis*. 2010;10:15.

16. Lau LL, Cowling BJ, Fang VJ, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. *J Infect Dis*. 2010;201:1509–1516.
17. Loeb M, Singh PK, Fox J, et al. Longitudinal study of influenza molecular viral shedding in Hutterite communities. *J Infect Dis*. 2012;206:1078–1084.
18. Takeyama A, Hashimoto K, Sato M, et al. Respiratory syncytial virus shedding by children hospitalized with lower respiratory tract infection. *J Med Virol*. 2016;88:938–946.
19. Bhattarai A, Villanueva J, Palekar RS, et al. Viral shedding duration of pandemic influenza A H1N1 virus during an elementary school outbreak--Pennsylvania, May-June 2009. *Clin Infect Dis*. 2011;52(suppl 1):S102–S108.
20. Winther B, Hayden FG, Hendley JO. *Picornavirus* infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. *J Med Virol*. 2006;78:644–650.
21. Tsang TK, Cowling BJ, Fang VJ, et al. Influenza A virus shedding and infectivity in households. *J Infect Dis*. 2015;212:1420–1428.
22. Loeffelholz MJ, Trujillo R, Pyles RB, et al. Duration of rhinovirus shedding in the upper respiratory tract in the first year of life. *Pediatrics*. 2014;134:1144–1150.
23. Chen WJ, Arnold JC, Fairchok MP, et al. Epidemiologic, clinical, and virologic characteristics of human rhinovirus infection among otherwise healthy children and adults: rhinovirus among adults and children. *J Clin Virol*. 2015;64:74–82.
24. Fuller JA, Njenga MK, Bigogo G, et al. Association of the CT values of real-time PCR of viral upper respiratory tract infection with clinical severity, Kenya. *J Med Virol*. 2013;85:924–932.
25. Wishaupt JO, Ploeg TV, Smeets LC, et al. Pitfalls in interpretation of CT-values of RT-PCR in children with acute respiratory tract infections. *J Clin Virol*. 2017;90:1–6.
26. Levine OS, O'Brien KL, Deloria-Knoll M, et al. The pneumonia etiology research for child health project: a 21st century childhood pneumonia etiology study. *Clin Infect Dis*. 2012;54(suppl 2):S93–S101.