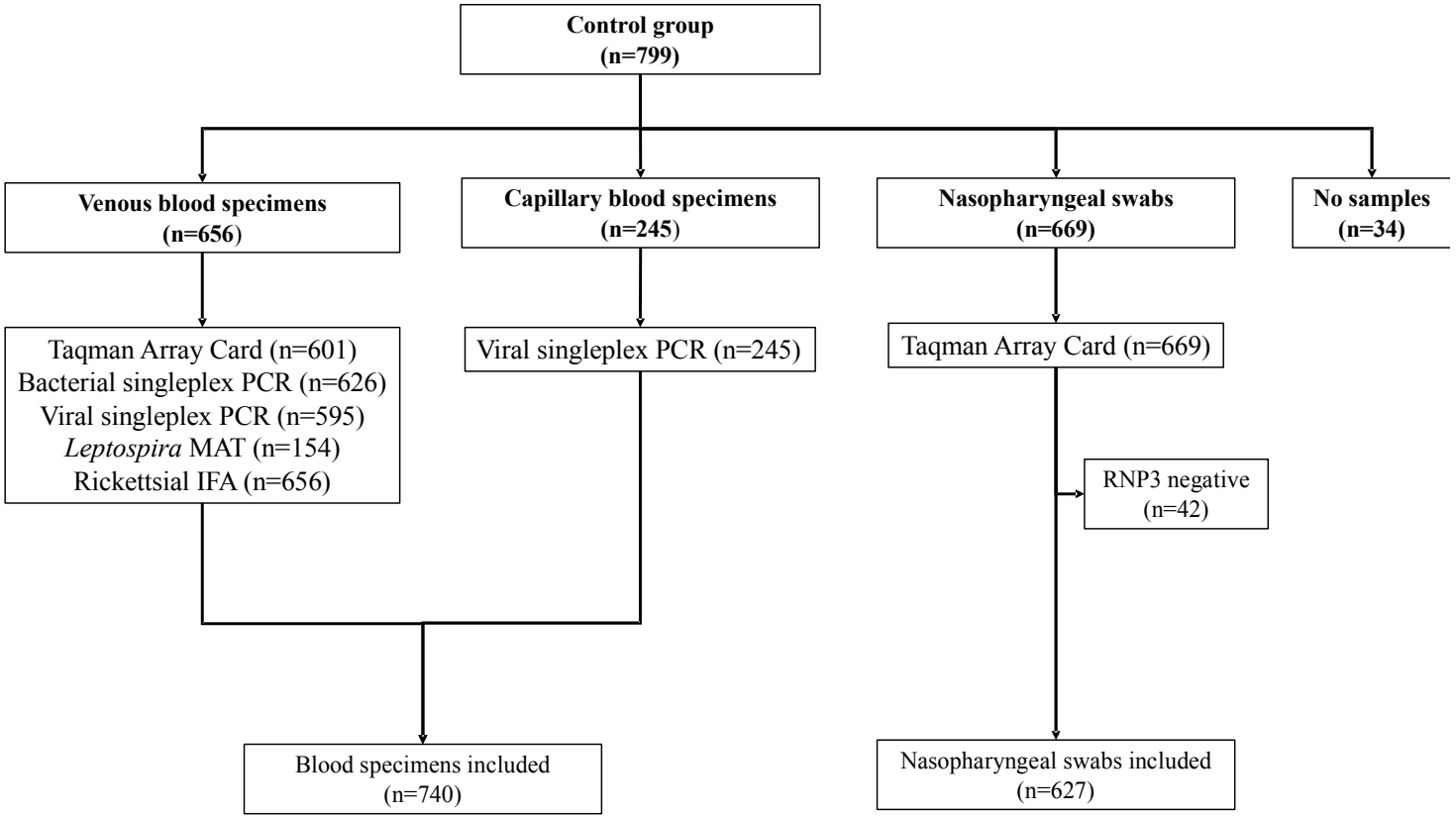
Causes of fever in primary care in Southeast Asia and performance of C-reactive protein for discriminating bacterial from viral pathogens

Supplementary material

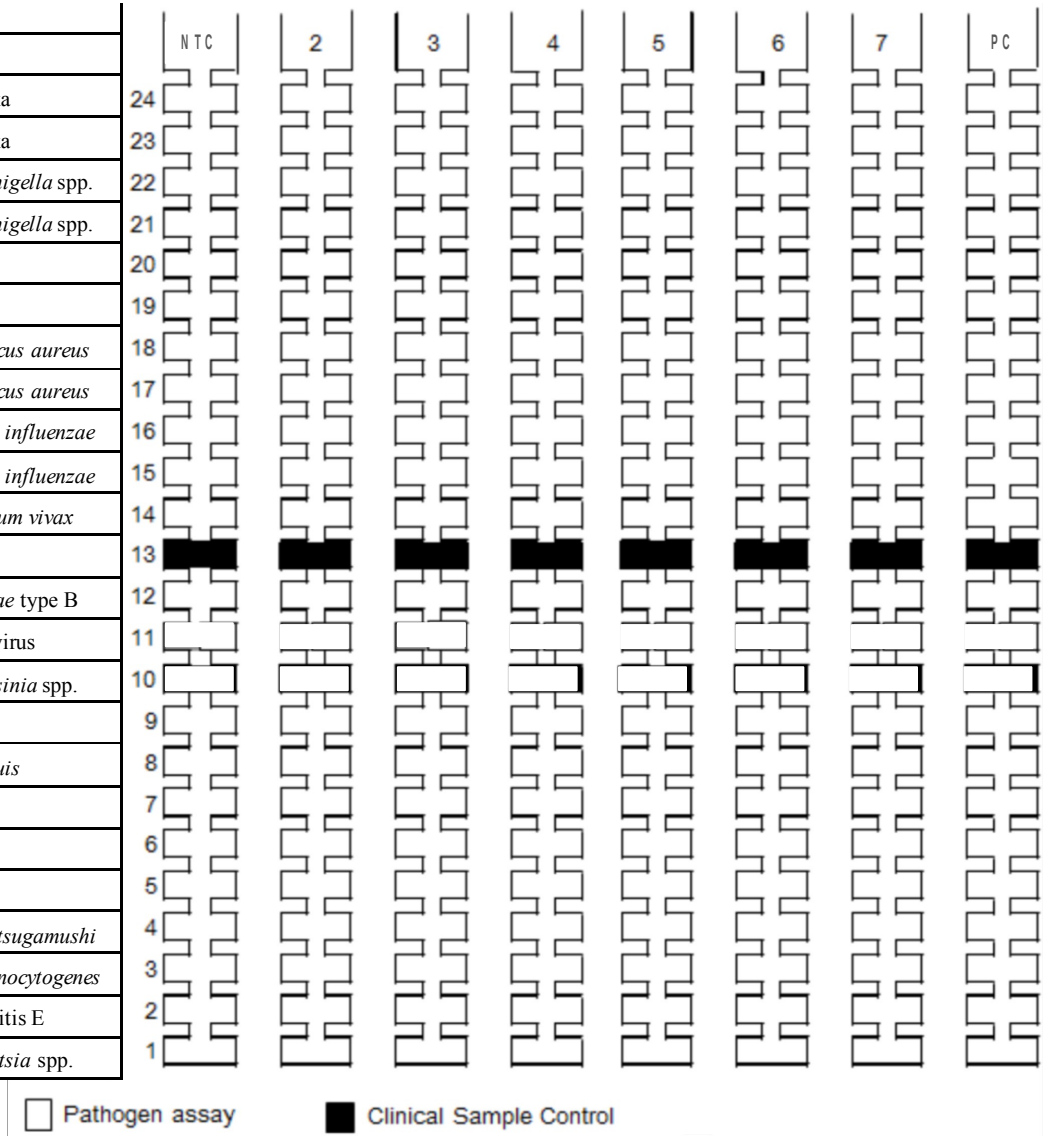
Supplementary figure 1. Diagnostic tests by type of specimen



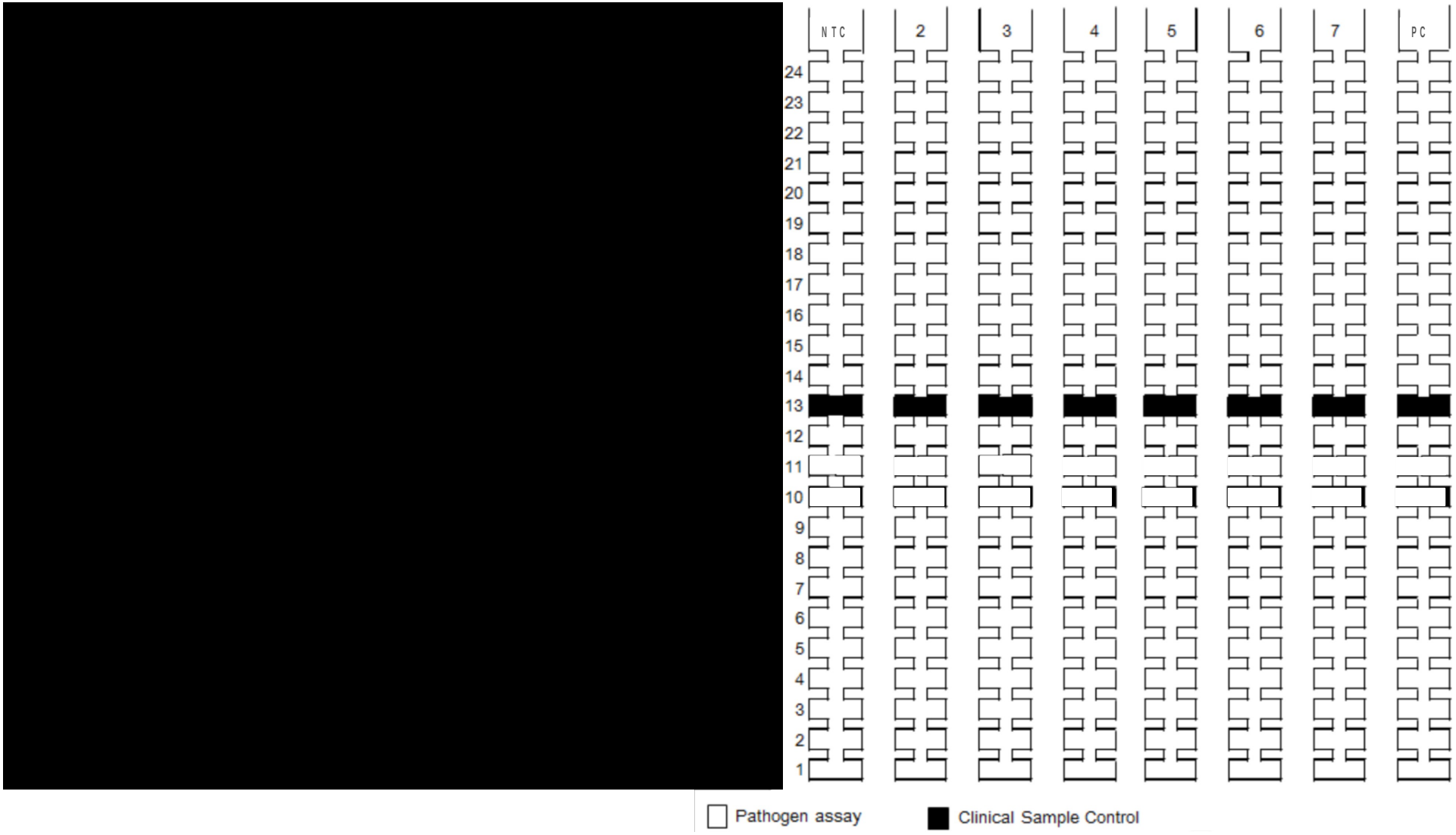
IFA – Indirect immunofluoresence assay
MAT – Microagglutination test
PCR - Polymerase chain reaction

Supplementary figure 2 A. Configuration of the TaqMan Array Card for detection of the organisms in blood specimens

Port	
Left	**Right**
Dengue / Chikungunya / Zika	Dengue / Chikungunya / Zika
Dengue / Chikungunya / Zika	Dengue / Chikungunya / Zika
Salmonella spp. / *Escherichia coli* / *Shigella* spp.	*Salmonella* spp. / *Escherichia coli* / *Shigella* spp.
Salmonella spp. / *Escherichia coli* / *Shigella* spp.	*Salmonella* spp. / *Escherichia coli* / *Shigella* spp.
Burkholderia pseudomallei	*Burkholderia pseudomallei*
Burkholderia pseudomallei	*Burkholderia pseudomallei*
Group A *Streptococcus* / *Staphylococcus aureus*	Group A *Streptococcus* / *Staphylococcus aureus*
Group A *Streptococcus* / *Staphylococcus aureus*	Group A *Streptococcus* / *Staphylococcus aureus*
Klebsiella pneumoniae / *Haemophilus influenzae*	*Klebsiella pneumoniae* / *Haemophilus influenzae*
Klebsiella pneumoniae / *Haemophilus influenzae*	*Klebsiella pneumoniae* / *Haemophilus influenzae*
Plasmodium falciparum / *Plasmodium vivax*	*Plasmodium falciparum* / *Plasmodium vivax*
Internal Positive control (IPCO)	Human control (RNP3)
Group B *Streptococcus* / *H. influenzae* type B	Group B *Streptococcus* / *H. influenzae* type B
Varicella zoster virus/Measles virus	Varicella zoster virus/Measles virus
Bartonella spp. / *Brucella* spp. / *Yersinia* spp.	*Bartonella* spp. / *Brucella* spp. / *Yersinia* spp.
Enterovirus / Adenovirus	Enterovirus / Adenovirus
Nipah virus / *Streptococcus suis*	Nipah virus / *Streptococcus suis*
Japanese encephalitis virus	Japanese encephalitis virus
Rhinovirus	Rhinovirus
Bocavirus	Bocavirus
Salmonella enterica Paratyphi A/*O.tsutsugamushi*	*Salmonella* enterica Paratyphi A/*O.tsutsugamushi*
Salmonella enterica Typhi/*Listeria monocytogenes*	*Salmonella* enterica Typhi/*Listeria monocytogenes*
Leptospira (pan serovar) / Hepatitis E	*Leptospira* (pan serovar) / Hepatitis E
Parechovirus / Rubella virus / *Rickettsia* spp.	Parechovirus / Rubella virus / *Rickettsia* spp.

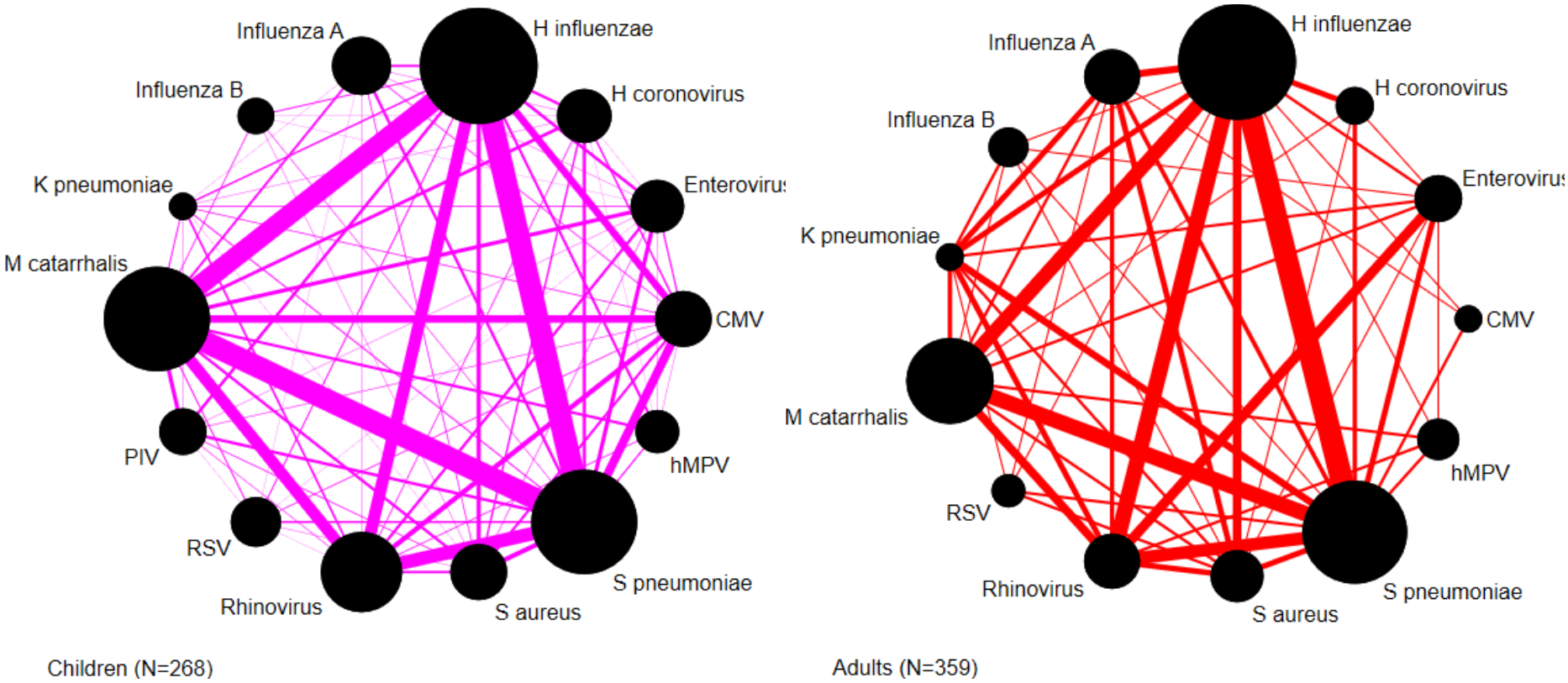


Supplementary figure 2 B. Configuration of the TaqMan Array Card for detection of the organisms in nasopharyngeal swabs.



Supplementary figure 3. Network analysis for bacterial and viral detection and combination in nasopharyngeal swabs

among children and adults in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.



The size of the black dot represents the frequency of organism detection in nasopharyngeal swabs. Only organisms detected in more than 10 specimens are represented.
Each of the axes between the dots represents the frequency of combination between two organisms.
Bacterial organisms: *K pneumoniae* is *Klebsiella pneumoniae; S pneumoniae* is *Streptococcus pneumoniae; M catarrhalis* is *Moraxella catarrhalis; S aureus* is *Staphylococcus aureus;* and *H influenzae* is *Haemophilus influenzae.*
Viral organisms: CMV is cytomegalovirus; H coronavirus is human coronavirus (229E; NL63; OC43 and HKU1); hMPV is human metapneumovirus; RSV is respiratory syncytial virus; and PIV is parainfluenza virus (type 1-3).

Supplementary table 1. Details of microbiological management

Sample collection Blood specimens were collected using ethylenediamine tetra-acetic acid (EDTA) tubes (Vacuette EDTA K3 tube, Greiner Bio-One International GmbH, Austria) in each primary care centre and transported in a cool box to a local laboratory. Plasma was obtained after centrifugation on the day of collection and stored at -80˚C until analysis. A dried blood spot (DBS, Whatman 31ET CHR, Merck, Germany) was obtained from capillary specimens when venous blood was not collected, primarily in children under 5 in Myanmar. In addition to blood specimens, patients from the control group had a nasopharyngeal (NP) swab, using the Sigma VCM® (Wiltshire, England), which includes a cellular foam bud and a transport medium suitable for nucleic acid preservation and molecular amplification [1]. The study staff were trained for the swab procedure according to the WHO standards (https://www.who.int/influenza/rsv/rsv_collection_transport_storage_samples/en/). Swabs were then stored at in a cool box after collection, and transported to a local laboratory on the same day for aliquoting and storage at -80˚C.
Total nucleic acid preparation for the Taqman Array Card assay The total nucleic acid (TNA) was prepared from each 350 µl of plasma or NP swab using the MagNA Pure Compact Extractor (Roche Applied Sciences, Indianapolis, IN, USA), following manufacturer's instructions. For the pre-lysis step, Bacterial Lysis Buffer (BLB) (Roche, Germany) and proteinase K were added at the same specimen volume and 10% of the specimen volume, respectively.
Taqman Array Card assay procedure The TaqMan Array Card (TAC) (Life Technologies, Foster City, USA) is a multiple-pathogen detection method based on real-time PCR evaluated in detecting various respiratory, enteric pathogens in children and adults from both high income countries and LMICs [2-5]. TAC offers several advantages: i) the panel of pathogens targeted can be customized, ii) a minimal volume of specimen is sufficient to test for up to 48 targets, iii) each card test for six specimens at the same time, iv) only general laboratory skills needed, v) positive and negative controls in each card and within each patient ensure the quality of the PCR reaction, and vi) low risk of contamination due to the hermetic well plate format. In this study, we customised the blood TAC assay to target nineteen bacteria, fourteen viruses and two parasites, as illustrated in supplementary figure 2 A. In addition, there were two extrinsic controls: one with the human RNase P (RNP3) gene as an extraction and specimen integrity control, and another one as an internal positive control for the amplification (IPCO) [6-8]. For NP swabs, we customized the respiratory TAC assay to target sixteen viruses, sixteen bacteria and one fungus, as illustrated in supplementary figure 2 B. Micro-organisms were selected in the panel according to those commonly found in Southeast Asia [9-14]. All assays were performed in duplicate to maximise sensitivity, and a coefficient of variation between the duplicates was calculated to ensure the TAC measurement consistency (supplementary table 2). In addition to the 6 channels for patient's specimen, one channel with sterile water was used as a negative control ensuring the absence of contamination in the PCR reaction, and one channel with TAC positive control, with single-use aliquots of combined RNA transcript for positive amplification of all targets on all TAC formats, provided by CDC. Each 50 µl of patient TNA, or negative control, or positive control was mixed with 50 µl of Quanta qScript XLT One-step RT-qPCR ToughMix, low ROX (Quanta Biosciences, USA). The ToughMix enzyme mix contains all reagents, including the polymerase and reverse transcriptase enzymes, and has been demonstrated to enhance stability during

PCR reactions with storage requiring refrigeration temperatures only [2].
Cards were centrifuged to homogeneously distribute the reaction mix to all wells (1 minute at 1,200 rpm twice) and sealed, following the manufacturer's instructions. Cards were run on the ViiA 7™ real-time PCR system (Life Technologies) using PCR cycling conditions comprising 10 min at 50˚C and 20 s at 95˚C followed by 45 two-step cycles of 3 s at 95˚C and 30 s at 60˚C as described previously [15].

Singleplex PCR assay

Dengue, chikungunya and zika viruses were further screened by a real-time RT-PCR assay, using the TaqMan® Fast Virus One-Step RT-PCR Master Mix (Applied Biosystems, Foster City, California) as described previously [16]. Performance of real-time RT-PCR on DBS for detecting these three arboviruses has been validated when compared with blood specimens [17].

Three probe-based real-time PCR assays further investigated *O. tsutsugamushi* (47 kDa *htrA* gene), *Rickettsia* spp. (17 kDa gene) as well as pathogenic *Leptospira* spp. targeting the *rrs* gene, as described previously [18-21]. We also screened for 16S RNA using an in-house real-time PCR targeting the 16S rRNA region V1 to V3 [22, 23]. For positive PCR, the PCR products were undertaken for Sanger DNA sequencing (Macrogen, Korea). We blast the resulted 16S rRNA sequences to NCBI data, OTU equal to or more than 97%.

Extraction for the singleplex PCR assay

We used an in-house DNA extraction method (Gentra Puregene Blood kit, Qiagen, Norway) as we previously published, we used each 1 mL of whole blood for nucleic acid preparation. 3 mL of red blood cell (RBC) lysis solution was added to the whole blood and centrifuged at 3,000 rpm for two minutes [11]. After discarding the supernatant, the remaining liquid was mixed with 1mL of cell lysis solution and 5 µL of RNase mixture. We added 333 µL protein precipitation solution (Gentra Puregene Blood kit, Qiagen, Norway), centrifuged at 3,000 rpm for 6 minutes and transferred the supernatant into a tube with undiluted isopropanol using a pasture pipette. We then centrifuged and discarded the supernatant, added 1 mL of 70% ethanol and inverted twice to wash the DNA pellet. Then, we centrifuged at 3,000 rpm for 1 minute at 25˚C, discarded the supernatant, centrifuged again at 3,000 rpm for 10 seconds and discarded all the solution using fine tip pasture pipette. We dried the pellet by leaving the tube open for 5 minutes, added a 100 µL preheated DNA Hydration Solution (Gentra Puregene Blood kit, Qiagen, Norway) at 65˚C, mixed and incubated at 65˚C for 1 hour.

Serology

Leptospirosis was also screened by IgM antibodies detection on a single acute specimen using a commercially available *Leptospira* IgM ELISA (Panbio Pty., Ltd., Queensland, Australia). *Leptospira* IgM ELISA positive specimens were then confirmed by microscopic agglutination test (MAT), in microtiter plates and using reference strains of 24 *Leptospira* serovars [24]. The serum titre was defined as the final dilution that showed 50% agglutination. Reciprocal agglutination titres of greater than or equal to 50% were considered positive reactions. We regarded leptospiral MAT as positive for a titre ≥1:800 following the 2013 CDC recommendations (https://wwwn.cdc.gov/nndss/conditions/leptospirosis/case-definition/2013/).

Scrub typhus, typhus group (TG) and spotted fever group rickettsiosis (SFG) were also screened on a single acute specimen by IgM ELISA (InBios International Inc., Seattle WA, USA). Scrub typhus was serologically confirmed by indirect immunofluorescence assay (IFA) using slides coated with *O. tsutsugamushi* (strains Karp, Kato, and Gilliam) as previously described [25]. A stringent diagnostic positivity criterion was an admission IgM titre ≥1:3200 [26]. Typhus group and SFG were only considered probable cases

using IFA by a single titre to ≥1:400, as no paired specimen was available [27].

Supplementary table 2. Diagnostic tests performed for bacterial, viral and fungal organisms

Blood specimens	Specimen	Assay*	Comments on diagnostic tests
Leptospira spp.	DNA from plasma	TAC	All serovars of all species from all genus
	DNA from plasma	Singleplex PCR	Real-time PCR targeting *rrs* gene [21]
	Plasma	ELISA	*Leptospira* IgM ELISA (Panbio Pty., Ltd., Queensland, Australia) [28]
	Plasma	MAT**	Using the following 24 serovars representing strains from *Leptospira interrogans*: Australis, Autumnalis, Ballum, Bataviae, Canicola, Cellidoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis. Icterohaemorrhagiae, Javanica, Louisiana, Manhao, Mini, Panama, Pomona, Pyrogenes, Ranarum, Sarmin, Sejroe, Shermani, Tarasovi, Semaranga [29]
Orientia tsutsugamushi	DNA from plasma	TAC	Real-time PCR targeting the 47 kDa outer membrane protein of *Orientia tsutsugamushi* [30]
	DNA from plasma	Singleplex PCR	Real-time PCR targeting the 47 kDa outer membrane protein of *Orientia tsutsugamushi* [30]
	Plasma	IFA	IFA of IgM against *Orientia tsutsugamushi* [31]
Rickettsia spp.	DNA from plasma	Singleplex PCR	Real-time PCR targeting 17 kDa antigen for the genus *Rickettsia* [30, 32]
Rickettsia typhi	Plasma	IFA	IFA of IgM against *Rickettsia typhi* [31]
Spotted fever group	Plasma	IFA	IFA of IgM against *Rickettsia conorii, Rickettsia rickettsii* or *Rickettsia australis* or *Rickettsia helvetica* [33]
Rubeola virus	DNA from plasma	TAC	All subtypes of measles virus
Rubella virus	DNA from plasma	TAC	
Parechovirus	DNA from plasma	TAC	All genotypes
Japanese encephalitis virus	DNA from plasma	TAC	
Dengue virus	DNA from plasma	TAC	Serovars 1-4
		Singleplex PCR	Real-time PCR [16]
Chikungunya virus	DNA from plasma	TAC	
		Singleplex PCR	Real-time PCR [16]
Zika virus	DNA from plasma	TAC	
		Singleplex PCR	Real-time PCR [16]
Varicella-zoster virus	DNA from plasma	TAC	
Enterovirus	DNA from plasma	TAC	All serotypes
Rhinovirus	DNA from plasma	TAC	
Bocavirus	DNA from plasma	TAC	
Nipah virus	DNA from plasma	TAC	

Salmonella enterica Paratyphi A	DNA from plasma	TAC	
Salmonella spp.	DNA from plasma	TAC	
Escherichia coli/ Shigella spp.	DNA from plasma	TAC	Does not detect *Shigella dysenteriae* type 1
Klebsiella pneumoniae	DNA from plasma	TAC	
Haemophilus influenzae type B	DNA from plasma	TAC	May cross-react with *Haemophilus influenzae* type A
Streptococcus suis	DNA from plasma	TAC	
Group A *Streptococcus*	DNA from plasma	TAC	
Group B *Streptococcus*	DNA from plasma	TAC	
Bartonella spp.	DNA from plasma	TAC	
Burkholderia pseudomallei	DNA from plasma	TAC	
Brucella spp.	DNA from plasma	TAC	
Staphylococcus aureus	DNA from plasma	TAC	
Yersinia spp.	DNA from plasma	TAC	
Plasmodium falciparum & vivax	DNA from plasma	TAC	
Respiratory specimens			
Viruses	NP swabs	TAC	Influenza (A & B), Adenovirus (all serotypes except 40 & 41), Enterovirus, Respiratory syncytial virus (A & B), human metapneumovirus, Rhinovirus, Parainfluenza virus (1-3), Coronavirus (NL63, HKU1, 229E, OC43), MERS Coronovirus, Bocavirus, Cytomegalovirus, Hepatitis E virus (all genotypes), Rubella virus, Varicella-zoster virus and Parechovirus
Bacteria	NP swabs	TAC	*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Moraxella catarrhalis; Bordetella pertussis, Bordetella parapertussis, Pseudomonas aeruginosa, Acinetobacter baumannii, Burkholderia pseudomallei, Chlamydia trachomatis*, *Klebsiella pneumoniae, Leptospira* spp., *Escherichia coli*, *Shigella* spp., *Streptococcus pneumoniae*, group A *Streptococcus*, *Staphylococcus aureus, Corynebacterium diphtheriae* and *Haemophilus influenzae*
Fungi	NP swabs	TAC	*Pneumocystis jirovecii*

*Molecular assays using PCR: polymerase chain reaction, serology assays including MAT: micro-agglutination test; IFA: immunofluorescence antibody assay; ELISA: enzyme-linked immunosorbent assay; NP: nasopharyngeal; TAC: Taqman array card

**Microagglutination test was only carried out in specimens positives for the *Leptospira* immunofluorescence antibody assay (immunoglobulin M ≥11 Panbio units)

Supplementary table 3. Diagnostic methods: number of testing and positives in total, in children and in adults.

	Blood specimens					Nasopharyngeal swabs
	Bacterial singleplex PCR	**Viral singleplex PCR**	**Taqman Array Card**	***Rickettsia* genus IFA**	***Leptospira* spp. MAT**	**Taqman Array Card**
Total	Tested: 626 Positive: 11 (1.8%)	Tested: 678 Positive: 26 (3.8%)	Tested: 601 Positive: 63 (10.1%)	Tested: 656 Positive: 1 (0.2%)	Tested: 154 Positive: 2 (1.3%)	Tested: 627 Positive: 468 (74.6%)
Children	Tested: 225 Positive: 5 (2.2%)	Tested: 283 Positive: 18 (6.4%)	Tested: 208 Positive: 26 (12.5%)	Tested: 254 Positive: none	Tested: 55 Positive: none	Tested: 268 Positive: 230 (85.8%)
Adults	Tested: 401 Positive: 6 (1.6%)	Tested: 395 Positive: 8 (2.0%)	Tested: 393 Positive: 13 (3.3%)	Tested: 402 Positive: 1 (0.3%)	Tested: 99 Positive: 2 (2.0%)	Tested: 359 Positive: 238 (66.3%)

Bacterial singleplex polymerase chain reaction (PCR) included 16S, *O. tsutsugamushi, Rickettsia* genus and *Leptospira* spp.
IFA: immunofluorescence assay
MAT: microagglutination test
NP swabs: nasopharyngeal swabs
PCR: polymerase chain reaction
Viral singleplex PCR included dengue, chikungunya and zika virus

Supplementary table 4. Evidence review for classifying influenza virus A & B, respiratory syncytial virus, human metapneumovirus and *Bordetella pertussis* as causal in nasopharyngeal swabs

Organism	Methods	Findings	References
Influenza virus A & B	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 3.6, 95% CI (2.4-5.3)	[34]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	Influenza A: OR 1.5, 95% CI (0.8-2.7) Influenza B: OR 3.8, 95% CI (1.2-12.4)	[35]
	Multi-site case-control study among children and adults with ILI	Children: RRR 24.0, 95% CI (9.5-60.7) Adults: RRR 12.3, 95% CI (7.5-20.3)	[36]
	Nested case-control study among children 0-42-month-old with pneumonia	OR 4.13, 95% CI (2.06-8.26)	[37]
Respiratory syncytial virus (RSV)	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 14.0, 95% CI (11.4-17.1)	[34]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	OR 6.3, 95% CI (4.2-9.4)	[35]
	Multi-site case-control study among children and adults with SARI	Children: RRR 9.9, 95% CI (6.2-15.8) Adults: RRR 2.2, 95% CI (1.2-3.9)	[36]
	Nested case-control study among children 0-42-month-old with pneumonia	OR 8.05, 95% CI (4.21-15.38)	[37]
	Multi-site case-control study among children and adults with a community-acquired pneumonia	AF 93%, 95% CI (87-97%) OR 15.2, 95% CI (7.92-29.2)	[38]
Human metapneumovirus (hMPV)	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 6.3, 95% CI (4.8-8.2)	[34]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	OR 1.3, 95% CI (0.5-3.2)	[35]
	Multi-site case-control study among children and adults with SARI or ILI	AF 85.6%, 95% CI (72.0-92.6%)	[36]
	Nested case-control study among children 0-42-month-old with pneumonia	OR 1.12, 95% CI (0.67-1.88)	[37]
	Multi-site case-control study among children and adults with a community-acquired pneumonia	AF 90%, 95% CI (80-95%) OR 10.4, 95% CI (5.02-21.6)	[38]
Para-influenza virus (PIV1-3)	Multi-site case-control study among children and adults with a community-acquired pneumonia	Children: OR 2.29, 95% CI (1.11-4.69) Adults: p-value .09, OR NC	[38]
	Multi-site case-control study among children and adults with SARI	Children: PIV-1: OR 4.1, 95% CI (1.7-10.0) PIV-2: OR 5.6, 95% CI (0.6-49.4) PIV-3: OR 2.8, 95% CI (1.5-5.5) Adults: PIV-1: OR 1.7, 95% CI (0.3-10.3)	[36]

	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	PIV-1: OR 7.52, 95% CI (4.79-11.80) PIV-2: OR 0.98, 95% CI (0.63-1.51)	[34]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	PIV-1: OR 1.3, 95% CI (0.6-3.0) PIV-2: OR 0.4, 95% CI (0.1-1.0) PIV-3: OR 1.5, 95% CI (0.9-2.6)	[35]
Coronavirus (HKU1; NL63; OC43; 229E)	Multi-site case-control study among children and adults with a community-acquired pneumonia	Children: aOR 3.17, 95% CI (1.44-6.99) Adults: aOR 3.19, 95% CI (0.59-17.1)	[38]
	Multi-site case-control study among children with an ARI	All coronavirus species:	[39]
	Prospective longitudinal study among children with an ARI	All coronavirus: asymptomatic 8% *versus* symptomatic 9%, p-value 0.38	[40]
	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	NL63: OR 0.77, 95% CI (0.58-1.03) HKU1: OR 0.84, 95% CI (0.60-1.18)	[34]
Rhinovirus (RV)	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 0.94, 95% CI (0.82-1.08)	[34]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	OR 0.8, 95% CI (0.7-0.9)	[35]
	Multi-site case-control study among children and adults with a community-acquired pneumonia	Children: OR 1.13, 95% CI (0.84-1.51) Adults: OR 13.4, 95% CI (3.04-59.1)	[38]
Bordetella pertussis	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 3.3, 95% CI (1.6-7.2)	[34]
	Nested case-control study among 0-42-month-old children with pneumonia	OR 11.08, 95% CI (1.33-92.54)	[37]

Supplementary table 5. Patient characteristics by organism detected in blood specimens in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017. Patients are presented by incremental C-reactive protein (CRP) concentrations.

Country Myanmar (n=50)	Methods	CRP (mg/L)	Age (years)	Comorbidit	Symptom onset (days)	T t e m	Clinical presentation	Health worker diagnosis	Antibiotic prescription
Aerococcus spp.	Singleplex PCR	15	22	Hepatitis B	3	36	Neurological & Respiratory	URTI	No
Leptospira spp.	Singleplex PCR	8	8	No	2	37	Undifferentiated	Acute viral infection	No
	Singleplex PCR	8	33	Asthma	4	37	Undifferentiated	Acute viral infection	No
	TAC	62	15	No	2	37	Respiratory	URTI	No
	MAT	63	29	No	3	37	Respiratory & Digestive	URTI	Azithromycin
	Singleplex PCR	200	13	No	3	37	Respiratory & Digestive	URTI	Amoxicillin
	Singleplex PCR	200	14	No	5	37	Neurological & Digestive	-	No
Klebsiella pneumoniae	TAC	8	6	No	2	37	Respiratory	URTI	No
	TAC	10	11	No	3	37	Respiratory	URTI	No
	TAC	10	56	No	4	37	Neurological	-	Amoxicillin
	TAC	18	25	HIV	6	37	Undifferentiated	HIV infection stage I	No
	TAC	28	37	No	3	37	Respiratory	Acute viral infection	Amoxicillin
	TAC	44	7	-	1	37	Digestive	Acute viral infection	No
	TAC	53	8	No	3	37	Digestive	-	No
	TAC	98	7	No	1	37	Respiratory	URTI	No
Rickettsia genus	IFA	29	32	No	5	37	Neurological & Digestive	Acute gastritis	No
Salmonella Paratyphi A	TAC	26	32	No	3	36	Digestive	Acute viral infection	No
Streptococcus suis	TAC	16	12	No	4	37	Respiratory	URTI	Amoxicillin
	TAC	200	27	No	7	37	Respiratory	LRTI	Amoxicillin
Streptococcus spp.	Singleplex PCR	12	28	No	2	37	Respiratory & Digestive	URTI	Amoxicillin
Bocavirus	TAC	97	43	No	5	38	Respiratory	-	Amoxicillin
Dengue virus	Singleplex PCR	-	2	No	1	39	Digestive	Acute gastroenteritis	Ciprofloxacin

	Singleplex PCR	-	2	No	2	4	Digestive	Chronic suppurative	Cloxacillin
	Singleplex PCR	-	4	No	1	3	Digestive	Food poisoning	Ciprofloxacin
	Singleplex PCR	-	4	No	2	3	Neurological & Digestive	Acute viral infection	No
	Singleplex PCR	-	4	No	4	3	Neurological, Respiratory & Digestive	URTI	No
	TAC	8	9	No	4	3	Undifferentiated	Acute viral infection	Amoxicillin
	Singleplex PCR & TAC	8	8	No	2	3	Digestive	Acute gastroenteritis	No
	Singleplex PCR & TAC	8	13	No	1	3	Respiratory	URTI	No
	TAC	8	13	No	1	3	Neurological	URTI	No
	Singleplex PCR & TAC	8	16	No	1	3	Respiratory	Acute viral infection	No
	Singleplex PCR & TAC	8	20	No	1	3	Neurological & Respiratory	URTI	Amoxicillin
	TAC	9	6	No	5	3	Neurological, Respiratory & Digestive	Acute viral infection	Cephalexin
	Singleplex PCR & TAC	10	7	No	5	3	Undifferentiated	-	No
	Singleplex PCR	10	13	No	5	3	Respiratory & Digestive	URTI	Azithromycin
	Singleplex PCR & TAC	10	6	No	2	3	Respiratory	LRTI	Amoxicillin
	TAC	11	11	No	1	3	Neurological	-	No
	Singleplex PCR & TAC	14	21	No	7	3	Respiratory	Acute viral infection	No
	Singleplex PCR & TAC	15	8	No	2	3	Digestive	Acute viral infection	No
	Singleplex PCR & TAC	15	12	No	1	3	Respiratory & Digestive	Acute viral infection	No
	Singleplex PCR & TAC	17	18	No	3	3	Digestive	Acute viral infection	No
	Singleplex PCR & TAC	20	10	No	5	3	Undifferentiated	LRTI	Amoxicillin
	Singleplex PCR & TAC	31	7	No	1	3	Neurological & Digestive	Head trauma	No
	Singleplex PCR & TAC	32	7	No	1	4	Respiratory	LRTI	Amoxicillin
	Singleplex PCR & TAC	32	9	No	2	3	Respiratory & Digestive	Acute viral infection	No
	Singleplex PCR & TAC	36	7	No	1	3	Undifferentiated	Acute viral infection	No
	Singleplex PCR & TAC	43	5	No	2	3	Undifferentiated	URTI	No
	Singleplex PCR & TAC	48	25	No	5	3	Neurological	Non-specific fever	Azithromycin
	Singleplex PCR & TAC	105	8	No	1	4	Neurological	-	No
Enterovirus	TAC	8	6	No	3	3	Respiratory & Digestive	URTI	Amoxicillin

Thailand (n=29)	Methods	CRP (mg/L)	Age (years)	Comorbidit	Symptom onset onset (days)	T t e	Clinical presentation	Health worker diagnosis	Antibiotic prescription
Haemophilus influenzae	TAC	28	4	No	1	3	Respiratory	Common cold	No
Leptospira spp.	MAT	30	≥12	No	1	3	Neurological & Respiratory	Acute pharyngitis	Amoxicillin
	Singleplex PCR	112	64	Hypertension	4	3	Respiratory & Digestive	Common cold	No
	Singleplex PCR	158	8	No	1	3	Neurological, Respiratory & Digestive	Common cold	No
Rickettsia genus	Singleplex PCR	10	8	No	2	3	Neurological, Respiratory & Digestive	Acute tonsillitis	Amoxicillin
	Singleplex PCR	28	7	No	1	3	Respiratory & Digestive	Acute pharyngitis	Amoxicillin
Salmonella Paratyphi A	TAC	8	11	No	3	3	Neurological & Respiratory	Common cold	No
	TAC	18	10	No	3	3	Respiratory	Acute pharyngitis	Amoxicillin
Salmonella Paratyphi A & *Rickettsia* genus	TAC	8	11	No	1	3 9	Respiratory	Acute pharyngitis	Amoxicillin
Salmonella spp.	TAC	9	62	Hypertension	7	3	Respiratory	Common cold	No
	TAC	14	20	No	2	3	Neurological & Respiratory	Acute pharyngitis	No
Streptococcus suis	TAC	8	5	No	1	3	Respiratory	Common cold	No
	TAC	20	9	No	2	3	Respiratory & Digestive	Common cold	No
Dengue virus	Singleplex PCR & TAC	12	3	No	2	3	Neurological, Respiratory & Digestive	Acute pharyngitis	Amoxicillin
	Singleplex PCR & TAC	23	9	No	2	3	Neurological, Respiratory & Digestive	Fever of unknown cause	No
Enterovirus	TAC	10	12	No	1	3	Neurological & Respiratory	Acute tonsillitis	Amoxicillin
	TAC	12	8	No	1	3	Respiratory	Acute pharyngitis	Amoxicillin
	TAC	13	4	No	3	3	Respiratory	Common cold	No
	TAC	19	5	No	3	3	Neurological & Respiratory	Common cold	No
	TAC	20	6	No	1	3	Undifferentiated	Acute pharyngitis	Amoxicillin
	TAC	32	9	No	1	3	Undifferentiated	Acute tonsillitis	Amoxicillin
	TAC	47	1	No	2	3	Respiratory	Acute pharyngitis	Amoxicillin
Rhinovirus	TAC	8	1	No	1	3	Respiratory	Common cold	No
	TAC	8	2	No	1	3	Respiratory	Common cold	No
	TAC	9	11	No	2	3	Respiratory	Acute pharyngitis	Amoxicillin
	TAC	33	3	No	2	3	Respiratory	Common cold	No
Rubella virus	TAC	8	15	No	3	3	Neurological, Respiratory & Digestive	Acute tonsillitis	Amoxicillin
Varicella-Zoster virus	TAC	8	11	No	1	3	Digestive	Common cold	No
	TAC	13	7	No	1	3	Neurological	Varicella	Amoxicillin

Supplementary table 6. Outcome characteristics among patients with a bacterial organism by antibiotic prescription in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017

Bacterial organism detected (n=36)	No/No effective antibiotic prescribed (n=28) *	Effective antibiotic prescribed (n=8)	p-value
Outcome characteristics			
Symptom resolution at day 5, n (%)	16 (57.1)	7 (87.5)	0.170
Symptom severity at day 5, median (IQR)	1 (1-1)	1 (1-1)	1.000
Documented fever at day 5, n (%)	2 (7.1)	0 (0)	0.430
Elevated CRP at day 5, n (%)	1 (3.6)	0 (0)	0.583
Symptom severity at day 14, median (IQR)	1 (1-2)	-	-
Symptom resolution at day 14, n (%)	27 (96.4)	8 (100.0)	0.747
Symptom severity at day 14, median (IQR)	1 (1-1)	-	-
Documented fever at day 14, n (%)	0 (0)	0 (0)	1.000
Occurrence of SAE, n (%)	0	0	1.000
Unscheduled visits, n (%)	0	0	1.000

*No/No effective antibiotic indicates the presence of a bacteria and either absence of antibiotic prescription (n=24/36, 66.7%) or prescription of ineffective antibiotic (n=4/36, 11.1%). In our study, ineffective antibiotic prescription included the prescription of cotrimoxazole or metronidazole to *Leptospira* spp.; macrolide or tetracycline to *Streptococcus suis*; beta-lactam antibiotics to *Rickettsia* genus; amoxicillin, quinolones, or cephalosporin to *Bordetella pertussis*.

The prescription of antibiotics at the facility was considered between the enrolment at Day 0 until Day 14 of the follow-up

Severity was ranked from 1-4 with severity=1 being the less severe presentation.

CRP: C-reactive protein

Elevated CRP defined as ≥50 mg/L in children and ≥100 mg/L in adults

SAE: serious adverse event including hospitalisation or death within 14 days of enrolment

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