Epidemiology of Respiratory Infections Caused by Atypical Bacteria in Two Kenyan Refugee Camps

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Abstract Chlamydia pneumoniae, Mycoplasma pneumoniae, and Legionella spp. are common causes of atypical pneumonia; however, data about these atypical pathogens are limited in the refugee setting. Paired nasopharyngeal and oropharyngeal specimens were collected from patients with respiratory illness presenting to healthcare centers in two refugee camps in Kenya. The specimens were tested for C. pneumoniae, M. pneumoniae, and Legionella spp. as well as eight respiratory viruses. Atypical pathogens were detected in 5.5% of the specimens of which 54% were co-infected with at least one of the eight viruses tested. Patients positive for atypical bacteria co-infected with virus were significantly more likely to have severe acute respiratory illness than patients infected with only atypical bacteria ($P = 0.04$). While the percentage of atypical pathogens identified was lower than expected, we found a significant relationship between atypical bacterial-viral co-infection and severity of disease in this refugee population.

Keywords Refugee health · Atypical bacteria · Atypical pneumonia · PCR

Introduction

The most common atypical pneumonias caused by non-zoonotic pathogens are Chlamydia pneumoniae, Mycoplasma pneumoniae, and Legionella spp. Unlike typical respiratory bacteria, these atypical pathogens often involve extrapulmonary organ systems. Their importance is highlighted by diagnostic difficulties and nonresponsiveness to beta-lactam antibiotics [1]. C. pneumoniae and M. pneumoniae are both frequently involved as causative agents in pediatric upper and lower respiratory illnesses [2].

C. pneumoniae and M. pneumoniae have been documented to circulate in Africa. Gray et al. found serologic evidence of acute infections with C. pneumoniae and M. pneumoniae among Somali refugees in a Djibouti refugee camp [3]. However, serologic assays with paired serum samples are by nature retrospective, not practical for rapid diagnosis, and less sensitive than molecular methods such as polymerase chain reaction. Real-time polymerase chain reaction (qPCR) assays have been developed to identify atypical bacteria, but systems have not been developed that use qPCR to detect atypical pathogens for diagnostic or surveillance purposes in refugee settings. We determined the presence of atypical bacterial etiologies diagnosed by qPCR in patients from two refugee camps in Kenya. As these camps are part of a viral respiratory disease surveillance system, we also assessed the rate of atypical bacterial and viral co-infection in this population.
Methods

Participants

The study was conducted at health care centers in the Dadaab and Kakuma area refugee camps from October 5, 2006 to April 2, 2008. The camps are located in the Northeastern and Rift Valley provinces of Kenya, respectively, and are part of a national influenza sentinel surveillance system run jointly by the Kenya Ministry of Public Health and Sanitation and the Kenya Medical Research Institute/U.S. Centers for Disease Control and Prevention-Kenya (KEMRI/CDC-K). Pediatric and adult patients whose illness met the case definition for influenza-like illness (ILI) or severe acute respiratory infection (SARI) were enrolled after informed consent was obtained from adults, older minors, and from guardians of all minors. Case definitions for ILI and SARI were adapted from the World Health Organization (Table 1) [4, 5]. The number of eligible ILI patients was limited to three per day for each camp; there was no limit to the number of SARI patients tested. As part of routine surveillance, nasopharyngeal (NP) and oropharyngeal (OP) swabs were taken from ILI or SARI patients, and a brief questionnaire with information about demographics and clinical presentation was administered.

Specimen Collection

NP and OP swab specimens were collected from patients with ILI and SARI by trained surveillance officers. For the NP specimen, a polyester-tipped flexible aluminum-shafted applicator (Puritan, Guilford, Maine, USA, cat# 25-801D) was inserted into one of the nostrils until resistance was felt at the nasopharynx, then rotated 180 degrees and withdrawn. For the OP specimen, a nylon flocked plastic shafted applicator (Copan Diagnostics, Murrieta, CA, USA, cat# 503CS01) was used to sample the posterior oropharyngeal mucosal membrane. After the samples were obtained, both swab applicator tips were cut and placed into a single vial containing 1 ml of viral transport media. The vials were refrigerated at 4°C and shipped within 72 h on ice packs to the KEMRI/CDC-K laboratory in Nairobi. Specimens were immediately divided and stored at −80°C. An aliquot of each specimen was subsequently shipped on dry ice to CDC-Atlanta for testing.

qPCR Testing for Atypical Bacteria

Total nucleic acid was extracted from 100-μl specimen aliquots at CDC-Atlanta by using the MagMAX™-96 Viral RNA Isolation Kit (Applied Biosystems, Carlsbad, California, USA) on the Kingfisher 96 Magnetic Particle Processor (Thermo Fisher Scientific, Waltham, Massachusetts, USA) following the manufacturers’ instructions. qPCR assays for C. pneumoniae, M. pneumoniae, and Legionella spp. were performed as described [6]. Briefly, the qPCR mixture was prepared in a total volume of 25 μl. Each qPCR mixture contained the following per reaction: 12.5 μl of Platinum® Quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, California, USA, cat# 11730-025), 1.5 μl of 50 mM MgCl2, 0.5 μM final concentrations of each primer, 0.1 μM final concentration of the probe, 1.25 U Platinum® Taq DNA Polymerase (Invitrogen, cat# 10966-034, 5 U/μl), 1 μl of 10 mM PCR Nucleotide Mix (Promega, Madison,

### Table 1 Case definitions of influenza-like illness and severe acute respiratory infection adapted from World Health Organization

| Influenza-like illness (ILI) | Severe acute respiratory infection (SARI) |
|-----------------------------|------------------------------------------|
| All ages (all of the following): | All ages (all of the following): |
| (1) Temperature ≥38°C | (1) Temperature ≥38°C |
| (2) Cough or sore throat | (2) Cough or sore throat |
| (3) Does not meet criteria for SARI | (3) Does not meet criteria for SARI |
| For children ages 2 months to <5 years: | For children ages 2 months to <5 years: |
| (1) Cough or difficult breathing | (1) Cough or difficult breathing |
| (2) AND any one of the following: | (2) AND any one of the following: |
| • Respiratory rate greater than 50 per minute for infant aged 2 months to <1 year | • Respiratory rate greater than 40 per minute for infant aged 1 to <5 years |
| • Respiratory rate greater than 40 per minute for child aged 1 to <5 years | • Chest indrawing or stridor in a calm child |
| • Temperature ≥38°C | • Unable to drink or breastfeed |
| • Temperature <35.5°C | • Vomits everything |
| • Pulse oxygenation <90% | • Convulsions |
| For persons ages ≥5 years (all of the following): | For persons ages ≥5 years (all of the following): |
| (1) Temperature ≥38.0°C | (1) Temperature ≥38.0°C |
| (2) Cough or sore throat | (2) Cough or sore throat |
| (3) Shortness of breath or difficulty breathing | (3) Shortness of breath or difficulty breathing |

World Health Organization. Handbook: IMCI Integrated Management of Childhood Illness 2005. Available from: [http://whqlibdoc.who.int/publications/2005/9241546441.pdf](http://whqlibdoc.who.int/publications/2005/9241546441.pdf)

World Health Organization. WHO Regional Office for Europe guidance for influenza surveillance in humans. 2009. Available from: [http://www.euro.who.int/__data/assets/pdf_file/0020/90443/E92738.pdf](http://www.euro.who.int/__data/assets/pdf_file/0020/90443/E92738.pdf)
Wisconsin, USA, cat# C1141), and 5 μl of extracted nucleic acid from each specimen. qPCR for each target was performed by using the ABI 7500 (Applied Biosystems, cat# 4351104) with the following conditions: initial activation of 95°C for 2 min, followed by 45 cycles of 95°C for 10 s and 60°C for 30 s. All specimens were tested in duplicate and considered positive when both replicates had positive Ct values. No specimens displayed discordant results.

Reverse Transcription qPCR (qRT-PCR) Testing for Respiratory Viruses

qRT-PCR assays for adenovirus, human metapneumovirus, parainfluenza viruses 1, 2 and 3, and respiratory syncytial virus [7] were performed at CDC-Atlanta with the same total nucleic acid extracts used above. All specimen extracts were also tested by qRT-PCR for the human RNase P gene to assess specimen quality. Testing for influenza A and B viruses was carried out at the KEMRI/CDC-K laboratory by using qRT-PCR primer/probe kits, positive controls, and procedures provided by CDC-Atlanta. RNA was extracted from 100-μl aliquots of each sample by using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, California, USA) according to the manufacturer’s instructions. qRT-PCR assays for all respiratory viruses were performed by using the AgPath-ID™ One Step RT-PCR Kit (Applied Biosystems).

Analysis

Data were analyzed with SAS software version 9.2 (SAS Institute, Cary, NC, USA). We used the chi-square test to compare variables between groups. Statistical significance was set at a P-value < 0.05.

Ethical Considerations

Ethical approval for the surveillance activities for respiratory diseases was obtained from the KEMRI Ethical Review Committee (protocol number 1161). After formal human subjects determination, this surveillance activity was determined to be nonresearch that did not require CDC Institutional Review Board approval.

Results

During the study period, 2,158 NP/OP swabs were collected. Atypical pathogens were detected in 118 (5.5%) of the specimens: C. pneumoniae was identified in 81(3.8%) patients, M. pneumoniae in 36 (1.7%), and Legionella spp. in only 1 patient. The percentage of atypical pathogens detected was similar after the data were stratified by illness category (outpatient ILI vs. hospitalized SARI cases—5.2 and 5.8%, respectively) and camp (Dadaab vs. Kakuma—6.3 and 5%, respectively). Both C. pneumoniae and M. pneumoniae were identified in Dadaab (in 33 and 15 specimens, respectively) and Kakuma (48 and 21, respectively). The one Legionella spp. case was identified in Dadaab Refugee Camp.

Demographic, clinical, and viral data were available for 113 of the 118 cases with atypical bacteria detected from NP/OP swabs (Table 2). As there were no significant differences in demographic variables for patients infected with C. pneumoniae or M. pneumoniae, their results are presented together here. The median age of these patients was 1.7 years (range: 2 months to 60 years); 41.6% were females. More than 95% of the patients were Somali (n = 51) and Sudanese refugees (n = 41) or Kenyans from the local community (n = 18); Rwandan, Ethiopian, and Congolese refugees the rest of the study group. In addition, demographic, clinical, and viral data were available for 1,960 of the 2,040 cases negative for atypical bacteria. The mean age was 1.5 years (range: 1 month to 84 years); 45.1% were female. More than 95% of the patients were Somali (n = 886) and Sudanese refugees (n = 756) or Kenyans from the local community (n = 227). There were no significant differences in demographic variables between patients infected with atypical bacteria and patients negative for atypical bacteria.

No patients were co-infected with more than one atypical bacterium. However, of the 113 atypical bacteria-positive specimens, 61 (54%) were co-infected with at least one of the eight viruses tested, and 20 (32.8%) of these had multiple viruses. The viral co-infection rate was similar for C. pneumoniae and M. pneumoniae (51.8 and 52.8%, respectively). Of the 1,960 specimens negative for atypical bacteria, 909 (46.4%) were positive for at least one virus and 236 (26.0%) of these had multiple viruses. The viral infection rates between specimens with and without atypical bacteria did not differ significantly (P = 0.14). For the specimens co-infected with an atypical bacterium and virus(es), adenovirus was the most common virus identified: Adenovirus was detected as the only virus in 21 (34.4%) specimens; it was identified along with one or more other viruses in 12 (19.7%) specimens. Adenovirus was also the most common pathogen for specimens that were negative for atypical bacteria but positive for at least one virus.

When differences in symptoms (fever, weight loss, convulsions, lethargy, loss of consciousness, headache, sore throat, cough, difficulty breathing, nausea, vomiting, muscle pain, and diarrhea) were compared between patients who were infected with C. pneumoniae versus those with M. pneumoniae, patients with M. pneumoniae reported more frequent loss of consciousness (P = 0.04). Pooling the clinical data for the atypical bacteria, patients
who were positive for atypical bacteria only had significantly more headaches, sore throats, and difficulty breathing ($P = 0.04$, $<0.01$, and 0.04 respectively) than patients who were co-infected with atypical bacteria and virus(es) (Table 2). Patients without atypical bacterial infection who were positive for more than one virus had significantly more loss of consciousness and difficulty breathing ($P = 0.01$ for both) than patients with only one virus identified.

Of the 113 specimens positive for atypical bacteria, 41 (36.3%) were from patients with ILI, and 72 (63.7%) were from patients with SARI. Likewise, of the 1,960 specimens negative for atypical bacteria, 661 (33.7%) were from patients with ILI, and 1,299 (66.3%) were from patients with SARI. While patients positive for atypical bacteria co-infected with virus were significantly more likely to have SARI than patients infected with only atypical bacteria ($P = 0.04$), no significant difference was seen between cases positive for multiple viral co-infections versus single virus infection. Neither C. pneumoniae nor M. pneumoniae patients were more likely to have SARI or ILI.

Examining the time period May 2007 to March 2008, when data sets are available for both camps, shows that M. pneumoniae patients peaked during March ($n = 6$) in Dadaab and during October in Kakuma ($n = 7$); July had the most C. pneumoniae patients in Dadaab ($n = 9$), while there was no clear peak in Kakuma (Fig. 1).

**Discussion**

In our study, atypical bacteria were found in 5.5% of ILI and SARI patients, which is lower than estimates (15–50%)
in the literature [1, 2, 8–11]. A prospective multicenter study using serology and PCR to follow hospitalized children showed that nearly half the cases of community-acquired acute bronchitis, wheezing, and pneumonia in this pediatric population were associated with *M. pneumoniae* and/or *C. pneumoniae* infections [12]; they are also frequent causes of upper respiratory tract infections [2]. While Bii et al. demonstrated *M. pneumoniae* by PCR in one-third of pediatric patients diagnosed with pneumonia in a Nairobi hospital [8], atypical pathogens circulated less frequently in the Kenyan refugee camps during our study period. The one patient with *Legionella spp.* (who survived) detected in our study is of interest, given the lack of documented *Legionella spp.* cases in Kenya [13].

We detected viral co-infection in 54% of specimens positive for atypical bacteria. In previous studies, 44–77% of cases of *M. pneumoniae* and *C. pneumoniae* were co-infected with viruses [9, 10]. The most common co-pathogen in our study was adenovirus; however, adenovirus (species C) may persistently shed from the lingual tonsils and adenoids [14], raising the possibility that our high percentage of adenoviral positivity may reflect both latent and acute infections. The question remains whether one or multiple pathogens are causing the symptoms of the respiratory tract infection.

A notable finding from this study was that patients with atypical bacteria co-infected with viruses were significantly more likely to have severe disease (SARI) than patients with single atypical bacterial infection. Although patients with atypical bacteria only had significantly more symptoms than patients with atypical bacteria co-infected with viruses, a simple count of symptoms may not reflect severity of disease. Similarly, in a study of community-acquired pneumonia in hospitalized pediatric patients, Michelow et al. found children with bacterial and viral co-infections had the greatest degree of disease severity, although typical as well as atypical bacteria were included in this analysis [10]. In contrast, when we compared patients with multiple viral infections and single viral infections, illness category (ILI vs. SARI) did not differ.

![Distribution of C. pneumoniae and M. pneumoniae cases—Dadaab and Kakuma refugee camps, May 2007–March 2008](image-url)

**Fig. 1** Distribution of *C. pneumoniae* and *M. pneumoniae* cases—Dadaab and Kakuma refugee camps, May 2007–March 2008
significantly. The clinical significance of multiple respiratory viral infections has not been resolved in the literature; some studies suggest higher morbidity than single viral infections while others do not [9, 15–17].

While the small number of cases precludes any robust statistical analysis, we noted that cases of atypical pneumonia peaked during July and March in Dadaab and during October in Kakuma. As July is the coldest month in Dadaab and March is the hottest, these weather changes may play a role in the seasonality trends of atypical pneumonias in Dadaab; October is usually the start of the short rain or dust storm season in Kakuma.

This study had several limitations. First, while we tested for eight viruses, we did not include some common viruses, such as coronaviruses, in our testing; we also did not test for zoonotic pathogens that may cause atypical pneumonias or other bacteria that typically cause community-acquired pneumonias. Second, our sampling technique included only NP and OP swabs. While OP swabs are the preferred specimens of choice to test for *M. pneumoniae* by PCR, nasopharyngeal aspirates or nasal washes are recommended to test for *C. pneumoniae* by PCR [18]; however, as our yield of *C. pneumoniae* was more than double that of *M. pneumoniae*, we feel that the NP swab was an adequate sampling technique for *C. pneumoniae*. In addition, the lack of a control group limits our interpretation of the positive results, as symptomless infection with either atypical bacteria or viruses is possible. Finally, the number of adults (*n* = 5) with atypical bacterial infection in this study was relatively small, accounting for just 4.4% of the patients, limiting generalizations primarily to the pediatric population.

In conclusion, while the percentage of atypical pathogens identified was lower than expected, the co-infection rate with viruses was consistent with published reports. Furthermore, we found a significant relationship between atypical bacterial-viral co-infection and severity of disease. These findings may have implications for the choice of antimicrobial therapy when treating patients with clinical pneumonia in refugee camps in Kenya. Although routine testing for atypical agents is impractical in this setting, for patients who do not respond to beta-lactam antibiotics or who are severely ill, covering for atypical pathogens may be warranted.

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