RESEARCH ARTICLE

Acute Respiratory Infections Epidemiology and Etiology in Hospitalized Moroccan Children under 15 Years

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ABSTRACT

Introduction: Acute respiratory infections (ARIs) are serious infections with multiple etiologies. Viral etiology is mostly common worldwide, especially in pediatric patients.

Materials and methods: This study aims to describe epidemiology and etiology of ARIs in Moroccan children under 15 years old, through patients' clinical features analysis and genetic detection of pathogens within 84 patients admitted to Hassan II regional hospital of Agadir, Morocco, from September 2015 to September 2016. Detection of pathogens was carried out using Multiplex real-time reverse transcription polymerase chain reaction (rRT-PCR) with Fast Track Diagnostic kit respiratory pathogens 21 plus.

Results: Results gave 61.9% of positive viral and bacterial infections and 19.23% of co-infections by at least two pathogens. Males were mainly infected and the respiratory syncytial virus (RSV) was the most common in 53.8% of patients. Children aged less than 2 years were the most infected by ARIs pathogens, and especially by RSV virus. Concerning seasonality pattern, all ARIs pathogens showed a seasonal spreading mainly in winter.

Conclusion: This study summarizes a great insight about epidemiology and etiology of ARIs among children under 15 years in Morocco. We observed that ARIs constituted a real health problem in infancy and the viruses are the commonest cause.

KEYWORDS: Acute Respiratory Infections, Multiplex PCR, TaqMan.

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INTRODUCTION

Acute respiratory infections (ARIs) are ubiquitous, transmissible, and very common in both adults and children. It may affect any part of respiratory tract. ARIs are among the leading causes of morbidity and mortality, in particular, within children under five years old worldwide [1]. Mortality of children with acute respiratory infections is about 1.9 million deaths per year [2]. According to the World Health Organization (WHO), approximately 290,000 to 650,000 deaths annually are caused by influenza virus infection alone [3]. ARIs were responsible of 13% of pediatric deaths during 2012 in Morocco [4]. ARIs have numerous etiologies (viral or bacterial), and they are classified into upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs). The majority of ARIs have viral etiologies [5]. Respiratory syncytial virus (RSV) and Influenza are the most implicated viruses in these illness [6]. Several techniques are employed for pathogens detection. Conventional methods like cultures and immunofluorescence are generally time-consuming and lack sensitivity. In contrast, molecular biology techniques
such as polymerase chain reaction (PCR) and real time PCR are sensitive and specific tools for pathogens detection. Multiplex real time PCR methods have been significantly developed over the past several years. This method is based on detection of a large panel of respiratory pathogens in a single sample [7].

Morocco is among the countries that established epidemiological surveillance for influenza-like illness (ILI) and/or severe acute respiratory infections (SARI) [8]. Our study falls within the scope of influenza surveillance at the national level. Our investigation consist on a retrospective study of epidemiological, clinical, and virological aspects of ARIs patients under 15 years old hospitalized in Hassan II regional hospital of Agadir, Morocco. It aims to investigate the exposure to different pathogens according to distinct factors such as age, sex, symptoms and seasons of infection.

**MATERIALS AND METHODS**

**Case definition and samples collection:** This study was performed in the medical analysis laboratory of Hassan II regional hospital, Agadir, Morocco. A total of 84 nasopharyngeal aspirates samples were collected from patients with symptoms of SARI and age under 15 years old. A standardized SARI case definition according to the WHO was followed, which defines SARI as an acute respiratory infection with history of fever or measured fever ≥ 38°C and cough, with onset within the last 10 days; and requires hospitalization [9]. Children with SARI symptoms and age under 15 years were included in the study while children without SARI symptoms and age upper than 15 years were excluded from the study. Nasal or nasopharyngeal swabs were collected for pathogens detection using Delta-Swab ViCUM®.

**Data collection:** Demographic and clinical data was collected from each patient using standardized case investigation form. This form includes four sections. The first section is related to patient identification data as age, sex and residence. The second one concerns clinical data including date of hospitalization and symptom’s onset, symptoms and risk factors. A third section provides epidemiological data such as vaccine statute of the patient against influenza during the 2015/2016 season and the last one is about sample collection and laboratory test results.

**Pathogens detection methods:** Bacterial and viral nucleic acids were extracted from 140µl of each sample using extraction kit QIAamp® viral RNA mini (QIAGEN). Multiplex real time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) was performed to detect nucleic acids of pathogens using the kit Fast-Track Diagnostics Respiratory Pathogens 21 plus (Fast-track Diagnostics, Luxembourg). This kit allows the detection of 21 respiratory viruses and bacteria causing upper respiratory tract infections, including influenza A, influenza A (H1N1) swl, influenza B, coronaviruses NL63, 229E, OC43 and HKU1, parainfluenza 1, 2, 3 and 4, human metapneumoviruses A and B, rhinovirus, respiratory syncytial viruses A and B, adenovirus, enterovirus, parechovirus, bocavirus, Mycoplasma pneumonia, Chlamydia pneumonia, Streptococcus pneumonia, Haemophilus influenza B and Staphylococcus aureus.

The Fast Track Diagnostic multiplex real time PCR assay for pathogens detection is provided in six separate mixes. Each mix contains primers and probes detecting four different viruses and/or bacteria. The kit also provides 25x RT-PCR enzyme mix and 2x RT-PCR buffer as well as bacterial and viral positive, negative and internal controls. These later were included in all runs to monitor assay performance.

The reaction volume for each test was 25µl, made up of 10µl of each extracted or controls nucleic acid combined to 1.5µl of probes and primers mix, 12.5µl of 2x RT-PCR buffer and 1µl of 25x RT-PCR enzyme mix. The PCR reactions were performed by an ABI 7500 PRISM real-time PCR system. Thermal cycling was initiated with reverse transcription incubation at 42°C for 15 min, followed by Taq polymerase activation at 94°C for 3 min, and 45 cycles of 8 sec at 94°C, and an annealing at 60°C for 34 seconds. A fluorescence reading was taken at the 60°C/34 seconds step in each cycle and threshold cycle (Ct) values were determined by manual adjustment. The positive controls and any positive samples showed an exponential fluorescence trace that crossed the Ct within 40 cycles.

**Statistical analysis:** To evaluate the epidemiologic profile, data from clinical records of each patient were assessed using IBM SPSS Statistics 25 and Epi Info™. Statistical significance was performed at p≤0.05.

**RESULTS**

**Demographics:** This study was conducted in 84 ARIs patients admitted to regional Hospital Hassan II, Agadir, Morocco, from September 2015 to September 2016. Males and females represented 63.1% and 36.9% respectively. All patients included in the study were under 15 years old. Patients were classified in three groups, a first group composed by 77.38% of patients all under two years old, a second group aged from 3 to 5 years old presenting 16.67% of patients, and a last group from patients of 6 to 15 years old presenting 5.95% (Figure 1).

**Clinical characteristics:** We investigated thirteen clinical symptoms (Figure 2) including fever, cough, sore throat, breathing difficulties, wheezing, stridor, chest indrawing, being unable to drink or breastfeed, vomiting, convulsion, consciousness disorder, diarrhea and hypothermia. Fever and cough were present in 87.27% and 78.18% of patients, respectively, while 58.18% of patients had breathing difficulties. Wheezing and chest indrawing constituted 47.27% and 45.45% of clinical symptoms respectively. As for other symptoms, they were detected in less than 35% of patients.

**Pathogens detection:** From all analyzed samples, 61.9% were positive for at least one virus or bacteria. Results for positive samples showed a predominance of respiratory syncytial virus (RSV) found in 53.8% of cases. Rhinovirus (RV) and coronavirus (CoV) were detected in...
5.8% of patients each. While nucleic acids of Para Influenza (PIV), Streptococcus pneumoniae (S.P.) and Staphylococcus aureus (S.A.), were found in 3.8% of cases each. Adenovirus (AdV) and Influenza A (InfluA) were found in 1.9% each. Co-infection with at least two pathogens was detected in 19.2% of patients (Figure 3).

**Figure 1:** Age distribution of studied patients

**Figure 2:** Clinical symptoms in ARI patients

**Figure 3:** Prevalence of pathogens in positive patients
The most infected patients were under two years old. Results from this patients group showed a predominance of respiratory syncytial virus (RSV) with 50% of positive cases against 3.8% in children of 3 to 5 years and null in the last group (6 to 15 years). The other pathogens were distributed mainly between the two groups (0-2) and (3-5) years. While PIV was the only virus detected in the group of 6 to 15 years (1.9%) (Figure 4).

Patterns of seasonality: Concerning monthly distribution of ARIs infections, all studied respiratory agents show a strong seasonal peak during the fall and winter period (Figure 5). RSV infection showed the most pronounced seasonality trends. Indeed, it typically exhibits a clear seasonality pattern with obvious peak in January and lowest activity in September and November. Concerning AdV and RV viruses, peaks were in December and November respectively, while the low number of cases infection by the other respiratory pathogens unable detection of any seasonality (Figure 6).
DISCUSSION

Our study takes part of a national influenza surveillance framework recommended by WHO to monitor circulating influenza viruses. This study involved a pediatric patient aged under 15 years old with acute respiratory infections. Pediatric population is the most sensitive according to many previous reports [7, 10]. The children’s sensitivity to respiratory infections is explained by physiological reasons such as immaturity of both respiratory and immune systems [11].

The present study was conducted in 84 patients from Hassan II regional Hospital, Agadir, Morocco. Males were more represented in our patients group (53 males and 31 females). Masculine gender predominance was also reported in other studies [12, 13].

Basing on case investigation forms, several clinical signs were detected. Indeed, fever was the major clinical feature with 88% of cases followed by cough with 81%. Same results were reported in Indian patients under 5 years old presenting 100% of fever and 98.8% of cough cases [14]. However, another Indian study gives Rhinorrhea as the most common ARI’s symptom observed in 94.8% of patients followed by cough with 81%, while fever represented only 35.4% of cases [15]. The prevalence of other clinical symptoms differs according to the infection. Indeed, clinical signs of respiratory infections are not specific to pathogens. These signs are found in several infectious diseases of the respiratory tract, making difficult to diagnose the cause of severe acute respiratory infections based solely on clinical examination.

Molecular detection of ARIs pathogens gave positive results in 62% of analyzed samples. Our results agree with other investigations about all age groups from numerous countries. For instance, positive ARIs pathogens were reported in 65.1% of patients in Cameroon [10], in 65% of patients in north of Morocco [7], 61.6% in Sweden [16], and 59.6% in Iran [13]. Relatively lower rates of positive cases were reported in Italy with 41.7% [17], and in Peru (42.6%) [18]. However, a previous study conducted in Morocco showed a higher positive rate (92%) [19].

According to our results, RSV was the predominant ARIs pathogens in our patients. Indeed, RSV was responsible for 53.85% of infection in all positive case and 50% of patients under two years old. Our results highlighted the high frequency of RSV infections among pediatric Moroccan population as many other countries. Indeed, RSV were also the major cause of ARI in Kuala Lumpur, Malaysia, with 70.6% of RSV infection in patients under 5 years old [20]. A study conducted on pediatric population in Morocco reported RSV and RV viruses as the most common ARIs etiologies [19]. However, a Vietnamese study within a population with all ages included, reported 32.1% of infections by Influenza virus against only 0.1% for RSV [21]. Actually, almost all studies reported RSV as the most common ARIs virus in children. This finding might be explained by immaturity of child immune system and inefficiency of the maternal antibodies in RSV preventing [20]. RSV is an enveloped non-segmented negative-sense RNA virus from the Paramyxoviridae family [22]. It’s the most important viral agent of lower respiratory tract (bronchiolitis and pneumonia) in children worldwide [23]. Globally, RSV is responsible for 33.1 million lower respiratory infections yearly, and was responsible of 59,600 of hospital deaths in 2015 in children under 5 years old [24]. In developing countries, young children RSV-associated death was about 118,200 cases in 2015 [24]. The incidence of RSV in developing countries is much higher than developed countries, with 91% of all RSV-associated hospitalizations and 99% of deaths [25]. RSV is a highly infectious and ubiquitous virus transmitted by aerosols requiring close contact. However, the virus remains...
infectious on various environmental surfaces [26]. Overall the circulation of RSV seems favored by the cold and especially the humidity [27]. At present, there is no effective antiviral or vaccine for this virus [28]. Although, a monoclonal antibody, Palivizumab, is available for passive prevention and recommended for only high risk population [29]. In this study, multiple viral or bacterial infections were found in 19.23% of positive cases. The group of patients under two years old was mostly co-infected with at least two pathogens (15.38%). Different co-infections rates were reported in several studies with conflicting findings. Indeed, 19% of Romanian children were co-infected by ARIs pathogens [30]. This rate was about 16.1% in Germany [31] and 11.3% in northeast Brazil [32]. Although, two studies from Switzerland and Morocco showed a relatively high rate of co-infections in young patients (24% and 40.4% respectively) [19, 33]. The other studied viruses presented a frequency less than 6% each. This fits with a study in Paris about hospitalized patients aged between 8 days to 16 years reporting 0.9% of PIV, 1.98% of AdV and 4.47% of Influenza virus [34]. Streptococcus pneumonia and Staphylococcus aureus were the two detected bacteria in our patients with 3.8% for each. A study from Bangladesh in children aged under 5 years old detected Streptococcus pneumonia in 9% of ARIs cases [35]. Another study found 17.9% of Streptococcus pneumonia, 15.43% of M. catarrhalis, and 8% of H. influenzae in children under 5 years in Senegal [36]. Although, a high rate (56%) of Streptococcus pneumoniae was observed in Niger among children under 5 years, Staphylococcus aureus was detected in 11.3% [37]. Our results reported that bacteria are less implicated in ARIs infections than viruses. Therefore, antibiotic treatments are not welcome in all ARIs infections cases. Hence, the interest of molecular diagnosis and etiologic investigations for better treatment decisions and patients’ outcome. Concerning seasonal distribution, the pathogens were detected especially in the autumn/winter period (November to February). Thus, the RSV exhibits a very pronounced seasonality. Previous findings in other temperate and Mediterranean climates observed similar circulating pattern, where RSV infection begins in late fall or early winter [38, 39]. While in tropical regions, infections with RSV were observed in March and August and infections with Influenza A virus had peaks on June, December–January [40]. Climatic factors play a pivotal role in seasonal trends [41]. This seasonal variation could be explained by variation of host physiologic characteristics including immunity [40]. Climate may moderates viability of pathogens in any surfaces and affects Human social behaviors favoring transmission.

CONCLUSION
Acute respiratory infections are a real public health problem, especially in children under 15 years old. This retrospective investigation, in ARIs patients from south of Morocco, reported viruses as the most common etiology of ARIs. Among all genetically detected pathogens, RSV was mostly common especially in children under two years old. Therefore, more interest must be given to develop safe and effective RSV vaccines for populations at risk. Concerning seasonality, all ARIs infections showed a seasonal distribution, especially RSV that exhibits a pronounced peak during winter season.

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AUTHORS’ CONTRIBUTIONS
The participation of each author corresponds to the criteria of authorship and contributorship emphasized in the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals of the International Committee of Medical Journal Editors. Indeed, all the authors have actively participated in the redaction, the revision of the manuscript and provided approval for this final revised version.

COMPETING INTERESTS
The authors declare no competing interests.

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