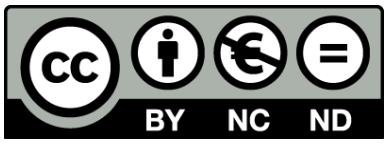
UNIVERSITAT DE BARCELONA

Epidemiology and aetiology of severe respiratory infections among children under five, admitted to the children hospital of Rabat, Morocco

Epidemiologia y etiología de las infecciones respiratoria agudas graves en niños menores de cinco años ingresados en el hospital de niños de Rabat, Marruecos

Imane Jroundi



TESIS DOCTORAL

Epidemiology and aetiology of severe respiratory infections among children under five, admitted to the children´s hospital of Rabat, Morocco

Epidemiologia y etiología de las infecciones respiratoria agudas graves en niños menores de cinco años ingresados en el hospital de niños de Rabat, Marruecos.

Imane Jroundi

Universitat de Barcelona

Epidemiology and aetiology of severe respiratory infections among children under five, admitted to the children´s hospital of Rabat, Morocco

Epidemiologia y etiología de las infecciones respiratoria agudas graves en niños menores de cinco años ingresados en el hospital de niños de Rabat, Marruecos.

Tesis presentada por **Imane Jroundi**

Para optar al grado de Doctor en Medicina

Director de tesis: **Pedro L. Alonso Fernández**

Co-Director de tesis : **Quique Bassat Orellana**

Línea de investigación: Salut Internacional

Programa de Doctorado, Medicina, Facultad de Medicina

Septiembre 2015

ISGlobal, Insituto de salud Global de Barcelona

Hospital Clínic, Universitat de Barcelona

Je dédie cette thèse

À mes parents, mes nièces

À mes amis homosapiens et à mes amis à quatre pattes

À la mémoire de Yoda

Index page

1. Glossary

ARI	Acute Respiratory Infection
ADV	Adenovirus
CFR	Case fatality rate
CHU	Centre Hospitalier Universitaire
CRESIB	Centre de Recerca en Salud Internacional de Barcelona
Flu	Virus influenza
GAVI	Global Vaccine Alliance
Hib	Haemophilus Influenzae type b
hMPV	Human metapneumovirus
HER	Hôpital d´Enfants de Rabat
IMCI	Integrated management of childhood illness
ICU	Intensive Care Unit
IPD	Invasive Pneumococcal Disease
OR	Odds Ratio
PCR	Polymerase chain reaction
NPA	Nasopharyngeal Aspirate
PCV	Pneumococcal conjugate vaccines
RISC	Respiratory Index of Severity in Children
RR	Relative Risk
RNA	Ribo Nucleic Acid
UNICEF	The United Nations Children's Fund
WBC	White Blood Cell
WHO	World Health Organization

2. Summary (English)

Despite of intense efforts in recent years towards the reduction of the burden of mortality from pneumonia, this disease remains one of the major public health problems threats to child survival in large parts of the world. The existing control measures are clearly insufficient to reduce globally the impact of this disease. In fact, the burden of this disease in terms of morbidity and mortality remains unacceptably high with almost one million children dying every year as a result of pneumonia episode, or what is the same, around one death every thirty four seconds. After neonatal conditions, pneumonia is the second most important killer of children, accounting for over 15% of all pediatric deaths. Over 90% of these pneumonia-associated deaths in young children occur in developing countries and are mainly secondary to bacterial infections. While clinical disease can affect any age group, mortality from pneumonia is essentially limited to children under five in poor countries.

The availability of effective vaccines against bacterial pneumonia, coupled with adequate and efficacious antibiotic treatments, should be sufficient to reduce this burden. Nevertheless, the identification and early treatment of clinical episodes and access to health centers seem to be an often insurmountable challenge in poorest countries, and children who would mostly benefit from such measures do not always receive them at all. Additionally, other important variables including malnutrition and low socioeconomic status, frequent in low and middle income countries where the major burden of these infections prevails, can adversely affect the prognosis of such episodes.

Knowledge of the locally specific determinants of pediatric respiratory disease in such settings would help to better understand why we are still far away from adequately controlling these infections, and why they still cause such a major impact in child health.

The first article of this thesis attempts to comprehensively review what was the available knowledge regarding pediatric pneumonia in Morocco, prior to the initiation of this project. Indeed, scarcity of data often hinders the implementation of measures to prevent and better manage these infections. This review confirms the alarming lack of recent data regarding pediatric pneumonia one of the major killers of children in Morocco.

The second article of this thesis is a general overview on the epidemiology, etiology and the clinical presentation of acute respiratory infections in Moroccan children under the age of five years. Through our study, we were able to show a high prevalence of viral infections, with wheezing as the major clinical symptomatology. These findings are similar to what can be found in wealthy countries and markedly differ from the high bacterial burden that can be

found in poorer settings, although overall case fatality rates remained unacceptably high in our setting.

As a result of afore mentioned high case fatality rates in our series, we decided to investigate the specific risk factors upon admission for a bad prognosis during hospitalization. The results of this analysis are presented in the third article of this thesis. The article concluded that the early identification of factors associated with a poor prognosis could improve management strategies and the survival likelihood of Moroccan children with severe pneumonia.

In the fourth article of this thesis, we chose to focus on two highly prevalent and potentially hazardous viruses causing acute respiratory infections in our setting, namely respiratory syncytial virus (RSV) and Human metapneumovirus (hMPV). In this analysis, we compared the epidemiological, clinical and laboratory features of these two infections, and concluded that despite the clinical presentation of those two pathogens was almost indistinguishable, hMPV tented to be highly more severe and significantly associated to a poor outcome. An early recognition of these viruses and good management of the cases is important to guarantee a better outcome.

The fifth article of this thesis specifically addresses the use of antibiotics to treat acute respiratory infections in Morocco. By analyzing data on pre-admission antibiotics use, and intra-hospital antibiotics utilization, we discuss whether such valuable drugs are used rationally in the country and whether a tighter control on antibiotics usage should be required, in a country such Morocco where over the counter drugs sale are common. This analysis also reflects on whether antibiotic usage may have had or not an impact on antimicrobial resistance rates.

Finally, the last article of this thesis examines data on the distribution of serotypes among *streptococcus pneumoniae* isolates from the nasopharynx of healthy children and compares it to those isolated among admitted children with clinical severe pneumonia closely to the time of the implementation of the *streptococcus pneumoniae* 13-valent conjugate vaccine in Morocco. These data will be used as a baseline to help assessing the impact of the vaccine and to monitor any potential serotype replacement phenomena.

Altogether, this thesis tries to offer a comprehensive snapshot of the situation of pediatric pneumonia in Morocco. A more precise understanding of the current situation will allow a better design of preventive and curative strategies and potentially the alleviation of the significant burden that respiratory infections still impose to children in this country.

3. Summary (Spanish)

A pesar de los esfuerzos hechos para reducir la carga de mortalidad causada por las infecciones respiratorias agudas, estas enfermedades se mantienen como uno de los principales peligros para la salud infantil en amplias zonas del mundo. Las medidas de control existentes son claramente insuficientes a la hora de reducir globalmente el impacto de infecciones tan comunes en la infancia. De hecho, la carga de estas enfermedades en términos de morbilidad y mortalidad persiste inaceptablemente alta con, anualmente, cerca de un millón de muertes anuales, lo que es lo mismo, una muerte cada 34 segundos. Las infecciones respiratoria agudas siendo las neumonías, su forma más paradigmática, son responsables del 15% de las muertes de niños menores de 5 años en el mundo. Más del 90% de estas infecciones respiratorias agudas se producen en los países en vías de desarrollo, fundamentalmente asociadas a neumonías bacterianas. Mientras que la enfermedad clínica puede afectar a cualquier grupo de edad, la mortalidad por neumonía se ve esencialmente circunscrita a los niños menores de cinco años en los países pobres.

La disponibilidad de vacunas efectivas contra las neumonías bacterianas, sumada al uso adecuado de los tratamientos antibióticos, debería ser suficiente para reducir esta carga. Sin embargo, la identificación y tratamiento precoz de los episodios clínicos y al acceso a los centros de salud parecen constituirse como barreras infranqueables en la mayoría de los países pobres. Adicionalmente, la malnutrición y el bajo nivel socio económico imperante en muchos de estos entornos dónde las neumonías son un mayor problema, contribuyen desfavorablemente al mal pronóstico de estas infecciones.

El conocimiento de los determinantes locales específicos de la enfermedad respiratoria aguda pediátrica en esos entornos ayudaría a entender o mejor por qué todavía estamos muy lejos de controlar adecuadamente estas infecciones, y profundizar sobre las razones por las cuales siguen causando un impacto tan importante en la salud del niño.

El primer artículo de esta tesis busca e copila de forma detallada el conocimiento existente sobre las neumonías pediátricas en Marruecos antes de la iniciación de este proyecto. De hecho, la escasez de datos a menudo dificulta la implementación de medidas para mejor prevenir y controlar estas infecciones. Esta revisión pone en evidencia la alarmante escasez de datos recientes sobre una de las primeras causas de morbi-mortalidad pediátrica en Marruecos.

El segundo artículo de esta tesis ofrece una visión general sobre la epidemiología, la etiología y la presentación clínica de las infecciones respiratorias agudas en niños marroquíes menores de cinco años. A través de nuestro estudio, hemos sido capaces de mostrar una alta prevalencia de infecciones virales, siendo las sibilancias la sintomatología clínica predominante. Estos resultados son similares a lo que se puede encontrar en los países ricos, y marcadamente diferentes de la elevada carga de bacterias que se pueden encontrar en los entornos más pobres, aunque las tasas de letalidad general se mantuvieron inaceptablemente altas en nuestro estudio.

Precisamente como consecuencia de esta elevada letalidad, decidimos investigar los factores de riesgo específicos de un mal pronóstico durante la hospitalización. Los resultados de este análisis se presentan en el artículo tercero de esta tesis, cuya conclusión principal es que la identificación temprana de los factores asociados con un mal pronóstico podría mejorar las estrategias de manejo y la probabilidad de supervivencia de los niños marroquíes con neumonía grave.

En el cuarto artículo de esta tesis, decidimos centrarnos en dos virus altamente prevalentes y potencialmente peligrosos que causan infecciones respiratorias agudas en nuestro medio: el virus respiratorio sincytial (VRS) y el metapneumovirus humano (hMPV). En este análisis, se compararon las características epidemiológicas, clínicas y de laboratorio de estas dos infecciones, y se pudo concluir que, a pesar de presentar una sintomatología clínica prácticamente indistinguible, el hMPV tendía a ser un poco más grave y asociándose en nuestra serie significativamente con un mal pronóstico. Un reconocimiento temprano de estos virus y la buena gestión de los casos es fundamental para garantizar una mejor evolución.

El quinto artículo de esta tesis aborda específicamente el uso de antibióticos para tratar las infecciones respiratorias agudas en Marruecos. Mediante el análisis de datos sobre el uso de antibióticos antes del ingreso, y la utilización de antibióticos intra-hospitalarios, se discute si estos medicamentos valiosos están -o no- siendo usados de forma juiciosa y racional; y si un control más estricto sobre su uso debería exigirse, en un país como Marruecos, donde la venta sin receta y de forma incontrolada son prácticas comunes. Este análisis también reflexiona sobre si el uso indiscriminado de antibióticos puede haber tenido o no un impacto en las tasas de resistencia a los antimicrobianos.

Finalmente, el último artículo de esta tesis examina los datos de la distribución de los serotipos de Streptococcus pneumoniae aislados de la nasofaringe de niños sanos y lo compara con aquellos aislados entre los niños ingresados con neumonía clínica grave, precisamente en el

contexto de la reciente introducción en Marruecos de la vacuna conjugada 13-valente contra este microorganismo. Estos datos serán utilizados como base de referencia para ayudar a evaluar el impacto de la vacuna y para monitorizar los posibles fenómenos de reemplazo de serotipos.

En conclusión, esta tesis trata de ofrecer una visión global de la situación de la neumonía pediátrica en Marruecos. Una comprensión más precisa de la situación actual permitirá un mejor diseño de estrategias preventivas y curativas, y, potencialmente, el alivio de la carga significativa que las infecciones respiratorias siguen imponiendo a los niños en este país.

4. General Introduction

4.1. The global burden of acute respiratory infections in children under five years of age

As of today, Pneumonia remains the leading infectious cause of death among children under-five, killing nearly 2,600 children a day. Pneumonia accounts for 15 per cent of all under-five deaths and killed about 940,000 children in 2013 [1, 2]. Most of its victims were less than 2 years old. The global incidence of ARI in children is estimated to be 154 million cases per year with each child suffering an estimated 4-6 episode per year (ALRI ~0.4 per year). Nearly 10% of these episodes are severe enough to require hospitalization [3]. The mean cost for the subset of patients requiring hospitalisation for ARI was estimated to 12000 US$ per episode [4-5]. The United Nations General Assembly Millennium Development goal number 4 (MDG4), which aimed at reducing under 5 mortality rate by two-thirds between 1990 and 2015, was the decisive move to put pneumonia at the forefront of the global health agenda. [4-6]

Children living in developing countries have especially high morbidity and mortality rates, resulting in a substantial burden imposed to the already fragile and often undersupplied health care systems [6-8]. These weaknesses possibly contributes indirectly to the inherent already high mortality associated to acute respiratory infections, in relation to challenges in the access to health, limitations in diagnostic and management tools, and the existing inequities in access to care and preventives services for all. Additionally, other frequently coexisting factors in developing settings, such as undernutrition, crowding and inadequate immunization, all well-established prognostic markers for ARIs, contribute to the adverse outcomes seen in poor countries [6-10].

In 2007, WHO and UNICEF jointly launched the Global Plan for Pneumonia (GAPP) [11], based on 3-pronged platforms of prevention, protection and treatment. This plan focused on improving nutrition, reducing exposure to indoor and outdoor air pollution, increasing vaccine coverage and delivering on a timely manner effective antimicrobial treatment.

Respiratory infections cause a variety of clinical syndromes of which pneumonia, perhaps the most pragmatic and severe of it all, is the most commonly associated with an adverse outcome, causing over 90% of all deaths [12].

A substantial proportion of pneumonia cases and deaths are attributable to two bacterial pathogens, *streptococcus pneumonia* [13], and *Haemophilus influenzae* type b (Hib) [14],

although their burden and geographical distribution has significantly decreased, since the progressive implementation of the two available conjugate vaccines against them. [15].

Indeed, the implementation in many developing countries as a result of the GAVI initiative´s support can prevent childhood pneumonia and its severity by as much as 50% [16-17]. Other common clinical syndromes affecting the respiratory lower tract include bronchiolitis and bronchitis, more typically associated to viral infections.

In this respect, the burden of viral-related ARI, and paradigmatically that of viruses such as respiratory syncytial virus (RSV) is increasingly recognized as a

Cause of respiratory track-associated mortality [18-19]. Indeed, RSV, is believed to cause an estimated 3.4 million annual hospital admissions, and between 66.000-199,000 deaths, accounting for at least one in every five ARI episodes among children younger than 5 years of age [18].

Whichever the underlying cause, case fatality rates among under-fives hospitalized with pneumonia are reported to be around 19% [3].

In rural setting of low or middle income countries, diagnosis of the most common paediatric diseases such as ARI generally depends on the recognition by primary health care workers of clinical symptomatology. Based on this rationale, the WHO developed in the 90´s the integrated management of childhood illnesses (IMCI) guidelines [20], a set of highly sensitive clinical algorithms providing guidance for diagnosis and management of common paediatric conditions. While at the peripheral level these highly sensitive clinical algorithms have contributed to saving thousands of lives [21], their poor specificity, makes them insufficiently reliable at the hospital level unless complemented by laboratory determinations [22-24]. As a result of the wide use of IMCI guidelines in Morocco, and under the guidance at the peripheral level of clinical signs rather than laboratory –confirmed diagnoses, antibiotic misuse in on the rise. Indeed, diagnostic uncertainty often leads to overtreatment, wasting the limited existing resources and accelerating antibiotic resistance level in the community, but more importantly, misdiagnosis (between viral and bacterial pneumonia) may divert the specific treatment from the real aetiology, putting in danger the patient's life. As the quality care and services in many African referral hospitals is already notoriously poor and necessarily constrained by limited resources [25-26], distinguishing the real aetiology causing their admission becomes critical to improve patient's survival chances.

Despite the pandemic character of acute respiratory infections in children, and its significant global burden (accounting for 115 227 000 disability-adjusted life years (DALYs) in 2010 [27]),

investments to improve this situation remain modest, with only about one percent (US$32 million) of all pharmaceutical research and development funding being spent on research and development for ARIs in 2007, compared with US$1.1 billion spent on HIV-related research. Clearly, investment in pneumonia research is low relative to its burden [28-29] and may require significant strengthening to adequately address its major impact in child survival [28-30].

4.2. Definition and classification of acute respiratory infection

Definition of pneumonia

In the poor resource regions in the world, the definition of pneumonia adopted is the WHO definition [31], which correspond to the presence of cough or difficulties of breathing and age adjusted tachypnea (age 2-11 months,≥ 50 / min;1-5 years, ≥40/ min). Severe pneumonia is defined as "cough or difficulties of breathing, plus one of the following: Lower chest indrawing, nasal flaying, or grunting". Very severe pneumonia is defined as severe pneumonia plus one of the following: Cyanosis, severe respiratory distress, inability to drink, or vomiting, or lethargy/ unconsciousness/ convulsions".

Definition of bronchiolitis

Bronchiolitis is a lower respiratory viral infection, which is typically most severe in young infants, occurs in annual epidemics and is characterized by airways obstruction and wheezing. It often corresponds to the first episode of airways obstruction in young infants [31].

Definition of bronchitis

Acute bronchitis is a lower respiratory tract infection that causes reversible bronchial inflammation. In up to 95% of cases, the cause is viral. While antibiotics are often prescribed for patients with acute bronchitis, little evidence shows that these agents provide significant symptomatic relief for shorten the course of the illness.

Bronchitis is defined clinically by the combination of a more or less productive cough, moderate fever, and in auscultation: sub-crackles and / or rhonchi. This clinical presentation is often associated with upper respiratory infection: rhinitis or nasopharyngitis. Unlike in adults, there is no sputum. The disease generally progresses favorably in one to two weeks, or could be complicated by a secondary bacterial infection [32].

Definition of asthmatic bronchitis or wheezing

Asthmatic bronchitis or wheezing are terms used to describe episodic wheezing in infants and young children. They design an inflammatory condition with reversible airway obstruction, and

are characterized by recurrent episodes of wheezing, often with cough, which respond to treatment with bronchodilators and /or anti inflammatory drugs [31].

In spite of these definitions, these clinical syndromes are not clear-cut, with frequent overlapping, or co-existence. Clinician's diagnosis, not necessary in agreement with epidemiological definitions, and given at discharge after the evaluation of all available diagnostic test results, is often used in clinical studies to guide the attribution of the symptoms to a specific syndrome.

4.3 Geographic distribution

Figure 1: Acute respiratory infection in under five years old children end 2014. Source: UNICEF analysis based on WHO-CHERG estimates for child causes of death 2000–2013.

More than 99% of all pneumonia mortality occurs in low- and middle-income countries (LMIC). South Asia and sub-Saharan Africa bear the burden of more than half of the total number of cases of suspected pneumonia among children under five worldwide [4].

4.4 Risk factors for severity and mortality from ARI

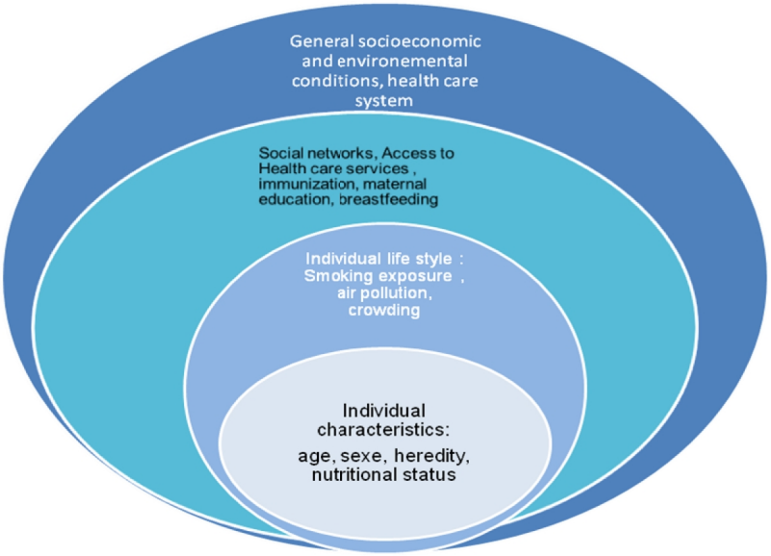


Figure 2: Acute respiratory infection determinants' among children

Figure 2 illustrates some of the determinants for severity and a poor outcome among children with ARI. These determinants can be divided in those depending on the host, those related to the pathogen, those associated to the health system and those associated to the socioeconomic status and the environment.

Host risks factors

Age: The risk of severity of pneumonia and the need for hospital admission is greater in infants. Children aged< 2 months have a significant higher risk of dying 5.2; (95% CI 1.7 to 16.0) [10].

Gender: The association between male sex and severe ARI has been reported but it is inconsistent. Only studies from developing countries have shown an association of OR1.5; (95% CI 1.0 to 2.3). Female gender in the other hand, has been associated to an increase by 15% in mortality [9].

Low birth weight: The pooled studies from developing countries reported a significant increased RR meta estimate of 3.2; (95% CI 1.0 to 9.9) of severe ARI for children being born with low birth weight [9-10].

Undernutrition: Undernutrition is clearly associated with severe ARI. The meta odd ratio estimated from ten pooled studies conducted in developing countries was 4.5; (95% CI 2.1 to 9.5) [10].

Breastfeeding: It was reported in studies from developed and developing countries that the lack of exclusive breastfeeding (defined as only breast milk in the first six months of life), was associated to severe ARI. Meta odds ratios were estimated at 2.7; (95% CI 1.7 to 4.4) in developing countries and 1.3 (95% CI 0.2 to 8.4) in industrialized countries [10].

Previous illness: The following diseases have been associated with an increased risk of severe ARI [9].

Chronic respiratory disease:

-Asthma,

-Cystic fibrosis, bronchial ciliary disease,

-Bronchopulmonary dysplasia in the context of prematurity,

-Recurrent bronchitis,

-Severe viral disease squeals (adenovirus, measles).

Co-morbidity:

-Congenital heart disease,

-Sickle cell disease,

-Immunosuppression,

-Renal failure (and / or nephrotic syndrome)

-Neuromuscular diseases.

Hypoxemia: Low oxygen hemoglobin saturation (< 90%) is a well established risk factor for a poor outcome in children and infants. SPo2 measurements of < 90% at the initial visit has also been shown to be predictive of failure of outpatient oral amoxicillin treatment. Cyanosis is the clinical sign that has the higher specificity of predicting hypoxemia [9, 33].

Pathogen risk factors

The understanding of the pathogenesis of the respiratory infectious agents is crucial for the development of effective treatment and public health preventive and control measures.

Whether the underlying infectious agent is of viral or bacterial origin, its capacity to infect the host and to develop disease depends on its entry route, its ability to gain access to host, the size of its inoculum, its ability to use host substrates and to circumvent host responses. Bacterial products can also cause damage of cells or tissues, or can alter host physiology [34].

Respiratory viral infections: The pathogenesis of viral respiratory infections is much poorly understood. Nevertheless, it is accepted that it depends on the interaction between the virus, the host and the environment [35].

The host characteristics, including its gender, age, immunological and nutritional status and the presence of specific cellular receptors for a given virus, can determine the resistance or its susceptibility to infection.

The disease severity depends on a series of factors, including the virus species, the expression of surface proteins, and the speed of its replication; its spreading potential, its virulence or the mutation of its proteins. Each virus can cause different cytopathic effects in the host cell, which may lead to several symptoms and diseases. Regarding the pathogenic influence of the viral co-infections, it has been reported that it may depends on the specific viruses involved, and the timing of their acquisition and its interval.

By increasing bacterial adherence, altering tracheobronchal clearance and by inhibiting the immunity process, respiratory viruses are associated to the promotion of secondary bacterial infection. The most well-known viral–bacterial interaction is the synergism between influenza virus and *S. pneumoniae* [34].

The infectivity of the viruses and their propagation can be optimized in certain environmental conditions such as high temperature for enteric viruses or low temperature for respiratory viruses. Also, the moisture and the aeration influence the viability of the virus before reaching their target organ. [35,36].

Bacterial respiratory agents: *Haemophilus influenzae*, *S. pneumoniae,* and *M. catarrhalis* are commensal agents in the human upper respiratory tract, which occasionally, can turn into pathogens and cause infectious diseases after the colonization of the nasopharyngeal niche [34]. All of those pathogens have several pathogenic properties in common. They are able to interact with mucus, to exert ciliotoxic activity, to adhere to bronchial epithelial cells, and to invade airway epithelium [37].

Pneumococci have many virulence factors, including capsular polysaccharides, pneumolysin, pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), and pneumococcal surface adhesin A (PsaA). Among these, capsular polysaccharides are considered to be the key determinant of its virulence (promoting adhesion to epithelial surfaces and playing a crucial role in the escape from host defences by complement-dependent and -independent phagocytosis). Interestingly, our accurate understanding of virulence factors has been critical for vaccines development [38].

Among 94 *S. pneumoniae* serotypes currently identified, it is estimated that only 20, are responsible for the majority of invasive diseases. It has been reported that the invasive property of pneumococci seems to be determined by capsular serotype rather than genotype [39]. According to the literature review, serotypes 1, 4, 5, 7F, 8, 12F, 14, 18C, and 19A are more likely to cause IPD. Serotypes 1 and 19A are the predominant causes of invasive pneumococcal pneumonia. While, Serotypes 1, 3, and 19A pneumococci are likely to cause empyema and hemolytic uremic syndrome [38-40]. Serotype 1 that causes sepsis and empyema in Europe and North America, causes meningitis in the African meningitis belt [41].

The increasing resistance of pneumococcal infections to the antibiotics commonly used to treat such infections is compromising the Management of the pathogen and it is responsible for a significant amount of its associated deaths [42, 43].

Health services

Few studies have reported the association between health care related factors and risk of death from ARI in children. **Absent or Incomplete immunization** is associated to a high risk of severity of ARI and increasing mortality rate. While previous consultations and/or antibiotics before admission, were significantly associated with decreased odds of dying [9].

Socioeconomic risks factors

Maternal education: The degree of maternal education has been associated to severity of ARI in developing countries 1.6; (95% CI 1.0 to 2.6), while this association was not found in developed countries [9].

Physical environment

Crowding doubles the risk of severity of ARI in developing countries [44].

Environmental risks factors

Indoor air pollution: Exposure to solid and biomass fuel is associated to death. The overall meta odds ratio estimate of 1.6 ;(95% CI 1.1 to 2.3) [10, 45-47].

Smoking: passive smoking was found in a meta analysis as an inconsistent risk factor of severity of ARI. When the variable considered was the presence of smokers in the house, the odds ratio found was 2.4 (95% CI 1.0 to 5.8), while considering maternal smoking, the odds ratio was about 2.7 (95% CI 1.0 to 7.8) [10].

4.5 Aetiologies

Identifying the aetiologic agent(s) responsible for pneumonia remains a challenge particularly in low or middle income settings. Primarily, because of difficulties in obtaining adequate samples from children, but also on account of the challenges related to differentiating infection from colonization and finally, because of lack of reliable diagnostic methods [7-8, 48-49] in addition to the significant overlap of clinical symptomatology for the different etiologies. Currently, there is no sensitive and specific gold standard for the diagnosis of associated viral or bacterial infections in patient with pneumonia. The WHO, proposed the use of radiographic endpoint of pneumonia (pleural effusion or lobar consolidated infiltrates) to diagnose bacterial pneumonia in studies of the pneumococcal vaccine, despite the limited inter and intra-rather reliability of chest X-rays [50-57].

Laboratory tests for viruses and bacteria have low sensitivity, or are not readily available. Positive blood or pleural fluid cultures are highly specific to diagnose bacterial pneumonia but have low sensitivity [58], and require specialized laboratory facilities. Additionally, blood culture results only become available 24 hours or more after blood collection and are often inconclusive due to the contamination [59-60]. Bacteria and virus polymerase chain reaction (PCR) tests may have low sensitivity and/or specificity [61-63], and results are also delayed, decreasing the utility of these tests for decisions regarding initiation of treatment. Sputum samples are difficult to obtain from children. Antigen detection in the urine has limited utility in children [64-65]. Other laboratory tests associated with the three pathogens including white

blood cells (WBC) counts in bacterial and lymphocytes counts in viral infections can be used to support medical decision but are inaccurate as clinical features. Blood inflammatory markers, such as C reactive protein (CRP) and Pro-calcitonin also have limited accuracy, in spite being commonly used in emergency departments of the developed world. Hence, the differential diagnosis of respiratory distress is particularly challenging precisely in those low or middle income countries that harbor most morbidity associated with lower respiratory tract infections, where specialized microbiology laboratories and radiology facilities are least available [22,25].

In addition to the limitation inherent to the available diagnostic methods, many children with ARI are infected simultaneously with multiple pathogens, including combination of both viruses and bacteria [1, 32-34]. Attributing the causality or the relative contribution of each pathogen to the clinical syndrome is therefore complex [66,67].

Bacterial infection is believed to play a far greater role as a cause of pneumonia in children in developing countries than it does in developed ones, but etiologic diagnosis is difficult to determine and is shifting. Apart from measles, which is often complicated by viral pneumonia or bacterial pneumonia, the main agents responsible for ARI in children include *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Respiratory syncytial virus* (RSV). However, in areas of high HIV prevalence this pattern is changing to include *Pneumocystis jiroveci* and a higher proportion of gram-negative bacteria organisms [1].

Bacterial infections

Streptococcus pneumoniae

Streptococcus pneumoniae is the first agent causing bacterial pneumonia, meningitis and sepsis in the world, leading to 826.000 annual deaths, or twhat is equivalent, an estimated 11% of all global annual deaths, despite the availability of vaccines and antibiotics [13].

Pneumococci are spherical, Gram-positive bacteria, which have many virulence factors, including capsular polysaccharides, pneumolysin, pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), and pneumococcal surface adhesin A (PsaA). Among these, capsular polysaccharides are considered to be the most important virulence factor. There are 94 distinct pneumococcal serotypes. Each serotype is distinguished by serological response, the chemical structure of capsular polysaccharides, and other related genetic mutations. Only a few serotypes produce the majority of pneumococcal infections. It was reported that serotypes 1, 4, 5, 7F, 8, 12F, 14, 18C, and 19A are more likely to cause IPD [38-40]. These 10 most common serotypes are estimated to account for about 62% of invasive disease

worldwide [38]. The ranking and serotype prevalence differ by patient age group and geographic area [41], although common serotypes are consistently identified throughout the world [68]. Vaccination against the *S.pneumoniae* is based on capsular polysaccharides. The widespread use of immunization may potentially alter the serotype distribution -by either serotype replacement or switching-, resulting of selective pressure on the pneumococcal population [69].

Pneumococci are transmitted by direct contact with respiratory secretions from patients and /or healthy carriers. Nasopharyngeal colonization is essential for the propagation of pneumococcal diseases. Disease is caused either by contiguous spread to the sinuses or the middle ear, aspiration into the lower respiratory tract causing pneumonia or by invasion of the bloodstream with or without seeding of secondary sites [68].

The clinical picture is generally homogenous, but may also depend on the specific serotype involved. Globally, serotypes 1, 4, 5, 7F, 8, 12F, 14, 18C, and 19A are more likely to cause IPD, while serotypes 3, 6A, 6B, 9V, 14, 19A, 19F, and 23F are reported to cause acute otitis media, and serotypes 1 and 19A are shown to be responsible for invasive pneumococcal pneumonia. In term of outcome, serotypes 3, 6A, 6B, 9N, and 19F are associated with an increased mortality and.1, 7F, and 8 associated with lower mortality [38].

Resistant pneumococci have unfortunately spread globally. a worldwide distribution now, reduced susceptibility to penicillin has been detected in *S. pneumoniae* in all WHO regions, and its rate can even exceed 50% [70], accounting for 4% ($91 million) of direct medical costs and 5% ($233 million) of total costs including work and productivity loss[71]. Indeed, the prevalence of strains resistant to penicillin-related compounds and to co-trimoxazole is increasing, posing a threat to the effective treatment of pneumococcal disease. The two primary genetic mechanisms which confer pneumococcal resistance are the transformation (the uptake of free DNA from the environment) and conjugative transposons (the transfer of segments of genomic DNA during bacterial fusion). A key factor influencing the emergence and spread of its resistant is the unnecessary antibiotic use for viral respiratory illnesses in humans [72, 73].

Surveillance systems for tracking the prevalence of *S. pneumonia* resistance includes the use of culture data from clinical isolates causing invasive disease, which give an indication of the resistance patterns of strains causing clinical infection, and tests of nasopharyngeal swabs from individuals without clinical illness to determine the community *S. pneumoniae* carriage resistance rate [74].

Haemophilus influenzae

Haemophilus influenza type b has traditionally been considered the second major cause of pneumonia and meningitis, globally, being responsible for 371.000 annual deaths, which correspond to around 5% of all global annual deaths among children under five years of age [13].

This aerobic gram negative bacteria is known to possess six different serotypes (a-f) of polysaccharide capsule [75]; almost all are preventable with current vaccine. 95% of invasive diseases are caused by serotype type b. The organism enters the body through the nasopharynx.and colonize it, and may remain only transiently or for several months, or can penetrate the epithelium of the nasopharynx and invade the blood capillaries directly. Their capsule allows them to resist phagocytosis and complement-mediated lysis in the nonimmune host. Nontypable (non encapsulated) strains are less invasive, but they are apparently able to induce an inflammatory response that causes disease.

Infection with *Haemophilus influenzae* type b (Hib) can result in meningitis and other severe infections such as pneumonia, bacteremia, cellulitis, septic arthritis, and epiglotitis, primarily among infants and children <5 years of age [76]. Most of Hib meningitis survivors can suffer of permanent disabilities such as mental retardation or deafness.

The contagious potential of invasive Hib disease is considered to be limited. However, certain circumstances, particularly close contact with a case-patient can lead to outbreaks.

Hib conjugate vaccines are highly immunogenic, safe and effective [75]. Hib conjugate vaccines can prevent childhood meningitis mortality and reduces the risk of all forms of invasive Hib disease [77-79].

Regarding Haemophilus influenza antibiotic susceptibility, β-Lactamase production is highly prevalent worldwide and is associated with resistance to ampicillin and amoxicillin. Effective antimicrobial therapy consists in third-generation cephalosporin (cefotaxime or ceftriaxone), or chloramphenicol in combination with ampicillin [76].

Mycoplasma pneumoniae

Mycoplasma pneumoniae is a very small bacterium in the class Mollicutes. It is a primarily mucosal pathogen, living a parasitic existence in close association with epithelial cells of its host, usually in the respiratory tract. Persons with active mycoplasmal infection will carry the organisms in the nose, throat, trachea, and sputum, it can be transmitted through aerosols from person to person. [80].

M. pneumoniae infection can involve both the upper and the lower respiratory tracts and occur both endemically and epidemically worldwide in children. M. pneumoniae causes up to 40% or more of cases of community-acquired pneumonias, as many as 18% of cases requiring hospitalization in children [81]. Recent studies showed it can occur also from the age of 2 years and, exceptionally before the age of one year. The frequency of this infection depends on the diagnosis technique used- serology or molecular methods-, and the occurrence of outbreaks [80-82].

The clinical presentation of *M. pneumoniae* respiratory disease is often similar to what is also seen with other atypical pathogens, particularly *Chlamydia. pneumoniae,* various respiratory viruses, and bacteria such as S. pneumonia. While the disease can persist for weeks or months, it is frequently mild and self-resolving. The organism may persist for several weeks in the oropharynx despite completion of recommended antimicrobial therapy and resolution of clinical symptoms [81, 82].

The disease is treated with macrolide, tetracycline, or fluoroquinolone antibiotics. Because its structure is lacking a cell wall it is inherently resistant to beta-lactam antibiotics. There is no vaccine to prevent *M. pneumonia*e infection. [81].

Staphylococcus aureus

Staphylococcus aureus is a very virulent gram-positive bacterium that can be carried asymptomatically in the anterior of the nares. Colonization precedes the infection that can spread locally or gain access to the blood [83].

S. aureus accounts for 3-5 % of CAP infections and is a complication of seasonal and pandemic influenza in children and young adults. Reports of *S. aureus* infection associated with influenza-related deaths in children have raised concerns that this syndrome is increasing in frequency, indeed the epidemiology of *S. aureus* infections has changed dramatically, predominantly because of the epidemic spread of a strain of community associated methicillin-resistant *S.aureus* [84].

The diagnosis of staphylococcal pneumonia is challenging. No specific symptoms, clinical signs, or imaging or laboratory findings have been identified as having high specificity for staphylococcal pneumonia. However, the suspicion for *S. aureus* should be required in children with CAP, especially those who are severely ill, has current or recent influenza, or whose symptoms do not improve with beta-lactam or macrolide antibiotic therapy [85]. vancomycin is used for its treatment.

Other bacterial agents

Other organisms, such as *Chlamydia* spp**,** *Pseudomonas* spp, *Pneumocystis jirovecii* and *M. tuberculosis* can cause pneumonia in children. Indeed the HIV epidemic has also contributed substantially to increases the vulnerability of children to these microbial agents, often leading to substential mortality from childhood pneumonia. The incidence and severity of HIV-associated pneumonia can be reduced in through the prevention of HIV infection, and the use of co-trimoxazole prophylaxis and with antiretrovirals treatment. [86].

Viral infections

Paramyxoviridae

Respiratory syncytial virus: Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections (LRTI) in children worldwide. In 2005, Its global burden was estimated at 33·8 (95% CI 19·3–46·2) million new episodes in children younger than 5 years (22% of ALRI episodes), with at least 3·4 (2·8–4·3) million with severe cases necessitating hospital admission. In term of mortality, almost 66 000–199 000 children younger than 5 years died from RSV-associated ALRI. 96 % of these deaths occurred in developing countries [18,87].

The RSV virus is a member of the family *Paramyxoviridae* and the subfamily *Pneumovirinae*. It is an enveloped RNA virus and two strains are recognized (subgroups A and B) [88].

The usual mechanism of RSV transmission is direct or close contact with contaminated secretions. A latent period of 4–6 days precedes the clinical infection. The first signs of RSV infection in infants are those of a common upper respiratory tract infection, such as nasal discharge and congestion, cough, fever and decreased appetite, but in up to 30% of infected children aged ≤ 2 years the infection spreads over the lower airways leading to a LRTI with varying degrees of severity, ranging from mild to life threatening respiratory failure. Numerous studies have reported increased wheezing following Bronchiolitis [88].

Several different types of laboratory tests are available for diagnosis of RSV infection, Rapid diagnostic assays performed on respiratory specimens, Antigen detection tests and culture and RT-PCR assays.

No infant vaccine candidates are near commercialization. Two passive immunization agents have been developed: RSVintravenous immune globulin (IVIG) and palivizumab. In the last few years, the commercialization of an intramuscular humanized monoclonal antibody, palivizumab, has added a new important tool to the therapeutic options available for RSV

prophylaxis. Passive immunization with palivizumab has proven to be safe and effective for preventing RSV hospitalization in infants at higher risk of acquiring severe RSV infection [89].

Parainfluenza virus: Human parainfluenza viruses (HPIV) are common community-acquired respiratory pathogens without ethnic, socioeconomic, gender, age, or geographic boundaries.

Human parainfluenza viruses are negative-sense, single-stranded, enveloped RNA viruses. They comprise five members; HPIV1, HPIV2, HPIV3, HPIV4A and HPIV4B. Of the human pathogens, HPIV1 and HPIV2 are generally more associated with croup, upper RTI and pharyngitis, whereas young infants (younger than 6 months) are particularly vulnerable to infection with HPIV-3. Unlike the other HPIV, 40% of HPIV-3 infections are in the first year of life. Brochiolitis and pneumonia are the most common clinical presentations [90].

Parainfluenza viruses are transmitted by inhalation of virus-laden droplets expelled into the air from lower respiratory or nasal secretions of infected individuals. After an incubation period of 2–8 days, viral replication occurs in the nasopharyngeal epithelium and 1–3 days later it is spread throughout the tracheobronchial tree. Recurrent infection is a common event.

Identification of parainfluenza viruses in clinical specimens can be achieved by detecting whole virus, viral proteins and viral RNA in clinical samples, as well as

by investigating host antibody responses. Nasopharyngeal secretions or throat swabs constitute the preferred specimens for all PIV detection methods.

Current therapy includes supportive care; children are usually placed in plastic tents supplied with cool, moist oxygen (croupette), and recovery usually occurs after 1–2 days. Vaccination against these important respiratory pathogens is not, as yet, available for clinical use. Also, there is no specific antiviral treatment for HPIV illness [91].

Human metapneumovirus: The hMPV was discovered in 2001and it is a virus associated with acute respiratory illness among infants and children worldwide [92].

hMPV is a member of the *Metapneumovirus* genus of the *Paramyxoviridae* family, a group of negative-stranded RNA viruses. Genetic studies on hMPV have demonstrated the presence of two distinct hMPV serotypes each divided in two subgroups.

Initially, human metapneumovirus (hMPV) was isolated from children with clinical symptoms of respiratory syncytial virus (RSV) infection in whom RSV could not be detected. Since then, numerous reports have described the detection of hMPV in clinical specimens from children, (both immunocompetent and immunocompromised patients), diagnosed with an acute respiratory illness all over the world.

Diagnosis is made by RT-PCR assays on respiratory secretions. Rapid antigen detection tests are not yet available and its growth in cell cultures is fastidious.

No vaccines, antibodies (monoclonal or polyclonal), or chemotherapeutic agents are currently licensed for use to prevent or treat hMPV infections. The contribution of hMPV to pediatric respiratory tract morbidity and mortality supports the development of a vaccine against this virus in combination with those being developed for RSV and parainfluenza viruses [93-95].

Orthomyxoviridae

Virus influenzae: Influenza is one of the most significant causes of acute upper respiratory tract infections worldwide. It has been reported that in 2008, there were almost 90 million (95% CI 49-162 million) of new cases of influenza, 20 million (13-32 million) cases of influenza-associated ALRI (13% of all cases of paediatric ALRI), and 1 million (1-2 million) cases of influenza-associated severe ALRI (7% of cases of all severe paediatric ALRI) occurring worldwide in children younger than 5 years. Deaths in children younger than 5 years attributable to influenza-associated ALRI were estimated between 28,000-111,500, with 99% of deaths occurring in developing countries [95].

There are three types of influenza virus a, b and c. the surface of the virus is coated with haemagglutinin and an enzyme neuraminidase. Influenza a is the least stable, b is more stable and c has low pathogenicity. Antigenic drift in both A and B is responsible for frequent outbreaks that occur at regular intervals.

The clinical feature of influenza is fever, malaise, upper respiratory symptoms with dry cough and muscles aches. Influenza viruses are highly contagious and can cause seasonal epidemics, manifesting as an acute febrile illness with variable degrees of severity, ranging from mild fatigue to respiratory failure and death. Severe influenza complications are most common in children younger than 2 years of age. Children with chronic health problems like asthma, diabetes and disorders of the central nervous system are at especially high risk of developing serious flu complications.

Direct immunofluorescence, ELISA or RNA amplification can be used to make the diagnosis.

The seasonal flu vaccine protects against the influenza viruses. A standard dose quadrivalent nasal spray is recommended for children from 2 to 8 years old. The trivalent flu vaccine is recommended for children from 6 months. [96]

Picornaviridae

Adenovirus: Adenoviruses are one of the causative agents of acute respiratory tract infections at all age groups worldwide. It has been reported that between 1995 and 2011 the proportion of adenovirus infection in all hospitalized children under age of five years with acute lower respiratory infections reached-5.9% [87].

Adenoviruses are non-enveloped double-stranded DNA viruses that can infect a variety of human tissues. Serotypes HAdV type 14 and subgroups B and C are more common to the respiratory tract.

Adenoviruses can cause an array of clinical diseases, including conjunctivitis, gastroenteritis, hepatitis, myocarditis, and pneumonia Most of these occur in children under 5 years of age and are generally self-limiting illnesses. Seasonal patterns of infection have been demonstrated in the general paediatric population.

Antibodies to adenovirus (mainly secretory IgA) are present in the upper respiratory tract within 3 days of infection. Approximately 7 days post-infection, antibodies can be detected in serum, and nasal secretions. The aetiology can be confirmed by different diagnostic approaches, such as cell culture, antigen detection, and PCR.

Of today, there is yet no efficient antiviral drug against HAdV [97-98].

Rhinovirus: Human rhinovirus (hRV) is often the most commonly isolated virus in children with respiratory symptomatology and is also the major cause of the common cold [99].

There are 99 recognized types of human rhinoviruses that differ according to their surface proteins. Advances in molecular diagnosis techniques led to the identification of a third (besides RV-A and RV-B) previously unidentified species RV-C, in 2006 [100-101] subsequently, there have been several investigations on RV-C but these studies have largely been limited to developed countries. The majority of studies investigating RV species in children hospitalized with ALRI found that RV-C is the most prevalent RV species and is often associated with more severe illness. [100,102-108]

The role of hRV in acute exacerbations of asthma and other airway disease has been demonstrated. Rhinovirus infections occur year round with seasonal peaks of incidence occurring in different moments according to the continent. There are two modes of transmission: via aerosols of respiratory droplets and from contaminated surfaces, including direct person-to-person contact. Children may have six to twelve colds a year. Rhinoviruses are implicated in acute and chronic bronchitis, bronchiolitis and pneumonia. Symptoms appear

after a 24–48 h incubation period, reach their peak 2–3 days later and last for 5–7 days in total, persisting occasionally for as long as 2–4 weeks. Symptom severity is highly variable, and may depend on infecting species. The presence of Rhinovirus, either alone or in combination with RSV, may be related to a more severe clinical presentation of acute bronchiolitis in infants.

The diagnosis is made by PCR. Interferon-alpha used intra nasally was shown to be effective against *Human rhinovirus* infections, but important side effects were noticed.

There are no vaccines against Rhinovirus available currently [109].

4.6 Diagnosis

Blood culture

Detection of pathogenic bacteria in the blood or pleural effusion of a child with pneumonia is considered, in the absence of a true gold standard diagnostic method, the best possible diagnostic approximation to the underlying etiology of the pneumonia. However, sensitivity of these methods is low. It has been accepted (based on antibacterial pneumonia clinical efficacy vaccines trials), that only one of five radiological pneumonia cases will have a confirmatory blood isolation [110]. in spite of this low sensitivity, blood cultures should be routinely performed as the only possible way for establishing an etiological surveillance of potential underlying mechanisms. However, uptake of such recommendation is low and challenging in settings where routine microbiology facilities are lacking. Blood cultures should precede the initiation of treatment and not to delay it [110].

Sputum culture

The cyto-bacteriological examination of sputum is difficult to implement in children and has little interest. Its reflects mostly the oropharyngeal and nasopharyngeal flora [48].

Serology

Serological methods are useful for the identification of certain pathogens (for instance Mycoplasma pneumoniae) when IgM are high. However, the delay in obtaining results is long (> 10 days) in the vast majority of cases. They do not therefore contribute to an initial therapeutic decision or early treatment change, which will depend on clinical and radiological features. In the presence of positive results, clinicians can modify the initial treatment and its duration. Moreover, these methods can however have epidemiological research interest [80].

Chest radiography

Posteroanterior and lateral chest radiography are commonly indicated in patients with suspected or documented hypoxemia or significant respiratory distress and in those with

failed initial antibiotic therapy. The x ray will document the presence, the size and the character of parenchymal infiltrates and identify complications of pneumonia, including pneumothorax, parapneumonic effusions and necrotizing pneumonia. The repeated chest radiography is only obtained in children who failed to demonstrate clinical improvement and in those who have progressive symptoms or clinical deterioration within 48-72 hours after initiation of antibiotic therapy [50].

Chest radiography is often used as the gold standard for diagnosis of pneumonia, even though it is neither sensitive nor specific, is difficult to interpret and shows a high inter-observer variability. Moreover, is not able to precisely differentiate between viral and bacterial etiologies, because viral and bacteria or mixed viral and bacterial infections may cause similar radiological findings. As a screening tool, however, it can often help determining the need (or not) for empiric antibiotic treatment [49].

Recent meta analysis showed that lung ultrasounds has a good performance for the diagnosis of childhood pneumonia and can be useful in setting where x ray is not available. It requires however, good previous expertise in its interpretation [111].

Testing for viral pathogens

These tests are based on the detection and recognition of viral nucleic acids. They provide results within a clinically relevant time frame, are not affected by prior antibiotic administration.

Multiplex nucleic acid amplification tests (NAATs) are promising tools to detect non cultivable pathogens as human metapneumovirus (hMPV) or low quantity of pathogens. However, their cost and technical support make them difficult to implement in resource -limited setting [67, 112-114].and their use in is normally restricted to research efforts.

Pulse oxymetry

Pulse oximetry should be performed in all children with diagnosis of pneumonia and those with suspected hypoxemia. While it seldom informs on the underlying etiology, it does have a value in terms of helping clinicians decide the need for admission and /or supplemental oxygen [31].

Biomarkers

In clinical practice of developed countries increased levels of C-reactive protein (CRP) and procalcitonin (PCT) are routinely used as indicators of bacterial infections [115-116]. regarding pneumonia, it has also been suggested that these biomarkers could help in their diagnosis. Authors from South Africa, showed an improvement of the specificity of this definition of

pneumonia, when using CRP and PCT in the efficacy evaluation of pneumococcal conjugate vaccine [117]. In a similar study conducted in Gambia, improvement of specificity was less marked, the presence of malaria in this country possibly affecting the specificity of pneumonia definition [118]. Besides CRP and PCT, other cytokines and proteins have been assessed for the diagnosis of severe bacterial infections in clinical management [119]. Even though the use of such biomarkers may be routine use for screening purposes, it remains to be demonstrated that they can accurately differentiate between bacterial and viral aetiology of pneumonia in children. However, recent meta-analyses have suggested that despite its low sensitivity, CRP could be useful for the diagnostic of serious bacterial infections, including pneumonia, in children presenting with fever [120,121]. Nevertheless, further work is needed before recommending the use of biomarkers as a diagnostic tool to identify the precise etiology of children with pneumonia.

4.7 Clinical management

The WHO and the UNICEF now recommend that the management of pneumonia should start at the community and health facility levels. These general recommendations include the implementation of case management at all levels, vaccination, prevention and management of HIV infection, improvement of nutrition and reduction of low birth weight and control of indoor air pollution [11]. These strategies include activities for treating, preventing and protecting from pneumonia. It has been estimated that, if correctly implemented, these measures could potentially reduce pneumonia mortality and morbidity by more than half.

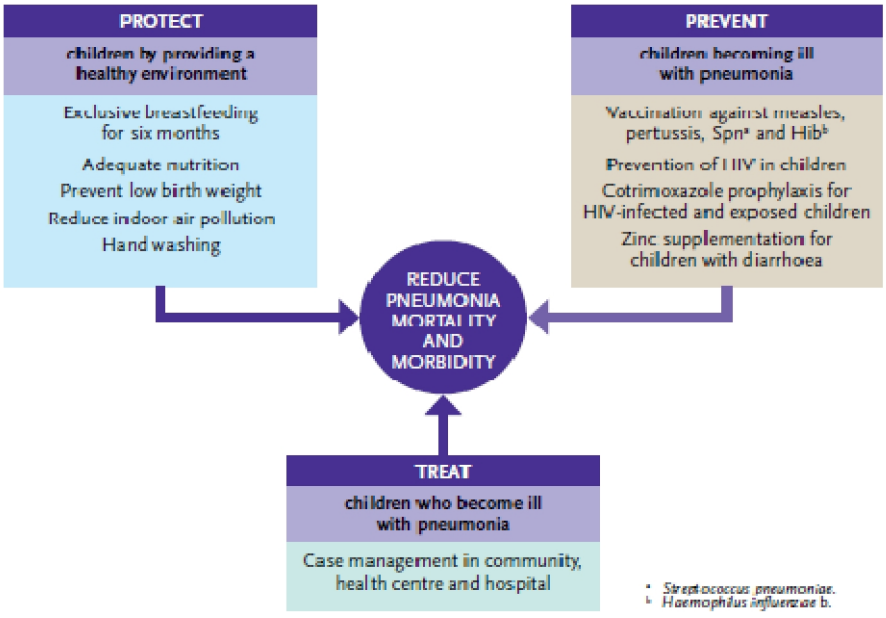


Figure 3: Framework of pneumonia control (Figure published in Global action Plan for Prevention and control of Pneumonia (GAPP). World Health Organization/The United Nations Children's Fund (UNICEF); Geneva 2009 [11].

However hospitalization may be required under the following situations [31,123]:

- Infants and children with respiratory distress or hypoxemia (oxygen saturation <90%);
- Suspicion of infection caused by community-acquired methicillin-resistant *Staphylococcus aureus* or any pathogen with high virulence;
- Infants aged under the age of six months;
- Or for families unable to provide appropriate care.

Regarding antibiotic prescription, the choice of antibiotics is influenced by the epidemiology of the infecting organisms in the area, the prevalence of drug resistance, underlying HIV prevalence in the area and available resources.

Worldwide, amoxicillin remains the antibiotic used as first-line therapy for previously healthy, appropriately immunized infants and preschool children with mild to moderate CAP suspected to be of bacterial origin [31,122,123]. Indeed, most bacterial pneumonia is responsive to amoxicillin, making it the antibiotic of choice. It provides appropriate coverage for *Streptococcus pneumoniae*, it is well tolerated and cheap [122,123]. When atypical pathogens are suspected, then macrolide antibiotics are the antibiotic drug class of choice, with azithromycin begin the preferred first-line agent [122,123].

In low resources settings, WHO recommend the use of Amoxicillin as the first line antibiotic in appropriately immunized and previously healthy children with mild to moderate pneumonia suspected to be of bacterial origin. Macrolides are prescribed when atypical pathogens such as Mycoplasma, Bordetella or Legionella are suspected [31].

Antibiotics can be administered orally for children presenting uncomplicated or even severe CAP. It has been demonstrated that they are safe and effective. When the patient is unable to tolerate oral fluids or to absorb oral antibiotics - because of vomiting or in the presence of signs of septicaemia or complicated pneumonia, the Intravenous administration of antibiotics is indicated [123]. Ampicillin or penicillin G will be then used for children fully immunized. But, in areas with high rate of penicillin resistance, the third-generation parenteral cephalosporin such as ceftriaxone or cefotaxime will be administred. If *S. aureus* is the suspected microorganism vancomycin or clindamycin should be associated with a beta-lactam agent [123].

It has been demonstrated through a clinical trial that Oral amoxicillin (50 mg/kg/day) given twice daily is as efficacious as thrice dailyin children under five years of age with non severe pneumonia [124], the current recommendation as given by WHO in the guideline for management of CAP [31]. Regarding the length of the antibiotic therapy there is no strong

evidence yet to support that the short course (three days) of antibiotic therapy is as effective as a longer treatment courses(five or seven days) for non-severe pneumonia in children under five years of age [125].

In addition to antibiotics, careful supportive management is required for children with CAP. Oxygen therapy is used when there is: cyanosis; lower chest in drawing; inability to drink or feed; and respiratory rate > 70 breaths per minute [126]. In children who are dehydrated, appropriate rehydration is required [122].

4.8 Antimicrobial susceptibility patterns

Worldwide there is a serious concern about the increasing emergence of antibiotic resistance among pneumococci, primarily against beta-lactams and macrolides, and thus, its potential impact on the treatment of pneumonia and invasive pneumococcal disease [127]. The antimicrobial resistance of *s. pneumoniae* is due inter alia to the selection pressure resulting from the misuse of antibiotics, the poor-quality of medicines marketed in some countries, and the insufficient regulation of the use of antimicrobial medicines [70]. Geographical variation of Pneumococcal penicillin non-susceptibility rate, range from 4 % in UK, to 25% in France and 50% in Spain [123] and 30% in North African countries [128].

The use of pneumococcal conjugate vaccines (PCV) leads to the reduction in antibiotic use, as the rate of invasive pneumococcal disease due to PCV concerned serotypes decline significantly. But, the serotypes not covered by the current PCV may also tend to increase [129].

However, Despite the increasingly wide literature on antibiotic resistance, there is less evidence of the impact of this on clinical outcomes for children who suffer from penicillin-resistant compared to those who had sensitive pneumococal pneumonias in term of duration of fever or tachypnoea, bacteraemia incidence, mean duration of therapy or length of hospital stay [123, 130, 131].

4.9 Preventives strategies

Vaccines

Vaccination has made a real impact on pneumonia and child survival worldwide. The WHO estimates that, in 2003, more than 2 million deaths were averted by immunisation [132]. Vaccination is a safe, effective, and cost-effective tool for preventing pneumonia. Currently, several vaccines are available and recommended for universal adoption; these include pertussis, measles, Flu, Hib, and pneumococcal vaccines, that the WHO recommends to be

include in all routine childhood immunization programs [133]. Indeed, in the last 10 years, great advances have been made in developing and introducing new vaccines and expanding the reach of immunization programmes. Many low-income countries have already introduced the Hib vaccine, and pneumococcal conjugate vaccines (PCVs) are increasingly becoming available in developing countries. However despites this progress in the availability on these vaccines, there is still a concern regarding the vaccine coverage rate between countries and within some region in the countries [134].

Hib Vaccine

Most low and middle-income countries have now introduced the Hib vaccine as a part of their routine EPI schemes, mostly as a result of the support provided by the GAVI alliance [134]. In Morocco, Hib vaccine was introduced in 2006.

The Hib vaccine has been shown to have protective efficacy greater than 90% against both laboratory-confirmed invasive meningitis and bactaeremic and non-invasive pneumonia [79, 135-136].It has been well documented in several studies in industrialized and developing countries [135,136]. Resulting in their capacity to reduce the carrier state in vaccinated children and to reduce the pool of Hib infected children in the community. Additionally, the hib conjugate vaccine can also provide herd immunity for unvaccinated children [137].

WHO recommends 3 doses of Hib vaccines associated to DTP or 2 or 3 doses with booster at least 6 months after last dose in children, aged > 6 weeks and < 59 months. In a recent WHO publication, the Global coverage with 3 doses of Hib vaccine has been estimated at 52%. There is great variation between regions. In the Americas, coverage is estimated at 90%, while it is only 18% and 27% in the Western Pacific and South-East Asia Regions respectively [138].In view of this, to improve the adhesion of parents to vaccination schedule and to reduce the workload of health professionals in developing countries, a Hib vaccines in 2 doses regime could be adopted, knowing that a recent analysis showed that the Hib conjugate vaccination strategy including two dose is as good as the three dose regime [139].

Pneumococcal Vaccines

Pneumococcal Conjugate vaccines (PCV) are safe and effective in preventing IPD,X-ray defined pneumonia, clinical pneumonia and deaths caused by *Streptococcus pneumoniae* among HIV-1 negative and HIV-1 positive children under two years of age [140]. The vaccine elicits a T cell-dependent response and produces an anamnestic reaction that makes the vaccine more effective in infants and children younger than two years of age.

PCV was first licensed in 2000 as a formulation that provided protection against seven of the most common pneumococcal serotypes. Currently there are three PCVs available globally:

- **PCV7** (the 7-valent CRM197 conjugated vaccine)
- **PCV10** (has the same serotypes as PCV7 plus serotypes 1, 5, and 7F, but different carrier proteins: protein D, diphtheria toxoid and tetanus toxoid)
- **PCV13** (has the same serotypes as PCV7 plus serotypes 1, 3, 5, 6A, 7F, and 19A each conjugated to CRM197). The PCV 13 offers better coverage for serotypes commonly causing disease in low- and middle-income countries [13].

Recommendations for PCV use from the World Health Organization (WHO) and funding from the GAVI Alliance have resulted in an increase in PCV uptake into national immunization programs, especially in lower-income countries. As of December 2012, a total of 86 (44%) WHO member states have added PCV to the routine infant immunization schedule of their national immunization programs; among those, 23 have introduced PCV with GAVI Alliance support. The proportion of the world's birth cohort living in countries with PCV in national immunization programs increased from 1% in 2000 to 31% in 2012 [141]. In Morocco, pneumococcal conjugate (13-valent) vaccine was introduced in November 2010.

It has been documented worldwide, that after the introduction of the PCV, the incidence of vaccine-type (VT) invasive pneumococcal disease (IPD) and the asymptomatic carriage caused by vaccine serotypes, decrease in all age groups and in parallel there is a slight increase in the non-vaccine (NVT) serotype IPD incidence (serotype replacement) [142-144]. This phenomenon has been suggested to be a result of vaccine selective pressure offering a relative advantage to non-vaccine types [142].

This suggests the importance of the continuous monitoring of IPD and nasopharyngeal *S. pneumoniae* carriage, so the adaptations of vaccine constitution recommendations can be promptly issued [145].

Nutritional interventions

Most of likely preventable deaths caused by infectious diseases and specially pneumonia occur in low-resource settings and are strongly linked to poverty, inadequate access to health care and undernutrition. Indeed, inadequate nutrition and acute lower respiratory infection (ALRI) are overlapping and interrelated health problems affecting children in developing countries [146].

Breastfeeding

Studies suggest that optimal breastfeeding practices, including exclusive breastfeeding during the first six months of life and continued breastfeeding until 24 months of age, are critical for reducing the burden of pneumonia among infants and young children. The protective effect of human milk against respiratory infection is attributed to its numerous immunobiological components [147]. Based on that, the WHO actively promotes exclusive breastfeeding for at least the first 6 months of life.

Vitamin A

It has been shown that vitamin A has a preventive effect on all-cause and disease specific mortality in children under five [148].It plays an important role for the integrity and the regeneration of respiratory epithelia, and it is involved in the production, growth and differentiation of red cells, lymph cells and antibodies .

Several observational studies indicated that a high dose vitamin A supplement was associated to a reduction of the morbidity and the mortality in children with acute respiratory infection. However, clinical trials have failed to find out that benefit. Current recommendations are issued by WHO in relation to vitamin A supplementation are provided mostly in the context of the treatment of measles and malnutrition

Vitamin D

Vitamin D has an important immunomodulatory role, which may contribute to reduce the incidence and risk of respiratory infection in children. Used in prophylaxis, as vitamin supplementation in association with *Streptococcus pneumoniae* vaccines it would strength the immune system [149]. However, studies did not show any evidence to support this potential preventive effect [150].

Zinc supplementation

Evidence suggests that preventive zinc supplementation may reduce child mortality and morbidity from infectious diseases, particularly pneumonia by correcting any deficiency leading to immunodeficiency [148]. However, results of systematic reviews recently conducted are insufficient to recommend the use of zinc as an adjunct to standard antibiotic therapy for pneumonia in children aged 2 to 35 months [151].

Environmental interventions

WHO declared in a recent report that each year, at least 3 million children under the age of five die due to environment-related diseases. As much as 60 percent of acute respiratory infections

in children worldwide are related to environmental conditions [152]. Indeed, many studies have assessed the strong role of the indoor and outdoor air pollution and smoking exposure with death or with an increasing risk of severe pneumonia [10, 45-47, 153]. While other studies have demonstrated that rates of bronchitis declined in areas where air pollution concentrations particles have fallen [154].

Regarding the interventions for creating a smoke free home environment, there is a need for developing a range of interventions to support the families and more research is needed to identify successful elements of interventions and the contexts in which they are most effective [155], beside the implementation of the regulations that protect children from smoking exposure in public areas

4.10. Public health implications

ARI contributed 67 million disability adjusted life years (DALYs) in 2000, more than any other disease entity including diarrhea (45 M) or malaria (32 M). In addition to the high mortality and suffering, it consumes significant health sector resources and the largely empiric treatment of ARI contributes to the worldwide pressure of emerging antimicrobial resistance [1,2].

In the last decade, pneumonia mortality in children under 5 years of age has fallen from 1.7 million cases globally in 2000 to approximately 1.3 million cases in 2011, with most deaths occurring in low- income countries. As a results of the widespread implementation of protein-polysaccharide conjugate vaccines *against Haemophilus influenzae type B* and *Streptococcus pneumoniae* and the implementation of case-management algorithms, additionally to the public health interventions targeting the Improvement of the access to health care and the living conditions, the reduction of the exposure to indoor pollutants and cigarette smoke and the promotion of the breast feeding [1,2,8].

The decline of pneumococcus and Hib associated disease in developing countries will raise two therapeutic problems. With their improved vaccine uptake, the importance of these two pathogens is anticipated to diminish by 50%, while it is expected that a greater proportion of cases may occur due to *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Mycobacterium* tuberculosis in tuberculosis (TB) endemic areas. This switch may in practice imply the need to use a third-generation cephalosporin,a macrolide, or a quinolone to treat efficiently CAP in the future. However, these drug classes are substantially more expensive than the present first line treatment in developing countries which includes benzylpenicillin, amoxicillin, gentamicin,and/ or chloramphenicol. Furthermore, the potentially massive scale of their use in childhood pneumonia may lead to widespread antibiotic resistance, compromising the

treatment of other important endemic infectious diseases such as typhoid, and tuberculosis [156,157].

Regarding the clinical management of ARI, the use of highly sensitive multiplex molecular diagnostics tools for screening purposes may lead to an active detection of multiple etiological agents in each individual. In practice, this means that the majority of the identified agents in these patients can be truly present but not necessarily involved critically in the development of disease. This will pose a considerable challenge to the clinicians in charge of determining the therapeutic management, while trying to determine the relative contribution of each pathogen in the development of the current ARI episode [156,157].

While these significant progresses have been made, considerable research is still required to maximize the implementation of known preventive interventions, and also to establish more supportive evidence and novel tools and approaches for the prevention, diagnosis and treatment of childhood ARI [8].

4.11 Acute respiratory infections in Morocco

By subscribing to the Millennium Development Goals, Morocco has pledged to reduce, by 2015, two-thirds the mortality of children under 5 years in relation to 1990 figures. Thanks to programs for the prevention and control of diseases, infant and child mortality, which accounted for 138 per thousand live births in 1980, has been reduced to 30.5 per thousand live births in 2011. This decline in mortality is usually attributed to increasing income, improvements in living standards and nutrition, progress in preventive and curative medicine and to national health programs [158,159]. In 1978, Following the Alma Ata declaration, the Moroccan MOH, adopted the 'community approach' and the 'primary health care strategies. They aimed at reducing all-causes mortality through cause specific actions. The main actions promoted included among others, the implementation of universal vaccination to prevent some leading causes of death, such as measles, whooping cough, tetanus, diphtheria, poliomyelitis, and tuberculosis. The National Program on Immunization started in 1981 (PEV) and was reinforced in 1987 (PNI). Vaccination is provided, free of charge, to the entire population [159].

The nutrition program has been running for many years and includes the promotion of exclusive breastfeeding, child growth monitoring, prevention of vitamin D, vitamin A and iron deficiencies, and also aims to improve the detection and treatment of severe malnutrition. Since 1998, vitamin A is distributed at the time of routine EPI vaccination.

Since 1996, Morocco adopted in some pilot sites within rural areas the Integrated Management of Childhood Illnesses' (IMCI) strategy. For communicable diseases, the main

focus was on the diagnosis and treatment of acute lower respiratory infections (ALRIs). The ALRI program was set up only in 1997. The Moroccan Ministry of Health (MoH) promoted the training of its health professionals in the adequate management of pneumonia cases and provided sufficient antibiotics'. Additionally the MoH introduced in 2007 and in 2011 the *hib* and the pneumococcal (13-valent) vaccines, respectively. Since that time, an important decrease of the death rate and the severity of pneumonia cases were detected by the national health statistics [159]. In parallel to that, the access to antibiotics' and the general vaccine coverage were improved.

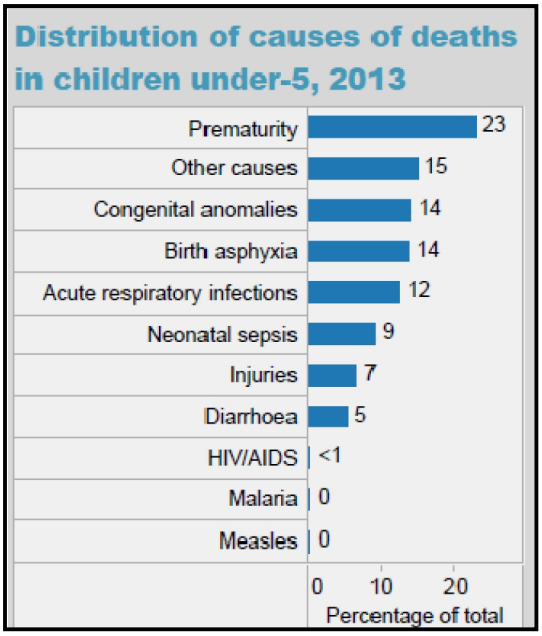


Figure 3: Causes of under-five deaths in Morocco. (Source: WHO-Country profile 2013) *,[160].*

However and in spite these efforts, pneumonia in children under five years of age in Morocco remains as of today a major public health challenge, causing 12% of deaths among children under five and being the leading cause of death in this age category [160], (Figure 3). The main etiological agents underlying these infections are the commonly described, but bacterial pathogens, predominant in the past, have now slowly been replaced by viral pathogens [159]. However, scarcity of data has been the rule in Morocco, and thus, the etiology and epidemiology of pneumonia remains very poorly characterized.

Also, physical and financial access to infant child care in suburban and rural areas, the inadequate quality of pediatric health services and others related social determinants, including environmental exposures (atmospheric pollution and indoor pollution, tobacco smoking), remain clearly poorly explored through the operational research at national level in Morocco [159].

5. Specific introduction to this thesis

This thesis is based on work undertaken through a partnership between Barcelona's centre for International Health Research (CRESIB) and HER, Hôpital d´Enfants de Rabat (HER), in Morocco. The partnership benefited from collaborations with the Moroccan Ministry of health. The studies received support from the Spanish Agency for International Cooperation (AECI).

The first paper is a systematic review which describes data available on ARI in Morocco at the moment of initiating the study and identifies the principal knowledge gaps.

The second, third, fourth and fifth papers presented here report data obtained among admitted children with severe acute respiratory infections to a university hospital in Morocco. These are four thorough analyses describing the epidemiology and aetiology of these infections disease in the area, the importance of coexisting infections, the role of antibiotic usage prior to the admission to hospital and factors involved with a poor prognosis.

The sixth and last paper is a brief report showing data on *Streptococcus pneumoniae* carriage and its serotype distribution among healthy children from the community and sick ones with clinical severe pneumonia in a period close to the implementation of the pneumococcal 13-valent conjugate vaccine in Morocco.

Despite these data coming from a single hospital in Morocco, they may still be highly informative and relevant for this country.

5.1 First paper

The first paper documents the knowledge gaps on pediatric respiratory infections in Morocco.

The motivation for this study was to first provide an overview of the known burden of ARI among children in Morocco, to describe the data available in terms of the epidemiology of ARIs, including its incidence, its distribution, risk factors, trends and aetiology. And secondly, to analyse whether the preventive and management strategies used are coherent with the epidemiology of ARI.

This paper highlights the insufficiency and the dispersion of the information collected by the physicians in their management of outpatients and inpatients admitted for acute respiratory infections. Also, it pointed out the lack of the robustness of the national health statistics data, which does not allow an accurate assessment of the preventives strategies and the clinical management of ARIs.

5.2 Second paper

The second paper on this thesis describes features on admission to hospital of children under five years old meeting the World Health Organization clinical criteria for severe pneumonia over a period of 14 months.

The study shows that the most frequent clinical diagnoses included wheezing-related conditions, while bacterial pneumonia was infrequent and the nasopharyngeal detection of virus was highly frequent among cases. It also documents an inacceptable high in-hospital case fatality rate.

5.3 Third paper

The third paper reports a robust analysis on the risk factors associated with a bad prognosis among children admitted with clinically severe pneumonia.

These data provide invaluable guidance to help identify children who are at highest risk of death for severe pneumonia, in term of improving the preventive and management strategies.

5.4 Fourth paper

The fourth article describes and compares RSV and hMPV-associated cases of WHO-defined severe pneumonia in a paediatric population admitted to Morocco's paediatric reference Hospital

Previous reports had explored the similarity of both viruses in terms of clinical presentation. But, this comparison had never been done among Moroccan children.

This paper highlights the severity of clinical presentation and poor outcome related to hMPV in Moroccan children.

5.5 Fifth paper

The fifth paper reports data on antibiotic usage prior and during hospitalization for severe pneumonia in children under five years of age.

Data collected showed an important frequency of prescription of antibiotics before admission. The paper also describes antibiotic prescription trends during hospitalization, which was consensual and based on medical reasoning. This high antibiotic usage can be contextualized in terms of the prevailing antimicrobial resistance rates observed in pathogens detected as part of the general study.

5.6 Sixth paper

The sixth paper is a brief report that describes *Streptococcus pneumoniae* carriage rates and serotype distribution among admitted children with severe clinical pneumonia and among healthy children from the community. The goal of this paper was to provide baseline snapshot from both populations coinciding with the time of pneumococcal vaccine introduction in this country.

This baseline will allow future comparisons once implementation of the vaccine has been generalized, to assess the impact of the vaccine and to determine whether serotype replacement is (or not) occurring.

6. Hypotheses and objectives

6.1 Project hypotheses

1. A substantial amount of all paediatric admissions will be related to respiratory problems
2. Less than 50% of children with CSP will have X-ray changes compatible with radiologically-confirmed pneumonia.
3. The main bacterial cause of CSP will be pneumococcus.
4. Since Haemophilus influenzae type b (Hib) vaccine is already being used in Morocco, we expect to detect few cases of Hib pneumonia.
5. Evidence of viral infection will be found in more than 50% of CSP cases.
6. Clinical signs and symptoms will have limited utility in differentiating bacterial from viral CSP.
7. Bacterial CSP will have higher levels of CRP and PCT than viral CSP
8. Mortality from CSP will be higher among younger children, among those with bacterial isolates resistant to first line antibiotics, among those with more difficult access to hospital care, and among those with lower socioeconomic and educational status.
9. CSP caused by viruses such as respiratory syncytial virus (RSV) and influenza virus will have a strong seasonal pattern.
10. Prevalence of bacterial resistance to currently used first line antibiotics will be high.
11. Children with CSP will have a high prevalence of zinc deficiency.

6.2 General objectives

The overall objective of this hospital-based surveillance was to evaluate the epidemiology, aetiology and clinical presentation of CSP, as defined by World Health Organization criteria, among children under 5 of age admitted to the Hôpital d´Enfants de Rabat.

6.3 Specific objectives

1. To determine the number of children < 5 years of age admitted to Hôpital d'Enfants during a period of at least 12 consecutive months with CSP and the proportion of those requiring admission to the intensive care unit.

2. To determine the main infectious causes of CSP among children < 5 years of age.

3. To identify the microorganisms responsible for CSP-related admissions in the intensive care unit.

4. To describe the case fatality ratios, seasonal patterns, age-specific incidence and variations in clinical manifestations of CSP pneumonia.

5. To evaluate the diagnostic utility of C-reactive protein (CRP) and/or procalcitonin (PCT) in differentiating bacterial and viral pneumonia.

6. To determine the impact of access to care (such as time from illness onset to admission and distance from residence to hospital) as well as socioeconomic and educational status on severe pneumonia mortality.

7. To estimate the prevalence of antimicrobial resistance as well as the patterns and mechanisms of antimicrobial resistance among bacteria causing CSP among children in Rabat in order to better guide antimicrobial therapy.

8. To determine the proportion of CSP cases caused by pneumococcus serotypes that could be prevented by existing pneumococcal conjugate vaccines and to assess potential serotype differences between normal healthy carriers, and CSP cases.

9. To determine the prevalence of zinc deficiency in children admitted with CSP.

7. Materials and methods

7.1 Study site

The study was conducted at Children's Hospital of Rabat (HER). This hospital is located in the Southwest of the city of Rabat. This is a public and academic healthcare facility and is one of the 10 hospitals part of University Hospital Ibn Sina (CHIS). It represents 17% of the bed capacity of the University Hospital CHIS, 80% of the regional bed capacity in pediatric surgery and pediatrics (100 beds), and assumes 23% of the University Hospital admissions. HER's catchment population matches the Rabat-Salé-Zemmour Zaers Regions, Gharb Chrarda Beni Hssen and Tangier - Tetouan. At the regional level, the HER is the only hospital facility dedicated to children, located in a university hospital environment. Its mission is to provide medical care for children from 0 to 16 years, admitted for pediatric medical and surgical management, to ensure the academic clinical teaching, postgraduate medical teaching and the training of paramedical staff; and to develop medical research. It also contributes to achieving the objectives set by the Ministry of Health for newborn and infant health.

7.2. Study population and procedures at recruitment

This prospective study was conducted from November 2010 to December 2011 at the Hôpital d'Enfants de Rabat (HER) in Morocco's capital. Children aged 2–59 months admitted to HER with respiratory symptomatology were identified and approached for recruitment if they fulfilled the World Health Organization (WHO) definition of clinical severe pneumonia (CSP), namely, history of cough or reported breathing difficulty and increased respiratory rate (RR) according to age and chest indrawing. Provided parents had signed an informed consent form, recruited children underwent standardized procedures upon admission, including an anteroposterior chest X-ray, pulse oximetry (Bionics palm care), nasal and pharyngeal swabs for diagnosis of bacterial infection/carriage and nasopharyngeal aspirates (NPAs) for diagnosis of respiratory viruses.

A minimum of 2 ml of venous blood was also collected for haemoculture, full blood cell count and biochemical determinations, including C-reactive protein (CRP), procalcitonine (PCT) and plasmatic zinc.

7.3. Case definitions

- Admission and discharge diagnoses were coded using the International Classification of Diseases, 10th Revision.
- Hypoxemia implied an oxygen saturation of (SaO_2) <90%.
- Fever was defined as an axillary temperature of 37.5°C.

- Nutritional status was based on weight-for-age Z scores (WAZ), calculated using the least mean square method and the 2000 Centers for Disease Control and Prevention Growth Reference.
- Invasive bacterial disease. (IBD) implied the isolation of 1 non-contaminant bacteria in blood or pleural fluid.
- Case fatality rates (CFRs) were calculated as the number of patients who died with a specific diagnosis divided by the total number of patients with known outcome admitted with that diagnosis, and thus represent the in-hospital mortality.

7.4. Laboratory methods

HER research laboratory

Analyses of samples from the study were performed in the laboratory of medical research CHU Ibn Sina CHIS, opened in 2010. The laboratory was created as part of a twinning agreement in the fields of health assistance, education and research between the Hospital Ibn Sina (CHIS) the Hospital Clínic of Barcelona (HCB) and the Fundació Clínic for BioMedical Research (FCRB) with the collaboration of the Spanish International Cooperation Agency for Development (AECID). It is a laboratory fully dedicated to biomedical research in the area of maternal and child health, which hosts joint research projects between the teams of the CHU and CRESIB, the research branch of Hospital and Clínic in Barcelona-Spain. This research platform includes molecular biology equipment and classical microbiology equipment and can therefore support basic bacteriology, virology and parasitology surveillance. It has been designed according to the international standards for biosafety, and is governed by the standards of quality and good practices.

Laboratory techniques

Samples were analyzed in the medical research laboratory of HER.

PCT and CRP were determined using mini VIDAS (Biomerieux) and Microlab, respectively. Plasmatic zinc was assessed in a subsample of patients at the Centre National de Recherche Scientifique et Technique's laboratory (Rabat, Morocco) using inductively coupled plasma atomic emission spectroscopy.

Full blood counts were performed as part of routine clinical evaluations at HER centralized laboratory.

Blood samples were cultured using an automated blood culture system (BD Bactec), and bacterial isolates identified by BD Phoenix Automated Microbiology System (PHX system, Becton Dickinson) or colony morphology and biochemical tests. In addition, a standard

bacterial culture was also performed in NPAs. Samples were cultured on blood and chocolate agar plates for 72 hours at 37°C in an atmosphere of 5% CO2. Bacterial isolates were identified by biochemical conventional methods (Api 20E, Biomerieux laboratories).

To increase the detection yield of conventional microbiology, three sequential real-time polymerase chain reactions (RT-PCR) based on the detection of the bexA gene of Hib (only for a subsample of randomly selected patients), and the ply and wzg genes of *S. pneumoniae* were performed in plasma samples. Molecular capsular typing of pneumococcal strains was investigated using sequential multiplex RT-PCR targeting 24 common serotypes (1, 3, 4, 5, 6A, 6B, 7F/A, 8, 9V/A/N/L, 14, 15B/C, 18C/B, 19A, 19F/B/C, 23A and 23F). Detection of DNA/RNA of influenza A and B; RSV A and B; parainfluenza viruses 1, 2, 3 and 4; rhinovirus (RhiV); adenovirus (ADV); enterovirus; coronaviruses 229E, NL63 and OC43; human metapneumovirus (hMPV); Mycoplasma pneumonia; Chlamidophila pneumoniae and Bordetella pertussis in NPAs was investigated by means of the TrueScience RespiFinder Pathogen Identification Panel (Applied Biosystems).

Figure 5: HER Research laboratory

7.5. Ethical consideration

The protocol and informed consent documents were approved by the Ethics Committee of the Hospital Clinic (Barcelona, Spain) and by the Comité d'Ethique de la Recherche Biomédicale (Départ N°1252-16Déc 2009) of the Faculty of Medicine in Rabat.

7.6. Data management and statistical analyses

All study questionnaires were double entered into a study database using a program written in Filemaker Pro 12 (Filemaker Inc., Santa Clara, CA, USA).

Statistical analyses were done using Stata 11 (Stata Corp., College Station, TX, USA). Study variables were counted and summarized in frequency tables.

Means with corresponding standard deviations, or medians and interquartile ranges (IQRs) are presented, for normally or non-normally distributed variables, respectively. A probability of <0.05 was considered statistically significant.

8. Articles

Study 1

Knowledge gaps on paediatric respiratory infections in Morocco, Northern Africa

Imane Jroundi, Chafik Mahraoui, Rachid Benmessaoud, Cinta Moraleda, Badr Essaoud Benjelloun, Quique Bassat

Arch Public Health. 2015 Jun 15; 73(1):28

Study 2

The epidemiology and aetiology of infections in children admitted with clinical severe pneumonia to a university hospital in Rabat, Morocco

Imane Jroundi, Chafiq Mahraoui, Rachid Benmessaoud, Cinta Moraleda, Houssain Tligui, Myriam Seffar, Salma Ech-Cherif El Kettani , Badr Sououd Benjelloun, Saad Chaacho, Carmen Muñoz-Almagro, Joaquim Ruiz, Pedro L. Alonso, Quique Bassat

Journal of tropical pediatrics. 2014, 60 (4): 270-8

Study 3

Risk factors for a poor outcome among children admitted with clinically severe pneumonia to a university hospital in Rabat, Morocco.

Imane Jroundi ,Chafiq Mahraoui, Rachid Benmessaoud, Cinta Moraleda, Houssain Tligui, Myriam Seffar, Salma Ech-Cherif El Kettani , Badr Sououd Benjelloun, Saad Chaacho, Carmen Muñoz-Almagro, Joaquim Ruiz, Pedro L. Alonso, Quique Bassat.

International Journal of Infectious Diseases. 2014, S1201-9712(14) 01612-9.

.

Study 4

A comparison of human Metapneumovirus and Respiratory Syncytial virus WHO-defined Severe Pneumonia in Moroccan children

Imane Jroundi, Chafiq Mahraoui, Rachid Benmessaoud, Cinta Moraleda, Houssain Tligui, Myriam Seffar, Salma Ech-Cherif El Kettani, Badr Sououd Benjelloun, Saad Chaacho, Carmen Muñoz-Almagro, Joaquim Ruiz, Pedro L Alonso, Quique Bassat

Epidemiology and Infections. 2015 Jul 6:1-11

Study 5

Antibiotic Usage Prior and During Hospitalization for Clinical Severe Pneumonia in Children under Five Years of Age in Rabat, Morocco.

Imane Jroundi, Chafiq Mahraoui, Rachid Benmessaoud, Cinta Moraleda, Houssain Tligui, Myriam Seffar, Salma Ech-Cherif El Kettani , Badr Sououd Benjelloun, Saad Chaacho, Carmen Muñoz-Almagro, Joaquim Ruiz, Pedro L. Alonso, Quique Bassat.

Antibiotics 2013, 2, 450-464.

Study 6

Streptoccoccus pneumoniae carriage among healthy and sick paediatric patients before the generalized implementation of the 13-valent pneumococcal vaccine in Morocco, 2010-2011.

Imane Jroundi, Chafiq Mahraoui, Rachid Benmessaoud, Cinta Moraleda, Houssain Tligui, Myriam Seffar, Salma Ech-Cherif El Kettani, Badr Sououd Benjelloun, Saad Chaacho, Carmen Muñoz-Almagro, Pedro L Alonso, Quique Bassat

Submitted to the Journal of infection and public health (submitted and under review)

9. Summary of results and conclusions

Study 1

Knowledge gaps on paediatric respiratory infections in Morocco, Northern Africa

Results

- The systematic literature review collected publication regarding pediatric ARIs over a period of17 years. 33 documents were screened: 17 original articles published in peer review journals, 12 in English language and five in French, one original paper was published in French language in a Moroccan peer reviewed journal. Four abstracts were published in French language and two in English. And three MOH reports were found related to the topic. From the school of medicine libraries', eight unpublished doctoral theses were extracted.
- These documents are related to the description of cases admitted within the universities hospitals, the description of the samples obtained from hospitalized patients with ARI, studies conducted within the community and finally to the annuals reports of the national health statistics.

Data available regarding ARIs in Moroccan children

- The world health statistics reports indicated that in 2012, ARI remained the leading cause of pediatric (<5) mortality in Morocco, accounting for a total of 13% of all deaths in this age group. Additionally, only half of the children with suspected pneumonia were taken to an appropriate health provider, and 49% of the patients suffering from pneumonia received antibiotics.
- National health statistics annual reports from 2005 to 2012 showed an important decrease of ARI-associated deaths among children aged from 2 years to 59 months. However, no changes can be observed for infants during the same period. The most commonly notified cases during this period were pneumonia cases, followed by the severe pneumonia cases, which occurred more frequently in infants. Almost 54% of the cases were reported from rural areas.
- In 2000 ARI accounted for 30% of all pediatric emergency consultations and 20% of the total number of admissions in the pediatric infectious disease ward. In 2010, data collected from the same hospital and ward indicated an identical frequency regarding the number of visits with ARI, but 42% of them were admitted.
- The clinical patterns of ARI were described in descriptive studies, mostly case studies, in admitted patients in university hospitals. Throughout the past 17 years, the clinical

presentation of ARI has varied. In the past there were predominantly lobar pneumonia episodes of presumed bacterial origin, whereas nowadays cases have a milder clinical presentation, and are more often presumed viral origin.

- Before the pandemic episode of influenza H1N1 in 2008, few studies had described the burden of viruses as causes of ARI among children. In 2014, one study reported that the most commonly identified viruses included Rhinovirus followed by Respiratory syncytial virus (RSV) and Adenovirus in children under five of age admitted in tertiary level hospital.
- The active microbiological surveillance conducted by the university hospital of Casablanca from 1994 to 1998, reported that the most isolated germs included, in order of frequency, the *Staphylococcus aureus*, followed by *Streptococcus pneumoniae* and H*aemophilus influenzae* type b.
- Data of over 14 years of ongoing surveillance on antibiotic susceptibility of *Streptoccoccus pneumoniae*, showed an important decrease of the antibiotic susceptibility, which was more prevalent among children´s isolates. The lowest susceptibility was noticed for penicillin.
- The most prevalent *Streptoccoccus pneumoniae´*s serotypes isolated among these admitted patients were 6A, 19F and 6B. The 13-valent anti pneumococcal vaccine, introduced into the Moroccan national immunization program in 2011, would cover up to 85% of those invasive isolates.
- The main independent risk factors for death or the requirement of intensive care unit include prematurity, exposure to passive smoking at home, history of fever, cyanosis, pallor, ronchis at chest auscultation, unconsciousness on admission, and Human metapneumovirus infection.

<u>Knowledge gaps</u>

- Lack of consistent and homogeneous cases definitions for ARI within the different sources of data (primary health care, university hospital and laboratory)
- Dispersion and segmentation of the data related to ARI hinder the synthesis of ARI trends and the impact assessment of the preventive strategies and the clinical management.
- Absence of a systematic microbiological surveillance for the etiology of ARI in the hospital setting
- Generalized absence of data for the etiology of non-severe ARI within the community
- Lack of investigation on the social determinants of ARI and the access to care
- Lack of evidence on the role of air pollution as a contributor to the incidence and the severity of ARI

- There is no evaluation or control of the effectiveness and adequacy of the antibiotics prescribed for ARI within the IMCI program.
- The epidemiological indicators which could permit the study of the impact of the introduction of conjugate pneumococcal vaccines are not collected

Conclusions

- Data regarding epidemiological, clinical, and microbiological aspects of ARIs in Morocco are scarce. As a result, evidence based management and preventive strategies are difficult to implement and to evaluate.
- Efforts should be made towards the development of research strategies on all the fields of social health determinants of ARIs, to permit a holistic approach for understanding the determinants of ARI in Morocco.
- Moroccan researchers should attempt to disseminate more widely the data they generate so that it reaches the wider scientific community and can be used to provide reliable indicators for national and international policy makers to guide future preventives and therapeutic strategies based on solid evidence.

Study 2

The epidemiology and aetiology of infections in children admitted with clinical severe pneumonia to a university hospital in Rabat, Morocco

Results

Patient's characteristics and clinical presentation

- During the 14-month-long study period, 3202 children aged 2–59 months with respiratory symptoms visited the HER emergency room, 1334 (42%) were hospitalized, and 700 (52.4%) were included in the final analysis.
- Females represented 35.9% (251/700) of the hospitalized population, and mean age was 21.5 months
- Vaccination compliance according to Morocco's public expanded program of immunization calendar was generally high (87%), despite a poor uptake of the pneumococcal conjugate vaccine (117/700, 16.7%) which had recently been introduced into the EPI national calendar.
- Almost one-third of patients (206/700; 29%) reported having used antibiotics prior to admission.
- Median duration of illness prior to hospitalization was 2 days (IQR 2–4). Fever was present in over two-thirds of the patients.
- Wheezing was the more common respiratory sign (66%), followed by rhonchi (51%). Hypoxemia was present in 8% of the patient (53/671).
- Mild or more severe malnutrition was frequent (30% of patients had a WAZ score ≤1 SD)
- In 72% of cases of admitted children fulfilling the Who definition for clinical pneumonia, chest X-rays were considered normal. In 19% of these children, X-Ray showed typical alveolar consolidations, 1% showed pleural effusion and approximately 8% showed other non-alveolar infiltrates
- 23, 28 and 22% of the patients had respectively increased CRP, PCT or total white blood cell count values
- 9% of patients had low plasmatic zinc levels.

Aetiology

- 3.5% (24/690) were confirmed cases of Invasive Bacterial Disease, detected either by conventional blood culture or molecular techniques.

- Three invasive Hib episodes were detected during the whole study period (one by blood culture and two by PCR)
- Invasive pneumococcal disease was also infrequent, only 10 episodes, 80% of which were detected through the use of PCR in blood.
- Serotypes 6A, 19F/B/C and 6B accounted for almost two-thirds of all circulating serotypes of *Streptococcus pneumonia.*
- Nasal and/or pharyngeal carriage of either *S. pneumoniae* or Hib was common among recruited children (23 and 12%, respectively).
- Vaccine coverage of circulating serotypes with the currently implemented 13-valent pneumococcal conjugate vaccine was 40 (4/10) and 67% (113/169) for IBD and nasal/pharyngeal carriage, respectively.
- The nasopharyngeal carriage of at least one respiratory virus in the nasopharynx was almost universal (628/683, 91.9%) in these patients. Mixed (double, triple, quadruple or even quintuple) infections occurred in 40% of the hospitalized patients.
- Rhinovirus was the most commonly detected virus (360 cases, 52.7%), followed by RSV (124 cases, 18.2%) and adenovirus (17%), and 61 of the inpatients (8.9%) had an hMPV infection.
- Among RSV positive infections, 68 (54.8%) were single RSV-Ainfections, 9 (7.3%) single RSV-B infections, and the remaining 47 (37.9%) mixed RSV-A/RSV-B infections. A single case in this series presented with a mixed hMPV RSV infection.

<u>Epidemiological aspects</u>

- Bronchiolitis episodes were essentially restricted to the coldest season
- Pneumonia and bronchitis episodes did not show a clear seasonal variation.

<u>Patient's management</u>

- The Median duration of admission was 4 days (IQR 2–7).
- 41% (286/700) of the recruited patients received intra-hospital antibiotics
- The proportion of patients being prescribed corticosteroids was 57%. 72% received bronchodilators and 76% received oxygen.
- Eight percent (57/699) of the patients required admission to the intensive care unit, where CFRs was 42%.
- The overall Case fatality rate was 4.1 %.
- Only 28% of the patients at the time of discharge had a clinical diagnosis of bacterial pneumonia (associated CFR: 6.2%) according to the discharging physician.

Conclusions

1. The data collected shows the high burden of respiratory infections among Moroccan children, as detected in Rabat's HER, and raises a major public health alarm, particularly because of the unexpectedly high associated in-hospital CFRs.
2. Invasive IBD was highly infrequent and the majority of patients had viral infections and/or wheezing-related conditions. This can testify the epidemiological transition of the etiologies of ARI and justify the urgent need of the implementation of surveillance systems that will help the recognition of the etiology of ARI, so as to improve their management.
3. The low incidence of IBD could partly be explained by antibiotic usage prior to hospitalization and by the low diagnostic yield of the conventional blood culture method. This underlines the importance of using more sensitive diagnostic tests in this setting, such as PCR to increase the detection of the pathogens related to ARI in children.
4. This study provides the first series of comprehensive epidemiological, clinical and laboratory data describing Moroccan children admitted with CSP. Surveillance exercises such as this should be continued and considered as baseline for further longitudinal studies and research regarding paediatric respiratory infections in the area. This may enable the identification of the trend of ARI ´s etiologies, their distribution and clinical presentations, and eventually contribute to improve their management.

Study 3

Risk factors for a poor outcome among children admitted with clinically severe pneumonia to a university hospital in Rabat, Morocco.

Results

- During the 14-month-long study period, among 3202 children aged 2–59 months who visited the HER emergency room with respiratory symptoms, 1334 (42%) were hospitalized, and 700 (52.4%) complied with the WHO- severe pneumonia case definition.
- 27.2% (187/689) of these patients were assigned to the poor prognosis group. Corresponding to children who died, or needed ICU admission or had a RISC score ≥3. While, almost 72.8% (502/689) children were assigned to the good prognosis group, corresponding to child which were discharged from hospital with clinical improvement , or did not need to be admitted to the intensive care unit , or having a RISC score <3.
- 55/689 (8%) patient from this series required an admission to the intensive care unit.
- 28 deaths were registered among 689 (4.1%) patients admitted.

Clinical characteristics of patients with poor prognosis

- Patient in the age group < 12 months were almost three times more at risk to belong to the poor prognosis category of patients (OR 2.8, 95% CI 1.97–2.98).
- They were more frequently premature (OR 2.50, 95% CI 1.24–5.04), or had co-morbidities (OR 3.06, 95% CI 1.37–6.83).
- These patients experienced a longer history of symptoms, corresponding to an average of 5.56 ± 7.85 days versus 3.40 ± 4.29 days in the good prognosis group (p = 0.001).
- They had received more frequently antibiotics before their admission, (36.36% vs 27.09%, p = 0.001)
- They were more frequently exposed to tobacco smoking at home (OR 1.79, 95% CI 1.18–2.72).
- They presented on admission more severe symptoms, including fever (OR 2.25, 95% CI 1.32–3.83), cyanosis (OR 2.09, 95% CI 1.05–4.15), pallor (OR 2.27, 95% CI 1.34–3.84) and impaired conscientiousness OR 16.63; 95% CI 4.81–57.45).
- They had rhonchi on auscultation (OR 2.45, 95% CI 1.58–3.79) and lower oxygen saturation (93.31 ± 6.35 versus 95.37 ± 3.12. p = 0.001).

- The history of previous admission for respiratory infection and wheezing, where found to be protective factors, indeed the history of asthma was the only independent risk factor for a positive outcome (OR 0.46, 95% CI 0.25–0.84).
- 16.5% (30/182) patients with poor prognosis were severely malnourished.
- Radiologically confirmed pneumonia was much more frequent (32.9% vs. 14.5%, p = 0.001) amongst children with a poorer outcome.
- The indication of antibiotics during their hospitalization was eight times higher in patients with poor prognosis (OR 8.75, p < 0.001).
- The length of stay were significantly more prolonged in this group (9.96 days vs. 4.31 days, p = 0.001).

Laboratory finding among patients with poor prognosis

- Children were more anaemic, 24 (16.11%)
- They had elevated biomarkers of infection (PCT and CRP). The PCT was almost four times more elevated in poor prognosis group (OR 3.94; 95%CI 2.69–5.77) and CRP two times higher (OR1.91; 95%CI 1.30–2.80).
- Importantly, no differences were seen according to the outcome group in the mean white blood cell count or in the prevalence of leukopenia or leukocytosis.
- Bacteraemia was more frequent (7.50% vs2.03%, p = 0.001)
- hMPV was found two times more frequently among this group (OR 2.13; 95%CI 1.13-4.02). The distribution of the others viruses identified in this series, namely the *Adenovirus*, *influenza, parainfluenza*, *Respiratory syncitial virus* was not statistically different between both groups.

Conclusions

1. An unacceptable high case fatality rate among children admitted with severe acute respiratory infection was registered in this tertiary level hospital
2. Infants were more likely to be at risk for poor prognosis in this setting; they may need a more conservative approach in terms of management.
3. The risk factors of poor prognosis found in this series were similar to those already described in other settings and are more likely to be preventable and managed at the community level though enhanced maternal education to early recognize the severity of ARI episode, and to improve her child nutritional status and the community health education to avoid indoor smoking and

4. An early recognition and a suitable evaluation of the clinical risk factors by the clinicians would help to improve their prognosis and better orient the clinical management.
5. This study showed for the first time in this setting that hMPV was associated with poor outcomes. This may lead to suggest an active surveillance of this virus to better study its pathogenicity among children under five years of age.

Study 4

A comparison of human Metapneumovirus and Respiratory Syncytial virus WHO-defined Severe Pneumonia in Moroccan children

Results

- Among the 683 patients included in this study, 628 (91.9%) had at least one viral pathogen detected in their nasopharyngeal aspirate, and sixty-one (8.9%) were infected with hMPV and 124 (18.2%) with RSV.
- Among RSV positive infections, 68 (54.8%) were single RSV-A infections, 9 (7.3%) single RSV-B infections, and 47 (37.9%) had mixed RSV-A/RSV-B infections
- RSV cases presented a very clear seasonal pattern, with over 98% cases occurring between November and April (coinciding with the coldest and least humid months).
- hMPV were predominantly detected in two peaks, one occurring during the spring months and the other during the fall season.
- Both viruses were very similar in terms of demographics, patient history, past morbidity and co-morbidity, vaccination history, socio-economic background and family environment distribution and characteristics.
- The clinical presentation of hMPV cases referred a significantly commoner history of fever and runny nose on admission and had poorer nutritional status. They presented a higher proportion of elevated C-reactive protein (>5mg/dL) (36.1% vs. 21.8%, p=0.041).
- Cases of RSV were significantly more frequently pale on arrival (22.9% vs. 9.7%, p=0.015), wheezed more (70.2% vs. 52.5%, p=0.018) and had a significantly higher mean respiratory rate (64.6 vs. 59, p=0.009).
- RSV was more commonly associated to bronchiolitis episodes (41.1% RSV vs. 6.6% hMPV, p<0.001).
- hMPV was more frequently associated with pneumonia episodes (42.6% vs. 31.4%, p=0.135) or bronchitis/asthma (39.3% vs. 25.8%, p=0.060).
- Laboratory results showed with a higher proportion of anaemic patients in the RSV group (19.2% vs 5.9%, p=0.032), with a significantly lower mean haemoglobin value on admission (10.4 g/l vs 12.1 g/l, p=0.004) while hMPV cases presented a higher proportion of elevated C-reactive protein (>5mg/dL) (36.1% vs 21.8%, p=0.041).
- Normal Chest X-rays was found in 60.9% and 71.7% in RSV group and hMPV respectively. Pleural effusion/ lobar condensation were found in 24.3% in RSV cases and in 18.9%among hMPV cases.

- Both viruses coexisted frequently with other respiratory viruses (Rhinovirus, Coronavirus, Influenza and Parainfluenza or Adenovirus).
- hMPV cases carried twice the amount of pneumococci in their nasopharynx (29.5% vs. 12.9%, p=0.006), and were significantly more commonly viral monoinfections than RSV cases (49.2% vs. 29.0%, p=0.007).
- hMPV patients had a higher mean RISC severity score (1.8 vs 1.5, p=0.025), and were more commonly transferred to the intensive care unit (11.5% vs. 3.2%, p=0.026).
- RSV-infected patients required more bronchodilator treatment (p=0.011), while hMPV received more corticosteroids (p=0.001) and antibiotics (p=0.048).
- Four deaths were notified (4/124) in the RSV group: two cases were pneumonia cases, one was a bronchiolitis and one was bronchitis.
- Three deaths among 59 cases of hMPV were reported (two pneumonia cases and one bronchiolitis case).

Conclusions

1. This study confirms the high prevalence and the importance in this country of both RSV and hMPV infections as cause of paediatric respiratory-related admissions.
2. In our setting RSV and hMPV viruses show similar clinical symptomatology, but hMPV seems to be associated with a more severe evolution than RSV.
3. Long-term studies are needed to characterize disease pathogenesis of HMPV among children and to understand the host response. This will help design evidence-based identifying antiviral preventive and curatives strategies against these pathogens.

Study 5

Antibiotic Usage prior and during hospitalization for Clinical Severe neumonia in children under five years of age in Rabat, Morocco.

Results

Patient's characteristics

- 29.4% (206/700) of all children <5 years of age admitted during a 1 year-period in a tertiary hospital of Morocco for clinical severe pneumonia, had received antibiotics within the two weeks preceding their hospitalization.
- Antibiotics were principally prescribed by a physician (166/192; 86.5%), obtained directly at the pharmacy (21/192; 10.9%) and in 5 cases (2.6%) taken as self-medication.
- 30.6% (63/206) of patients having received pre-admission antibiotics had medical insurance.

Factors significantly associated to antibiotic prescription before the admission

- The mean age of children who received antibiotics was 19.7±13,8 in comparison to 22,3±14,8 among those who did not received antibiotics before their admission(p=0.03). 35.9% (74/206) of antibiotic prescription before admission occurred in children younger than 1 years of age.
- Children having received antibiotics prior to admission, had a shorter duration of breastfeeding: 8,4 ±7,5 vs 10.1±7,9 (p=0.03).
- They were not correctly vaccinated regarding the National Immunization program. (aOR 1.89; 95%CI 1.11-3.23)
- Their parents reported a history of fever (aOR 1.52; 95%CI 1.02-2.28), difficulties of feeding (aOR 1.59; 95%CI 1.11-2.28) and symptoms lasting over 7 days (aOR 3.98; 95%CI 2.17-7.31).

Characterization of patients which needed intra-hospital antibiotics prescription

- 40.9% (286/700) of the recruited children required antibiotic therapy.
- Among those who had received antibiotics before the admission 52.4% (108/206) needed intra-hospital antibiotics prescription.
- 31.6% (221/286) took one kind of antibiotics, while 7.2% (50/286); required two kind of antibiotics and further 2.1% (15/286) received three or more.
- Those children were young (25±15 ; 16,4 vs ± 12,2(p <0.01),they had severe clinical picture on arrival and longer duration of respiratory symptoms (3 ±4,3 vs 5.3±6,7 (p<0.01).

- The chest X-Ray of patients who needed antibiotics during their hospitalization showed pneumonia images, and they had a higher C - reactive protein or high procalcitonin plasmatic levels.
- Intra-hospital antibiotic prescription was clearly syndrome-oriented. Ninety-two percent (180/195) of the pneumonia cases of suspected bacterial origin received antibiotics. Only 13.8% (44/319) of the bronchitis episodes were treated with antimicrobials.
- Case fatality rate was higher among patients who required antibiotics during the admission (22/283; 7.8%) vs (6/407; 1.5%; $p < 0.001$).

Types and susceptibility of the antibiotic used in ambulatory setting and in the hospital setting

- Before the admission, at the community level, Amoxicillin/ clavulanic acid and oral amoxicillin were the two most frequently used antibiotics, respectively (73/206; 35.4%) and (50/206; 24.3%), followed by macrolides (47/206; 22.8%).
- In the hospital setting, the antibiotics more commonly prescribed included: cephalosporins (213/286; 74.5%), macrolides (60/286; 21%) gentamicin (39/286; 13.6%), Amoxicillin/ clavulanic acid (32/286; 11.2%) and amoxicillin (14/286; 4.9%)
- The *Streptococcus pneumoniae* isolated from blood culture and obtained from patient´s nasopharynx, showed a good susceptibility to most antibiotic tested. Resistance to amoxicillin was about 14.6%, regarding to Penicillin G it was about 10.3%, and 20.4% of resistance to erythromycin and 24.7% to Trimethoprim/ sulfamethoxazole.

Conclusions

1. In our population, 30% of the children admitted for severe ARI had received antibiotics before their admission. This low-to-moderate figure is lower to that reported in 2011at the national level for outpatient treatment of ARIs.
2. The high extra-hospital use of extended spectrum antibiotics (amoxicillin-clavulanic acid; macrolides, cephalosporins, identified in this series, raises some concerns about favouring the selection of antibiotic resistant pathogenic microorganisms.
3. Our series showed a virtually or even inexistent utilization of penicillin, a drug that remains first line recommendation for the treatment of most community-acquired upper respiratory tract infections in many developed countries.
4. Independent risk factors associated with pre-hospitalization usage of antibiotics in Rabat seem mostly related to the severity of the clinical picture or the duration of the illness.

5. During hospitalization, prescription of antibiotics seems to be syndrome-based, being more frequent in those cases oriented towards a bacterial origin, and much lesser among the wheezers.
6. The antibiotics used at hospital setting are based on currently existing and well-established international recommendations.
7. In this series, the susceptibility of common circulating respiratory bacteria to commonly used antibiotics remains reasonably high.

Study 6

Streptoccoccus pneumoniae carriage among healthy and sick paediatric patients before the generalized implementation of the 13-valent pneumococcal vaccine in Morocco, 2010-2011.

Results

- Seven hundred eligible patients for the study were admitted for severe clinical pneumonia during the one- year survey, and 200 healthy children were recruited from primary health care centers during one month.
- The proportion of infants among recruited children was 31% (214/700) in the admitted patients and 70% (138/195) in children from the community.
- Nasopharyngeal carriage *of streptococcus pneumonia* among admitted patients was 23% (159/697), and 40.5% (79/195) among children attending the primary care centers.
- Serotype distribution was assessed by molecular methods in 74.2% (118/ 159) in admitted children, and in 58.2 % (46 /79) cases of healthy children.
- The most frequent circulating serotypes included 6A, 6B and 19F/B/C. Representing respectively 29%, 18% and 18,6% in sick children and 16,5%, 12,6% and 14% in healthy children.
- Their proportion of *S.pneumoniae* carriage among healthy children was 2 to 8 times higher in children less than 12 months.
- While 19F/B/C was twice important among those who get the 13-valent pneumococcal conjugate vaccine, 6B was not isolated among unvaccinated children.
- The vaccines coverage of the circulating serotypes for which serotype data were available was 95.7% (113/118).

Conclusions

1. This study showed a moderately low nasal carriage of pneumococci, ranging from 23% to 40% in two very distinct paediatric populations, namely sick children with clinical severe pneumonia and healthy children. These proportions are lower than expected carriages rates of those serotypes most commonly described in the literature from developing countries.
2. Most of the serotypes identified are included in the current vaccine used by the national immunization program Introduced in November 2010.
3. Continuous monitoring and investigation for the serotype distribution of pneumococcal carriage and invasive disease remains necessary in Morocco, to adequately detect the emergence of new circulating serotypes, and prevent further morbidity and mortality as well to adequately as assess the impact of the recently implemented vaccine.

10. General conclusions

Study 1

1. Data on ARIs burden, aetiology and determinants in Morocco are sparse and insufficient to guide and to evaluate the preventive and curatives strategies conducted by the Ministry of Health and adopted by the physicians.
2. A national surveillance system on ARIs epidemiology, aetiology should be implemented and should involve both public and private sectors.
3. Research studies on the environmental determinants of ARI such as indoor and outdoor air pollution should be conducted and their results must be considered in the development of preventive health policies and regulation.
4. Moroccan researchers should attempt to disseminate more widely the data they generate, so that it reaches the wider scientific community and can be used to provide reliable indicators for national and international policy makers to guide future preventives and therapeutic strategies based on solid and updated evidence.

Study 2

5. The burden of respiratory infections among Moroccan children, as detected in Rabat's HER is significant. It is also particularly preoccupying because of the unexpectedly high associated in-hospital CFRs.
6. Morocco seems to have experienced an epidemiological transition, since the clinical presentations of ARI has shifted towards wheezing-related conditions, and also in the relation to the low incidence of IBD partly explained by the importance of the antibiotic usage practices prior to hospitalization. These conditions underline the importance of using more sensitive diagnostic tests in this setting, such as PCR to increase the detection of the pathogens related to ARI in children and to improve their management.

Study 3

7. Mothers/ caregivers and clinicians/ health workers at the community level should be taught to identify preventable factors of bad prognosis of ARI in order to trigger the appropriate action.
8. A suitable evaluation of the predictive factors of bad prognosis by the clinicians would orient the clinical management and will contribute to improve the prognosis of pneumonia in children.

Study 4

9. In Morocco, both RSV and hMPV infections are important causes of paediatric respiratory-related admissions.
10. hMPV is associated with a bad prognosis in our series.
11. As no specific curative or preventive treatment is available in Morocco for neither of those two viruses, an early recognition of these pathogens is important to improve the prognosis of the children affected by a good clinical management of the symptoms related to the disease.

Study 5

12. A rational use of antibiotics should be encouraged among health professionals who practice either in hospital or at the ambulatory setting, to slow the progression of the antibiotic resistance in Morocco, through continuing education in clinical pharmacology.
13. Stronger regulations on antibiotics sales would be a necessary measure to reduce the inappropriate use of Antibiotic.
14. Perceptions and attitudes of both antibiotic prescribers and users regarding antibiotics usage should be further investigated.

Study 6

15. Continuous monitoring and investigation of pneumococcal carriage and its serotype distribution and surveys of pneumococcal associated invasive bacterial disease remain necessary in Morocco, to adequately detect the emergence of new circulating serotypes as well as to assess the impact of the pneumococcal vaccines.

11. The way forward

The challenge now is to move forward from these six studies, and translate their conclusions into improvements in the national policies in the prevention and the control of ARIs among children. These data should guide on how to further design strategies to improve data identification, collection, analysis, dissemination and utilization, both at the clinical level and policy maker levels.

These studies make a contribution to the improvement of paediatric ARIs knowledge in Morocco by identifying and analysing the data collected systematically from the national health statistics and by underlying the knowledge gaps on the epidemiology and aetiology of ARI. Additionally, the specific surveillance conducted among admitted children provide a comprehensive picture of their clinical, epidemiological and etiological characteristics, as well to their prognosis factors and general response to available care. It highlights areas in which enhanced case management may be possible, and provides a first baseline for further research regarding paediatric respiratory infections.

These studies need to be pursued by more research in different urban, suburban and rural geographical areas within the country, at different health facility levels, and also should encourage the involvement of the private sector as an important new source of data. A wider research on all social determinants' of the disease, both in terms of accessibility to health care, atmospheric pollution exposure and to indoor pollution, should be explored for a holistic approach to the care and prevention of ARI. This would also facilitate the translation of the knowledge gained into policy and may guarantee the sustainability of the production of reliable data, which will orient the best preventive and management strategies to decrease the current impact of ARIs in child health in Morocco.

These next steps can be organized in 4 axes: epidemiological surveillance, research, Training and education and health policies regulations.

Epidemiological surveillance

The goal is to improve data availability, reliability and relevance, by:

1. Merging the available databases to provide a comprehensive view of the epidemiology and trends of ARI. Such as merging the database of the immunization program to the database of the health care facilities and hospitals

2. Reporting annually the synthesis of data regarding the incidence of ARI (cases of severe and very severe pneumonia) and their distribution by age, the hospitalization and mortality rates

4. Establishing a national ARI surveillance involving urban and rural sentinel health centers and private practitioners for monitoring the nasopharyngeal carriage of *S.pneumoniae*

5. Establishing a continuing microbiological surveillance of the antibiotic resistance by integrating clinical data from patients and community samples.

Research

The most urgent questions that still need to be addressed include:

1. Investigating the social and environmental determinants that influence the incidence and severity of ARI.

2. Investigating the physicians and patients practices of in terms of IRA related antibiotic prescribing and consumption

3. Conducting studies to explain the very high rate of in-hospital CRF, and investigating the attribuable rate of nosocomial infections and using post mortem evaluations and/or sampling to reach a“ gold standard” aetiological diagnosis.

4. Investigating the pathogenic role of hMPV in Moroccan child

5. Conducting case-control studies among children carrying viruses to identify the pathogenic role of these viruses

6. Conducting prospective cohort studies to identify the long-term effects of different serotypes of rhinovirus in infected child.

Training and education

Based on the evidence produced, and the local epidemiology of ARI, there is a need of:

1. Providing continuous training for physicians on the diagnosis and management of patients with ARI and in clinical pharmacology.

2. Train the laboratory technicians at the peripheral level for the diagnosis of AR.

3. Strengthen ARI prevention programs by educating the mothers to early recognize the signs of severity and by the promotion of stopping cigarette smoking in the community level.

Health policies regulations

Advocacy towards the regulation of antibiotics sale

12. Bibliography

1. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet 2015; 385: 117–71*
2. Zar HJ and Ferkol TW. The global burden of respiratory disease Impact on child health. *Pediatric Pulmon*, 2014; 49(5): 430-4
3. Nair H, Simoes E A F, Rudan I, Gessner BD, Baumgartner EA, Zhang JSF et al. Global and regional burdenof hospital admissions for severe acute lower respiratory infections in young children in 2010: A systematic analysis. *Lancet.* 2013; 381:1380-90.
4. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*. 2015;31;385(9966):430-40
5. Rajaratnam JK, Marcus JR, Flaxman AD, Wang H, Levin-Rector A, Dwyer L, Costa M, Lopez AD, Murray CJ. Neonatal, postneonatal, childhood and under-5 mortality for 187 countries, 1970-2010: a systematic analysis of progress towards Millennium Development Goal 4. *Lancet*. 2010 Jun 5;375(9730):1988-2008
6. World Health Assembly. Accelerated progress towards achievement of Millennium Development Goal 4 to reduce child mortality: prevention and treatment of pneumonia WHA 63.24. *World Health Organization*; Geneva, 2010.
7. Dagan R, Bhutta ZA, de Quadros C A, Garau J, Klugman KP et al. The Remaining Challenge of Pneumonia: The Leading Killer of Children. *Pediatr Infect Dis J* 2011; 30: 1–2.
8. Zar H J, Madhi S A, Aston S J, Gordon S B. Pneumonia in low and middle income countries:progress and challenges. *Thorax* 2013; 68:1052–1056.
9. Sonego M, Pellegrin MC, Becker G, Lazzerini M. Risk factors for mortality from acute lower respiratory infections (ALRI) in children under five years of age in low and middle-income countries. A systematic review and metanalysis of observational studies. *PLoS ONE* .2015;10(1):e0116380.
10. Jackson S, Mathews KH, Pulanić D, Falconer R, Rudan I, Campbell H, Nair H. Risk factors for severe acute lower respiratory infections in children – a systematic review and meta-analysis. *Croat Med J* 2013;54:110-21
11. Global action Plan for Prevention and control of Pneumonia (GAPP). *World Health Organization/The United Nations Children's Fund (UNICEF)*; Geneva 2009
12. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet.* 2010;375(9730):1969-1987.
13. O'Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. *Lancet.* 2009;374(9693):893-902.
14. Watt JP, Wolfson LJ, O'Brien KL, et al. Burden of disease caused by Haemophilus influenzae type b in children younger than 5 years: global estimates. *Lancet.* 2009;374(9693):903-911.

15. Obaro SK, Madhi SA. Bacterial pneumonia vaccines and childhood pneumonia: are we winning, refining, or redefining? *Lancet Infect Dis.* 2006;6(3):150-161.

16. Nieto Guevara J, Daza C, Smith R. Decrease in Hospitalizations for Pneumonia in Children under Five Years of Age in an Indian Reservation in Panama after the Introduction of the Heptavalent Pneumococcal Conjugate Vaccine (PCV7). *Int J Pediatr.* 2013;2013:514578

17. Theodoratou E, Al-Jilaihawi S, Woodward F, Ferguson J, Jhass Y, Balliet M, Kolcic I, Sadruddin S, Duke T, Rudan I and Campbell H. The effect of case management on childhood pneumonia mortality in developing countries. *Inter J of Epidemiology* 2010; 39:i155–i171

18. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet.* 2010;375(9725):1545-1555.

19. Falade AG, Ayede AI. Epidemiology, aetiology and management of childhood acute community-acquired pneumonia in developing countries--a review. *African journal of medicine and medical sciences.* 2011;40(4):293-308.

20. Gove S. Integrated management of childhood illness by outpatient health workers: technical basis and overview. The WHO Working Group on Guidelines for Integrated Management of the Sick Child. *Bull World Health Organ.* 1997;75 Suppl 1:7-24.

21. Arifeen SE, Hoque DM, Akter T, et al. Effect of the Integrated Management of Childhood Illness strategy on childhood mortality and nutrition in a rural area in Bangladesh: a cluster randomised trial. *Lancet.* 2009;374(9687):393-403.

22. English M, Berkley J, Mwangi I, et al. Hypothetical performance of syndrome-based management of acute paediatric admissions of children aged more than 60 days in a Kenyan district hospital. *Bull World Health Organ.* 2003;81(3):166-173.

23. Nadjm B, Amos B, Mtove G, et al. WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense Plasmodium falciparum transmission: prospective study. *BMJ.* 2010;340:c1350.

24. Simoes EA, Peterson S, Gamatie Y, et al. Management of severely ill children at first-level health facilities in sub-Saharan Africa when referral is difficult. *Bull World Health Organ.* 2003;81(7):522-531.

25. Reyburn H, Mwakasungula E, Chonya S, et al. Clinical assessment and treatment in paediatric wards in the north-east of the United Republic of Tanzania. *Bulletin of the World Health Organization.* 2008;86(2):132-139.

26. English M, Esamai F, Wasunna A, et al. Assessment of inpatient paediatric care in first referral level hospitals in 13 districts in Kenya. *Lancet.* 2004;363(9425):1948-1953.

27. Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; 380: 2197–223.

28. Institute for Metrics and Evaluation. Pushing the pace. Progress and challenges in fighting childhood pneumonia. Institute for metrics and evaluation. Seattle: Institute for Metrics and Evaluation, 2014.

29. Head MG, Fitchett JR, Cooke MK, et al. Investments in respiratory infectious disease research 1997–2010: a systematic analysis of UK funding. *BMJ Open* 2014; 4: e004600

30. Head MG, Fitchett JR, Newell ML, Scott JAG, Clarke SC, Atun R. Investment in pneumonia and pneumococcal research. *Lancet Infect Dis* 2014; 14: 1037–38

31. World Health Organization. Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. Geneva, 2013 revised edition.

32. Rambaud-Althaus C, Althaus F, Genton B, D'Acremont V. Clinical features for diagnosis of pneumonia in children younger than 5 years: a systematic review and meta-analysis. *Lancet Infect Dis* 2015;15: 439–50

33. Subhi R, Adamson M, Campbell H, et al. The prevalence of hypoxaemia among ill children in developing countries: a systematic review. *Lancet Infect Dis* 2009; 9: 219–27.

34. Bosch AATM, Biesbroek G, Trzcinski K, Sanders EAM, BogaertD. Viral and Bacterial Interactions in the Upper Respiratory Tract. *PLoS Pathog* 2013; 9(1): e1003057.

35. Briand S. Research needs for the battle against respiratory viruses. Geneva: World Health Organization;2013

36. Manjarrez-Zavala MA, Rosete-Olvera DP, Gutiérrez-González LH, Ocadiz-Delgado R and Cabello-Gutiérrez C (2013). Pathogenesis of Viral Respiratory Infection, Respiratory Disease and Infection - A New Insight, Dr. Mayank Vats (Ed.), ISBN: 978-953-51-0968-6, InTech, DOI: 10.5772/54287.Available from: http://www.intechopen.com/books/respiratory-disease-and-infection-a-new-insight/pathogenesis-of-viral-respiratory-infection

37. Kadioglu A, Weiser JN, Paton JC and Andrew PW. The role of Streptococcus pneumonia virulence factors in host respiratory colonization and disease. *Nature Rev Microb* 2008, 6.288-301

38. Joon Young Song, Moon H. Nahmand M. Allen Moseley. Clinical Implications of Pneumococcal Serotypes: Invasive Disease Potential, Clinical Presentations, and Antibiotic Resistance. *J Korean Med Sci* 2013; 28: 4-15

39. Weinberger DM, Harboe ZB, Sanders, Elisabeth A M, Ndiritu M, Klugman KP, Rückinger S, Dagan R, Adegbola R, Cutts F, Johnson HL, O'Brien KL, Scott JA, Lipsitch M: Association of serotype with risk of death due to pneumococcal pneumonia: a metaanalysis. *Clin Infect Dis* 2010, 51:692-9.

40. Rodrigo C, Lim WS: The relevance of pneumococcal serotypes. *Curr Infect Dis Rep* 2014, 16:403.

41. Blumental S, Granger-Farbos A, Moïsi JC, Soullié B, Leroy P, Njanpop-Lafourcade B-M, et al. Virulence Factors of Streptococcus pneumoniae. Comparison between African and French Invasive Isolates and Implication for Future Vaccines. *PLoS ONE* 2015, 10(7): e0133885

42. Feldman C, Anderson R: Antibiotic resistance of pathogens causing community-acquired pneumonia. *Semin Respir Crit Care Med* 2012, 33:232-43.

43. Navarro-Torné A, Dias JG, Hruba F, Lopalco PL, Pastore-Celentano L, Gauci AJ; Invasive Pneumococcal Disease Study Group. Risk factors for death from invasive pneumococcal disease, Europe, 2010. *Emerg Infect Dis.* 2015; 21(3):417-25.

44. Cardoso MR, Cousens SN, De Góes Siqueira LF, Alves FM, D'Angelo LA et al. Crowding: risk factor or protective factor for lower respiratory disease in young children? . *BMC Public Health*. 2004; 3(4):19

45. Kleimola LB, Patel AB, Borkar JA, Hibberd PL. Consequences of household air pollution on child survival: evidence from demographic and health surveys in 47 countries. *Int J Occup Environ Health*. 2015 6:2049396715Y0000000007. [Epub ahead of print]

46. Kilabuko JH, Nakai S. Effects of cooking fuels on acute respiratory infections in children in Tanzania.*Int J Environ Res Public Health*. 2007;4(4):283-8

47. Smith KR, Samet JM, Romieu I, et al. Indoor air pollution in developing countries and acute lower respiratory infections in children. *Thorax*. 2000; 55:518 –532.

48. Hammit LL, Murdoch DR, Scott JA et al. Specimen collection for the diagnostic of pediatric pneunmonia. *CID* 2012; 54(Suppl 2): S132-s9.

49. Lynch T, Bialy L, Kellner JD, Osmond MH, Klassen TP, et al. (2010) A Systematic Review on the Diagnosis of Pediatric Bacterial Pneumonia: When Gold Is Bronze. *PLoS ONE* 5(8): e11989.

50. Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. *Bulletin of the World Health Organization.* 2005;83(5):353-359.

51. Edwards M, Lawson Z, Morris S, et al. The presence of radiological features on chest radiographs: how well do clinicians agree? *Clinical radiology.* 2012;67(7):664-668.

52. Bloomfield FH, Teele RL, Voss M, Knight DB, Harding JE. Inter- and intra-observer variability in the assessment of atelectasis and consolidation in neonatal chest radiographs. *Pediatric radiology.* 1999;29(6):459-462.

53. Albaum MN, Hill LC, Murphy M, et al. Interobserver reliability of the chest radiograph in community-acquired pneumonia. PORT Investigators. *Chest.* 1996;110(2):343-350.

54. Hagaman JT, Rouan GW, Shipley RT, Panos RJ. Admission chest radiograph lacks sensitivity in the diagnosis of community-acquired pneumonia. *The American journal of the medical sciences.* 2009;337(4):236-240.

55. Johnson J, Kline JA. Intraobserver and interobserver agreement of the interpretation of pediatric chest radiographs. *Emergency radiology.* 2010;17(4):285-290.

56. Loeb MB, Carusone SB, Marrie TJ, et al. Interobserver reliability of radiologists' interpretations of mobile chest radiographs for nursing home-acquired pneumonia. *Journal of the American Medical Directors Association.* 2006;7(7):416-419.

57. O'Grady KA, Torzillo PJ, Ruben AR, Taylor-Thomson D, Valery PC, Chang AB. Identification of radiological alveolar pneumonia in children with high rates of hospitalized respiratory infections: comparison of WHO-defined and pediatric pulmonologist diagnosis in the clinical context. *Pediatric pulmonology.* 2012;47(4):386-392.

58. Murdoch DR, O'Brien KL, Scott JA, et al. Breathing new life into pneumonia diagnostics. *Journal of clinical microbiology.* 2009;47(11):3405-3408.

59. Banya WA, O'Dempsey TJ, McArdle T, Lloyd-Evans N, Greenwood BM. Predictors for a positive blood culture in African children with pneumonia. *The Pediatric infectious disease journal.* 1996;15(4):292-297.

60. Gonsalves WI, Cornish N, Moore M, Chen A, Varman M. Effects of volume and site of blood draw on blood culture results. *Journal of clinical microbiology.* 2009;47(11):3482-3485.

61. Ramirez M, Melo-Cristino J. Expanding the diagnosis of pediatric bacteremic pneumococcal pneumonia from blood cultures to molecular methods: advantages and caveats. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2010;51(9):1050-1052.

62. Avni T, Mansur N, Leibovici L, Paul M. PCR using blood for diagnosis of invasive pneumococcal disease: systematic review and meta-analysis. *Journal of clinical microbiology.* 2010;48(2):489-496.

63. Resti M, Moriondo M, Cortimiglia M, et al. Community-acquired bacteremic pneumococcal pneumonia in children: diagnosis and serotyping by real-time polymerase chain reaction using blood samples. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2010;51(9):1042-1049.

64. Dowell SF, Garman RL, Liu G, Levine OS, Yang YH. Evaluation of Binax NOW, an assay for the detection of pneumococcal antigen in urine samples, performed among pediatric patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2001;32(5):824-825.

65. Charkaluk ML, Kalach N, Mvogo H, et al. Assessment of a rapid urinary antigen detection by an immunochromatographic test for diagnosis of pneumococcal infection in children. *Diagnostic microbiology and infectious disease.* 2006;55(2):89-94.

66. Martínez-Roig A, Salvadó M, Caballero-Rabasco MA, Sánchez-Buenavida A, López-Segura N, Bonet-Alcaina M. Viral Coinfection in Childhood Respiratory Tract Infections. *Arch Bronconeumol*. 2014 Mar 22. pii: S0300-2896(14)00069-6

67. Obasi CN, Barrett B, Brown R, Vrtis R , Barlow S, Muller D, Gern J. Detection of viral and bacterial pathogens in acute respiratory infections. *Journal of Infection* 2014; 68: 125e130.

68. Mitchell A. M. and Mitchell T.J. Streptococcus pneumoniae: virulence factors and variation. *Clin Microbiol Infect* 2010; 16: 411–418

69. Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, Konradsen HB, Nahm MH. Pneumococcal Capsules and Their Types: Past, Present, and Future. *Clin Microbiol Rev.* 2015; 28(3):871-99.

70. World Health Organization. Antimicrobial resistance: global report on surveillance 2014. Geneva;2014

71. Reynolds CA, Finkelstein JA, Ray GT, et al. Attributable healthcare utilization and cost ofpneumonia due to drug-resistant *Streptococcus pneumoniae*: a cost analysis. *Antimicrob Resist Infect Control* 2014; 3: 16.

72. Schrag SJ, Beal B, Dowell S. Resistant pneumococcal infections. Geneva: *World Health Organization*; 2001.

73. Kronman MP, Zhou Chuan, Mangione-Smith Rita. Bacterial prevalence and antimicrobial prescribing trends for acute respiratory tract infections. *Pediatrics.* 2014;134;e956

74. Billeter M; American Society of Health-System Pharmacists. ASHP therapeutic position statement on strategies for identifying and preventing pneumococcal resistance. *Am J Health Syst Pharm.* 2004. 15; 61 (22):2430-5.

75. Stephen L. Cochi J, Ward I. *Haemophilus influenzae* Type b. Bacterial Infections of Humans.1991, pp 277-332

76. Centers for Disease Control and Prevention. Epidemiology and Prevention of Vaccine-Preventable Diseases: Haemophilus influenzae type B. Pinkbooks 2015, 13th Edition**.**

77. Davis S, Feikin D, Johnson HL. The effect of Haemophilus influenzae type B and pneumococcal conjugate vaccines on childhood meningitis mortality: a systematic review. *BMC Public Health*. 2013;13 Suppl 3:S21.

78. C. O. Obonyo. J. Lau. Efficacy of Haemophilus influenzae type b vaccination of children: a meta-analysis. *Eur J Clin Microbiol Infect Dis* 2006, 25: 90–97

79. Braikat M, Barkia A, El Mdaghri N, Rainey JJ, Cohen AL, Teleb N. Vaccination with Haemophilus influenzae type b conjugate vaccine reduces bacterial meningitis in Morocco. *Vaccine*. 2012;30 (15):2594-9.

80. Wang K, Gill P, Perera R, Thomson A, Mant D, Harnden A. Clinical symptoms and signs for the diagnosis of *Mycoplasma pneumoniae* in children and adolescents with community-acquired pneumonia. *Cochrane Database of Systematic* Reviews 2012, Issue 10. Art. No.: CD009175

81. Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of *Mycoplasma pneumoniae*infections. *FEMS Microbiol Rev*. 2008;32:956–73

82. Waites KB and Talkington DF. Mycoplasma pneumoniae and its Role as a Human Pathogen *Clinical Microbiology Reviews*. 2004 ; 17 (4): : 697–728

83. Archer G L. Staphylococcus aureus: A Well-Armed Pathogen. Clin Infect Dis1998;26:1179–81

84. Kallen AJ, Reed C, Patton M, Arnold KE, Finelli L, Hageman J. Staphylococcus aureus community-onset pneumonia in patients admitted to children's hospitals during autumn and winter of 2006–2007.*Epidemiol Infect.* 2010; 138(5):666–672.

85. Stuckey-Schrock K, Hayes BL and. George CM. Community-Acquired Pneumonia in Children. *Am Fam Physician.* 2012;86(7):661-667

86. Rudan I,Boschi-Pinto C, Biloglav Z, Mulhollandd K & Campbell H. Epidemiology and etiology of childhood pneumonia. *Bull World Health Organ* 2008; 86:408–416.

87. Lukšić I,Kearns PK,Scott F,Rudan I,Campbell H,Nair H. Viral etiology of hospitalized acute lower respiratory infections in children under 5 years of age -- a systematic review and meta-analysis. *Croat Med J.* 2013 Apr;54(2):122-34

88. Surveillance for Respiratory Syncytial Virus in CDC's Global Disease Detection Network: Epidemiology, Disease Burden and Clinical Characteristics. *J Infect Dis.* 2013;208(S3):S165-246

89. Del Vecchio A, Ferrara T, Maglione M, Capasso L and Raimondi F. New perspectives in Respiratory Syncitial Virus infection. *J Matern Fetal Neonatal Med,* 2013; 26(S2): 55–59

90. Henrickson K J. Parainfluenza Viruses. *Clin. Microbiol. Rev*. 2003, 16(2): 242-264

91. Psarras S, Papadopoulos NG, Johnston SL, Parainfluenza virus. In: Zuckerman JA, Banatvala JE, Pattison JR, Grifiths PD, Schob BD, eds. Principles and practices of clinical virology: John Wiley& Sons ldt, 2004.

92. Edwards K M, Zhu Y, Griffin MR, Weinberg G A, Hall CB , Szilagyi PG. and al. Burden of Human Metapneumovirus Infection in Young Children. *N Engl J Med*. 2013 February 14; 368(7): 633–643

93. Panda S, Mohakud NK, Pena L, Kumar S. Human metapneumovirus: review of an important respiratory pathogen. *Int J Infect Dis*. 2014 ;25:45-52

94. Warris A and de Groot R. Human metapneumovirus infection. 317. Pediatric infectious diseases revisited. Edited by Horst Schroten and Stefan Wirth. Germany; 2007

95. Nair H, Brooks WA, Kartz M et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet*. 2011; 378: 1917–30.

96. Potter CW. Influenza. In: Zuckerman JA, Banatvala JE, Pattison JR, Grifiths PD, Schob BD, eds. Principles and practices of clinical virology: John Wiley& Sons ldt, 2004

97. Ghebremedhin B. Human adenovirus: Viral pathogen with increasing importance.*Eur J Microbiol Immunol*. 2014 ;4(1):26-33

98. Kunz AN, Ottolini M. The role of adenovirus in respiratory tract infections. *Curr Infect Dis Rep.* 2010 ;12(2):81-7

99. Hayden FG. Rhinovirus and the lower respiratory tract. *Reviews in Medical Virology.* 2004;14(1):17-31.

100. Lamson D, Renwick N, Kapoor V, et al. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza- like illness in New York State during 2004-2005. *J Infect Dis.* 2006;194:1398-1402.

101. McErlean P, Shackelton L, Lambert S, Nissen M, Sloots T, Mackay I. Characterization of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. *J Clin Virol.* 2007;39:67 - 75.

102. Lau SKP, Yip CCY, Lin AWC, et al. Cinical and molecular epidemiology of human rhinovirus C in children and adults in Hong Kong reveals a possible distinct human rhinovirus C subgroup. *J Infect Dis.* 2009;200(1):1096-1103.

103. Arden KE, McErlean P, Nissen MD, T.P. S, Mackay IM. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *J Med Virol.* 2006;78(9):1232-1240.

104. Renwick N, Schweiger B, Kapoor V, et al. A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. *J Infect Dis.* 2007;196:1745 - 1760.

105. Bizzintino JA, Lee WM, Laing IA, et al. Association between human rhinovirus C and severity of acute asthma in children. *Eur. Respir. J.* 2011;37(5):1037-1042.

106. Miller EK, Edwards KM, Weinberg GA, et al. A novel group of rhinoviruses is associated with asthma hospitalizations. *J Allergy Clin Immun.* 2009;123:98-104.

107. Miller EK, Khuri-Bulos N, Williams JV, et al. Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. *J Clin Microbiol.* 2009;46(1):85-89.

108. Linsuwanon P, Payungporn S, Samransamruajkit R, et al. High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. *J Infection* 2009;59(2):115-121.

109. Gern JE. The ABCs of Rhinoviruses, wheezing and asthma. *J Virol* 2010; 84:7418-26.

110. McCulloh RJ, Koster MP, Yin DE, Milner TL, Ralston SL , Hill VL , Alverson BK , Biondi E A. Evaluating the Use of Blood Cultures in the Management of Children Hospitalized for Community-Acquired Pneumonia. *PLoS ONE* 2015; 10(2): e0117462.

111. Pereda MA , Chavez MA, Hooper-Miele CC , Gilman RH , Steinhoff MC. Lung Ultrasound for the Diagnosis of Pneumonia in Children: A Meta-analysis. *Pediatrics.* 2015;135(4):714-722

112. Zumla A, Al-Tawfiq JA, Enne VI, Kidd M, Drosten C, Breuer J. Rapid point of care diagnostic tests for viral and bacterial respiratory tract infections—needs, advances, and future prospects. *Lancet Infect Dis* 2014; 14: 1123–35

113. Rhedin S, Lindstrand A, Rotzén-Östlund M, Tolfvenstam T, Ohrmalm L, Rinder MR etal. Clinical utility of PCR for common viruses in acute respiratory illness. *Pediatrics.* 2014; 133(3):e538-45.

114. Selva L, Benmessaoud R, Lanaspa M, Jroundi I, Moraleda C, Acacio S,and al. Detection of Streptococcus pneumonia and Haemophilus influenzaeType B by Real-Time PCR from Dried Blood Spot Samples among Children with Pneumonia: A Useful Approach for Developing Countries. *PLoS ONE* 2013; 8(10): e76970

115. Schneider HG, Lam QT. Procalcitonin for the clinical laboratory: a review. *Pathology.* 2007;39(4):383-390.

116. Polverino E, Torres A. Diagnostic strategies for healthcare-associated pneumonia. *Semin Respir Crit Care Med.* 2009;30(1):36-45.

117. Madhi SA, Heera JR, Kuwanda L, Klugman KP. Use of procalcitonin and C-reactive protein to evaluate vaccine efficacy against pneumonia. *PLoS Med.* 2005;2(2):e38.

118. Cheung YB, Zaman SM, Ruopuro ML, et al. C-reactive protein and procalcitonin in the evaluation of the efficacy of a pneumococcal conjugate vaccine in Gambian children. *Trop Med Int Health.* 2008;13(5):603-611.

119. Díez-Padrisa N, Bassat Q, Roca A. Serum biomarkers for the diagnosis of malaria, bacterial and viral infections in children living in malaria-endemic areas. *Drugs Today* (Barc). 2011 Jan;47(1):63-75

120. Van den Bruel A, Thompson M.J, Haj-Hassan T, Stevens R, Moll H, Lakhanpaul M et al. .Diagnostic value of laboratory tests in identifying serious infections in febrile children: systematic review. *BMJ* 2011; 342:d3082

121. Xiao X, Long X, Sheng H-L and Xiao Z-H. Correlation between serum levels of C-reactive protein and infant pneumonia: A meta-analysis. *Exp Ther Med*. 2015 Jun;9(6):2331-2338

122. Bradley JS, Byington CL, Shah SS, Alverson B, Carter ER, Harisson C et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: Clinical practice Guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *CID*. 2011; 53 (7): e25-e76

123. Harris M, Clark J, Coote N, Fletcher P, Harnden A, McKean M, Thomson A. Community Acquired Pneumonia in Children Guideline Group On behalf of the British Thoracic Society Standards of Care Committee. *Thorax.* 2011; 66 (S2): ii15.

124. Vilas-Boas AL, Fontoura MS, Xavier-Souza G, Araújo-Neto CA, Andrade SC, Brim RV et al. Comparison of oral amoxicillin given thrice or twice daily to children between 2 and 59 months old with non-severe pneumonia: a randomized controlled trial. *J Antimicrob Chemother.* 2014 ;69(7):1954-

125. Lassi ZS, Imdad A, Bhutta ZA. Short-course versus long-course intravenous therapy with the same antibiotic for severe community-acquired pneumonia in children aged two months to 59 months (Review), The Cochrane Library 2015, Issue 6

126. Onyango FE, Steinhoff MC, Wafula EM, Wariua S, Musia J, et al. Hypoxaemia in young Kenyan children with acute lower respiratory infection. *BMJ*. (1993); 306: 612-615.

127. Reinert R. R. The antimicrobial resistance profile of Streptococcus pneumoniae. *Clin Microbiol Infect* 2009; 15 (Suppl. 3): 7–11.

128. Zerouali K, Ramdani-Bouguessa N, Boye C, Hammami A; The Active 2 Group. Multicentric study in five African countries of antibiotic susceptibility for three main pathogens: Streptococcus pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa. *J Chemother.* 2014 (3):1973947814Y0000000220

129. Dagan R, Klugman KP.Impact of conjugate pneumococcal vaccines on antibiotic resistance. *Lancet Infect Dis*. 2008; 8(12):785-95.

130. Ginsburg AS, Tinkham L, Riley K, Kay NA, Klugman KP, Gill CJ.Antibiotic non-susceptibility among Streptococcus pneumoniae andHaemophilus influenzae isolates i dentified in African cohorts: a meta-analysis of three decades of published studies. *Int J Antimicrob Agents.* 2013;42 (6):482-91

131. Paganini H, Guinazu JR, Hernandez C, et al. Comparative analysis of outcome and clinical features in children with pleural empyema caused by penicillin-nonsusceptible and penicillin-susceptible Streptococcus pneumoniae. *Int J Infect Dis* 2001;5:86e8

132. Oish iK, Tamura K and Akeda Y. Global Control of Pneumococcal Infections by Pneumococcal Vaccines. *Trop Med and Health*. 2014; 42(2S):83–86

133. World Health Organization, Recommendations for routine immunization. Geneva, 2011. (http://www.who.int/immunization/policy/immunization_tables/en/, accessed 15 May 2015).

134. World health organization. Global Vaccine Action Plan 2011-2020: The immunization landscape today. Geneva, 2014. (http://www.who.int/immunization/global_vaccine_action_plan/GVAP_doc_2011_2020/en/, accessed 15 May 2015).

135. Davis S, Feikin D, Johnson HL. The effect of Haemophilus influenzae type B and pneumococcal conjugate vaccines on childhood meningitis mortality: a systematic review. *BMC Public Health.* 2013;13: S3-S21

136. Swingler GH, Michaels D, Hussey GGD. Conjugate vaccines for preventing Haemophilus influenzae type B infections. *Cochrane Database of Syst Rev*, 2007, Issue 2. Art. No.: CD001729

137. Vernon J. MC, Ramsay M. E AND Lean AR MC. Understanding the impact of Hib conjugate vaccine on transmission, immunity and disease in the United Kingdom. *Epidemiol. Infect.* 2008 ; 136 : 800–812

138. World health organization. Immunization coverage,fact sheet N 378, 2015 (http://www.who.int/mediacentre/factsheets/fs378/en/, assessed 15 may 2015)

139. Kiran K. Thumburu, Meenu Singh, Rashmi Ranjan Das, Nishant Jaiswal, Amit Agarwal, Ajay Kumar and Harpreet Kaur. Two or three primary dose regime for Haemophilus influenzae type b conjugate vaccine: meta-analysis of randomized controlled trials. *Ther Adv Vaccines* 2015, Vol. 3(2) 31–40

140. Lucero MG, Dulalia VE, Nillos LT, Williams G, Parreño RAN, Nohynek H, Riley ID, Makela H. Pneumococcal conjugate vaccines for preventing vaccine-type invasive pneumococcal disease and X-ray defined pneumonia in children less than two years of age (Review) The Cochrane Library 2009, Issue 4

141. Centers for disease control and prevention. Progress in Introduction of Pneumococcal Conjugate Vaccine — Worldwide, 2000–2012. *MMWR Morb Mortal Wkly Rep*. 2013; 62(16);308-311

142. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. *Lancet.* 2011;378:1962–73

143. Steens A, Bergsaker MA, Aaberge IS, Rønning K, Vestrheim DF.Prompt effect of replacing the 7-valent pneumococcal conjugate vaccine with the 13-valent vaccine on the epidemiology of invasive pneumococcal disease in Norway. *Vaccine*. 2013;31(52):6232-8.

144. Gladstone RA, Jefferies JM, Tocheva AS, Beard KR, Garley D, Chong WW, Bentley SD, Faust SN, Clarke SC. Five winters of pneumococcal serotype replacement in UK carriage following PCV introduction. *Vaccine.* 2015 Apr 21;33(17):2015-21.

145. Flasche S, Le Polain de Waroux O, O'Brien KL, Edmunds WJ. The serotype distribution among healthy carriers before vaccination is essential for predicting the impact of pneumococcal conjugate vaccine on invasive disease. *PLoS Comput Biol*. 2015; 11(4):e1004173.

146. Roth DE, Caulfield LE, Ezzati M, et al. Acute lower respiratory infections in childhood: opportunities for reducing the global burden through nutritional interventions. *Bull World Health Organ*. 2008; 86:356 –364.

147. Lamberti LM, Zakarija-Grković I, Fischer Walker CL, Theodoratou E, Nair H, Campbell H, Black RE. Breastfeeding for reducing the risk of pneumonia morbidity and mortality in children under two: a systematic literature review and meta-analysis. *BMC Public Health*. 2013;13 Suppl 3:S18.

148. Imdad A, Yakoob MY, Sudfeld C, Haider BA, Black RE, Bhutta ZA. Impact of vitamin A supplementation on infant and childhood mortality. *BMC Public Health* 2011, 11(Suppl 3):S20

149. Del Fiol F de Sá, Barberato-Filho S, Cruz Lopes L, Bergamaschi C de Cássia. Vitamin D and respiratory infections. *J Infect Dev Ctries* 2015; 9(4):355-361.

150. Das RR, Singh M, Panigrahi I, Naik SS. Vitamin D supplementation for the treatment of acute childhood pneumonia: a systematic review. *ISRN Pediatr.* 2013; 19:459160

151. Haider BA, Lassi ZS, Ahmed A, Bhutta ZA. Zinc supplementation as an adjunct to antibiotics in the treatment of pneumonia in children 2 to 59 months of age. Cochrane Database of Systematic Reviews 2011, Issue 10. Art. No.: CD007368

152. World health organization. Global Plan of Action for Children's Health and the Environment. Geneva 2014.(http://www.who.int/ceh/global_plan/en/, assessed the 26/08/2015)

153. Bircher J and Kuruvilla S. Defining health by addressing individual, social, and environmental determinants: New opportunities for health care and public health. *Journal of Public Health Policy,* 2014; 35(3): 363–86.

154. Schwartz J. Air pollution and children's health. *Pediatrics.* 2004 Apr;113(4 Suppl):1037-43

155. Brown N, Luckett T, Davidson PM and Michelle Di Giacomo M. Interventions to Reduce Harm from Smoking with Families in Infancy and Early Childhood: A Systematic Review. *Int J Environ Res Public Health*. 2015 Mar; 12(3): 3091–3119.

156. Esposito S, Principi N.Unsolved problems in the approach to pediatric community-acquired pneumonia. *Curr Opin Infect Dis.* 2012;25(3):286-91

157. Scott J AG, English M What are the implications for childhood pneumonia of successfully introducing Hib and pneumococcal vaccines in developing countries? *PLoS Med*. 2008, 5(4): e86.

158. Garenne M, Darkaoui N, Braikat M, and Azelmat M. Changing Cause of Death Profile in Morocco: The Impact of Child-survival Programmes. *J Health Popul Nutr*. 2007 Jun; 25(2): 212–220.

159. Jroundi I, Mahraoui C, Benmessaoud R, Moraleda C, Benjelloun BS and Bassat Q Knowledge gaps on paediatric respiratory infections in Morocco, Northern Africa. Archives of Public Health 2015, 73:28

160. World health organization. Morocco: WHO statistical profile (http://www.who.int/gho/countries/mar.**,** assessed the 27/04/2015)

Acknowledgements

Ce paragraphe je l´ai écris dans ma langue maternelle le français, car c´est la langue que je maitrise le mieux pour exprimer mes émotions.

J ai choisi d´illustrer ma thèse par un tapis marocain rouge, tissé à la main, non pas pour son cote esthétique, mais pour le symbole qu'il représente pour la femme marocaine. Chaque tapis est unique et correspond à une tranche de vie, ou un événement important. Les dessins qui y figurent et ses couleurs, traduisent des sentiments, des espérances intimes et surtout transmettent un message, ou des messages, d´interprétation parfois complexe.

C´est pour signifier que cette thèse au delà d être un document et un titre académique, pour moi elle représente bien plus que cela. Combien d´obstacles, de concessions et de défis j ai du braver pour pouvoir suivre ce programme.

Ce programme fut une expérience capitale dans ma carrière, tant il a eu un impact dans ma vie professionnelle en tant que praticienne de santé publique et en tant qu´enseignante, mais aussi a influencé ma vie privé, en m´ouvrant à de nouvelles langues, l´espagnol et le portugais et l´anglais, à de nouvelles cultures et à surtout élargir mes contacts professionnels.

Mon séjour dans une institution aussi prestigieuse, et l´ observation de la structuration de la recherche, de la formation et des moyens utilisés pour la translation de l´évidence en politiques de santé publique, était très important à cette étape de mon développement professionnel.

Alors je ne remercierai jamais assez mes maitres, le professeur Pedro Alonso, Jordi Sunyer et Quique Bassat, lesquels ont été, ou à l´origine de mon embarcation dans cette aventure professionnelle ou ils m´ont accompagné durant sa réalisation jusqu`a sa concrétisation.

Jordi, tout à commencé grâce à vous, et je tenais à vous exprimer ici toute ma gratitude pour votre bienveillance, vos encouragements, votre écoute pendant mon année de master à BCN. Notre rencontre au Mexique lors du congrès d épidémiologie environnementale a été déterminante pour envisager la possibilité d´une formation en Espagne, un pays dont je ne parlais pas la langue. Dès lors je me suis sentie prête pour oser interpeller Pr Alonso, quelques mois après, qui était en déplacements au Maroc à la recherche de jeunes cadres pour les former à la recherche en santé publique.

Pedro, je vous remercie infiniment de m´ avoir tendu la main de m´ avoir invité à faire partie de votre équipe, et de m avoir donné accès à une aussi si riche expérience professionnelle. Veuillez croire en ma profonde admiration pour vos œuvres en santé internationale.

Quique, j ai été très chanceuse de t´avoir comme mon co-directeur, je te remercie très sincèrement d avoir bien accepté de m´encadrer tout au long de ces années et pour tes efforts à mener à bien ce projet au Maroc, qui a permis une meilleure connaissance des infections respiratoires au Maroc.

Je dédie aussi un spécial remerciement à Enric Grau qui a accompagné mon atterrissage en Espagne et m´a expliqué avec beaucoup de subtilité la culture espagnole et catalane et surtout a été un bon catalyseur pour la mise en œuvre de ce projet doctoral au Maroc.

Je tiens aussi à remercier Nuria Casamitjana, par sa disponibilité et son écoute.

Un grand remerciement au Professeur Chafik Maharaoui, à Cinta à Moraleda et Rachid Benmessaoud, mes compagnons de route et mes partenaires proche dans ce projet. Ainsi qu´à toute l´équipe de la Fundacio clinic de Rabat et de la OCI de BCN, Yolanda Amat et Surriel dont l´assistance a été très précieuse.

Mes pensées vont à Ned Haye et à Marcella qui sont partis trop tôt, qu'ils reposent en paix.

Et je n´oublie surtout pas mes chers amis Betuel, Elisa, Claudia, Irene, Miguel, Mamta, Julia, Isabel, Gladys, Nathalie, Christopher, Magda, Raquel, Monste, Golbahar, Denise, pour leur disponibilité, gentillesse, générosité et qui ont rendu à chaque fois, très agréables mes séjours Espagne.

Il n´existe aucun mot qui puisse exprimer ma profonde gratitude pour ma mère, une femme extraordinaire, qui m´a toujours soutenue dans mes décisions professionnelles et a toujours trouvé les mots justes pour m´encourager à persévérer dans mes études.

Imane Jroundi, Summer 2015

UNIVERSITAT DE BARCELONA

ISGlobal Instituto de Salud Global Barcelona