Acute respiratory infections among children in the Democratic Republic of the Congo – nasopharyngeal pathogens, antibiotic resistance and vaccination

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Dedication

To all youth health workers and researchers working in the field of child health in South Kivu and everywhere else in the Democratic Republic of the Congo, may this work inspire you to move forward to improve the health of children in the DR Congo.

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ABSTRACT

Acute Lower Respiratory Infections (ALRI) remain a leading cause of morbidity and mortality among children in the Democratic Republic of the Congo (DR Congo). The pneumococcal conjugate vaccine PCV13 was introduced in the in the South-Kivu region in 2013. The aim of this thesis was to investigate the epidemiologic of ALRI, nasopharyngeal bacteria and viruses, pneumococcal serotypes and antibiotic resistance among children after the PCV13 introduction.
In **paper I** 2,007 children hospitalised with ALRI during 2010-2015 were retrospectively reviewed and the case fatality rate among these children was 5%. The number of severe ALRI cases per year decreased after the vaccine introduction, while the total number of ALRI cases per year remained unchanged. Five percent of the cases were treated with non-recommended, broad-spectrum antibiotics.
In **paper II**, 794 children from the general population attending health centres during 2014 and 2015 were sampled from nasopharynx. The prevalence of pneumococci was higher among children who had not received PCV13, and among those who lived in a house with an open fire used for cooking and with open access to the living areas**.** Multi-resistance among the isolated pneumococci was high (43%), and almost all isolates were resistant to trimethoprim-sulfamethoxazole.
Multiplex PCR performed directly on 375 of the nasopharyngeal samples (**paper III**), showed a high load of bacteria and viruses although respiratory syncytial virus (RSV) was rare. Approximately 50% of the pneumococci were identified to a serotype not included in PCV13.
Paper IV included 116 hospitalised children with radiologically confirmed pneumonia. High levels of any virus or any bacteria in nasopharynx were associated with severe pneumonia, and having a congenital disease as an underling condition was associated with fatal outcome.
Conclusions: There were a high prevalence of bacteria and viruses in the upper respiratory tract of both healthy and sick Congolese children, and the level of antibiotic resistance in carried pneumococci was high. There is a need to modify current treatment guidelines in DR Congo and to reduce the prevalence of pathogens by interventions, including improved living conditions.

Key words: Acute respiratory infections, nasopharynx, culture and vaccination.

SAMMANFATTNING PÅ SVENSKA

Akuta luftvägsinfektioner hos barn i Demokratiska Republiken Kongo – bakterier och virus i luftvägarna, antibiotikaresistens och vaccination

Lunginflammation och andra allvarliga nedre luftvägsinfektioner orsakar hög sjuklighet och dödlighet hos barn under fem år i Demokratiska Republiken Kongo (DR Kongo). Särskilt drabbade är barnen i Södra Kivu provinsen i de östra delarna av landet, där mångåriga konflikter lett till försämrad folkhälsa och stora brister i sjukvårdssystemet. Allvarliga luftvägsinfektioner kan orsakas både av bakterier och virus, och särskilt viktiga hos barn är pneumokockbakterien och viruset RSV (respiratory syncytial virus). År 2013 introducerades ett nytt vaccin mot 13 olika typer av pneumokocker i barnvaccinationsprogrammet i Södra Kivu. Det finns över 95 olika pneumokocktyper och ingen vet vilka pneumokocktyper som fanns i landet före vaccinintroduktionen. Inte heller finns data på antibiotikaresistens hos pneumokocker som bärs av barn i regionen, vilka övriga mikrober som cirkulerar eller hur sjuklighet och dödlighet i allvarliga luftvägsinfektioner förändrats efter vaccinintroduktionen.

I detta arbete undersöktes barn som sjukhusvårdades pga svår luftvägsinfektion i östra DR Kongo före och efter introduktionen av det nya pneumokockvaccinet. De mest allvarliga fallen minskade efter att vaccinet introducerades i regionen, men dödligheten var oförändrad och lika många barn vårdades på sjukhus med luftvägsinfektion före som efter vaccinstarten. Många barn behandlades med typer av antibiotika som inte rekommenderas för luftvägsinfektioner, och som riskerar att öka antibiotikaresistensen i samhället. Efter vaccinintroduktionen giordes även en mer noggrann undersökning av sjukhusvårdade barn med konstaterad lunginflammation. De allra flesta barnen hade behandlats med antibiotika före sjukhusvistelsen och det var relativt vanligt med onödigt bred antibiotikabehandling. Barnen hade ofta både bakterier och virus i övre luftvägarna, och höga mängder sågs ofta hos de med svårast sjukdom. De barn som avled hade i större utsträckning pneumokocker och/eller RSV i övre luftvägarna än barn som tillfrisknade. Det gick inte att avgöra vilken bakterie eller vilket virus som orsakade infektionen och när friska barn undersöktes hade också de en riklig förekomst av både bakterier och virus i övre luftvägarna. Pneumokocker som bars av de friska barnen var ofta resistenta mot antibiotikapreparat som vanligen används inom vården. Minst hälften av pneumokockerna var typer som inte täcks av det nya pneumokockvaccinet, vilket överensstämmer med andra studier som visar att pneumokocktyper som inte ingår i vaccinerna blir allt vanligare vid både sjukdom och bärarskap. Bärarskap av pneumokocker var vanligare hos barn på landsbygden jämfört med barn som bodde i tätorter. Likaså var pneumokocker vanligare hos barn som bodde i hushåll där maten lagades över öppen eld och där husets konstruktion inte hindrade rök från elden att spridas till sovrum och andra ytor där man ofta vistades, jämfört med barn som inte exponerades för rök i hemmet på detta sätt.

Sammantaget visar avhandlingen att förekomsten av bakterier och virus som kan orsaka svår luftvägssjukdom är hög hos både friska barn och barn med sjukhuskrävande luftvägsinfektion i DR Kongo. Irrationell användning av antibiotika är vanligt och antibiotikaresistensen oroväckande hög hos pneumokockbakterier hos de friska barnen. Det finns ett stort behov av att uppdatera och modifiera vårdprogram och behandlingsrekommendationer för luftvägsinfektioner och lunginflammation som drabbar barn i Södra Kivu provinsen. Likaså finns ett stort behov av att höja levnadsstandarden och förbättra tillgången till god sjukvård för barnfamiljer i DR Kongo.

RESUME EN FRANCAIS

Les infections respiratoires basses aiguës (IRBa) font partie des principales causes de morbidité et de mortalité chez les enfants de moins de cinq ans dans la région du Sud-Kivu en République Démocratique du Congo (RD Congo). Le vaccin conjugué contre le pneumocoque, PCV13 a été introduit dans la région vers l'année 2013.

Le but de cette thèse était d'étudier l'épidémiologie des IRBa, les bactéries et virus retrouvés dans le nasopharynxe, les sérotypes de pneumocoque circulant et leur résistance aux antibiotiques chez les enfants de moins de cinq ans en RD Congo après l'introduction du PCV13.

Dans le premier article, 2 007 enfants hospitalisés pour une IRBa au cours de la période allant de 2010-2015 ont été rétrospectivement analysés, le taux de létalité parmi ces enfants était de 5%. Le nombre de cas d'IRBa sévère par année avait sensiblement diminué après l'introduction du vaccin PCV13, tandis que le nombre total de cas d'IRBa par année était resté inchangé. Cinq pour cent des cas ont été traités avec des antibiotiques à large spectre non recommandés.

Dans le deuxième article, 794 enfants venant de la communauté, reçus aux différents centres de santé pour une vaccination de routine en 2014 et 2015 ont bénéficié d'un prélèvement nasopharyngien. La prévalence des pneumocoques était significativement élevée chez les enfants qui n'avaient pas reçu de PCV13, et parmi ceux qui vivaient dans une maison avec une cuisine utilisant le bois et ayant un contact direct au salon et/ou aux chambres à coucher. Les pneumocoques isolés avaient démontré une multi-résistance élevée (43%) et presque tous les isolats étaient résistants au triméthoprime-sulfaméthoxazole.

Dans le troisième article, la PCR réalisée directement sur 375 prélèvements nasopharyngiens, avait révélé une densité bactérienne et virale très élevée bien que le virus respiratoire syncytial (VRS) était rare. Environ 50% des sérotypes de pneumocoque identifiés appartenaient aux sérotypes non inclus dans le PCV13.

Le quatrième article, avait prospectivement analysé 116 enfants hospitalisés pour une pneumonie confirmée par radiographie des poumons. A l'admission, la pneumonie sévère était significativement associée à une augmentation du taux des globules blanc >20,000/μL. Une forte détection nasopharyngienne des virus et des bactéries était associée à la sévérité de la pneumonie pendant qu'une forte densité de VRS ou de pneumocoques était associée à une issue fatale.

Conclusions : Les voies respiratoires supérieurs des enfants Congolais malades et non malades ont une forte prévalence de bactéries et de virus pathogènes dont un groupe de pneumocoque a résistance très élevée aux antibiotiques. Ce qui implique la nécessite d'ajuster les directives de traitement actuel des IRB en RD Congo et de mettre sur pied des mesures ou interventions, y compris l'amélioration des conditions de vie pour réduire la morbidité des IRB ainsi que la prévalence des agents pathogènes.

MUHTASARI KWA KISWAHILI

Magonjwa ya kifuwa kwa watoto wadogo yaani chini ya umri wa myaka tano, yanaendelea kuwatatiza watoto wadogo na kusababisha vifo vingi jimboni mwa Kivu ya Kusini, Mashariki mwa Jamhuri ya Kidemokrasia ya Kongo (DR Kongo). Chanjo ya ndui ya kuepuka na magonjwa inayoletwa na pneumococcus ili anzishwa jimboni mwa Kivu ya Kusini karibuni mwaka wa 2013. Chanzo ya natharia hiyi ili kuwa kuchunguza kimangonjwa aina ya magonjwa ya kufuwa, kugunduwa aina za vijidudu vinavyo patikana puani kwenda kooni za watoto wadogo na vinavyo sababisha magonjwa ya kifuwa, aina za pneumococcus zinazo patikana inchini, na namna zinavyo jadiliana na nguvu za dawa kama vile antibiotics baada ya kuanzishwa chanjo ya magonjwa ya kifuwa(pneumococcus).

Kitika **Nakala ya Kwanza**, Baazi ya rekodi 2007 za watoto waliyo tuzwa hospitalini kwa ajili ya magonjwa ya kifuwa tangu mwaka 2010 hadi 2015 zili chunguzwa kwa makini, nazo kaweka wazi ya kwamba magojwa ya kifuwa yana sababisha baazi ya asili mia tano (5%) ya vifo vya watoto hospitalini. Uchunguzi huu uliyoonesha wazi mapunguko makubwa mwakani za idadi ya watoto waliyouguwa magonjwa ya kifuwa kikali ingawaje idadi kwa jumla ya watoto waliyouguwa kifuwa mwakani haikupungua baada ya matumizi ya chanjo la kuepusha magonjwa ya kifuwa. Asilimia tano ya kesi hizo zilitibiwa na dawa zisizopendekezwa.

Kwenye **Nakala ya Pili**, watoto wenye afya 794 waliohudhuria vituo vya afya mwakani 2014 pia 2015 kwa ratiba yakuchanjwa ndui, walichukuliwa wa sampuli kutoka puani kwenda kooni. Ili onekana kwamba, watoto ambao hawa kuwayi ku chanjwa nduyi ya PCV13, walipatikana kuwa na vijidudu vya pneumococcus kwa uwingi kuliko wale ambao walio chanjwa. Pia watoto ambao wanaishi katika nyumba ambazo zina jiko la kuni linalo sambaza moshi nyumbani na chumbani pote, walipatikana kuwa na vijidudu vya pneumococcus kwa uwingi kuliko wale ambao jiko hali tapanye moshi. Karibuni asilimia arobaini na tatu(43%) ya vijidudu vya pneumococcus vilivyo patikana vilisababisha upingamizi ya hali ya juu kwa dawa zinazo tumiwa ki kawaida. Ila aina dawa, kama trimethoprim-sulfamethoxazole ilionekana kutokuwa na uwezo wowote mbele ya aina za pneumococcus zilizopatika katika eneo.

Kwa Nakala ya Tatu, utafiti wa ki sasa (yaani polymerase chaine reaction) iliyofanywa moja kwa moja kwenye 375 ya sampuli za puani za watoto wenye afya. Huu utafiti ka onyesha kana kwamba wiani kubwa la vijidudu kana bacteria na virusi vimepatikana kwenye watoto wenye afya jimboni, ingawa virusi aina ya (RSV) vilikuwa ndogo. Karibu asilimiatano(50%) za aina za pneumococcus zilizogunduliwa jimboni hazipatikani miongoni mwa zili zilizondani ya chanjwa PCV13 inayotolewa jimboni kwa sasa.

Nakala ya ine ilijumuisha watoto 116 waliyolazwa hospitalini kwa sababu ya magonjwa ya kifuwa (nimonia) iliyothibitishwa kwa picha za x-ray. Watoto waliyotibiwa kwa trimethoprim-sulfamethoxazole baada ya kuletwa hospitalini walipatikana na aina kali mno ya nimania kuliko ambawa wali tibiwa na dawa zingine. Viwango vikubwa vya virusi au bakteria vilipatikana zaidi kwa watoto walioshikwa na nimonia kali mno, na watoto ambao walipatikana na viwango vingi vya aina virus RSV na viwango vingi vya pneumococcus walihatarishwa kiasi cha kufariki kuliko watoto walio patikana na vijidudu vinginevyo.

Hitimisho: Kumepatikana viwango vya juu mno za bacteria na virus zenyi kusababisha magonjwa ya kifuwa kwenye puwa kwenda kooni mwa watoto wenye afya na wenye kuuguwa inchini ya Kidemokrasia ya Kongo (DR Kongo). Vijidudu vya pneumococcus vilivyo vumbilika inchini vimekuwa vyenyi kupingana na dawa (antibiotic) zinazotumikishwa ki kawaida inchini. Hii husababisha haja kubwa ya kurekebisha mikakate ya matibabu ya magonjwa ya kifuwa inchini DR Congo na haja yakupangiliya njia za kupambana na magonjwa ya kifuwa kwa watoto pamoja na kuraisisha hali bora za kimaisha inchini

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. Birindwa AM, Tumusifu MJ, Mwinja A, Nordén R, Andersson R, Skovbjerg S**. Decreased number of hospitalized children with severe acute lower respiratory infection after introduction of the pneumococcal conjugate vaccine in the eastern Democratic Republic of the Congo**.
Accepted in The Pan African Medical Journal

II. Birindwa AM, Emgård M, Nordén R, Samuelsson E, Geravandi S, Gonzales-Siles L, Muhigirwa B, Kashosi T, Munguakonkwa E, Tumusifu MJ, Cibicabene D, Morisho L, Mwambanyi B, Mirindi J, Kabeza N, Lindh M, Andersson R, Skovbjerg S. **High rate of antibiotic resistance among pneumococci carried by healthy children in the eastern part of the Democratic Republic of the Congo.** BMC Pediatrics. 2018 Nov 19; 18(1): 361.

III. Birindwa AM, Kasereka KJ, Gonzales-Siles L, Geravandi S, Mambo Mwilo M, Kanku TL, Mwinja LN, Muhigirwa B, Kashosi K, Tumusifu MJ, Mungo C, Bugashane BE, Saili MS, Nordén R, Andersson R, Skovbjerg S. **High bacterial and viral load in the upper respiratory tract of children in the Democratic Republic of the Congo.** Revision submitted.

IV. Birindwa AM, Kasereka KJ, Gonzales-Siles L, Geravandi S, Mambo Mwilo M, Kanku TL, Mwinja LN, Muhigirwa B, Kashosi K, Tumusifu MJ, Mungo C, Bugashane BE, Saili MS, Nordén R, Andersson R, Skovbjerg S. **Bacteria and viruses in the upper respiratory tract of Congolese children with radiologically confirmed pneumonia**. Revision submitted.

Paper I and II were reprinted by permissions from The Pan African Medical Journal and BMC Pediatrics.

ABBREVIATIONS

ALRI: acute lower respiratory infection;
RNA: Ribonucleic Acid;
BCG: Bacillus Calmette –Guérin vaccine;
CARe: Center for Antibiotic Resistance Research, Gothenburg;
CDC: Centres for Disease Control and Prevention;
CI: confidence interval;
CRF: Case fatality rate;
CRP: C-reactive protein;
Ct: Cycle threshold;
DNA: Deoxyribonucleic acid;
DR Congo: Democratic Republic of the Congo;
DR Kongo: Demokrasiya Republika Ya Kongo;
ENA: Emergency Nutrition Assessment;
EUCAST: European committee on antimicrobial susceptibility testing;
ECDC: European Centre for Disease Prevention and Control;
FATH: Foundations for appropriate technologies in health;
HIV: human immunodeficiency virus;
Hi: *Haemophilus influenzae;*
Hib: *Haemophilus influenzae* type b;
IRB: infections respiratoires basses;
IMCI: Integrated Management of Childhood Illnesses;
IV: intravenous;
GDP: Gross Domestic Product;
MERS: Middle East Respiratory Syndrome coronavirus;
MIC: minimum inhibitory concentration;
MSF: Médecins Sans Frontières;
OR: odds ratio;
PCR: Polymerase chain reaction;
PNSP: penicillin non-susceptible pneumococci;
PCV: pneumococcal conjugate vaccine;
PCV7: 7-valent pneumococcal conjugate vaccine;
PCV10: 10-valent pneumococcal conjugate vaccine;
PCV13: 13-valent pneumococcal conjugate vaccine;
SARS: severe acute respiratory syndrome;
Sp: *Streptococcus pneumoniae;*
TMP-SMX: trimethoprim-sulfamethoxazole;
USD: United state dollars;
RSV : respiratory syncytial virus;
WHO: World Health Organisation

1. Table of Contents

1. INTRODUCTION

Health care in DR Congo

The Democratic Republic of the Congo (DR Congo) is the second largest country on the African continent, borders with nine countries, and has a population of over 80 million inhabitants. The country has experienced recurrent wars, and political and social instability during the last two decades, with sporadic fighting still occurring [1, 2]. The persistence and long duration of these conflicts have devastated the civilian population and collapsed the health care infrastructure and organization. The government expenditure on health per capita remains one of the lowest in the world, (4.0% of Gross Domestic Product (GDP) in 2017 [3], which means 40–45 USD/inhabitant/year) and too low to enable general access. For the available health care structure, the quality and accessibility remains a big problem in the country [1, 4]. Since the social security system is almost non-existent in the country, the child medical care induces enormous healthcare expenditures for already poor households as the health care access is enabled only upon direct payment [5, 6].

DR Congo has a primary healthcare system based on district health systems starting with local health centers, called "Centre de Santé" and "post de Santé", which are staffed by licensed and non-licensed nurses, graduated from government certified programs, but with minimum training. Many of these structures were affected during the last decades of conflicts and have not been rebuild until recently. The second health care system level consists of hospitals and clinics staffed by medical doctors. They provide most of the general treatments and perform basic clinical care, but also refer patients to regional or provincial hospitals. At the top of the system is a national hospital and university clinic in Kinshasa [1, 2]. The costs of the health care at this level are very high and are not accessible for most of the population. Modern medical equipment is very rare in the country, including molecular diagnostic methods for infectious diseases. Also traditional bacteriological methods such as bacterial culture are still rare at provincial hospitals, and very expensive at the few hospital that are able to provide them [4, 7].

The South-Kivu province is located in the Eastern DR Congo, covers 65,000 km^2, and has 5 million inhabitants. The province is divided into 34 health districts, including Ibanda, Kadutu, Kaziba and Miti-murhesa, in which our studies were performed. The main diseases and conditions in the province leading to morbidity and mortality in children below five years of age include acute lower respiratory infections (ALRIs), malnutrition, diarrhoea and malaria. The province has the highest burden of ALRI and malnutrition in the country and also

the largest damages on health infrastructures because of the war and several ongoing conflicts [8-10]. Modern medical equipment is very rare in the province. Nowadays, the province has started a residency pre-program for pediatricians in order to improve the children health care. An estimation of 10 certified pediatricians were working clinically in the province in 2016. The country had 0.9 physicians per 1000 population in 2013 [11, 12].

Acute Lower respiratory infections (ALRIs) in children

An ALRI can be defined as an infection of the lower respiratory tract that covers the continuation of the airway from the trachea and bronchi to the bronchioles and alveoli [13], and includes the diagnoses pneumonia and bronchiolitis [14]. ALRI remains a leading cause of mortality and morbidity in children below five years of age globally [14, 15]. In 2016, 68 million episodes of ALRIs were estimated, equivalent to 0.11 cases per child-year, with 5.1 million hospital admissions worldwide [14]. The greatest number of ALRI among children younger than five years of age occur in low-income countries in Asia and Africa [15]. In 2017, nearly 810,000 children younger than five years died from ALRI worldwide [15], and approximately 50% of these deaths occurred in Sub-Saharan Africa [16].

The burden of ALRIs in DR Congo

ALRI constitute the major cause of mortality and morbidity among under-five children in the DR Congo. In 2017 ALRI caused 20% of deaths among children aged 1-59 months with a death rate of 9.4 per 1,000 live births [17]. The highest morbidity and mortality due to ALRI were reported in the South-Kivu province in the Eastern part of the country [18]. In 2017 only about 40% of under-five Congolese children with pneumonia or ALRI symptoms were taken to an appropriate heath care facility, while the remaining children were brought to private pharmacies, or traditional practitioners [19]. The adherence to existing clinical guidelines for the management of severe very sick children including those with ALRIs among clinicians in DR Congo is low [9]. Only 42% of the clinicians were found to follow them, and no more than half of these clinicians were recently trained for use of updated guidelines recommending the use of amoxicillin instead of trimethoprim-sulfamethoxazole for the treatment of pneumonia in children aged from 2 to 59 months [2, 9, 20].

Risk factors for ALRI

In low- and middle-income countries including those located in Sub-Saharan Africa, risk factors for developing childhood pneumonia include crowding, malnutrition, incomplete immunization, prematurity, sickle cell disease and immune suppression including human immunodeficiency virus (HIV) infection [15, 21-25]. Smoke from use of solid fuel in the household is also identified as a significant risk factor for developing childhood pneumonia and is associated with the severity of the disease as well [26, 27]. The DR Congo harbours the second largest forest in the world and the rural electrification rate is only 1%. In the South-Kivu province, biomass fuel remains the most common fuel for cooking and also for heating during the night time in the mountain regions [28, 29]. The household air pollution has recently been reported as a risk factor for developing respiratory infections in the South-Kivu province [29-31].

Risk for death is increased in children with ALRI and symptoms such as tachypnea, grunting, central cyanosis, wheezing or asthma [32, 33]. Fatal outcome during hospitalization has also been associated with co-morbidities and development of lung infiltration, consolidation and pleural effusion [32, 33].

Clinical diagnose of ALRI and pneumonia

ALRI

ALRIs are defined in the International Classification of Diseases (ICD) as those infections that affect airways below the epiglottis and include acute manifestations of laryngitis, tracheitis, bronchitis, bronchiolitis, lung infections or any combination among them [34]. One of the most widely used, and still used in some countries, is the Integrated Management of Childhood Illnesses (IMCI), developed by WHO and UNICEF in 1995 to promote health and provide preventive and curative services for children under five in countries with more than 40 deaths per 1,000 live births [35, 36]. IMCI grouped pneumonia and bronchitis under the term of ALRIs. This approach is based on the identification of children with fast breathing and/or lower chest wall indrawings [34, 37, 38]. A simple clinical classification of ALRI in children below five years of age into three categories according to severity has been proposed. The first category, called mild ALRI, include children with cough for less than two weeks but no fast breathing or indrawings. The second category, named moderate ALRI, includes children with cough and fast breathing but no chest indrawings. The third category, classified as severe ALRI, includes children with cough and chest indrawings and not being able to drink or presenting additional sign of danger or stridor at rest [13, 34, 37].

Pneumonia

Community acquired pneumonia can be defined as an acute infection of less than 14 days duration, acquired in the community, and affecting the lower respiratory tract leading to cough or difficult breathing, tachypnea or chest-wall indrawings [39, 40]. The revised WHO classification and treatment of childhood pneumonia at health facilities from 2014 are derived from two previously WHO IMCI guidelines on the management of childhood pneumonia, published in 2010 and 2012 [41-43]. In the current classification two major changes have been made, the first one was to re-classify the three categories of pneumonia into two categories, namely pneumonia and severe pneumonia [44, 45]. In the first category, pneumonia, two previous categories "fast breathing pneumonia" and "chest indrawing pneumonia" were merged into one category. Pneumonia is now defined as fast breathing and/or chest indrawings in a child aged from 2 to 59 months [44]. This approach simplifies the management at outpatient level and reduces the number of referrals for hospitalisation. In the second category, severe pneumonia, there is any additional danger sign [44, 46]. Severe pneumonia can also clinically be defined as a child with cough or difficult breathing who also has central cyanosis or oxygen saturation ≤90% on pulse oximetry or severe respiratory distress [44, 46, 47].

Recently scientific comments on the IMCI clinical guideline and the revised WHO recommendations suggested that these guidelines should be reviewed and probably revised. This means to reconsider the symptom of chest wall indrawings and the role of clinical anaemia in malaria settings, which have been associated with fatal outcome in children with non-severe pneumonia [48].These continuous changes and revisions of the definition of pneumonia show the lack of clear-cut objective endpoint for pneumonia diagnostics. Radiologically confirmed pneumonia appears to be the golden standard in pneumonia research [49].There is, however, no strict radiological definition of pneumonia in children existing at present; instead, there is a spectrum of appearances that are consistent with the clinical and pathological diagnosis of pneumonia [50]. One is the typical appearance of severe lobar consolidation, which is known to be strongly associated with bacterial pneumonia and the second one is the mild interstitial and perihilar infiltrates that are often associated with viral infections [50].

Aetiology of ALRI

Bacteria in ALRI

Streptococcus pneumoniae (the pneumococcus) remains the main bacterium causing ALRI among under-five children in many countries in the world, including the DR Congo, followed by *Haemophilus influenzae* [23, 51, 52].

Streptococcus pneumoniae

S. pneumoniae are Gram-positive, facultative anaerobic bacteria and was first isolated by Pasteur in 1881 from the saliva of a patient with rabies [53, 54]. The association between pneumococci and lobar pneumonia was first described by Friedlander and Talamon in 1883, but pneumococcal pneumonia was confused with other types of pneumonia until the development of the Gram stain in 1884 [54]. *S. pneumoniae* is part of the normal nasopharyngeal flora, especially in young children. The carriage rate among children in African countries varies widely between different studies, which might be explained by geographical and seasonal variability, differences in socio-economic factors and methodological differences. Studies from Kenya reported a 17% nasopharyngeal carriage rate among children in Thika 2010, 53% during the dry season and 62% during the rainy season in Kilifi 2008 and 60% among children in Kibera, Nairobi in 1997 [55-57]. A Tanzanian study, which used the same method as in our study, reported a carriage rate of 31% among under-five healthy children sampled between 2013 and 2015 [58], while 72% was reported in Gambian children [59].

Transmission

Transmission of *S. pneumoniae* can be direct person-to-person contact via respiratory droplets through coughing or sneezing or indirect via hands or contaminated materials or surfaces [60-62]. The spread of the organism within a family or household is influenced by factors such as household crowding and co-existing viral respiratory infections [63]. Reports from Bangladesh and Nigeria show that pneumococcal and other respiratory infections are more common during rainy or high humidity seasons [64, 65]. The bacteria can spread locally from the respiratory niche to organs nearby such as the middle ear and cause acute *otitis media*, or by aspiration reach the lungs and cause pneumonia **(Figure1).** The bacteria may also reach the blood stream, either directly from the nasopharynx or from a mucosal infections such as pneumonia, and cause invasive pneumococcal disease, which include for example bacteremia and meningitis **(Figure1)** [60]. The incidence of under-five pneumococcal infections may be underestimated in Sub-Saharan African countries due to lack of

laboratory diagnostic facilities. The adoption of both culture based methods and molecular diagnostics my provide more precise estimates of disease [66]. Although the DR Congo was reported as one of the five countries in which 49% of global under-five pneumonia deaths occurred in 2015 [22], there is to our knowledge no publish data on the pneumococcal carriage among under-five year children in the country.

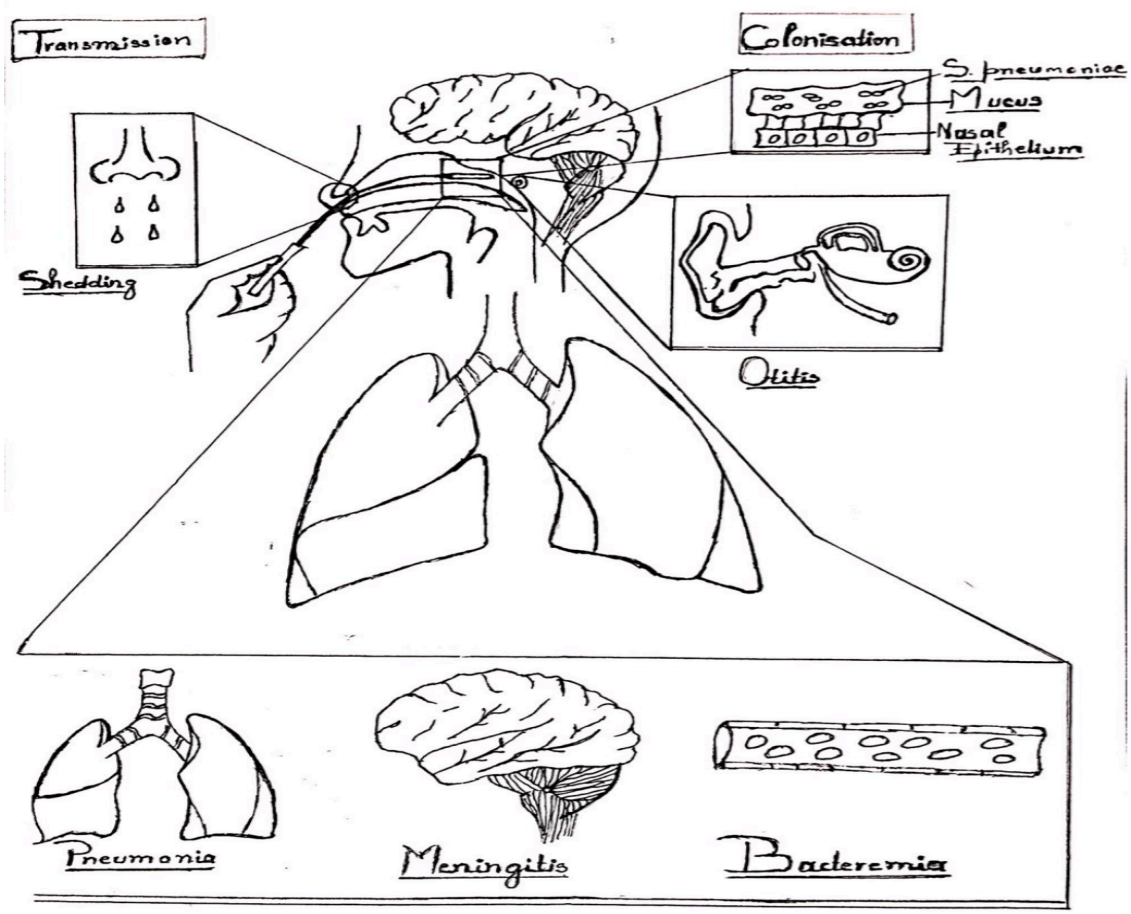

Figure 1: *Streptococcus. pneumoniae; colonization, transmission and invasion*

The pneumococcus is surrounded by a cell membrane, a thick cell wall and, most importantly, a polysaccharide capsule **(Figure 2).** This capsule plays a fundamental role in the pneumococcal virulence by protecting the bacteria from the host immune system. The capsule is also the basis for epidemiological categorization into different serotypes due to differences in the composition of the polysaccharides [67, 68]. At present, 98 different serotypes have been identified, based on their reaction with type-specific antisera [61, 67, 68]. Type-specific antibodies to capsular polysaccharide are

protective. These antibodies and complement interact to opsonize the pneumococci, which facilitates phagocytosis and clearance of the organism. Antibodies to some pneumococcal capsular polysaccharides may cross-react with related types, providing protection against additional serotypes. Most *S. pneumoniae* serotypes have been shown to cause severe disease, but only a few serotypes produce the majority of pneumococcal infections [67]. Ten serotypes are estimated to account for about 60% of all invasive diseases worldwide [69]. The serotype prevalence differs by patient age group and geographic area. In the DR Congo, there is no data on the carriage rate of different serotypes in the child population. Nor is there any data on the distribution and prevalence of serotypes in pneumococcal infections, including pneumonia and invasive disease.

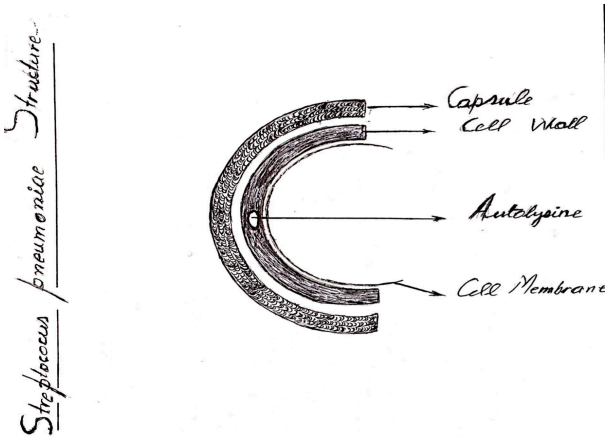

Figure 2: *Structure of S. pneumoniae*

Identification of pneumococci

Microbiological pathogen identification plays a key role in the management and surveillance of ALRI. There are several methods for the detection of pneumococci and conventional methods include culture from blood, sputum or nasopharyngeal samples, antigen detection in urine and nucleic acid amplification from respiratory samples.

Pneumococci can be cultured from normally sterile sites or from locations with commensal bacterial flora, including nasopharynx. In clinical laboratories, cultured isolates of *S. pneumoniae* can be identified by microscopic morphology (Gram-positive cocci, usually in pairs), colony morphology, optochin susceptibility and bile solubility [70, 71] **(Figure 3).** Nowadays, many clinical laboratories use Matrix Assisted Laser Desorption Ionization - Time Of Flight (MALDI-TOF) for identification of cultured pneumococci and other pathogenic bacteria [72]. The isolation of pneumococci is tricky due to the pneumococcal enzyme autolysin, encoded by the *lytA* gene. Upon activation the enzyme causes

the pneumococcus to lyse and die, thus not growing in bacterial cultures [73]. This might be overcome by use of molecular methods, in which both viable and dead bacteria can be detected.

Quantitative PCR, also called real-time PCR, couples amplification of a target DNA sequence with quantification of the concentration of that DNA species in the reaction. The *lytA* and *cpsA* are examples of target genes that have been used for identification of pneumococci in clinical samples [74]. *S. pneumoniae* is, however, genetically very close-related to other species in the Mitis-Group of the genus *Streptococcus*, and both the *lytA* and *cpsA* genes have been detected in other streptococcal species than *S. pneumoniae* [74]. Recently, the "*Xisco*" gene was described to be specific for *S. pneumoniae*, however, also this gene has been found in a non-pneumococcal species [75]. Thus, it has been proposed that pneumococcal identification by molecular methods should rely on detection of more than one gene [75].

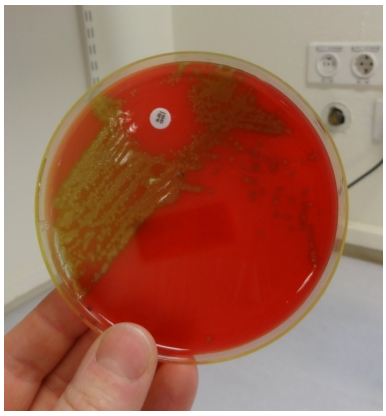

Figure 3: *Cultured pneumococci identified by susceptibility to optochin*

Pneumococci antibiotic susceptibility testing

The disk diffusion method is a culture-based method that is widely used for testing the antibiotic susceptibility of bacteria. Isolated bacterial colonies are suspended and inoculated onto a solid agar plate onto which antibiotic containing discs are applied. After incubation overnight the size of the inhibition zones formed around the antibiotic discs are measured and compared to published clinical breakpoints [76, 77] **(Figure 4)**.

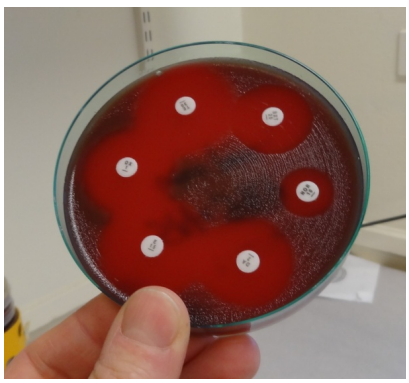

Figure 4: *Assessment of S. pneumoniae susceptibility to penicillin and other antibiotics using disk diffusion test*

The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that prevents visible growth of the bacterium and can be measured by the broth microdilution method. Gradient strips, so called E tests, are applied on bacterial seeded agar plates and are widely used for MIC determination, though no more recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[78, 79] **(Figure 5)**.

To assess the susceptibility of *S. pneumoniae* to penicillin a screening test using the disc oxacillin is usually performed. Pneumococcal isolates susceptible to oxacillin can be reported susceptible to several beta-lactam antibiotics including penicillin, ampicillin and ceftriaxone. Pneumococcal isolates with reduced sensitivity to oxacillin (diameter <20 mm) are regarded as resistant to phenoxymethylpenicillin, and are usually further tested by MIC determination for assessment of susceptibility against benzylpenicillin (penicillin G). MIC determination is also performed to assess susceptibility against ampicillin and ceftriaxone, at least in cases in which oxacillin <8 mm [78] **(Figure 5).**

When the susceptibility test has been performed, organisms are usually classified as S, R, or I which refers to a predicted *in vivo* situation, rather than *in vitro* susceptibility [79]. "S" denotes "Susceptible, standard dosing regimen", in which there is a high likelihood of therapeutic success using normal dosage regimens. "R" denotes "Resistant" in which there is a high likelihood of therapeutic failure. The "I" category was former known as "Intermediate", but since 2019 it denotes "Susceptible, increased exposure" and there is a high likelihood of therapeutic success if exposure to the agent is increased [79]. Accordingly, pneumococci categorized as I or R to benzylpenicillin according to the susceptibility test were previously regarded as penicillin non-susceptible pneumococci (PNSP), but may now be referred to as "non-wild-type" isolates, in contrast to the completely susceptible "S" isolates [78].

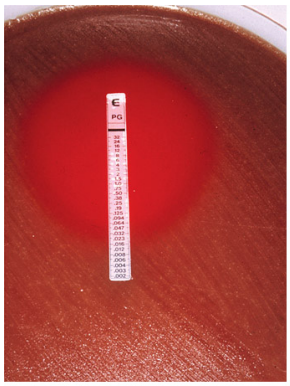

Figure 5: *Minimum inhibitory concentration (MIC) determination by using the E-test*

Serotyping

The Quellung reaction or Neufeld test is the traditional standard method for serotyping of pneumococcal isolates. It is based on the capsular reaction/swelling test reaction [71, 80]. This method involves testing a pneumococcal cell suspension with pooled and specific antisera directed against the capsular polysaccharide. The method is labor intensive and time consuming, and usually only performed at national reference laboratories. The latex agglutination reagent is created by the attachment of antibodies to latex particles [81]. In a positive reaction, a visible agglutination reaction is produced in the presence of specific pneumococcal serotype antigens [82]. Commercial latex reagents are available, able to rapidly detect up to 92 serotypes from cultured *S. pneumoniae* [83]. Latex reagents can also be prepared in-house using commercially available antisera [82]. Compared with Quellung, latex agglutination is less expensive, easier to learn, and does not require a microscope. It may therefore be more suitable for settings with limited budgets and training capacity.

Recently, a variety of new serotyping methods have been developed including phenotypic methods that rely on antigen detection or genotypic detection methods using multiplex real-time PCR or microarray [70, 71, 80]. Molecular methods can be used without previous culture and isolation of the bacterium, which might be an advantage in settings with limited bacteriological culture facilities.

Pneumococcal vaccines

Two different types of pneumococcal vaccines, polysaccharide and conjugate vaccines, are used in the prevention of severe pneumococcal disease [73]. Polysaccharide vaccines contain capsular pneumococcal polysaccharide antigens, which elicit a T-cell independent immune response in the host. Since children below two years of age have poor ability to produce a T-cell independent immune response, the polysaccharide vaccines are not possible to use in children below two years [60, 84]. On the contrary, the conjugate vaccines contain an immunogenic non-pneumococcal protein conjugated to the pneumococcal polysaccharides, which confers a strong prolonged immunity in children below two years of age [85-87].

The first pneumococcal conjugate vaccine (PCV7) was licensed in 2000. It includes purified capsular polysaccharide of seven serotypes of *S. pneumoniae* (4, 6B, 9V, 14, 18C, 19F and 23F) conjugated to a nontoxic variant of diphtheria toxin known as CRM197. The PCV10 contains the serotypes 1, 5 and 7F in addition to the PCV7 serotypes, while the PCV13 contains the serotypes 3, 6A

and 19A in addition to the PCV10 serotypes. The PCV13 vaccine has reduced the incidence of invasive pneumococcal disease in children aged 2-59 months by 55% in the Gambia [88]. A South African study reported a rate of 81% of pneumococcal colonization before and 65% two years after the introduction of PCV13 vaccination [89]. In 2013 DR Congo introduced the PCV13 in the routine vaccination program of children as a three-dose scheme, scheduled at six, ten and fourteen weeks without any catch-up campaign.

Haemophilus influenzae

Haemophilus influenzae is a Gram-negative rod-shaped bacterium colonizing the nasopharynx or throat of healthy children [90], and can be detected in respiratory specimens by culture or PCR. It is transmitted through respiratory droplets and can be either encapsulated (typable) or unencapsulated (nontypable) [91]. Although the pathogenesis of *H. influenzae* infections is not completely understood, the type b polysaccharide capsule is considered as the major virulence factor [92]. The major diseases caused by *H. influenzae* are childhood pneumonia, meningitis, epiglottitis and bacteremia, which are primarily caused by the capsulated type b (Hib) strains [91]. Four countries, including DR Congo, were reported to have 50% of all deaths due to *H. influenzae* from 2000 to 2015 worldwide [93]. Prior to introduction of *H. influenzae* type b (Hib) vaccines, *H. influenzae* was estimated to be responsible for three million serious illnesses worldwide and an estimated 386,000 deaths per year, chiefly in children through meningitis and pneumonia, with 95% of cases and 98% of deaths occurring in developing countries [94].

Hib vaccines are safe and exhibit good efficiency when given in early infancy. The vaccine has been included in the routine childhood vaccination program in the DR Congo since 2009. Invasive Hib disease has been practically eliminated in many industrialised countries, and its incidence has been dramatically reduced in the developing world including DR Congo as well [93]. A worldwide study estimated that deaths caused by Hib among children under five years declined by approximately 90% from 2000 to 2015 [93]. However, in 2015, the global burden of disease data reported that almost 65% of bacterial pneumonia deaths in children under five years were attributed to *S. pneumoniae* and *H. influenzae* [95]. A recent study from Brazil reported increased carriage of non-type b *H. influenzae* after the introduction of the Hib vaccine [96]. The overall hospitalisation rate due to *H. influenzae* in children in Texas was dominated by non-b types [97]. A Gambian study on new-born and infants reported an increasing carriage rate of both *S. pneumoniae* and *H. influenzae* from below 30% in the first week of life to 90% at 15 to 19 months of age [98]. The same study reported an average prevalence of 7% of encapsulated *H. influenzae* and 0.7% of *H. influenzae* type b in nasopharynx of newborns and infants aged 0-12 months

in areas where the vaccine is not widely available [98, 99]. Five years after the introduction of the Hib vaccine in the Gambia, the type b strain carriage among children under five years dropped from 12% to 0.25% [100]. A recent study reported PCR detected *H. influenzae* in the nasopharynx of approximately 50% children with radiologically confirmed pneumonia in Gambia, Kenya, Mali, South Africa and Zambia after the Hib vaccine was introduced in these countries [101].

Viruses in ALRI

Viral infections are very common among children and viral ALRI in children display significant seasonal variation, especially in areas with temperate climate. However, in tropical areas seasonal patterns are less clear, and viruses may circulate throughout the whole year with peaks during periods with lower temperatures, rainfall or higher humidity. Viruses causing ALRI among children below five years of age include respiratory syncytial virus (RSV), influenza A and B, parainfluenza, adenovirus, coronavirus, human metapneumovirus, rhinovirus, parainfluenza viruses and human bocavirus [52, 102].

Paramyxoviridae viruses

The *paramyxoviridae* family include six of the most important viruses causing the large portion of viral ALRIs among under-five children worldwide, namely RSV, human metapneumovirus, and parainfluenza virus 1-4. Paramyxoviruses contain non-segmented, single-stranded RNA genomes[103]. RSV is the most aggressive virus of the group, as it is the leading cause of viral ALRI hospitalisations among children and causes bronchiolitis in under one-year old children [51, 103-106]. RSV infection is thought to account for approximately 85% of bronchiolitis and 20% of pneumonia hospitalisations in infants [107]. In 2015, it was estimated that RSV was associated with 33 million episodes of ALRI among children under five years of age worldwide and 3.2 million hospitalisations [108]. A recent study from DR Congo reported that RSV is the predominant virus detected in the nasopharynx of children with ALRI treated at the provincial hospital in Bukavu [109]. Parainfluenza is mostly associated with croup and bronchiolitis [52]. Seroepidemiological studies indicate that more than 90% of children have been infected with metapneumovirus by five years of age and it has been reported as the second cause of bronchiolitis after RSV [110, 111]. A study from the USA reported that metapneumovirus was associated with up to 6% of hospitalisations of under five-years children 2003 to 2009 [112]. RSV and human metapneumovirus have been reported as two of the three most frequent detected virus among children under two years hospitalised for ALRI in Algeria [113]. The immunity is incomplete as after most viral respiratory infections, and might lead to recurrent infections with RSV and metapneumovirus [114].

Influenza A and B

Influenza A and B are the predominant influenza viruses that infect humans. They are enveloped, single stranded RNA virus with a segmented genome[115]. The influenza A viruses are divided into subtypes based on the variant of haemagglutinin (H1 to H18) and neuraminidase (N1 to N11), of which combinations of H1-H3 and N1-N2 have been reported to be involved in human disease. For influenza B virus there are two distinctly separate linages circulating in the human population. Influenza A and B cause annually epidemics, and accumulation of mutations in the HA genes allow the virus to successively escape the host immunity through antigenic drift. Sporadic events of reassortment within the genetic elements of the segmented RNA genome of influenza leads to the emergence of novel subtypes. These larger genetic events may result in pandemic outbreaks with increased severity due to the absence of immunity in the human population [114]. Currently, influenza B and influenza A viruses (H1N1 pdm09 and H3N2) are co-circulating causing yearly seasonal epidemics worldwide. In 2018, influenza virus accounted for 7% of ALRI cases, 5% of ALRI hospital admissions, and 4% of ALRI deaths in children under five years of age worldwide [116]. Recently, a study in a primary care setting in Senegal, reported influenza virus A and B among the main causes of ALRI among under-five years children [117].

Coronaviruses

Coronaviruses are enveloped and spherical containing a single-stranded RNA genome[118]. Until recently, the most frequent human respiratory coronaviruses were the types 229E, OC43, NL63, and HKU1 causing relative mild ALRIs in children, which less often require hospitalisation. The epidemic of severe acute respiratory syndrome (SARS) caused by the SARS-CoV virus started in China in 2002. A second outbreak occurred on the Arabian Peninsula in 2012 by Middle East Respiratory Syndrome coronavirus (MERS). MERS-CoV antibodies have been detected in archived blood samples from two Kenyan livestock handlers collected between 2013 and 2014, suggesting endemic presence of MERS coronavirus in the northeastern region of Africa [119, 120]. The most recent coronavirus, SARS-CoV-2 associated with the disease Covid-19 is currently causing a pandemic that started in Wuhan, China at the end of 2019. Covid-19 has low mortality among children [121]. From reports in China, more than 90% of infected children had asymptomatic, mild, or moderate symptoms of Covid-19 [122]. A Chinese study on >2000 children including under-five years with suspected or confirmed Covid-19, reported that 5% of virologically confirmed cases had dyspnea or hypoxemia and 0.6% progressed to acute respiratory distress syndrome or multiorgan failure [123, 124]. Moreover, under

five years children hospitalised in USA for Covid-19, had high amounts of SARS-CoV-2 viral RNA in their nasopharynx compared to older children and adults [125]. Recently a Norwegian study detected coronavirus in 10% of hospitalised children with respiratory tract infection [126]. Children in low- and middle-income countries develop predominantly mild diseases or asymptomatic infection of COVID-19 similar to that in high-income countries [127, 128]. The indirect effects of the pandemic on child health are substantial in low- and middle-income countries, such as disrupted schooling, lack of access to school feeding schemes, reduced access to health facilities and interruptions in vaccination and other child health programs [127, 129, 130]. On August 25th 2020, the WHO reported a total of 1,196,277 Covid-19 cases and 27,984 (CFR: 2%) deaths in 55 African countries, among them 9,842 (CFR: 2,3%) in DR Congo (number of children not specified) [131, 132].

Enterovirus and rhinovirus

Both enterovirus and rhinovirus belong to the family of *Picornaviridae.* ***Enterovirus and rhinovirus*** are small, non-enveloped, positive-stranded RNA viruses[133]. They are the most frequent viruses implicated in common cold and may be associated with the exacerbation of bronchiolitis and asthma in children [133]. They have been assigned to distinct types, rhinovirus A-C and enterovirus A-D, respectively [133]. Rhinovirus has been reported as the most common viral infection in young South-African and Bangladeshi children with mild or moderate clinical respiratory symptoms [134, 135].

Adenoviruses

Adenoviruses are non-enveloped, double-stranded deoxyribonucleic acid viruses[136]. Outbreaks of adenoviral respiratory infections can occur in closed communities such as daycare centers and boarding schools but most of the infections remain subclinical [114]. Hospitalization for severe ALRI due to adenovirus among children have been reported in China in a study performed between 2011 and 2014 [137].

Methods for viral detection

Viruses can be diagnosed by virus cell culture, serology, antigen detection, and nucleic acid amplification tests. The traditional golden standard method, virus isolation, takes days to weeks and many viruses remain uncultivable, making this method unsuitable for routine use [138].

Nucleic acid amplification tests including PCR have been developed for most respiratory viruses. They are highly sensitive and are used in routine clinical laboratories for detecting respiratory virus. Nucleic acid amplification methods has replaced cell culture isolation as the new golden standard for the detection of respiratory viruses [138]. Multiplex PCR assays introduced in the last decade, can detect up to 15-20 different viruses in a single test using numerous primer pairs [139]. Even if molecular detection has many proven advantages over standard virological methods, cell culture remains an important method for the detection of emerging novel viruses and for phenotypic characterization of viral isolates. Recently, DNA microarray testing has emerged; they represent an approach for massive virus detection and surveillance, and are capable of detection of all known virus, as well as novel viruses related to known viral families in a single assay [138]. Also, with the emergence of high trough-out massive parallel sequencing it is possible, in theory, to detect any novel virus or bacteria present in a clinical sample [138]. However, these methods are expensive and labor intensive, as they require highly specialized equipment and staff.

Treatment of ALRI and pneumonia

According to the WHO management of ALRI and childhood pneumonia management, the three groups of ALRI can be treated following their clinical categorisation as suggested by the revised WHO pneumonia management and the IMCI clinical management guideline [44, 46, 140].

Mild ALRI and no pneumonia

In this category of mild ALRI with cough and symptoms of common cold but without any signs of pneumonia, children should not be treated with antibiotics. Instead the parents/guardians should be given home care advice, including feeding and nutritional advices if needed. Some protocol suggest to treat the cough if indicated (an exhilarating cough which prevents the child from sleeping) and recommend a follow up after five days if no improvement [141, 142].

Moderate ALRI or pneumonia

In this clinical pneumonia group, children need oral antibiotic treatment with amoxicillin as recommended by the WHO [44]. In settings with high HIV infection rate oral amoxicillin should be given for five days while three days treatment is enough in settings with low HIV prevalence [13, 44, 141]. In DR Congo, trimethoprim-sulfamethoxazole has been the first line antibiotic treatment for pneumonia and it was used in 30% of cases with pneumonia in an

epidemiologic observation study done by the global health organisation Foundations for appropriate technologies in health (FATH) in 2018 [2, 20]. Trimethoprim-sulfamethoxazole, erythromycin, azithromycin and amoxicillin-clavulanic acid have all been proposed as the oral antibiotic treatment for pneumonia in low-income countries in studies from Asia and South America [143, 144]. However, these treatments have not proven to be as efficient as the oral amoxicillin based on the rational cost efficacy in low-income settings [13, 143]. Thus in the revised classification of pneumonia, oral trimethoprim-sulfamethoxazole was replaced as the first-line treatment of pneumonia by oral dispersible amoxicillin tablets, due to the high effectiveness of amoxicillin [44]. Amoxicillin is also a better choice in the aspects of antibiotic resistance development.

Severe ALRI or severe pneumonia

Severe ALRI or severe pneumonia in children should be treated in the hospital for five days with intravenous antibiotic, nasal oxygen, intravenous fluids and oro-gastric feeding if needed.
The recommended first line antibiotic treatment is the combination of ampicillin or benzylpenicillin with gentamicin and if there is no improvement after 48 hours of the treatment, a switch to the second line treatment ceftriaxone and gentamicin is recommended [141]. Before the discharge day an appointment for follow up and if needed catch-up immunisations should be given to the child.

ALRI Prevention

ALRI prevention is based on the vaccination against the most common pathogens that cause ALRI such as *S. pneumoniae H. influenzae* type b (Hib), measles, and *B. pertussis*. The current childhood vaccination program in DR Congo is shown in **Table 1.**

***Table 1** : Ongoing childhood vaccination program in DR Congo*

	BCG vaccine	***Oral polio vaccine***	***Diphtheria, tetanus, pertussis, hepatitis B and Hib (Pentavalent)***	***S. pneumoniae PCV13***	***Measles and yellow fever***
At birth	*X*	*X*			
6 weeks		*X*	*X*	*x*	
10 weeks		*X*	*X*	*x*	
14 weeks		*X*	*X*	*x*	
At 9 months					*x*

***BCG:** Bacillus Calmette –Guérin vaccine*
***Hib:** Haemophilus influenzae type b vaccine*
***Pentavalent:** Association of 5 vaccines in one (Diphteria, tetanus, pertussis, hepatitis B and Haemophilus influenzae type b)*
***PCV13:** 13-valent pneumococcal conjugate vaccine*
***Measles:** Monovaccine containing only the measles antigen*

In addition to the vaccination programs, ALRI among children can be prevented by improving living condition such as reduction of indoor air pollution or smoke from solid fuel, hygiene measures such as hand washing and portable water access [15, 26, 145, 146].

2. AIMS AND OBJECTIVES

Overall aims:

The overall aims of the thesis were to study acute lower respiratory infections (ALRI) among hospitalised children below five years of age in the Eastern DR Congo after the introduction of the pneumococcal conjugate vaccine (PCV13), to determine the prevalence of pneumococci and other respiratory pathogens in both healthy and sick children, and to assess antibiotic resistance rates and serotype distribution of carried pneumococci.

Specific aims:

1. To investigate if there was any reduction in ALRI among hospitalised children after PCV13 was introduced in DR Congo (**paper I**).
2. To determine risk factors for pneumococcal carriage, antibiotic resistance rates, serotypes, and vaccine coverage of carried pneumococci among healthy and sick children in DR Congo (**paper II, III and IV**).
3. To determine the presence of bacteria and viruses in nasopharynx of children from the general population and in children with radiologically confirmed pneumonia, and to investigate if there were any associations between detected pathogens and sociodemographic or medical factors, or severity of disease (**paper III and IV**).

3. MATERIAL AND METHODS

Study sites

Figure 6: *Map of the South-Kivu province with the 34 Health Districts (Zone de Santé), including Miti-Murhesa, the rural health district located in the north of Bukavu town, Kaziba and Nyantende located in the south, and a sub-view of Bukavu town health districts (Kadutu and Ibanda). Reprinted by permission from Inspection Provinciale de la Santé du Sud-Kivu.*

Data on children with ALRI were retrospectively collected at two general referral hospitals (Panzi Hospital and Ciriri Hospital) and two district hospitals (Miti-Murhesa and Nyantende) **(paper I) (Figure 6 and 7)**. The rural hospitals Miti Murhesa and Nyantede Hospital, covered a population of 231,000 and 132,000 inhabitants, respectively, at the time of the study **(Figure 6).** Ciriri Hospital is located in the suburban area of Bukavu, and covered a population of 337,000 inhabitants. Panzi Hospital is a teaching hospital located in Bukavu town in the district health of Ibanda, and served a population of 453,000 inhabitants at the time of the study. It has 81 paediatric beds, of which 12 are located in the paediatric emergency ward and 69 are for the stable patients. Panzi Hospital was also the study site for the prospective study on children with radiologically confirmed pneumonia **(paper IV) (Figure7).**

Data were collected from children at seven different health centers; three of them were located in urban areas of Bukavu city (Muhanzi health, Kadutu Health center and Malkia wa amani) and another four were located in rural or sub-urban areas of Bukavu (Panzi Health Center, Nyantende Health Centre, Muku Health Centre and Kaziba Health Centre) **(paper II) (Figure 6).** Children from four of these health centers (Panzi, Nyantende, Muku and Kaziba) were also included in **paper III (Figure 7)**.

Study population

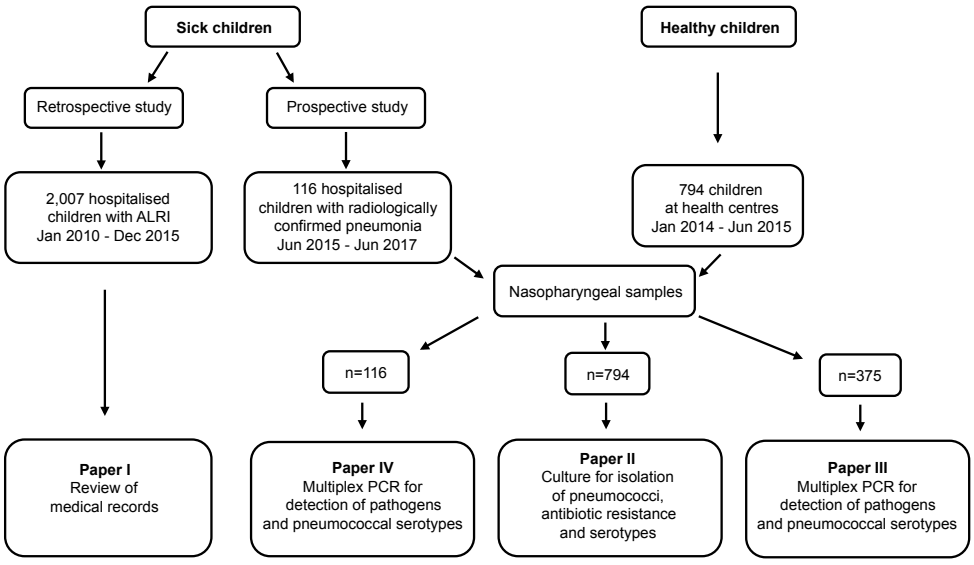

Figure 7: *An overview of the included patients and performed analyses*

Paper I

Out of nearly 21,500 hospitalised children aged from 2 to 59 months data were retrospectively collected from hand-written medical records of 2,007 children diagnosed with ALRI between January 2010 and December 2015 **(Figure7)**. In this study, cases were considered as ALRI if the medical records contained the discharge diagnoses of any types of pneumonia (pneumonia, severe pneumonia, bronchopneumonia, atypical pneumonia) or any types of bronchitis (bronchitis, rhino-bronchiolitis, rhino-pharyngo-bronchitis). Children with asthma or chronic obstructive pulmonary disease were not included. The data were divided in two groups, before the introduction of PCV13 and after the vaccine introduction. Data from children hospitalised in 2013, the year when PCV13 was introduced in the region, were included in the overall description of the cases, assessment of antibiotic treatment and the case fatality rate, but were not included in the comparison of ALRI cases before and after PCV13 introduction.

Paper II and III

From January 2014 to June 2015, 794 children from the general population aged two to 60 months attending health centres in the South-Kivu province for immunisation or growth monitoring were included in the study and sampled from nasopharynx for pneumococcal culture **(paper II) (Figure 7).** Information on immunisation status, socio-economic conditions and medical history were collected. From 375 children of these children, nasopharyngeal samples were available for molecular analyses of microbial pathogens **(paper III) (Figure 7).**

Paper IV

Children (n=116) between 2 months and 5 years of age with radiologically confirmed pneumonia at Panzi Hospital between June 2015 and June 2017 were included in the study **(Figure7)**. Data were collected on demographic and medical factors, clinical symptoms and signs, treatment and outcome. Patients were classified as having pneumonia or severe pneumonia according to the revised WHO classification of childhood pneumonia [44]. Nasopharyngeal samples were taken for pathogen detection, and venous blood samples were collected for white blood cell counts and C-reactive protein (CRP).

Microbiological analyses

Nasopharyngeal samples collected from both healthy and sick children were cultured at the Clinical laboratory at Panzi hospital for isolation of *S. pneumoniae* **(paper II and IV)**. Identified isolates were further tested for antibiotic

susceptibility using disk diffusion test and minimum inhibitory concentration (MIC) determination **(paper II)**. Comparative tests of antibiotic susceptibility on a fraction of isolates were also performed at the Department of Infectious Disease, University of Gothenburg, Sweden. Serotypes of cultured pneumococcal isolates were determined using a multiplex real-time PCR, capable of detecting 40 different pneumococcal serotypes or in some cases serogroups (**paper II**). This assay was also used for identification of pneumococcal serotypes and/or serogroups directly in the nasopharyngeal samples obtained from the children in the general population **(paper III)**, as well as from the children hospitalised due to pneumonia **(paper IV)**.

Another multiplex real-time PCR assay was used for detection of 16 different viruses and 5 bacterial species in the nasopharyngeal samples from the healthy **(paper III)** and the sick children **(paper IV)**. The assay included the following pathogens: adenovirus, bocavirus, coronavirus 229E, HKU1, NL63 and C43, enterovirus, influenza A and B, human metapneumovirus, parainfluenza 1-3, rhinovirus and RSV, *Bordetella pertussis, Chlamydophila pneumoniae, H. influenzae, Mycoplasma pneumoniae* and *S. pneumoniae*.

Ethical consideration

All studies were conducted in accordance with the consideration of ethical principles. All included children's guardians or parents were informed about the study and signed the consent form. Before study start, all study protocols, consent forms and questionnaires were approved by the Ethics Committees at the Université Catholique de Bukavu, DR Congo. The study parts performed in Sweden were ethically reviewed and approved by the Regional Ethics committee in Gothenburg, Sweden.

4. RESULTS

Children hospitalised with acute lower respiratory infection (ALRI) or radiologically confirmed pneumonia (paper I and IV)

ALRI accounted for 9.4% of all cases treated at the paediatric wards at four hospitals in the South-Kivu province between 2010 and 2015. Half of the hospitalised children spent at least one week in the hospital. Most cases occurred during the heavy raining months December and January. Out of all ALRI cases, 27% children had severe ALRI. Out of the 116 children diagnosed with radiologically confirmed pneumonia at Panzi Hospital during 2015-2017, 73% met the criteria of having severe pneumonia at admission. High CRP levels (>75 mg/dL) and white cell counts (>20,000 cells/μL) at admission were significantly associated with severe pneumonia. Fast or difficult breathing was significantly more frequently identified among children hospitalised with radiologically confirmed pneumonia (109/116, 94%) as compared to those hospitalised for ALRI (868/2007, 43%) (OR 8.4 CI 5.01-14.13; p<0.0001).

The proportion of severe ALRI cases decreased significantly in all children after the introduction of PCV13 as compared to the pre-vaccine period. The number of cases with rapid breathing decreased after the introduction of the PCV13. The case fatality rate in the hospitalised children with ALRI was 5% and the mortality rate was estimated to 0.08 children per 100,000 inhabitants a year. There was no difference in the fatality rate of the hospitalised children before and after the PCV13 introduction. The fatality rate was significantly associated with severe ALRI as well as having any of the underlying conditions malnutrition or congenital disease. The symptom of fatigue was four times more frequent among the children who died as compared to those who recovered. The case fatality rate among the hospitalised children with radiologically confirmed pneumonia was 9%, and having an underlying congenital disease was associated with fatal outcome.

Antibiotic use (paper I, II and IV)

According to the parent or guardian, 19% of the children coming to the health centre for growth monitoring or vaccination had been treated with antibiotics during the last month. Among the children with radiologically confirmed pneumonia at Panzi Hospital, 87% had been treated with antibiotics before hospital admission, in most cases with a penicillin derivate. However, 20% of the children with pre-hospitalisation antibiotic treatment had been given the broad-spectrum antibiotic trimethoprim-sulfamethoxazole. This antibiotic was

also more often given before hospitalisation to children with severe pneumonia than children with non-severe disease.

Antibiotic treatment during hospitalisation was assessed among the hospitalised children both in the retrospective and the prospective study. After the introduction of PCV13 more than half of the hospitalised children with ALRI were treated with ceftriaxone combined with gentamicin, which was a significant increase as compared with the period before PCV13 introduction. There was also a quite high use of non-recommended antibiotics for the treatment of ALRI such as chloramphenicol and ciprofloxacin. The prospectively collected data on children with radiologically confirmed pneumonia showed that the ceftriaxone and gentamicin combination was more frequently used than the ampicillin and gentamicin combination (53% versus 40%, p = 0.035), and the former combination was also more frequently used among the severe pneumonia cases as compared with the non-severe pneumonia cases.

Pneumococcal carriage and antibiotic resistance (paper II and IV)

Nasopharyngeal swabs were obtained and cultured for pneumococci both from from the general population children (n=794) and from the hospitalised children with radiologically confirmed pneumonia (n=116). The overall isolation rate was low though much higher among the children in the general population (21%) as compared with the hospitalised children, in which only one sample was culture positive for *S. pneumoniae*. From the 164 pneumococcal strains isolated from the children in the general population, 89% were shown to be non-susceptible to oxacillin and therefore regarded as resistant to phenoxymethylpenicillin. When these isolates were analysed by MIC determination, 18% were resistant (MIC >2 mg/L) against benzylpenicillin and 12% against ceftriaxone. Ninety-five percent of the isolates were resistant to trimethoprim-sulfamethoxazole, and 43% of the pneumococci were multidrug resistant (non-susceptible to ≥ 2 antimicrobials, including benzylpenicillin).

Potential pathogens detected by molecular methods in nasopharyngeal secretions from healthy and sick children (paper II, III and IV)

Collected nasopharyngeal samples were also subjected to bacterial and viral detection by real-time PCR analysis; 375 from children in the general population and 116 from children with radiologically confirmed pneumonia. *S. pneumonia*e and *H. influenzae* were frequently detected at high levels in both groups **(Table 2).**

Table 2: *Real-time PCR using the cycle threshold (Ct) level Ct<30 as cut-off level for detection of bacteria and viruses in nasopharyngeal secretions from children in the general population and children hospitalised for radiologically confirmed pneumonia*

Pathogens	Number of cases (%)		
	Pneumonia cases n=116	Children from general population n=375	*p-value (Fisher test)*
S. pneumoniae	61 (53)	290 (77)	< 0.0001
H. influenzae	23 (20)	190 (51)	< 0.0001
Any bacteria	72 (62)	313 (83)	0.0001
Rhinovirus	34 (29)	113 (30)	0.86
Enterovirus	2 (2)	26 (7)	0.051
Adenovirus	5 (4)	11 (3)	0.88
Parainfluenza virus	3 (3)	6 (2)	0.49
RSV	5 (4)	2 (0.5)	0.011
Any virus	53 (47)	147 (39)	0.21
Any bacteria and virus	78 (67)	343 (91)	< 0.0001

The detection rates of *S. pneumonia*e and *H. influenzae* were much higher in the samples obtained from the general population children as compared to the children with pneumonia **(Table 2).**

Rhinovirus was the most frequently detected virus in both groups **(Table 2).** Among all detected pathogens, RSV was the only pathogen significantly more often detected among confirmed pneumonia cases **(Table 2).** In the univariable analysis high nucleic acid levels (Ct <30) of pneumococci or RSV were associated with fatal outcome in the children with radiologically confirmed pneumonia, but the association was not significant in the multivariable analysis. Co-occurrence of bacteria and virus was common both among the children in the general population and the pneumonia cases, and the rate was highest among the children in general population. Co-occurrence of RSV and pneumococci was observed in the two RSV pneumonia cases that had a fatal outcome.

Occurrence of bacteria in relation to socio-demographic and medical factors (paper II and III)

Pneumococci were significantly more commonly isolated by culture from children aged more than 24 months than in children below 24 months. However, this was not confirmed in the nasopharyngeal samples directly analysed for pneumococci by real-time PCR. Living in rural area or living in a house with an open fire for cooking located inside the house and directly connected to the living room and/or the bed rooms were significantly associated with higher frequency of pneumococci either isolated by culture or detected at high levels by

real-time PCR. Moreover, medians Ct levels of detected pneumococci were significantly lower (i.e. higher bacterial load) in children living in a house with an open fire cooking without smoke protection as compared with those having a closed kitchen. Significantly higher bacterial load (lower Ct values), were detected for both pneumococci and *H. influenzae* in children living in rural areas, as compared to those living in urban areas.

Lower rates of pneumococci were found among vaccinated children who had received 2-3 doses of PCV 13 compared to non-vaccinated children both using culture (3% vs. 30%) and direct detection of nucleic acids at high levels (Ct<30) by PCR in the nasopharyngeal samples (71% vs. 81%). Children with malnutrition, current fever or those with recent antibiotic treatment were more commonly colonised with cultivable pneumococci than children without these factors, but this could not be confirmed by real-time PCR.

Pneumococcal serotype distribution (paper II, III and IV)

One or more serotype/serogroup could be identified in 58% of the cultured pneumococcal isolates from the children in the general population, and in 61% and 55% of the nasopharyngeal samples positive for pneumococci by real-time PCR and obtained from the general population children and the children hospitalised with pneumonia, respectively. In the living pneumococcal isolates, that could be re-cultured from the general population children, the most common serotype found among the non-vaccinated children was the PCV13 containing serotype 19F while the non-PCV13 serotypes 11A/D and 35B/35C were the most frequently identified serotypes in the PCV13 vaccinated children. Other prevalent serotypes/serogroups identified by PCR directly on the nasopharyngeal samples from the general population or the sick children were 5 and 6 (included in PCV13) and 15BC and 10A (not included in PCV13). Among the children hospitalised for pneumonia 63% of the identified serotypes/serogroups belonged to PCV13, i.e. a slightly higher proportion than identified in the children from the general population.

5. DISCUSSION

The health care system in the DR Congo still have major problems related to accessibility and quality. These health care problems are as much linked to the state of infrastructure in the country, including available medical equipment as financial resources allocated to health by the government [2]. Our studies on children hospitalised for acute lower respiratory infections (ALRI) or radiologically confirmed pneumonia in the South-Kivu province show that severe lung infection still remains an important cause of morbidity and mortality among children in the Eastern DR Congo.

We found that 9% of all hospitalised children were treated for ALRI during our study period which is lower compared to other African countries where the proportion of hospitalisation due to ALRI in under-five year old childrenwas more than 25% [16]. Our lower rate of hospitalisation can be explained by the fact that approximately only 40% of Congolese children with ALRI are taken to appropriate health care providers for the management [19]. Many hospitals in DR Congo still have accessibility problems due to the absence of government medical assurances and health care costs being on the charge of families. There is still much to do to improve the health care accessibility in the country. The differences might also be explained by the inclusion criteria or definition of cases. As expected the proportion of severe pneumonia was higher among the children hospitalised with radiologically confirmed pneumonia compared to the children with severe ALRI among the children hospitalised due to ALRI.

We found that high CRP (>75 mg/dL) and high white cell counts (>20,000 cells/μL) at admission were associated with severe pneumonia. Similar observations have been described in Sudan, China and Australia [147-150], when CRP and white cells counts are known biomarkers that predicted the prognosis of severe pneumonia [148, 151, 152]. Fast breathing was significantly more frequently identified among children hospitalised with radiologically confirmed pneumonia than children with ALRI (94% versus 43%). This was expected as fast breathing is one of the main clinical criteria for pneumonia in the revised WHO classification of pneumonia among children aged between 2 and 59 months [44], and used in our study.

There was a significant decrease in the proportion of ALRI cases among children aged below 24 months after the PCV13 introduction but not in older children. Moreover, our results show an association between a higher rate of culture positivity for pneumococci and age; pneumococci were significantly more often isolated from children aged more than two years than in children below two

years. We started collecting samples approximately 18 months after PCV13 was introduced in the South-Kivu province. There was no catch-up program, which means that most of the children older than two years were not vaccinated while most of the children below two years children had received at least 2 doses of PCV13 at the time of the sampling. We showed that vaccinated children carried less pneumococci than non-vaccinated both by nasopharyngeal pneumococcal culture (3% vs. 30%) and real-time PCR (71% vs. 81% using the cut-off level Ct<30). This strongly suggests a preventive effect of PCV13 on pneumococcal carriage as previously shown in Kenya, Malawi and other Sub-Saharan African countries[153-155].

The PCV13 impact, both on pneumococcal carriage and pneumonia among under-five year children is now well known, and for example described in a study from Burkina Faso where it significantly reduced the incidence of hospitalisation for all-cause pneumonia among under-five year children [156]. In Zambia the number of hospital admissions for pneumonia declined by 38% among children aged below one year and 29% among children aged between one and four years after PCV10 was introduced [157]. Substantial declines of childhood community acquired pneumonia were also observed in Israel after the introduction of PCV7 and later by PCV13 [158]. The impact of PCV10 or PCV13 on the proportion of under-five hospitalised children with ALRI or pneumonia has been reported in several other African countries as well, including Kenya, Malawi and Rwanda [153, 156, 159, 160]. Thus, PCVs are a corner stone in the fight against pneumococcal childhood infections [161].

The case fatality rates (CFRs) of 5% in the hospitalised children with ALRI and 10% among the cases with radiologically confirmed pneumonia were similar to the CFR described in Burkina Faso, where it was 8% among children hospitalised for pneumonia and 11% among children with severe pneumonia [156]. Similar CRFs as in our study have also been described for pneumonia cases in Malawi (7%), and in children hospitalised for ALRI in South-Africa, Kenya, Gambia and in Ghana, where it was 3% for severe ALRI including pneumonia cases and 6% for very severe ALRI [162, 163]. Higher CRFs have been reported in Khartoum in Sudan (17%), in Bangui in the Central African Republic (12%) and in Zimbabwe (15%) for children treated at hospitals for ALRI [147, 164, 165]. The CRF of children treated for ALRI or pneumonia at hospitals in the Eastern DR Congo could be expected to be higher than found in our studies, which might be explained by the limited healthcare accessibility. In the DR Congo health care system, parents take care of most of the medical expenses of their sick children; this incites some parents to not choose the referral hospitals because of the cost even after being referred by the health centres. This is supported by a previous study in Oriental Kassai in the central parts of the DR Congo, in which more than 80% of the Congolese households did not use modern health care because of the

cost [166]. A global study on the burden of ALRI suggested that up to 80% of deaths due to ALRI among under-five year children happen out of hospital in low-income countries including DR Congo [163].

The fatality rate was significantly associated with severe ALRI as well as having malnutrition or congenital disease. Underlying conditions including malnutrition and congenital diseases have been described as risk factors associated with death among under-five children hospitalised for ALRI or pneumonia in several low- and middle-income countries including the Central African Republic and Sudan [147, 164, 167]. Malnutrition with deficiencies in proteins, vitamins and calories among children is well known as affecting the immune response against pathogens [168]. Undernourished children are more vulnerable to infectious pathogens and more likely to die from infectious diseases, including pneumonia [168, 169]. The result from the cultured nasopharyngeal swabs support the evidence that malnutrition in children under five years of age is associated with higher rates of pneumococcal carriage as previously described in other low-income Sub-Saharan countries, in Venezuela and in India [170-173].

Antibiotic use and antibiotic resistance

We noted that there was a high use of ceftriaxone combined with gentamicin among the children hospitalised for severe ALRI and those hospitalised for radiologically confirmed pneumonia, and this treatment even increased after PCV13 introduction in the area. The WHO recommends the use of this combination only when there is no improvement with the use of ampicillin combined with gentamicin. In low-income countries the main goals are to reduce the mortality as much as possible but also to reduce the increasing rates of antibiotic resistance due to excessive use of broad spectrum antibiotics such as third-generation of cephalosporins [174]. A recent study from Tanzania report the excessive use of antibiotics for the treatment of pneumonia, including administration of ceftriaxone, cloxacillin or metronidazole in addition to the recommended ampicillin and gentamicin combination [175]. The inappropriate use of antibiotics results in increasing rates of antibiotic resistance worldwide [176]. Similar to our study on children with confirmed pneumonia, high rates of pre-hospitalisation antibiotic use with penicillin derivates and trimethoprim-sulfamethoxazole were found in Tanzania [175, 177, 178]. The excessive and inappropriate prescription of antibiotics is common in low-income countries in which healthcare providers have little or no etiological diagnostic facilities [175, 179]. Self-medication is very common in the DR Congo, and when there is no regulation of antibiotic accessibility, parents or children guardian easily access antibiotics without prescription through private pharmacies [180-182]. Lack of guidelines for antibiotic treatment, lack of laboratory competence and

equipment for antibiogram testing are factors linked to the risk of increasing antibiotic resistance in the DR Congo [183].

We found that four times as many children hospitalised for pneumonia had received pre-hospitalisation antibiotic treatment compared to the general population children having a history of antibiotic use during the last month. A high rate of antibiotic resistance in nasopharyngeal carried pneumococci among healthy children has been reported in Tanzania, Kenya and Botswana [58, 153, 184, 185]. A recent study from Tanzania, in which the same method as in our study were used, reported less pneumococcal resistance against benzylpenicilin and ampicillin than found here [58]. In opposite to our finding, a study from Bangladesh reported high susceptibility against benzylpenicilin and ampicillin in pneumococcal isolates from children under-five years with respiratory symptoms [135].

In our children from the general population, more than 95% of the *S. pneumoniae* isolates were resistant to trimethoprim-sulfamethoxazole, similar as found in Tanzania, Ethiopia and in the Gambia [58, 186, 187]. Moreover, pre-hospitalisation treatment with trimethoprim-sulfamethoxazole was more commonly used in children with severe pneumonia than in the children with non-severe disease. Previous studies found that trimethoprim-sulfamethoxazole was used in more than 40% of Congolese children with ALRI when there were no available guidelines recommending the use of amoxicillin or where there was no available amoxicillin in the health centres [2, 20]. Our finding of high level of resistance suggests avoiding the use of trimethoprim-sulfamethoxazole in the treatment of ALRI and pneumonia among under-five children in DR Congo.

Moreover, nearly 40% of pneumococcal strains were resistant to ceftriaxone, which is usually prescribed for the treatment of severe infections such as meningitis in DR Congo. We used breakpoints according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) from 2017 for screening for beta-lactam resistance during our study period [78]. We found that 80% of the isolates had reduced susceptibility to benzylpenicillin, of which 20% were resistant and 62% were classified as intermediate susceptible, now being classified as susceptible, increased exposure [79]. Although 80% of the pneumococcal isolates can no longer be classified as “non-susceptible”, they are still “non-wild-type” isolates, a term now used by the European Centre for Disease Prevention and Control (ECDC). In practice this motivate the use of high dose regimen of benzylpenicillin for the management of childhood pneumococcal pneumonia in DR Congo.

Bacteria and viruses detected by molecular methods in nasopharyngeal secretions from sick and healthy children

The most frequently detected bacteria in both children from the general population and children with radiologically confirmed pneumonia were *S. pneumoniae* and *H. influenzae.* Studies from other African countries have reported *S. pneumoniae* and *H. influenzae* as the most frequently detected bacteria with some geographical variability [52, 106, 188]. Our finding of high rates of bacterial carriage among children in the general population might be explained by poor living conditions with high exposure to indoor air pollution. This is supported by our results showing an association between a higher frequency of pneumococci either detected by culture or PCR (cut-off level Ct<30) and living in rural area or living in a house with an open fire located inside the house and directly connected to the living and/or the bedrooms. Biomass or vegetable residues or wood are the most important source of fuel for cooking in poor areas in low-income countries like DR Congo. A study from a peri-urban area in South Africa reported a high rate of bacterial carriage including pneumococci among children living in conditions with high exposure to indoor air pollution [189]. Crowding, indoor air pollution and living in rural areas have been reported as risk factors for pneumococcal carriage and ALRI or pneumonia in Sub-Saharan African countries [22, 190-192]. An *in vitro* study on indoor air pollution found that particulate matter <10μm in diameter (PM_{10}) promoted bacterial invasion of epithelial airway cells by attenuating innate defence mechanisms [193]. Black carbon has also been shown to induce bacterial colonisation as well as spread of bacteria from the nasopharynx to the lungs [194]. In Burkina Faso indoor air pollution was shown to be significantly associated with ALRI among under five years children [195]. In contrast to our findings, lower loads of bacteria in the nasopharynx of control children compared to children with pneumonia were reported in Gaborone, the capital of Botswana, in Dakar, the capital of Senegal, in Bangladesh and in Thailand [101, 117, 196, 197]. In neither of these studies, biomass was the most common fuel for cooking.

When comparing severe pneumonia to non-severe cases among admitted children with radiologically confirmed pneumonia, high loads of any bacteria regardless of bacterial species were significantly associated with severe pneumonia. In contrary to our results, an Indian study could not find any significant differences in density and frequencies of detected bacteria including *S. pneumoniae* and *H. influenzae* among hospitalised children with pneumonia compared to those with severe or very severe pneumonia [198]. A Tanzanian study reported no difference in densities or numbers of detected *S. pneumoniae* and *H. influenzae* among radiologically confirmed pneumonia children compared to non-radiologically confirmed pneumonia cases [49]. In contrary to our results

studies from Senegal, Kenya and the Gambia reported significantly higher rates of *S. pneumoniae* among sick children compared to the control group [117, 199, 200].

In the context of our study we could not establish the causality of the infection among the sick children, and we did not have a control group sampled during the same study period. RSV was the only pathogen significantly more often detected among the pneumonia cases compared to children in the general population, suggesting that RSV remains one of the most virulent pathogens causing pneumonia among under-five children. This is supported by observations from several other African countries [52, 106, 201-203].

Rhinovirus was the most frequently detected virus among all sampled children and the detection rate using a high cut-off level (Ct<30) was similar in the children from the general population as in the children hospitalised with pneumonia. . Similar rates of rhinovirus among confirmed pneumonia cases and control children were also found in a multi-center study from eight low-income countries between 2010 and 2014 [204]. In opposite to our results, Tanzanian and South-African studies reported significantly higher rates of enterovirus and parainfluenza among pneumonia cases compared to the control group, and higher rates of rhinovirus among the control group compared to the pneumonia cases [49, 201]. A Russian study also reported the predominance of rhinovirus among the healthy control group compared to children with community acquired pneumonia while enterovirus and coronavirus were similar in both groups [205]. We found that high nucleic acid load of any virus, regardless of type was significantly associated with severe pneumonia compared to non-severe pneumonia among the children hospitalised for radiologically confirmed pneumonia. A study from Bangladesh has reported the predominance of any virus among cases with severe pneumonia and malnutrition compared to malnourished without pneumonia [206].

We found that co-occurrence of any virus and any bacteria were significantly more common among children in the general population than in children with radiologically confirmed pneumonia. This finding can probably be explained by the high abundance of pathogens in the nasopharynx of Congolese children in the South-Kivu province. The co-occurrence of bacteria and virus in the nasopharynx of children with ALRI has been reported in India and among children with radiologically confirmed pneumonia in Senegal [117, 203, 207, 208]. A recent study from Tanzania reported that the co-occurrence of bacteria and virus in the nasopharynx of under five years children was strongly associated with community acquired pneumonia [209]. We found in univariable analysis that high loads of pneumococci or RSV in the nasopharynx of sick children were associated with fatal outcome among the under-five children hospitalised for

pneumonia, but we could not confirm this result in the multivariable analysis. Another study from Philippine showed that detection of RSV among children with pneumonia was significantly associated with a fatal outcome [32]. Our findings suggest that the pneumococci and RSV remain the main pathogens implicated in the pathogenicity of severe pneumonia in the South-Kivu province. Thus, we cannot focus only on bacteria such as pneumococci when planning ALRI preventions measures or management, but also to focus on viruses, especially RSV, which has an important role in the occurrence and morbidity of ALRI.

Pneumococcal serotype distribution

Our result showed that among the identified pneumococcal types approximately 50% could be categorized as non-PCV13 serotypes/serogroups. But when we add the number of pneumococcal cases in which our panel could not identify any serotype, the proportion of non-PCV13 serotypes would probably be much higher, as described in others studies in Sub-Saharan African countries, where the number of non-PCV13 serotypes are increasing after the introduction of PCV13 [210, 211].

The PCV13 serotypes/serogroups most often identified among children from the general population were 5, 6 and 19F, while 15BC, 10A, and 12F were the most common non-PVC13 serotypes, which is similar to other studies from Africa [211]. When we compared the serotypes/serogroups identified in the children from the general population with those identified in the children admitted to hospital with radiologically confirmed pneumonia, we found that the most prevalent serotypes/groups both included in PCV13 (5, 6ABCD, 19F and 19A) and non-vaccine types (15BC, 10A, 12F and 11) were equally common in both groups. A study from Mozambique described similar as our serotypes detected among healthy children compared to children with pneumonia [212]. We did not detect serotype 3 at all among the general population children. Serotype 3 has been described as one of the most prevalent serotypes among healthy Gambian children ten years after the introduction of PCV13 and among Malawian children after seven years [213, 214]. This finding can be explained by geographic variances in the serotype distribution or shorter time after implementation of PCV13 in DR Congo, but it is important to mention that the most prevalent PCV13 serotypes described in DR Congo are similar to the most prevalent PCV13 serotypes described in other Sub-Saharan countries where the PCV13 vaccine was recently introduced [211]. Different observations have been described in Morocco, where the serotype 5 was not identified either in cases with pneumonia nor in healthy children [215]. In our case we cannot evaluate the

serotype replacement phenomena, as there is no pre-vaccine study from DR Congo describing the circulating serotypes.

Limitations of the thesis project

A limitation of our research project was that our control group of children from the general population were not collected simultaneously with the radiologically confirmed pneumonia cases, although some of the samples were collected during the same time. The low cultured pneumococcal detection rate can be linked to methodological shortcomings and transportation delay; some samples reached the University Hospital laboratory for culture six hours after the sampling. Storage and transport of the nasopharyngeal samples may have affected the detection level of viral or bacterial nucleic acids, although high frequencies of both bacteria and viruses were indeed detected among the sampled children. Retrospective data were collected from for different hospitals with non-standardised classification of ALRIs. ALRI diagnoses were assessed by more than ten different physicians from various different medical backgrounds with limited access to diagnostic tools, including x-ray and microbiological diagnostic tools.

Strengths of the actual research

We detected a broad range of potential pathogens in the children, analysed pneumococcal serotypes, and performed epidemiological studies, which are very scarce in this resource-limited setting.
Being from the Eastern DR Congo, with clinical experience in the region, the author of the thesis was involved in all data collection, clinical examination as well as nasopharyngeal sampling of the included children. The samples for pathogen identification were collected from the general population children and hospitalised sick children from the same age group and same population areas. To our knowledge, this is the first study on common circulating pneumococcal serotypes and common viruses among under-five year's children in the Eastern DR Congo. Finally, our study was performed a few months after the introduction of PCV13 in the country with inclusion of both unvaccinated and vaccinated children of the some age groups from the same areas.

6. CONCLUSION AND SUMMARY OF MAIN-FINDINGS

Our thesis revealed:

- A significant reduction of children hospitalised due to severe acute lower respiratory infections (ALRI) was seen after introduction of the pneumococcal conjugated vaccine PCV13 in the general infant vaccination program in the Eastern DR Congo.
- Malnutrition, congenital diseases and severe ALRI were associated with case fatality in hospitalised children with ALRI;
- There was frequent inappropriate use of antibiotics for ALRI at the hospital,
- There was a high level of antibiotic resistance against commonly used antibiotics in *Streptococcus pneumoniae* carried by the children in the general population.
- About 50% of identified pneumococcal serotypes could be categorized as non-PCV13 serotypes/serogroups among children in the general population and 40% among children hospitalised with radiologically confirmed pneumonia.
- There was a high load of bacteria and viruses in the nasopharynx of both healthy and sick children

7. FUTURE PERSPECTIVES AND SUGGESTED CARE

From our results, needed interventions to control ALRI and pneumonia among children in DR Congo can be divided into five basic categories:

Improving the living conditions / indoor air pollution

Indoor air pollution can be reduced by electric stoves or improved biomass stoves that offer cooking without open fire or a cooking place connected to a chimney, better housing (i.e. houses with living room or sleeping room not connected to the cooking place with an open fire), education through local radio, churches, schools on the badness of indoor air pollution and promote the use of clean fuels for cooking.

Prevention of malnutrition

In order to reduce malnutrition, agricultural and micronutrient (Zinc, Vitamin D, selenium and vitamins) interventions, providing safe drinking water and sanitation, education about better diets for vulnerable groups (under five years children and pregnant women) and increased quality of health services are needed [216, 217].
Education about locally available proteins and plants that contain essential micronutrients is particularly effective and sustainable. There is a good WHO protocol for the management of malnutrition in DR Congo [218, 219]. This needs to be promoted and used effectively in health centers and district hospitals.

Healthcare system and accessibility in DR Congo

Establish a free-of-charge care policy as an effective way to expand the coverage and use of health services by vulnerable (poor) populations. This can be introduced progressively, starting by the three most killing diseases of under five years children (ALRIs, diarrhea and malaria). This includes the holistic management of the diseases including medicines and supplies, hospitalisation fees and supportive cares.

1. Immunisation program against *Haemophilus influenzae* type b (Hib) and pneumococci (PCV13) for children under five years of age.
2. Improve the management of ALRI in the country by earlier diagnostics and adapting of the recommended antibiotic treatment to the level of antibiotic resistance (revise the guideline for the management of ALRI).

My suggested ALRI Management

Simplify and generalise or systematise ALRI management for earlier clinical diagnosis and adequate treatment at primary care level. This will have the potential to significantly reduce the mortality in DR Congo, where access to paediatricians is very limited. For this strategy, I suggest:
To use the revised new WHO clinical classification of pneumonia for earlier diagnostic and antibiotic treatment with slight modification according to the local behaviour, culture and ongoing antibiotic resistance identified during our research studies. This means in practice:

At the community level:

Train community health workers (Rélais communautaire) including pharmacists and pharmacist assistants for the identification of pneumonia symptoms, treatment of non-severe cases and transfer severe cases to the first-level health care facility. The training will be based on the identification of the symptoms of pneumonia (fast breathing and/or chest indrawings by looking at the chest wall movement and listening to the breath sound for counting the respiratory rate), initiate the antibiotic treatment (oral amoxicillin) for the home care and identification of the signs of danger (e.g. not able to drink or breastfeed, persistent vomiting, convulsions, lethargy or loss of consciousness, stridor in a calm child or severe malnutrition) for the earlier transfer to the first-level health care facility or hospital after giving the first dose of antibiotic.

At first-level health care facility:

Management of non-severe pneumonia (Oral correct dose and duration of amoxicillin treatment, schedule an appointment for follow up, feeding advice and hygiene). Transfer to referral hospital after the first dose of antibiotic if there is any sign of danger.

At the referral hospital:

In hospital management with intravenous antibiotic (combination of ampicillin and gentamicin) for five days, supportive care such as intravenous (IV) fluids, nasal oxygen if oxygen saturation is < 90%. If there is no improvement after 48 hours of IV antibiotic treatment, switch the antibiotic treatment to ceftriaxone and gentamicin. If not improved after 5 days, transfer to the university hospital for more investigations.

Take advantage of hospitalisation for the nutrition, hygiene and vaccination advices to the parents or guardian. Before the discharge, check the immunisation schedule of the child and give a catch-up vaccination appointment if needed.

At the university hospital:

Same treatment as at the referral hospital with addition of radiological and microbiological investigation (Chest X ray, Ultrasound, or CT scan if needed, blood culture, possibly a nasopharyngeal swab culture, at the admission and adapt the treatment to the aetiology after exams (imaging and adjust the antibiotic treatment following the support of CRP and leukocytes count or microbiology if available).

Oral Amoxicillin doses and presentation

Table 3: Oral amoxicillin doses for outpatient treatment of non- severe pneumonia by community health workers and professional health workers

OUT PATIENT PNEUMONIA TREATMENT	AGE/WEIGHT OF CHILD	AMOXICILLIN DISPERSIBLE TABLETS (250 mg)
By community health workers in the community	2-12 months (4–<10 kg)	1 tab x 3/day x 5 days (15 tabs)
	12 - 5 years (10–19 kg)	2 tabs x 3/day x 5 days (30 tabs)
By health workers at the health centers	2 -12 months (4–<10 kg)	2 tabs x 2/day x 5 days (20 tabs)
	12 -3 years (10–<14 kg)	3 tabs x 3/day x 5 days (45 tabs)
	3 - 5 years (14–19 kg)	3 tabs x 3/day x 5 days (45 tabs)

Antibiotics treatment for children with severe pneumonia at district hospitals

Table 4: Doses of intravenous antibiotics for inpatient treatment of severe pneumonia at district hospitals

Lines of Treatments	Antibiotics association	Antibiotic doses
First line	Ampicillin	50mg/kg every 6 hours par days for five days
	Gentamicin	7.5mg/kg in one dose par day for five days
Second line (If there is no improvement after 48 hours of treatment with the first line)	Ceftriaxone	50 to 100mg/kg par day in one or two doses for five days
	Gentamicin	7.5mg/kg in one dose par day for five days

Nasal oxygen indications

Oxygen should be available at all times. The two main sources of oxygen are cylinders and oxygen concentrators. Oxygen therapy should be guided by the pulse oximetry:

Indications:

Give oxygen to children with an oxygen saturation < 90%
When a pulse oximeter is not available, clinical signs should guide the oxygen therapy for a child with severe pneumonia [141]:

- Central cyanosis
- Inability to drink (when this is due to respiratory distress)
- Severe lower chest wall indrawing
- Respiratory rate >70/min
- Grunting with every breath (in young infants)
- Depressed mental status

Set a flow rate of 1–2 liters/min to deliver an inspired oxygen concentration of up to 40% (0.5 liters/min for young infants). Humidification is not required with nasal prongs [44, 220, 221].

Supportive care

Remove by gentle suction any thick secretion at the entrance to the nasal passages or throat, which the child cannot clear, or process to the nasal lavage with normal saline solution. If the child has fever >38.5C give paracetamol. If the child has wheezing, give a rapid acting bronchodilator. Ensure that the child receives daily maintenance fluids appropriate for his or her age, but avoid over-hydration. Encourage breastfeeding and oral fluids. If the child can't drink, insert a nasogastric tube and give maintenance fluids in frequency small amounts. If the child is taking fluids adequately by month, do not use a nasogastric tube as it increases the risk for aspiration pneumonia and obstructs part of the nasal airway. If oxygen is given by nasal catheter at the same time as nasogastric fluids, pass both tubes through the same nostril. Encourage the child to eat as food can be taken.

Follow up and transfer

Give an appointment for follow up and catch-up immunisations schedule if applied before the discharge day. If not improved, the child will be referred to university hospital in the province for more investigation and special management.

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