Kwame Nkrumah University of Science and Technology

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College of Health Sciences

Department of Clinical Microbiology

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Viral Agents in Children Presenting with Signs and Symptoms of Respiratory Infections in the Ashanti-Akyem North District of Ghana

By

Augustina Angelina Annan

2011

VIRAL AGENTS IN CHILDREN PRESENTING WITH SIGNS AND SYMPTOMS OF RESPIRATORY INFECTIONS IN THE ASHANTI-AKYEM NORTH DISTRICT OF GHANA

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By

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Kwame Nkrumah University of Science and Technology
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
School of Medical Sciences,
College of Health Sciences

August 2011

DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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Supervisor's Name Signature Date

Certified by:

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Head of Dept. Name Signature Date

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DEDICATION

This work is dedicated to my adorable son John-Claude, my husband Dr. Justice Sylverken, my parents Mr. and Mrs. John Jude Kwamina Annan and my siblings for their love and support.

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ABBREVIATIONS

RI	Respiratory infection
RVI	Respiratory Viral infection
RTIs	Respiratory tract infections
LRT	Lower respiratory tract
URT	Upper respiratory tract
URTIs	Upper respiratory tract infections
LRTIs	Lower respiratory tract infections
LRI	Lower respiratory infection
AdV	Adenovirus
RSV	Respiratory syncytial virus
PIV	Parainfluenza virus
RhV	Rhinoviruses
ENT	Enteroviruses
hMPV	Human metapnuemovirus
MYC	Mycoplasma
CHL	Chlamydophila
HAdV	Human adenovirus
HIV	Human immunocompromised virus
EKC	Epidemic Keratoconjunctivitis
TB	Tuberculosis
SARS	Severe Acute Respiratory Syndrome
COPD	Chronic Pulmonary Disease
WBC	White Blood Cell
M - gene	Matrix gene

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N - protein	Nucleoprotein
G - protein	Glycoprotein
HA-gene	Hemagglutinin gene
NCR	Non-coding region
5'-UTR	5'- untranslated region
DNA	Deoxyribonucleic acid
cDNA	complementary DNA
RNA	Ribonucleic acid
cRNA	Carrier RNA
mRNA	messenger RNA
S	Sense
AS	Antisense
dNTPs	deoxynucleoside triphosphates
ATP	Adenosine triphosphate
GTP	Guanosine triphosphate
CTP	Cytidine triphosphate
TTP	Thymidine triphosphate
BSA	Bovine Serum Albumin
EDTA	Ethylenediaminetetraacetic acid
NaCl	Sodium Chloride
KCl	Potassium Chloride
$MgCl_2$	Magnesium Chloride
LB	Luria-Bertani
TBE	Tris- Hydroxymethyl–amino methane, Boric acid and Ethylenediamine tetraacetic acid Na_2– salt dehydrate

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GPS	Gene Power Supply
UV	Ultra violet
β-ME	β-mercaptoethanol
FRET	Fo¨rster resonance energy transfer
CPE	Cytopathic Effect
EM	Electron microscopy
EIA	Enzyme immunoassay
IFA	Indirect immunofluorescence
IgA	Immunoglobulin A
CAP	Community-acquired pneumonia
OPS	Oropharyngeal swab
NPS	Nasopharyngeal swab
NPW	Nasopharyngeal washing
NPA	Nasopharyngeal aspirate
CDC	Centers for Disease Control
WWW	World wide-web
EPA	Environmental Protection Agency
EPI	Expanded Programme for Immunization
CAH	Child and Adolescent Health and Development
UNICEF	United Nations International Children Educational Fund
IMCI	Integrated Management of Childhood illnesses
CDC	Centre for Diseases Control
USA	United States of America
WHO	World Health Organization
DFA	Direct immunofluorescent antibody

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FA	Fluorescent-Antibody
PCR	Polymerase Chain Reaction
RT-PCR	Real-time Polymerase Chain Reaction
RT-PCR	Reverse transcription Polymerase Chain Reaction
RT-RT-PCR	Real time-Reverse transcription Polymerase Chain Reaction
OR	Odds Ratio
CI	Confidence interval
KNUST	Kwame Nkrumah University of Science and Technology
SMS	School of Medical Sciences
CHRPE	Committee on Human Research, Publications and Ethics
APH	Agogo Presbyterian Hospital
GCP	Good Laboratory Practice
BNITM	Bernhard Nocht Institute for Tropical Medicine
OPD	Out-patients department
CWC	Child Welfare Clinic
CRF	Case Reporting Form
HPLC	High performance liquid chromatography
FAM	6-carboxy-fluorescein
MGB	Minor Groove Binder
TAMRA	6-carboxy-tetramethyl-rhodamine
MGBNFQ	Minor Groove Binder non-fluorescent Quencher
KCCR	Kumasi Centre for Collaborative Research in Tropical Medicine

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ABSTRACT

Respiratory Infections (RIs) constitute one of the major causes of morbidity and mortality among the pediatric population of developing countries. When caused by viruses, their manifestations are very difficult to detect on clinical grounds and most importantly by conventional diagnostic methods. Whilst studies on the viral causes of RIs are well documented in developed countries, there exist scanty information on them in most developing countries. The main aim of this study was to optimize and establish molecular diagnostic systems which are able to simultaneously detect several pathogens per clinical sample for respiratory viral infections. The viral aetiology and determinants of respiratory viral infections in children were also evaluated.

Three real time multiplex Polymerase Chain Reaction (m-RT-PCR) and five monoplex real-time polymerase chain reactions (RT-PCR) were optimized and established for the detection of twelve respiratory agents during the study. The three m-RT-PCRs included two duplexes for the simultaneous detection of respiratory syncytial virus and human metapneumovirus (RSV/hMPV), influenza A and B (influenza A/B) and a triplex for parainfluenza 1, 2 and 3 (PIV 1-3). The five monoplexes included three assays for Rhinoviruses (RhV), Enteroviruses (ENT), Adenoviruses (AdV) and two for non-viral agents *Mycoplasma pneumoniae* (MYC) and *Chlamydophilia pneumoniae* (CHL).

Nasopharyngeal swabs were taken from children who presented with at least two signs and symptoms suggestive of respiratory infection to the out patient department of Agogo Presbyterian Hospital in the Asante Akim North district in the Ashanti Region of Ghana between February 2008 and March 2009. Also collected were their sociodemographic, socioeconomic and clinical data. A predictive algorithm for the viral causes of respiratory infection was then developed.

A total of 1,191 children were enrolled in the study. The developed assays detected 476 pathogens in 429 (36.5%) children. RSV/hMPV was the most frequently detected pathogen in 154 (13.1%), RhV in 93 (7.9%), PIV 1-3 in 83 (7.1%) and influenza A/B in 68 (5.8%) of the children. Furthermore, AdV and ENT were

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detected in 40 (3.4%) and 29 (2.4%) respectively whilst MYC was found in 6 (0.5%) of the children. CHL was detected in only 1 (0.1%) child. More than one respiratory agent was detected in 47 (3.9%) of the study participants.

Despite the differences in the symptoms presented, most viral agents could not be associated with specific clinical signs or symptoms. The risk for RVIs decreased steadily with increasing age (OR=0.57, 95% CI: 0.49-0.67, $p<0.05$) with the youngest children (up to 12 months) at highest risk (OR=0.87, 95% CI: 0.69-1.10). This was true for RSV/hMPV, PIV 1-3 and AdV ($p<0.05$). For influenza A/B, there was an increased risk with increasing age ($p<0.05$). With an overall male-to-female ratio of 1.2:1, gender ($p=0.99$), religion ($p=0.89$) and ethnicity ($p=0.56$) were not associated with RVIs.

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High temperature (≥37.6°C) was also a significant determinant for viral respiratory infections ($p<0.05$). With the exception of maternal age ($p=0.02$) and nutrition ($p<0.05$), none of the maternal factors studied turned out to be predictors of RVIs in the children. Similarly, apart from associations between accessibility to health facilities ($p<0.05$), none of the socioeconomic factors were determinants of RVIs. While RSV/hMPV and influenza viruses were detectable during the rainfall seasons, PIV, RhV, ENT and AdV were detected sporadically throughout the year.

This study has defined the epidemiology of specific respiratory viruses and the clinical presentation of children with signs and symptoms of respiratory infection. With the advent of molecular diagnostic techniques such as multiplex RT-PCR, this study provides valuable information to clinicians and virologists confronted with children suffering from respiratory tract illnesses of viral etiology for possible understanding of the viral causes of these infections.

APPENDICES

Appendix 1: Ethical approval form

Appendix 2: Informed consent form

Appendix 3: Virology laboratory reporting form

Appendix 4: Case Reporting Form (CRF)

Appendix 5: Socioeconomics information form

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