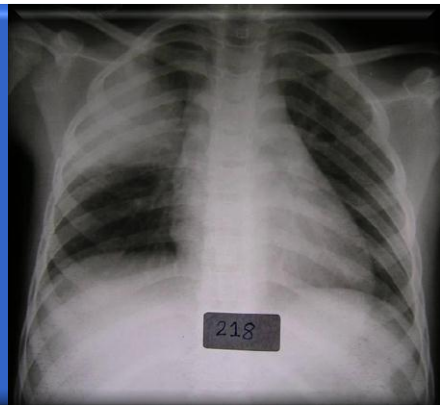


Pneumonia Aetiology

Why is it so difficult to distinguish pathogens from innocent bystanders?

David Murdoch
Department of Pathology
University of Otago, Christchurch

THEINFECTIONGROUP

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND

Outline

- Background
- Diagnostic challenges for pneumonia aetiology
- Research needs
  - Innovative new diagnostic tools
  - New approaches to data analysis
  - Alternative paradigms of pneumonia aetiology

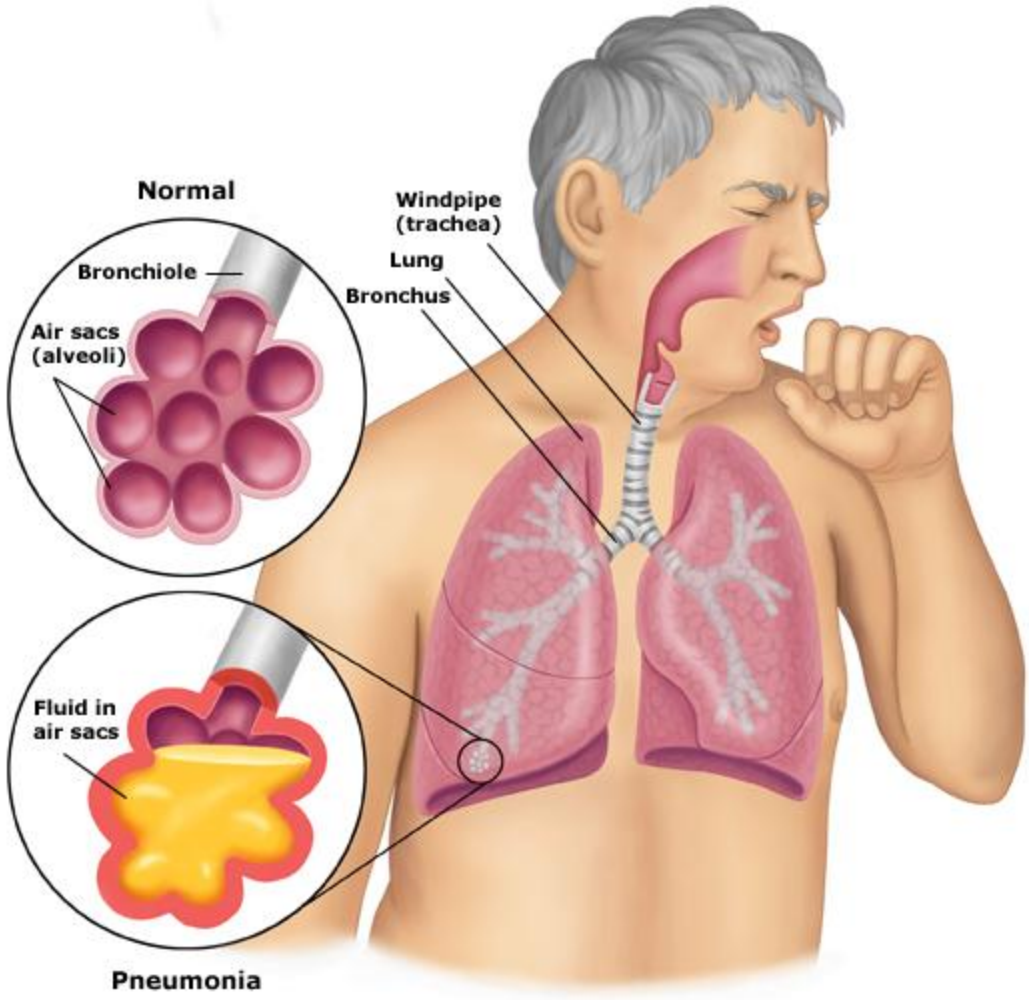
PERCH

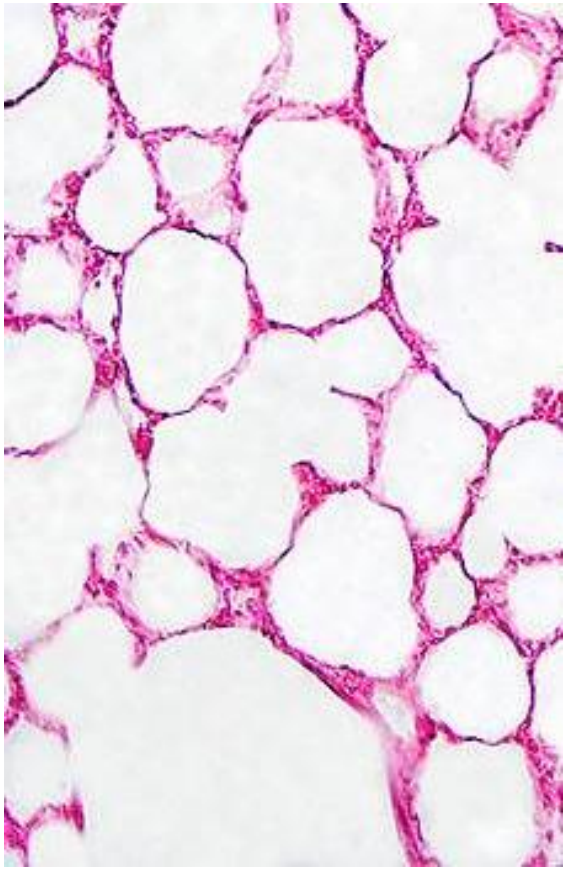
Pneumonia Etiology Research for Child Health

Updated: July 5, 2012

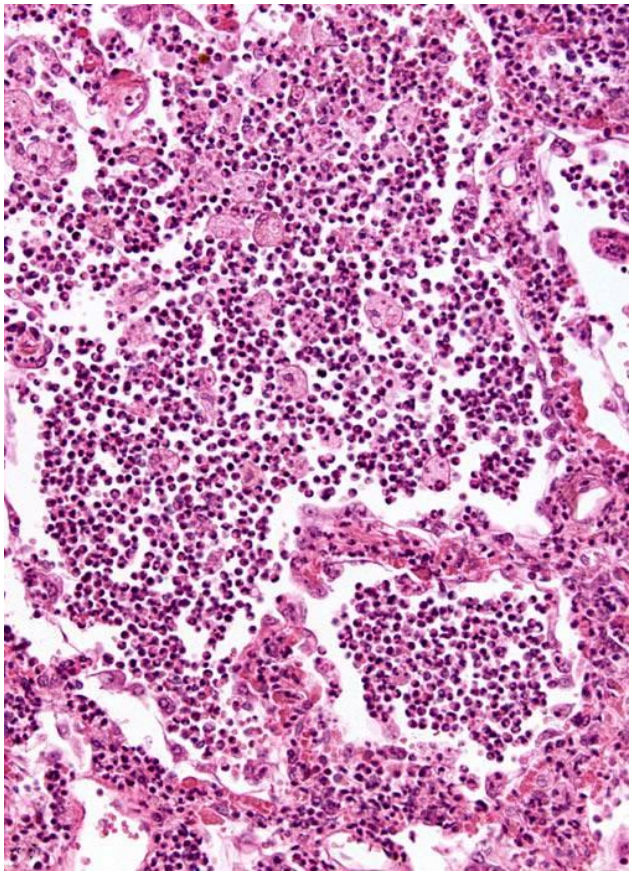
* PERCH Coordinating Center

* Core team members

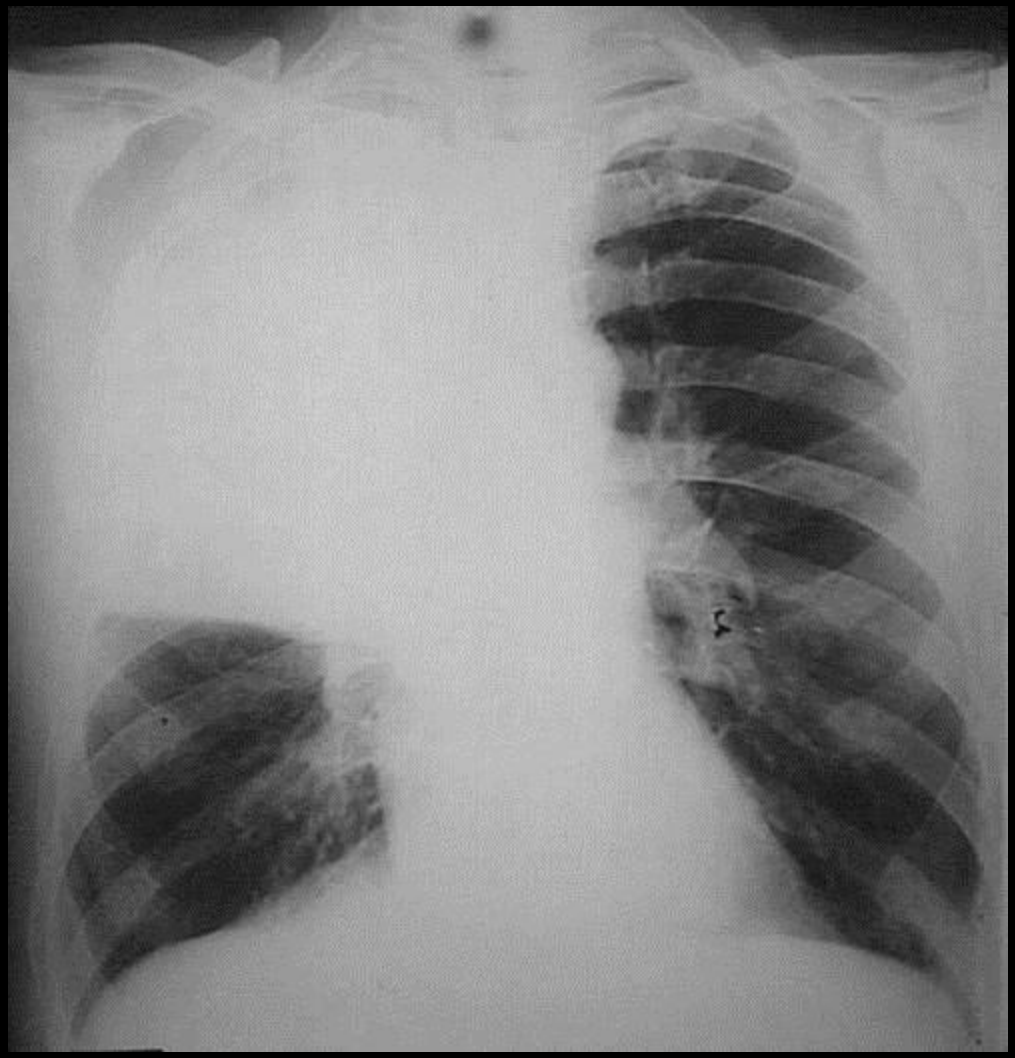

Normal
Bronchiole
Air sacs
(alveoli)
Fluid in
air sacs
Pneumonia
Windpipe
(trachea)
Lung
Bronchus

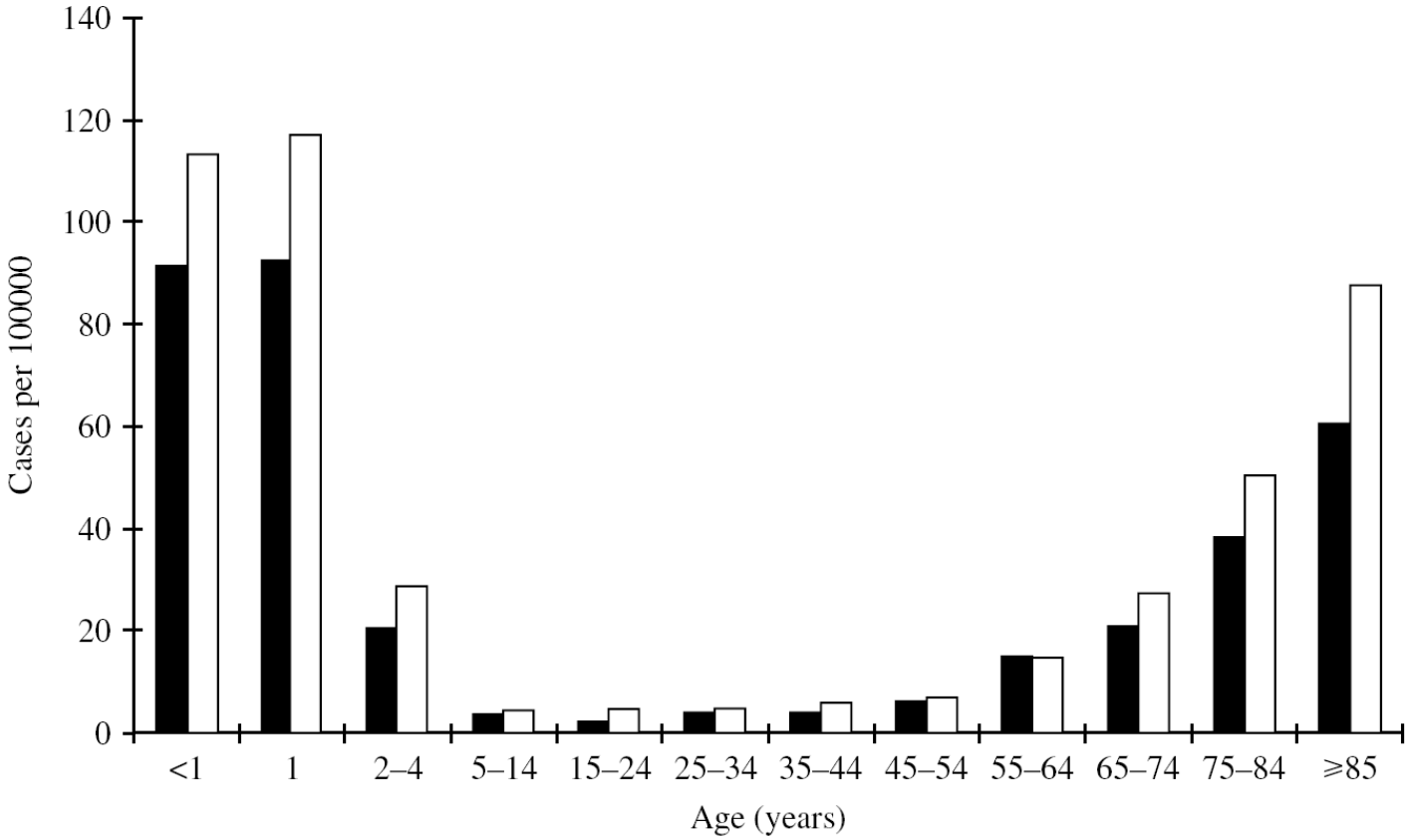


Normal lung tissue



Pneumonia





Average annual incidence of invasive pneumococcal disease by age and gender (■, female; □, male), 1998–2005.

Heffernan et al *Epidemiol Infect* 2008;136:352-9

PNEUMONIA

THE FORGOTTEN KILLER OF CHILDREN

unicef

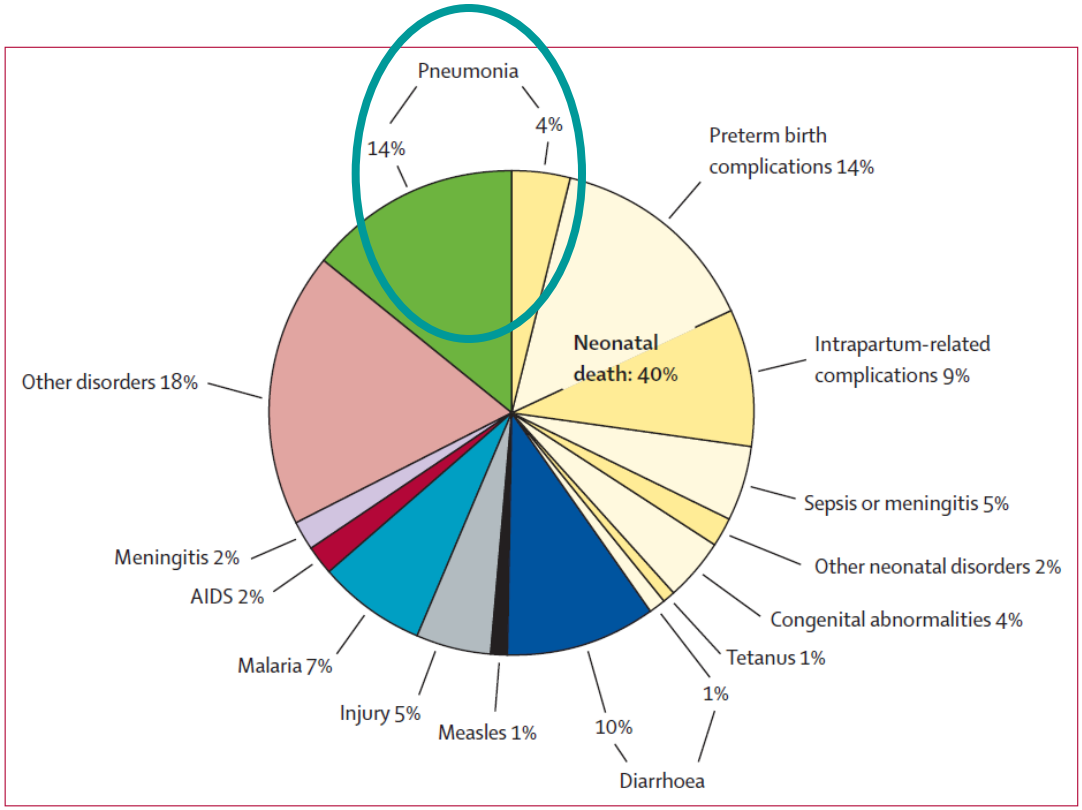
World Health Organization

Burden of Childhood Pneumonia

Deaths

- **7.6 million deaths** among children <5y in 2010
- **18% (1.4 million) were caused by pneumonia**

Liu et al. *Lancet* 2012; 379: 2151-61



Global causes of mortality in children <5 years

A Wide Variety of Organisms can Cause Pneumonia

Bacteria

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Mycoplasma pneumoniae*
- *Moraxella catarrhalis*
- *Legionella* species
- *Chlamydophila pneumoniae*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Acinetobacter* species
- *Pseudomonas* species
- *Coxiella burnetii*
- *Chlamydia psittaci*
- *Francisella tularensis*
- *Yersinia pestis*
- *Bacillus anthracis*
- *Burkholderia pseudomallei*
- Mycobacteria

Viruses

- Influenza A and B
- Respiratory syncytial virus
- Adenoviruses
- Parainfluenza viruses
- Coronaviruses
- Varicella zoster virus
- Measles virus
- Cytomegalovirus
- Herpes simplex virus

Fungi

- *Pneumocystis jiroveci*
- *Cryptococcus neoformans*
- *Coccidioides immitis*
- *Histoplasma capsulatum*

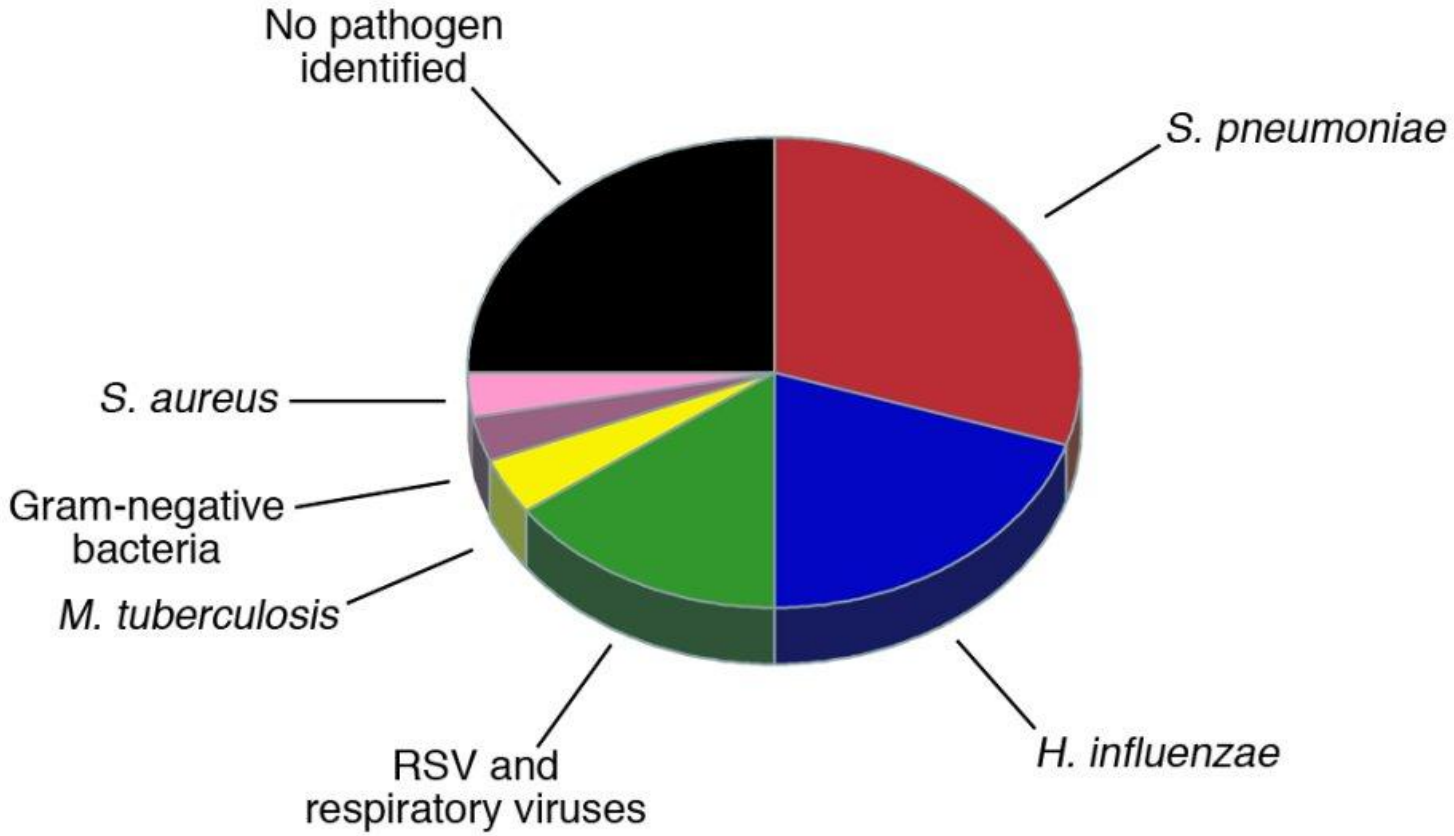
Parasites

- *Paragonimus westermani*

This list is incomplete!

Causes of Severe Pneumonia

HIV-negative children in developing countries
1995-2005



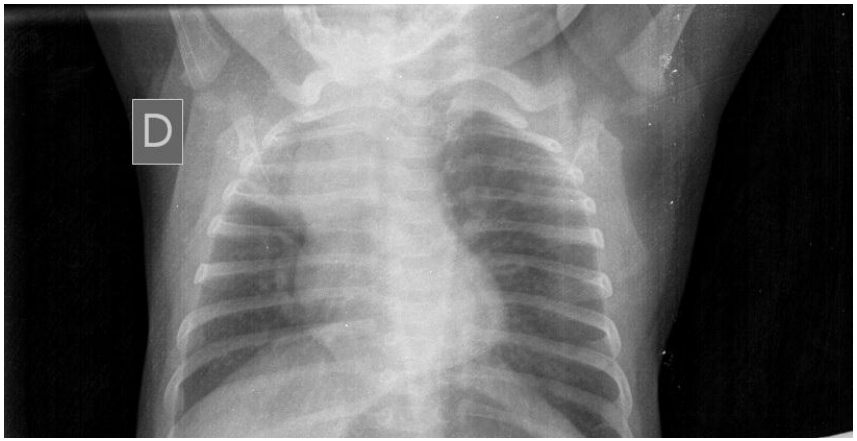
Scott et al. *J Clin Invest* 2008;118:1291-1300

Why is it Important to Know the Causes of Pneumonia?

- Better direct antibiotic treatment
- Identification of unexpected or unusual causes
- Detection of antibiotic resistance
- Surveillance
- Informing and vaccine design

Case Report

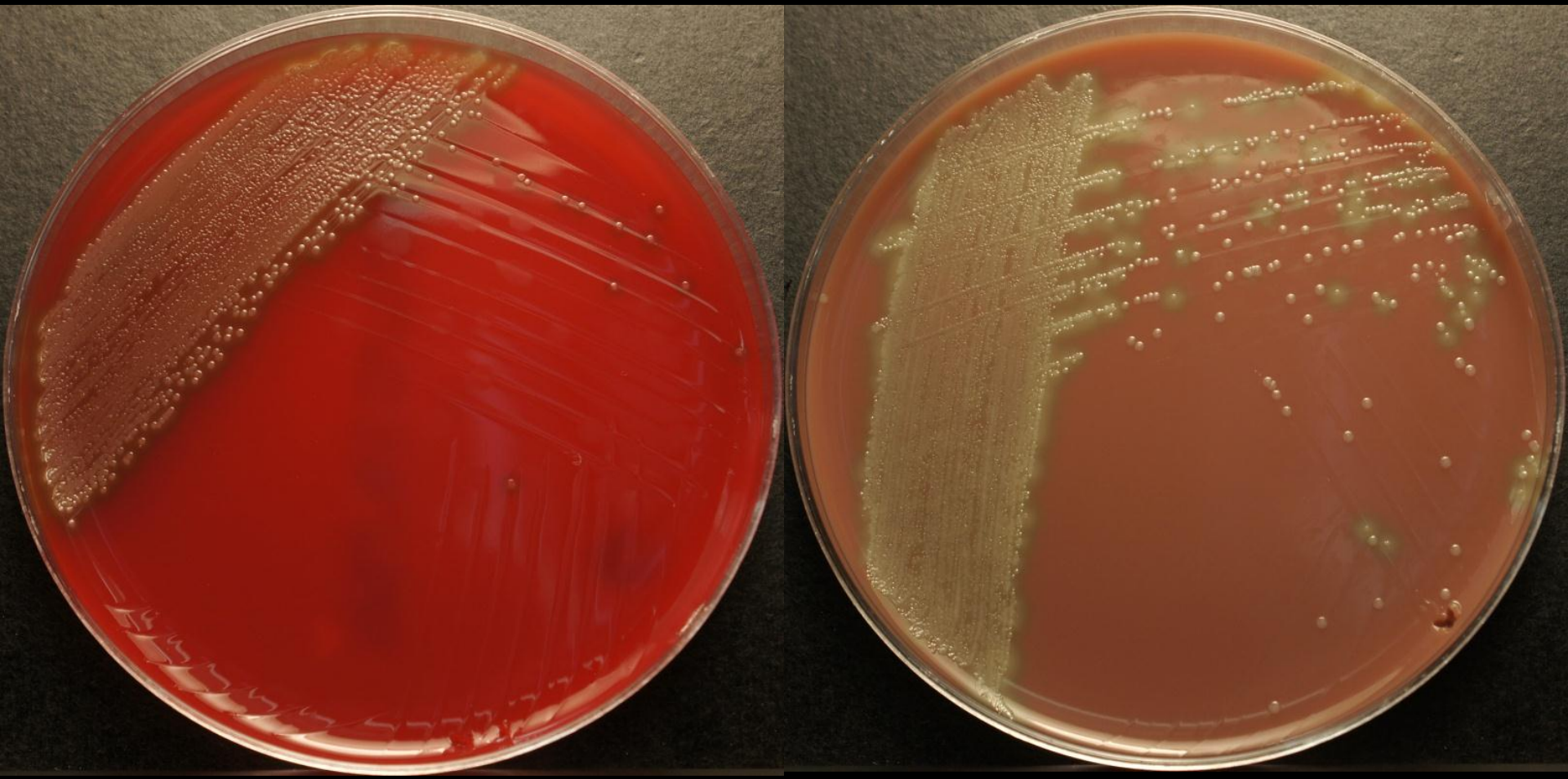
- 7 month old boy from Mali
- Admitted with very severe pneumonia
- Antibiotics given in the community 2 days previously



Induced sputum microscopy (100X)

Induced sputum microscopy (1000X)

Induced Sputum Culture

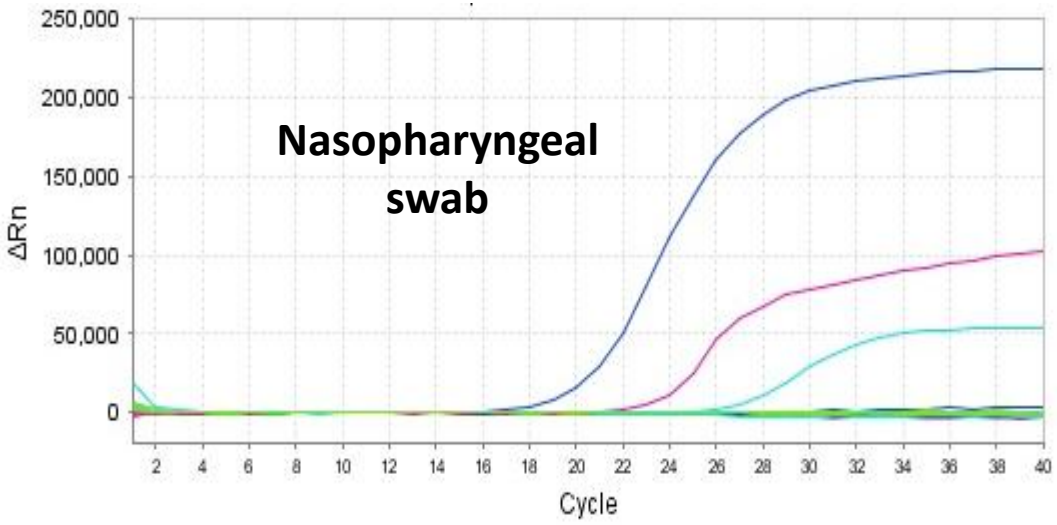


3+ *Streptococcus pneumoniae*

3+ *Haemophilus influenzae*

1+ Oropharyngeal flora

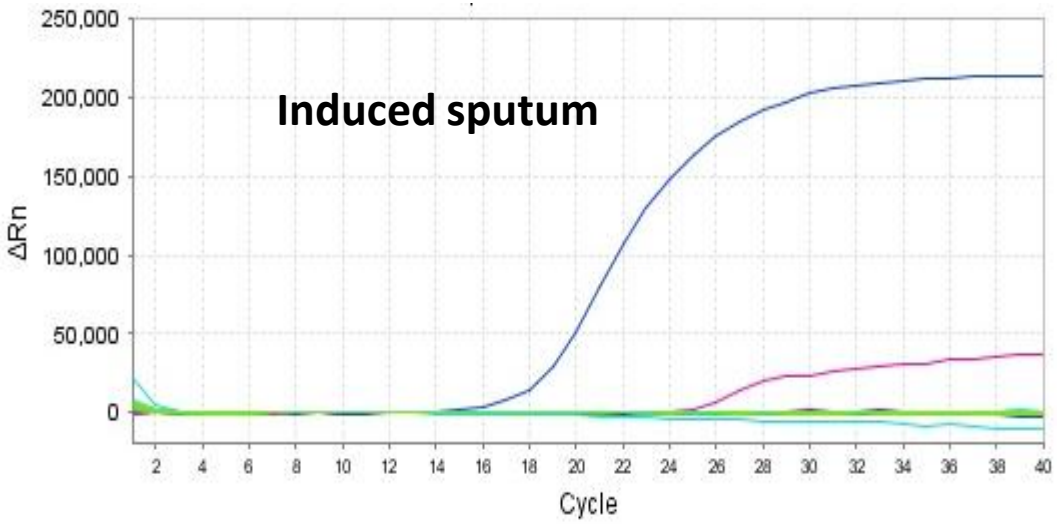
Multiplex PCR Results



Streptococcus pneumoniae

Haemophilus influenzae

Adenovirus



Streptococcus pneumoniae

Haemophilus influenzae

Summary of Results

Specimen	Test	Potential pathogen
Blood	Culture	None
Induced sputum	Microscopy	Gram-positive diplococci
	Culture	*S. pneumoniae, H. influenzae*
	PCR	*S. pneumoniae*, *H. influenzae*
Nasopharyngeal swab	PCR	*S. pneumoniae*, *H. influenzae*, adenovirus

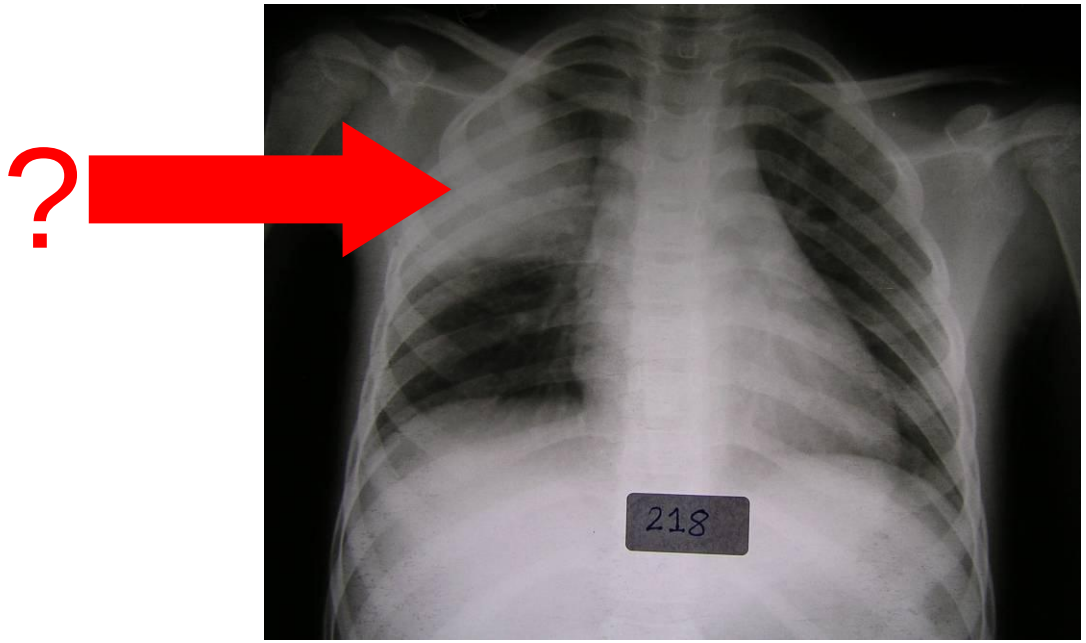
What is/are the causative pathogen(s) in this child's pneumonia?

Specimen	Test	Potential pathogen
Blood	Culture	Not collected
Induced sputum		Not collected
Nasopharyngeal swab	PCR	*S. pneumoniae*, *H. influenzae*, adenovirus

Fundamental Issues in Determining Pneumonia Aetiology

(1) Specimen collection

— Difficulty in obtaining specimens from the site of infection is a fundamental problem in pneumonia diagnostics

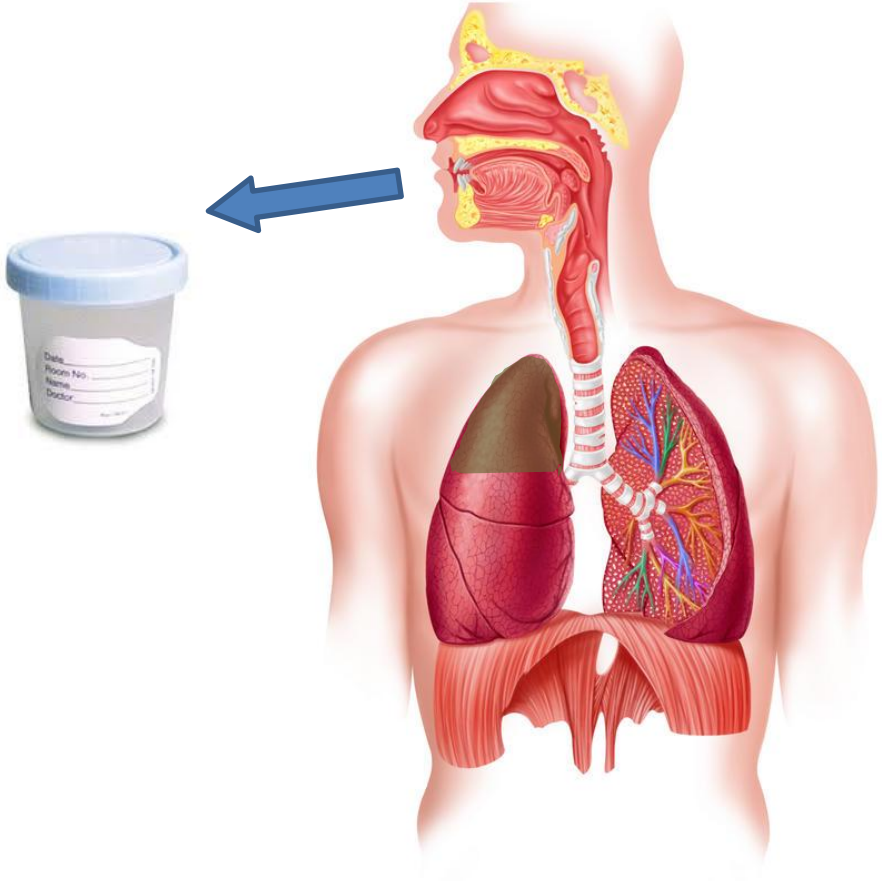


Lung Aspirates

- Specimen collected from the site of infection
- Is a normally sterile specimen
- Only collected if there is lobar illness in hospitalized patients
- Good safety profile, but perceived risk high
- Rarely done anymore

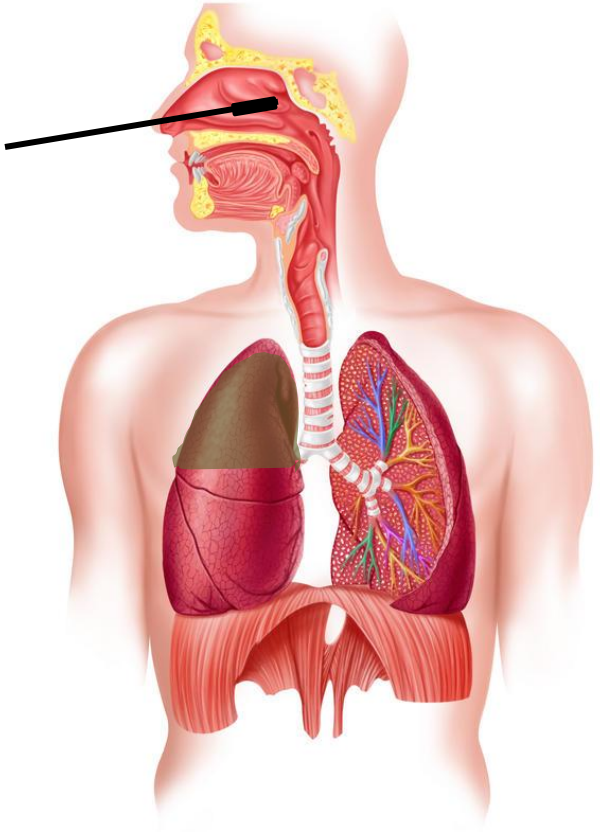
Sputum

- Comes from the lower respiratory tract
- But is often contaminated with oropharyngeal flora
- Difficult to obtain from young children



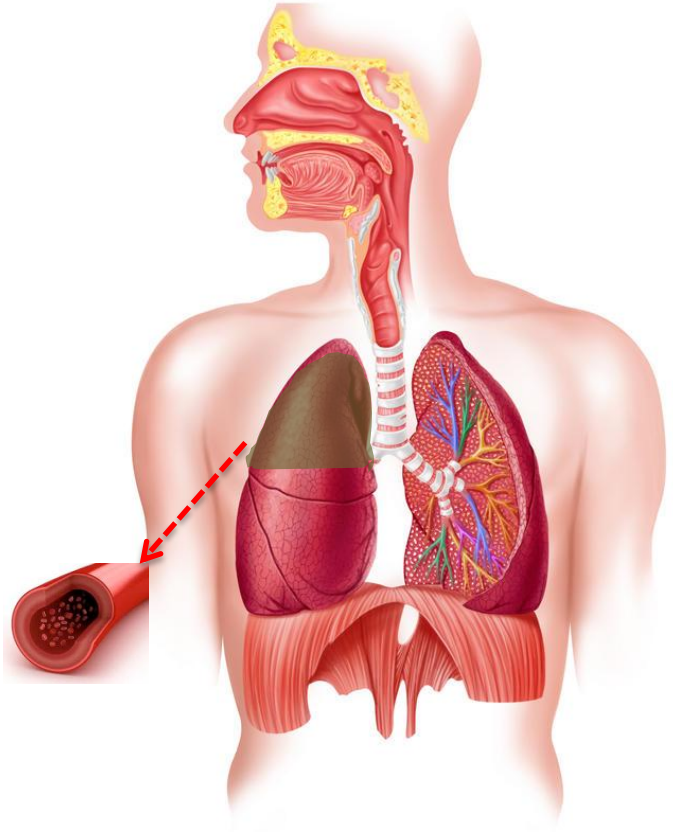
Nasopharyngeal Specimens

- Relatively easy to collect
- May not be representative of the lower respiratory tract
- Nasopharynx is the site of replication of most common respiratory viruses
- Colonization with bacteria is common



Blood

- Relatively easy to collect
- Highly specific for aetiology
- Only positive in 10% bacterial pneumonia

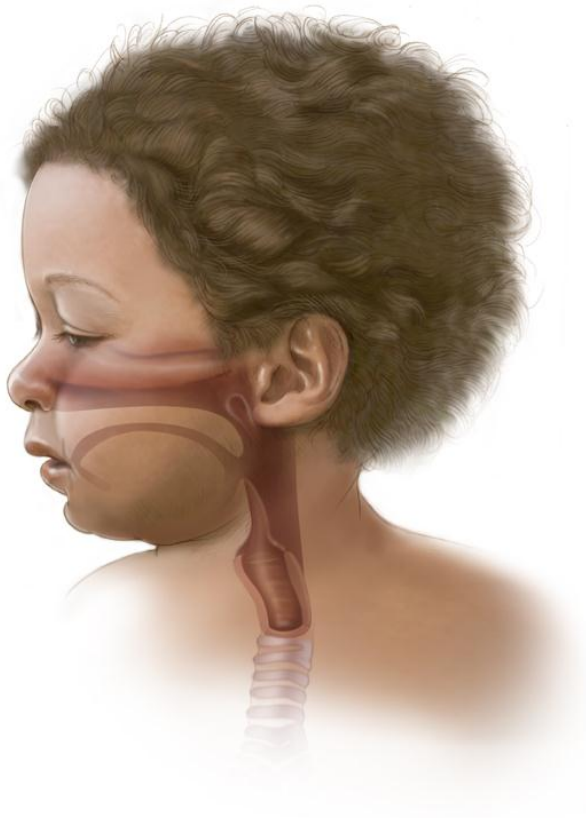


Fundamental Issues in Determining Pneumonia Aetiology

(2) Differentiating innocent bystanders from true pathogens

- colonisers
- viruses from coincidental recent upper respiratory infection

Some pneumonia pathogens can also colonise healthy people



Lancet Infect Dis 2004; **4:** 144–54

A Wide Variety of Organisms can Cause Pneumonia

Bacteria

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- *Coccidioides immitis*
- *Histoplasma capsulatum*

Parasites

- *Paragonimus westermani*

Detection of Respiratory Pathogens by PCR from Nasopharyngeal Swab Specimens in Kenyan Children with Pneumonia and Controls

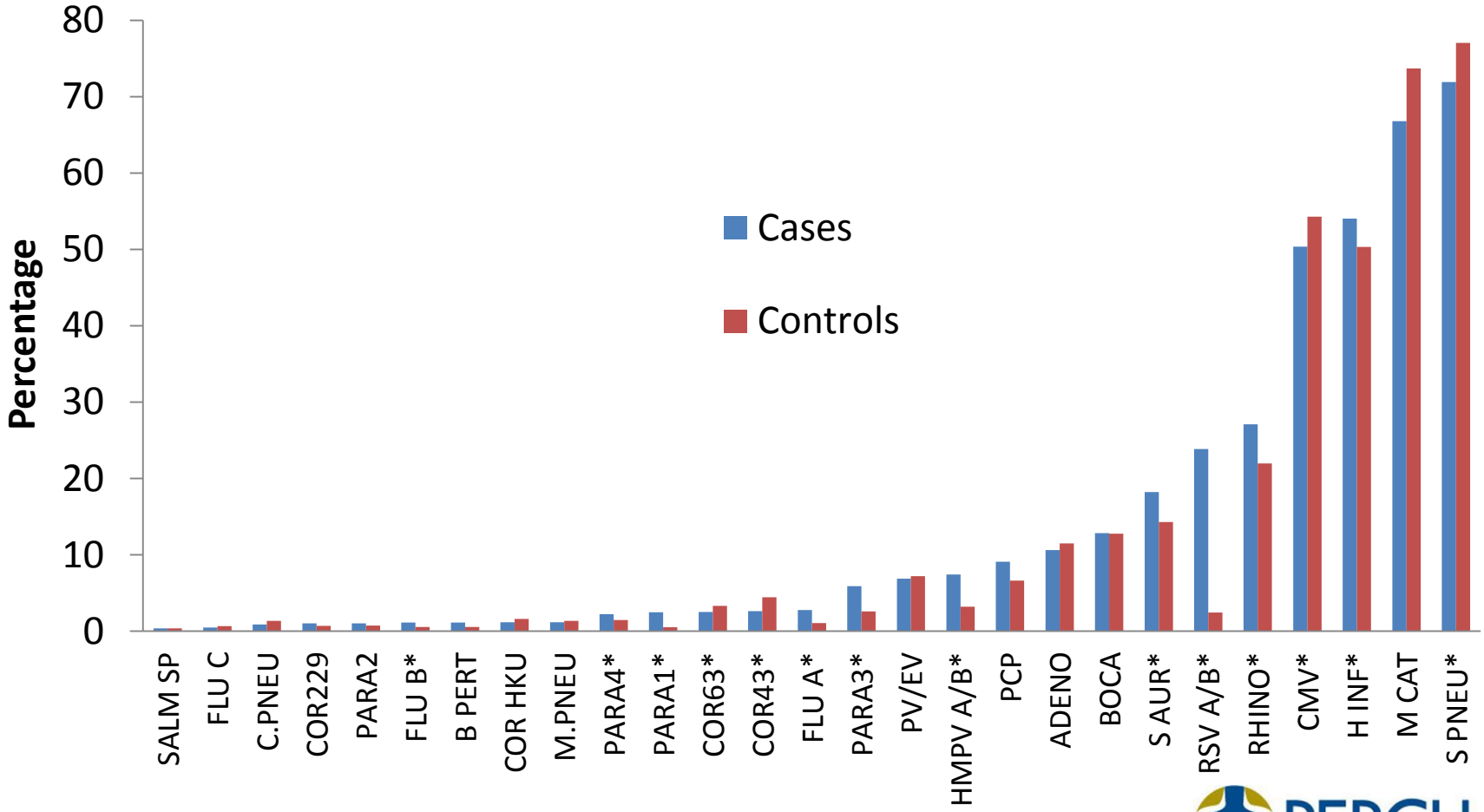
	aOR for Case Patients vs Controls Without URTI		aOR for Case Patients vs All Controls	
Pathogen	aOR	95% CI	aOR	95% CI
RSV A	**12.5**	3.1–51.5	**3.8**	2.2–6.6
RSV B	∞	<.001[a]	**11.9**	3.7–38.2
Adenovirus	0.5	.3–1.0	0.7	.4–1.2
Rhinovirus	1.0	.6–1.5	1.0	.7–1.3
Parainfluenza 1	1.4	.2–11.6	0.9	.3–2.7
Parainfluenza 2	0.8	.1–7.1	**0.3**	.1–.8
Parainfluenza 3	1.3	.6–3.2	0.9	.5–1.6
Parainfluenza 4	1.1	.2–5.0	1.4	.4–4.5
Influenza A	1.4	.2–11.4	0.7	.2–2.2
Influenza B	∞	1.0[a]	∞	1.0[a]
Influenza C	∞	1.0[a]	0.8	.1–4.8
Coronavirus 229E	0.7	.3–1.9	0.6	.3–1.1
Coronavirus OC43	2.0	.5–8.8	1.0	.5–2.1
Coronavirus NL63	∞	1.0[a]	1.0	.2–5.4
HMPV	4.6	.6–34.4	2.8	.9–8.1
Mycoplasma pneumoniae	0.3	.1–1.9	0.5	.1–2.1

Most pathogens were found in a similar proportion of cases and controls

Clinical Infectious Diseases 2012;54(S2):S190–9

Classical Case-Control Analysis
PERCH Study (NP/OP PCR)

~8000 children aged <5 years from developing countries



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Pneumonia Etiology Research for Child Health

Use of a Rapid Test of Pneumococcal Colonization Density to Diagnose Pneumococcal Pneumonia

W. C. Albrich,[1,5] S. A. Madhi,[1,2] P. V. Adrian,[1,2] N. van Niekerk,[1] T. Mareletsi,[1] C. Cutland,[1,2] M. Wong,[3] M. Khoosal,[4] A. Karstaedt,[3] P. Zhao,[6] A. Deatly,[6] M. Sidhu,[6] K. U. Jansen,[6] and K. P. Klugman[1,7,8]

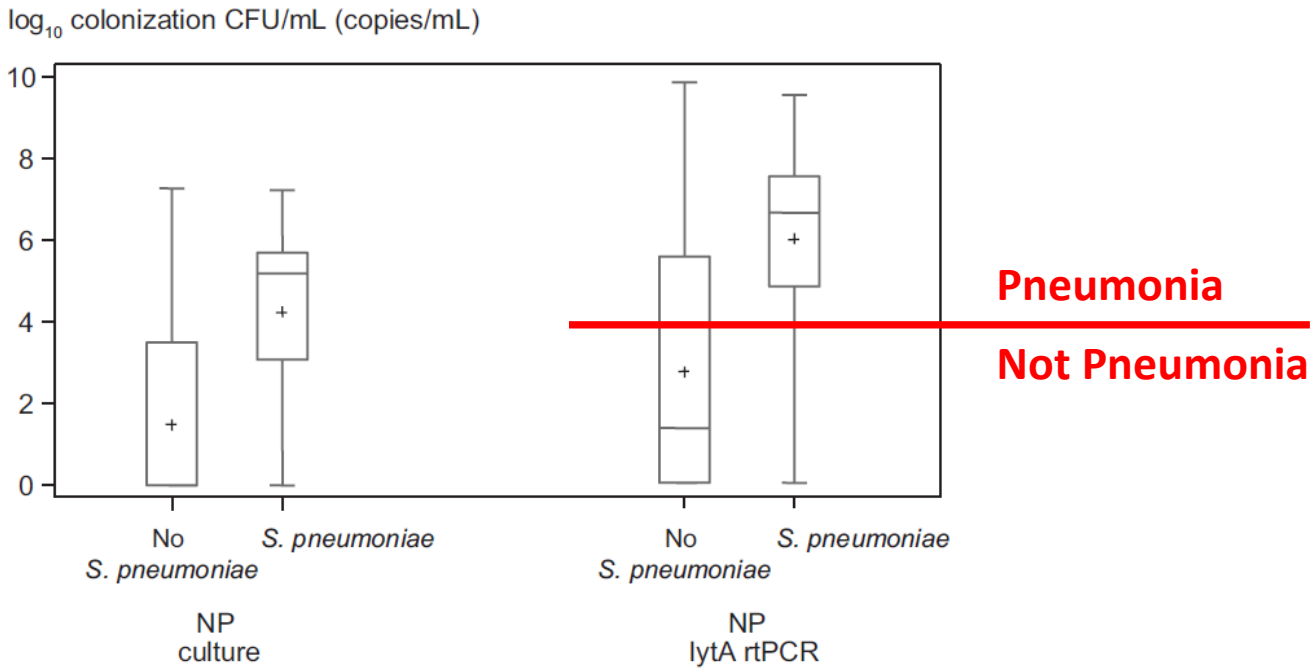
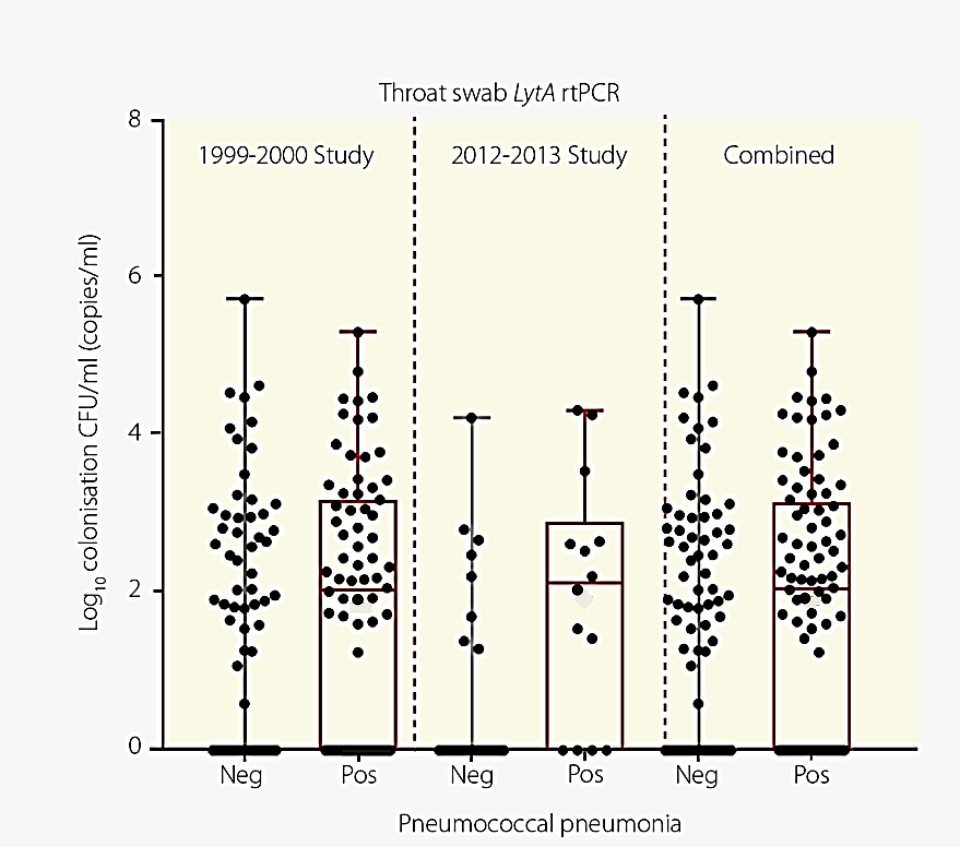


Figure 1. Quantitative colonization densities in human immunodeficiency virus–infected patients with community-acquired pneumonia.

Oropharyngeal Colonisation Density

- No cut-off in pneumococcal load clearly distinguishes pneumococcal pneumonia
- AUC 0.77 (95%CI 0.71, 0.82) for the combined dataset



Fundamental Issues in Determining Pneumonia Aetiology

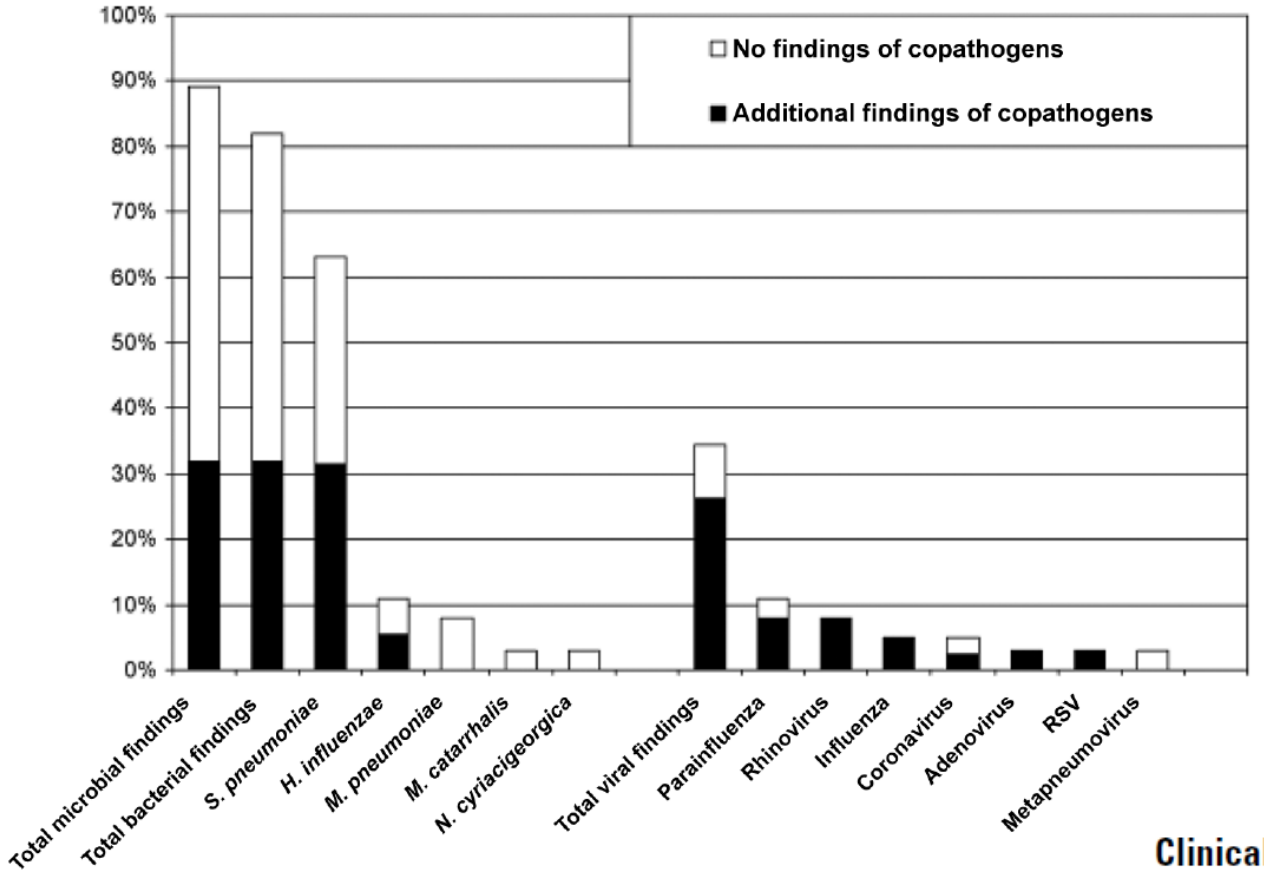
(3) Understanding polymicrobial infections

- —Multiple pathogens are being detected more frequently in individual pneumonia patients
- —This is a consequence of:
  - —Testing for a wider variety of targets (e.g. multiplex PCR)
  - —Testing of multiple specimen types
- —Most recent studies of pneumonia aetiology report about 25% polymicrobial infections

Etiology of Community-Acquired Pneumonia: Increased Microbiological Yield with New Diagnostic Methods

Niclas Johansson,[1,3,4] Mats Kalin,[1,3,4] Annika Tiveljung-Lindell,[2,5] Christian G. Giske,[2,5] and Jonas Hedlund[1,3,4]

Departments of [1]Medicine and [2]Microbiology, Tumor, and Cell Biology, Karolinska Institutet, and [3]Infectious Diseases Unit and Departments of [4]Infectious Diseases and [5]Clinical Microbiology, Karolinska University Hospital, Solna, Stockholm, Sweden



≥2 pathogens identified in 35%

Clinical Infectious Diseases 2010;50:202–9

Bacterial vs Viral Pneumonia

- This is a traditional aetiological classification of pneumonia
- However, there is increasing interest in bacterial-viral interaction in the pathogenesis of pneumonia

A 910120

[Printed by authority of the Registrar General.]

CERTIFIED COPY of an ENTRY OF DEATH.
Pursuant to the Births and Deaths Registration Acts, 1836 to 1874.

Registration District. ECCLESALL BIERLOW.

1918. Death in the Sub-district of ECCLESALL WEST CENTRAL in the County of SHEFFIELD, C. B.

Columns:— 1		2	3	4	5	6	7	8	9
No.	When and where Died.	Name and Surname.	Sex.	Age.	Rank or Profession.	Cause of Death.	Signature, Description and Residence of Informant.	When Registered.	Signature of Registrar.
4/5	Sixth November 1918 54 Lydgate Lane	Jessie Murdoch	Female	49 years	Wife of James Murdoch a Razor forger	(1) Influenza (2) Bronchitis Certified by Geo. Johnston M.D.	Mary Newsam sister present at the death 9 Bosville road Sheffield	Sixth November 1918	T. E. Dearden Registrar.

I, TALBOT TYDEARDEN Registrar of Births and Deaths for the Sub-district of ECCLESALL WEST CENTRAL, in the County of SHEFFIELD, C. B. do hereby certify that this is a true copy of the Entry No. 4/5 in the Register Book of Deaths for the said Sub-district, and that such Register Book is now legally in my custody.

WITNESS MY HAND this 7th ~~day of~~ November 1918.

T. E. Dearden
Registrar of Births and Deaths.

The Act 3 & 4 Geo. V., cap. 27, section 3, enacts that "FORGERY of the following documents, if committed with intent to defraud or deceive, shall be FELONY and punishable with Penal Servitude for any term not exceeding fourteen years:—Any register or record of Births, Baptisms, Marriages, Deaths, Burials.....which now is, or hereafter may be, by law authorised or required to be kept in the United Kingdom, relating to any Birth, Baptism, Marriage, Death, Burial.....or any part of any such Register, or any Certified Copy of any such Register, or of any part thereof."

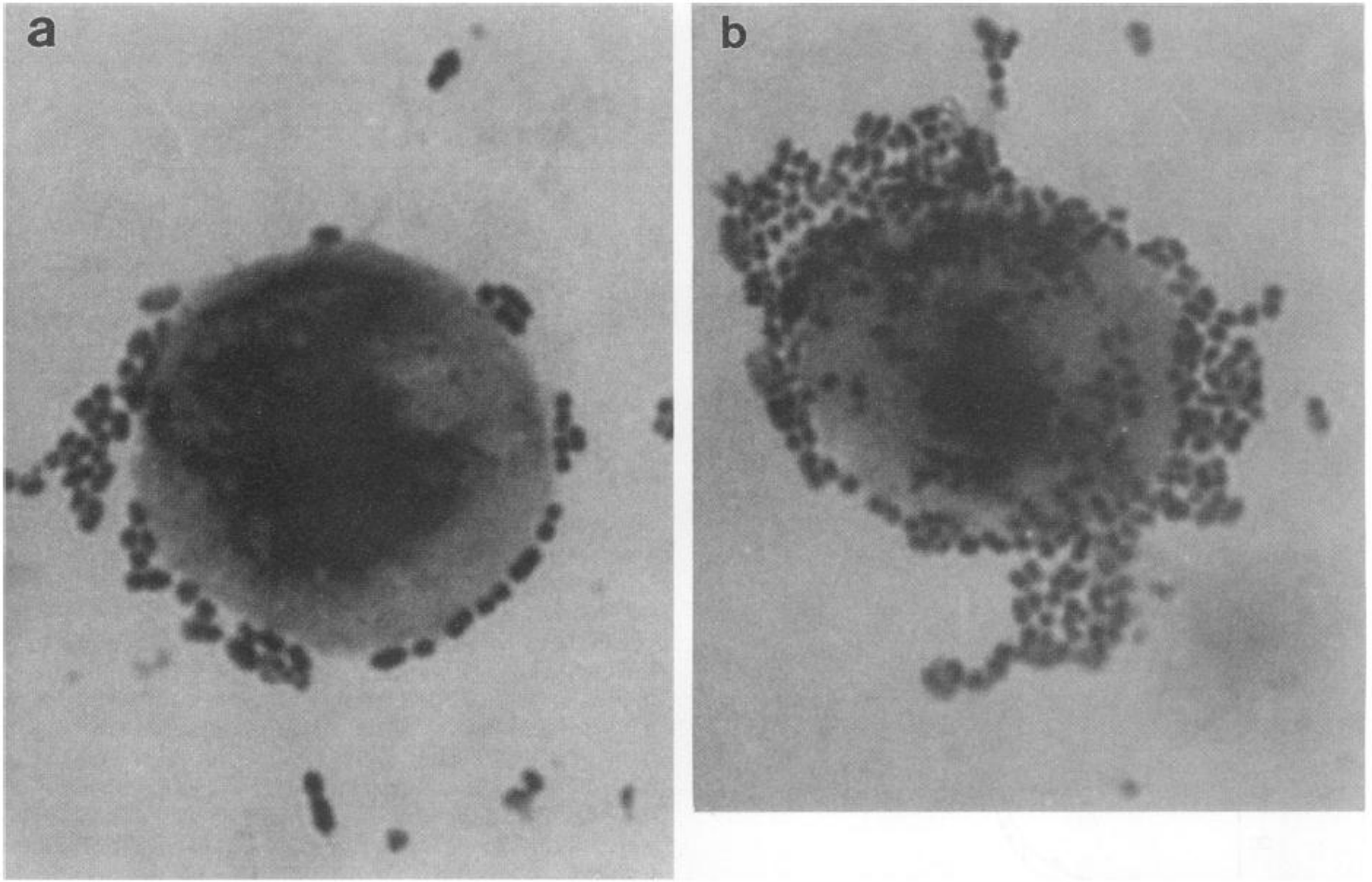
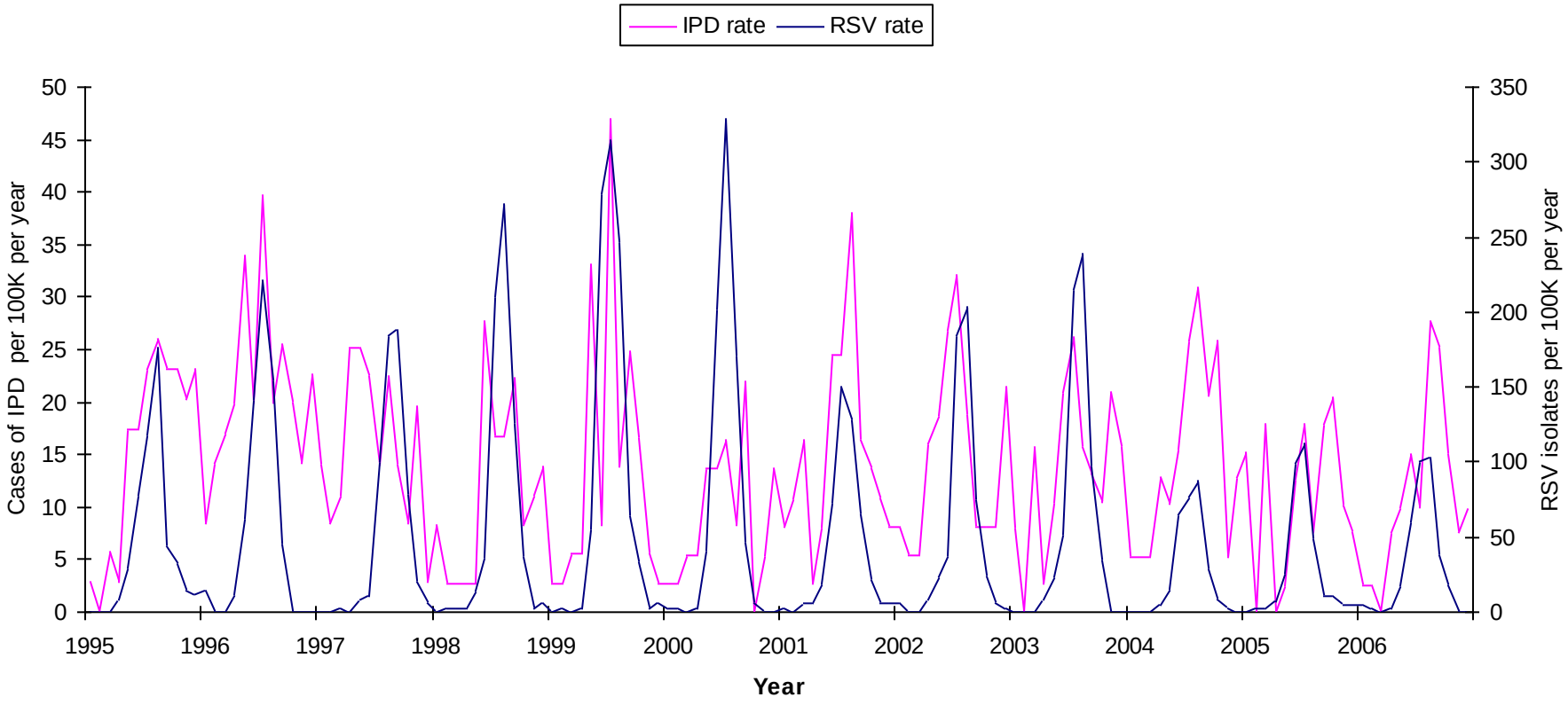


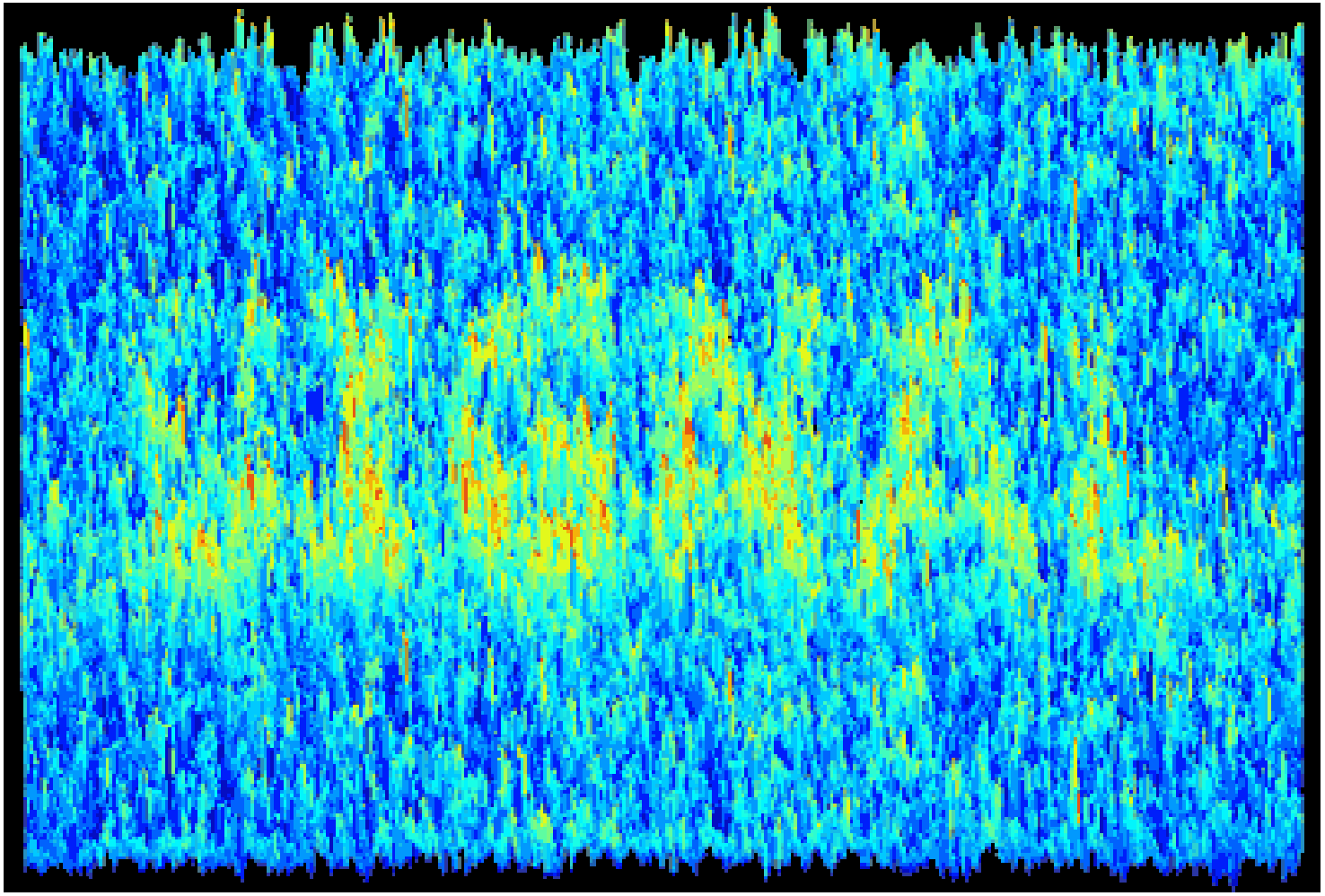
FIG. 2. Examples of adherence of *S. pneumoniae* CCUG 10175 to an uninfected A549 cell (a), an A549 cell infected with adenovirus type 5 (b), and an A549 cell infected with adenovirus type 9 (c).

Rates of Invasive Pneumococcal Disease and RSV Infection in Christchurch, NZ 1995-2006



Journal of Infection (2009) **58**, 37–46

How do we distinguish the pneumonia “signal” from among the diagnostic and analytic “noise”?

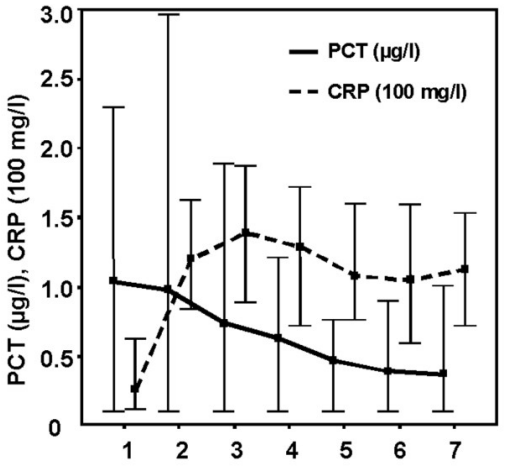


Pneumonia Aetiology Research

- **We need:**
  - Innovative new diagnostic tools
  - New approaches to data analysis
  - To think about alternative paradigms of pneumonia aetiology

New Diagnostic Tools

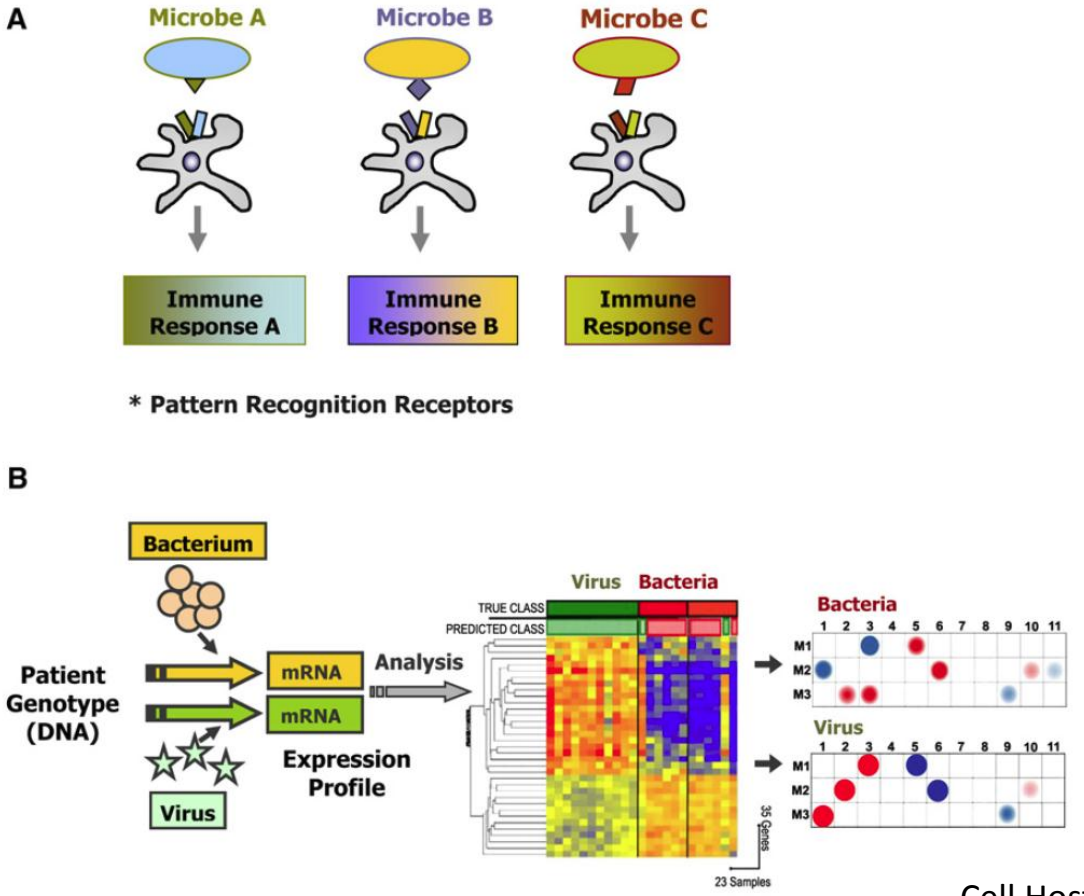
- Biomarkers
  - Aetiological diagnosis
  - Severity assessment



- Breath analysis

New Diagnostic Tools

- Host gene signatures



Cell Host Microbe 2009;6:199

New Approaches to Data Analysis

- Analytical challenges:
  - Indirect observation of infection site
  - Poor sensitivity of diagnostic tests
  - Similar detection in cases and controls does not rule out pathogen as a putative aetiologic agent
  - Not all specimen types from cases are collected from controls
  - Combining results from multiple specimens/tests
  - Measurement error in lab tests
  - Pathogen inter-relationships

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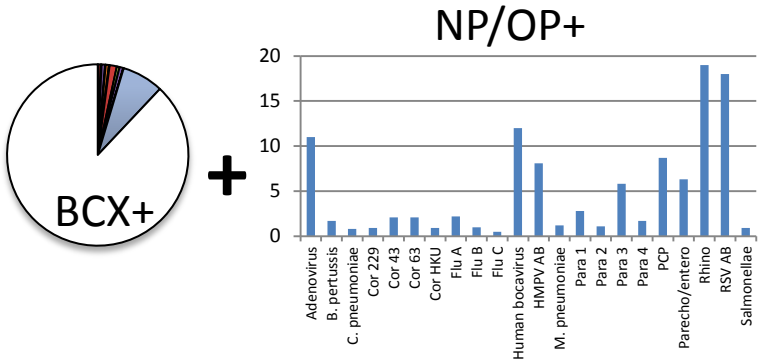
Analytic approaches to describing pneumonia aetiology

The PERCH Study Group

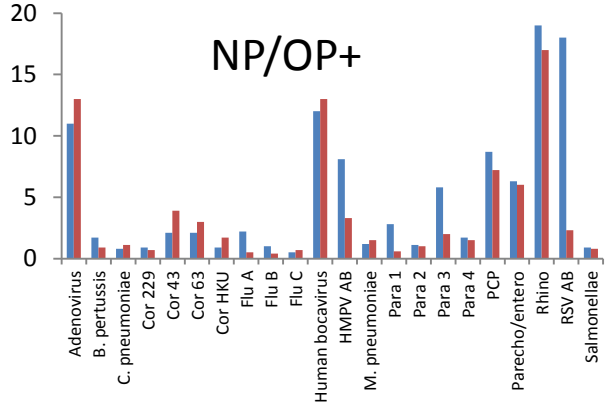
Analysis plan…

A series of descriptive analytic approaches

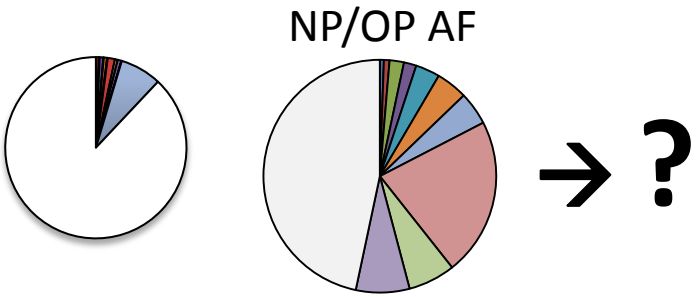
1. Cases only - descriptive



2. Classical case-control



3. Attributable Fraction case-control



4. PERCH Quantitative

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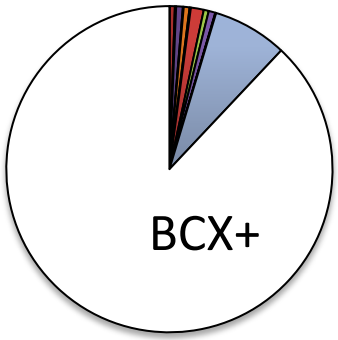
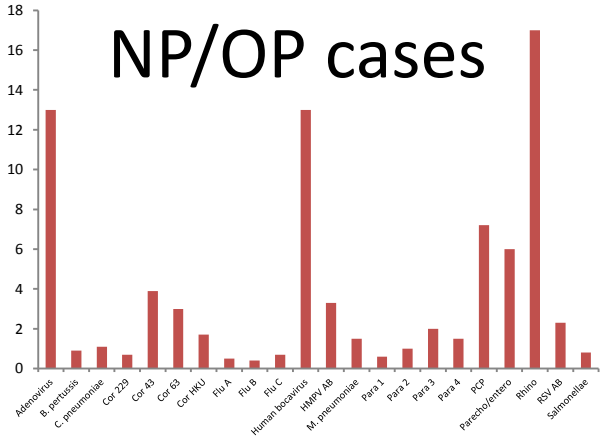
PERCH Quantitative (PQ) model (probabilistic model)



+

Sensitivity
Specificity

+



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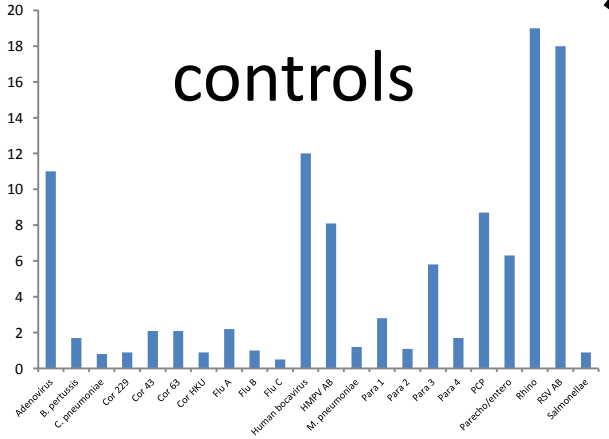
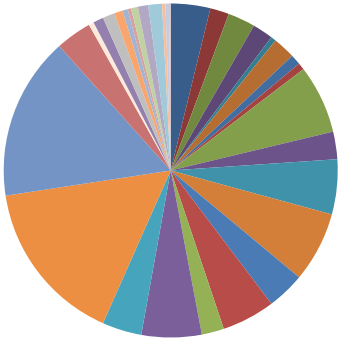
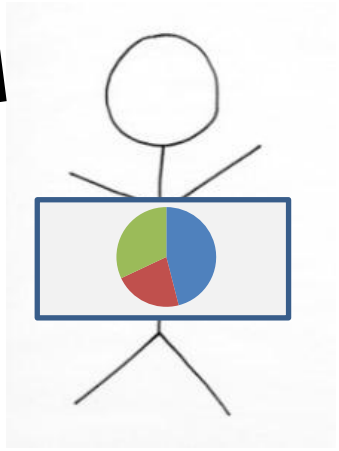
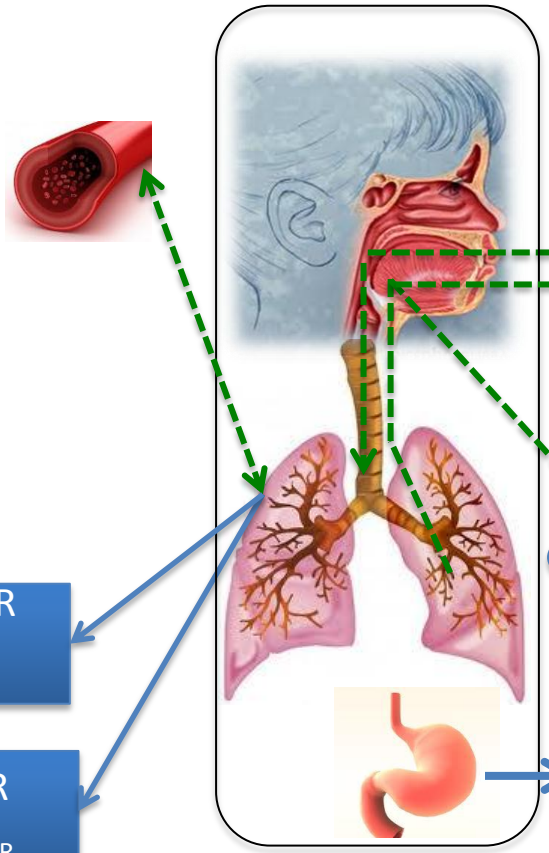






PERCH
Pneumonia Etiology Research for Child Health

Model Framework for Infections (I) and Pathogen Measurements (M)



Blood infection, I_B

Blood culture measure, $M_{B,CX}$

Blood *Lyt*A PCR measure, $M_{B,PCR}$

A/C antibodies measure M_{SERA}

External to the lung

NP I_{NP}

NP measure $M_{NP, PCR}$

NP measure $M_{np, CX}$

Sputum I_{SP}

Sputum measure $M_{SP, PCR}$

Sputum measure $M_{SP, CX}$

Lung aspirate culture and PCR measure*, $M_{L,CX}$ and $M_{L,PCR}$

Pleural fluid culture and PCR measure*, $M_{PF, CX}$ and $M_{PF, PCR}$

Gastric aspirate*§ $M_{GA, TB\ CX}$

* collected on a subset of cases

§ TB testing only

Measurement of pathogen/evidence of infection

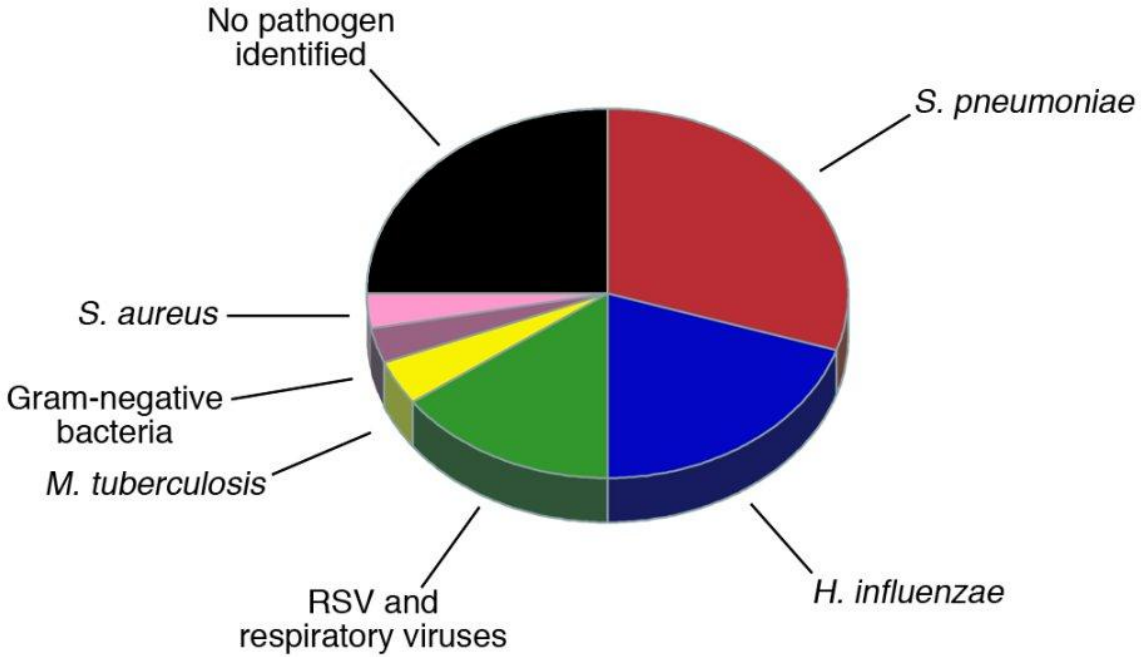
Pathogen direction of travel

Potential non-pneumonia causes of pathogen detection

Sample collected in both cases and controls

Alternative Paradigms of Pneumonia Aetiology

- Traditional view – one pathogen per pneumonia episode



J Clin Invest 2008;118:1291-1300

Is pneumonia a polymicrobial disease?

Polymicrobial Paradigm

- Pneumonia is (usually) a polymicrobial disease
- Pneumonia is a consequence of upper respiratory flora getting in the wrong place
- Precipitating/initiating factors may include:
  - Recent respiratory virus infection
  - Malnutrition
  - Other causes of immune suppression
- If the upper respiratory flora includes a more virulent pathogen (e.g. *S. pneumoniae*), that microbe may predominate

Implications of the Paradigm Shift

- It explains why:
  - There are big gaps in the aetiology pie chart
  - Two or more potential pneumonia pathogens are frequently detected in individual patients
  - Good quality sputum frequently contains "oropharyngeal flora" only

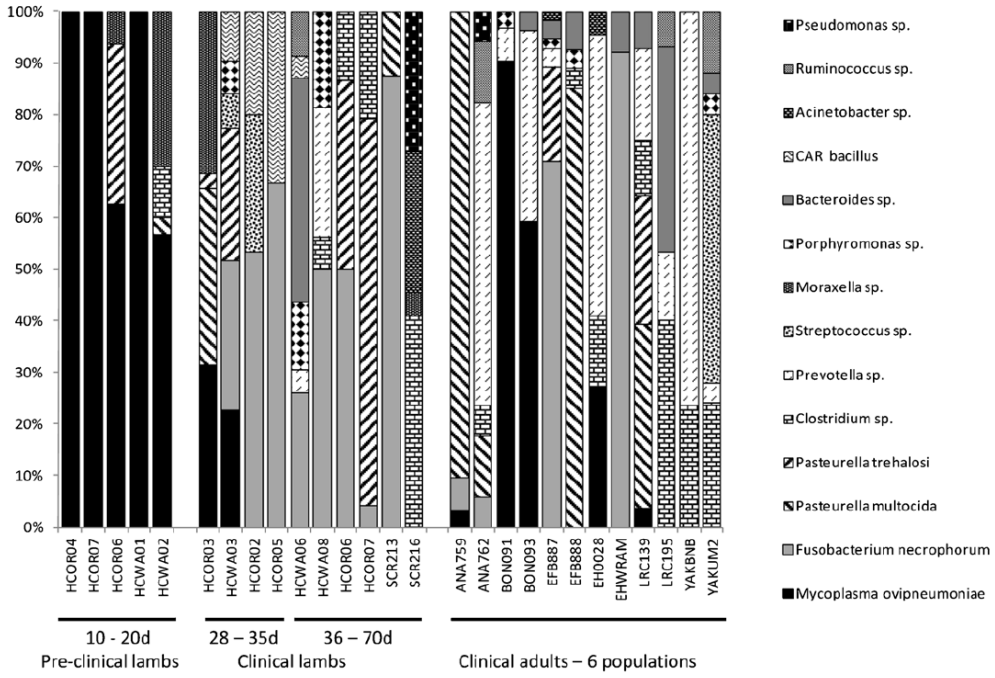
Implications of the Paradigm Shift

- Does not lessen the importance of key individual pathogens and prevention by vaccination
- Questions the validity of the simple bacterial versus virus concept of pneumonia aetiology
- Redirects research efforts to focus on:
  - the respiratory microbiota
  - triggers for pneumonia
  - Pathogen-pathogen interactions

Preventive Veterinary Medicine 108 (2013) 85–93

Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease

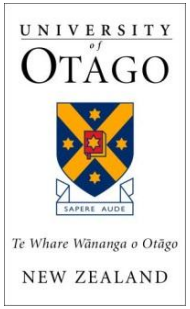
Thomas E. Besser[a,*], E. Frances Cassirer[b], Margaret A. Highland[a,c], Peregrine Wolff[d], Anne Justice-Allen[e], Kristin Mansfield[f], Margaret A. Davis[g], William Foreyt[a]

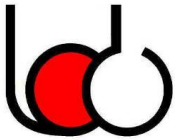


Concluding Comments

- Its sometimes hard being a pneumonia researcher
- It is important to challenge dogma
- Look out for the PERCH study publications

PERCH Collaborators





RMPRU

respiratory & meningeal pathogens research unit