دانشگاه علوم پزشکی
و خدمات بهداشتی درمانی تهران

Laboratory Diagnosis of Pneumonia

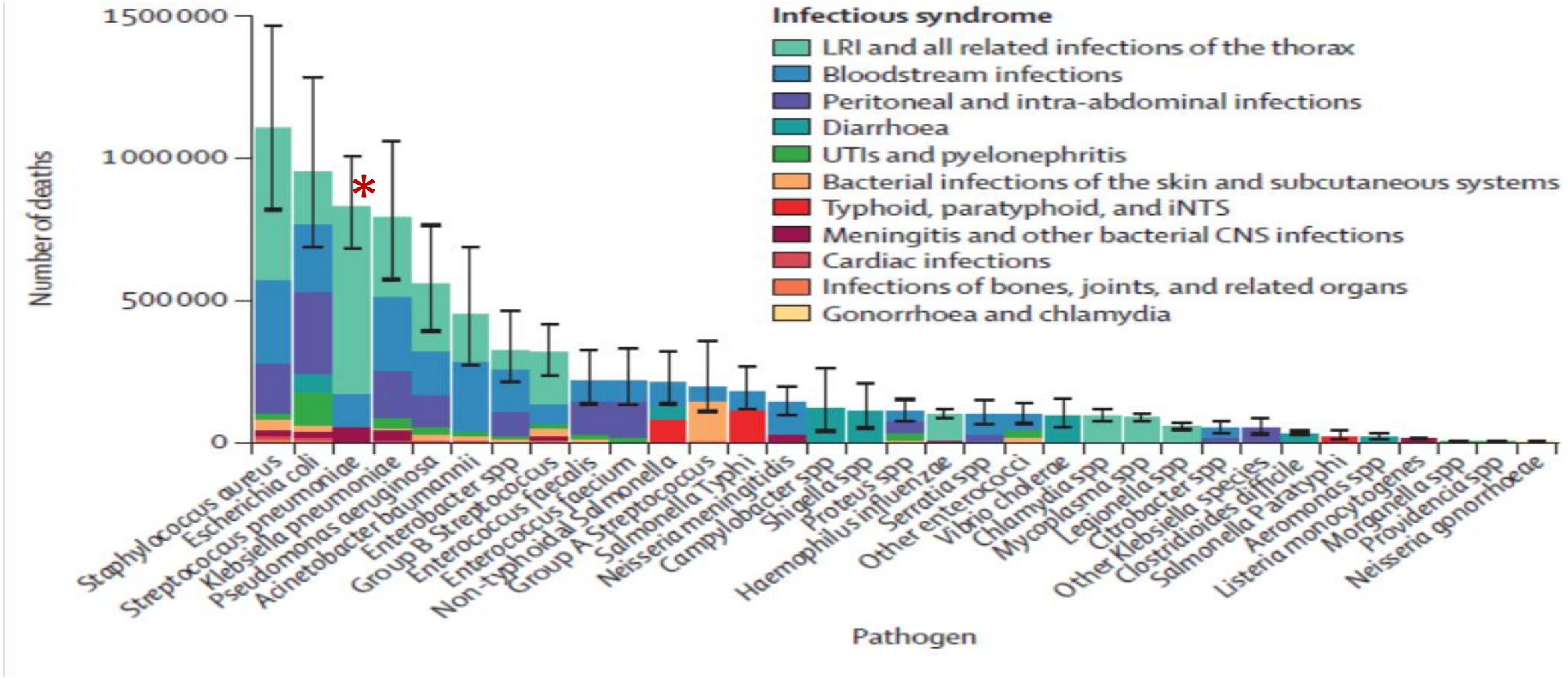
Dr. Masoumeh Douraghi, Ph.D

School of Public Health, Tehran University of Medical Sciences

mdouraghi@tums.ac.ir

3 rd Nov 2023

Global number of deaths by pathogen and infectious syndrome, 2019



GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2022 Dec 17;400(10369):2221-2248. doi: 10.1016/S0140-6736(22)02185-7. Epub 2022 Nov 21. PMID: 36423648; PMCID: PMC9763654.

Preventing and effectively treating infections

Achieving Sustainable Development Goal (SDG) 3

TARGET 3.2

By 2030, end preventable deaths of newborns and children under 5 years of age, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1,000 live births and under-5 mortality to at least as low as 25 per 1,000 live births

THE 17 GOALS - Sustainable Development Goals
https://sdgs.un.org

Accurate diagnosis and attribution of the causes of pneumonia

- Measuring the burden of disease,
- Implementing appropriate preventive or treatment strategies, and
- Developing more effective interventions

Pathogenesis of Pneumonia

Former thought

- Invasion of a sterile LRT by a single pathogenic organism

Recent evidence

- Healthy lung is not sterile,
- Normal lung microbiome exists in a dynamic state
- Dysbiosis in the normal microbial ecology
- Overgrowth of a single pathogen or multiple pathogens
- it is hard to distinguish colonizing flora from pathogenic organisms in respiratory samples.

Global childhood immunization programs of vaccines against bacterial pathogens have had an impact on the causes of childhood pneumonia

Current guidelines for microbiological diagnosis

- British Thoracic Society ((BTS) guidelines for community acquired pneumonia (CAP) recommend microbiological testing only in:

  - Children with severe pneumonia who need to be admitted to intensive care or those with complications.
  - In such cases, a variety of diagnostic tests are suggested including;
    - blood culture;
    - nasal specimens for viral detection;
    - acute and convalescent serology for viruses and atypical organisms; and
    - pleural fluid (if present) for microscopy, culture, antigen detection, and polymerase chain reaction.

Harris M, Clark J, Coote N, et al. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. *Thorax* 2011;

- Guidelines from those of the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America (IDSA), which include:
  - rapid diagnostic testing for influenza virus and other respiratory viruses in children with CAP who are being treated in the community or in hospital,
  - For children with CAP who are in hospital,
  - blood culture in those with complicated or moderate to severe disease and
  - sputum for Gram stain and culture in those who can produce a specimen.
  - tracheal aspirates for culture and detection of viral pathogens are recommended in children who have been intubated,
  - bronchoscopy, bronchoalveolar lavage, or lung biopsy are recommended only in those with severe pneumonia who have negative diagnostic tests and
  - targeted testing may be warranted in outbreak situations, or if infection with specific pathogens, such as *M tuberculosis* or *Bordetella pertussis*, is suspected.

Bradley JS, Byington CL, Shah SS, et al. The management of community acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin Infect Dis 2011;53:e25-76. doi:10.1093/cid/cir531.

Value of different specimen types and associated diagnostic tests

- Optimal specimen collection is key to accurate identification of the cause of pneumoniae.
- Diagnostic yield depends on:
  - the type and quality of the specimen collected,
  - timing of collection,
  - previous use of antimicrobials,
  - transport, storage, and laboratory processes for testing.

- It may be difficult to obtain a representative sample from the lower respiratory tract, and contamination with organisms such as *Streptococcus pneumoniae* or *Haemophilus influenzae*, which colonize the upper airways but are also important pathogens, is likely. This may lead to overestimation or underestimation of the contribution of these bacteria to pneumonia.

Specimen types and laboratory tests for the causes of pneumonia in children

- **Nasopharyngeal swab or aspirate**
  - Bacterial culture, molecular or antigen detection of bacteria and viruses
  - Sputum or induced sputum
  -

- **Bronchoalveolar lavage or aspirate**
  - Bacterial culture, molecular or antigen detection of bacteria and viruses

- **Transthoracic lung aspirate**
  - Bacterial culture, molecular or antigen detection of bacteria and viruses

- **Blood** (blood culture is indicated only in children who are in hospital, in intensive care, or have complicated pneumonia.)
  - Culture for bacterial pathogens
  - Molecular testing
  - Serology
  - Biomarker detection
- **Urine** **(**high sensitivity (close to 100%) for invasive pneumococcal infection but lacks specificity (60-80%), particularly in children with nasopharyngeal pneumococcal carriage)
  - Antigen detection

Laboratory tests in children with ambulatory or severe pneumonia

Test	Indications	Limitations
Children with mild or ambulatory pneumonia		
Serum for C reactive protein or procalcitonin	Limited evidence that use in an algorithm for children with mild or uncomplicated pneumonia may reduce antibiotic exposure	Data are very limited; studies include longitudinal monitoring of kinetics of response to treatment; requires a blood specimen; not currently recommended to distinguish bacterial disease
Upper respiratory tract samples for rapid viral detection of influenza, for example	During outbreaks of viral illness, rapid testing may reduce antibiotic prescription; guidelines differ on recommendation	Bacterial coinfection cannot be excluded; vigilance for coexisting bacterial disease required
Children with severe disease, complications, and those in hospital		
Blood culture	All children	Low yield but higher in HIV infected children; cost effectiveness questionable
Upper or lower respiratory tract samples for rapid viral testing (PCR or antigen detection)	Children with severe or complicated pneumonia	Difficult to infer causality for many pathogens; most useful for influenza virus and RSV
Induced or expectorated sputum for mycobacterial culture and PCR (Xpert MTB/RIF) for *Mycobacterium tuberculosis*	Children in whom pulmonary tuberculosis is clinically suspected	Repeated specimens needed to optimize microbiologic yield
Induced or expectorated sputum or upper respiratory tract sample for PCR for *Bordetella pertussis*	Children in whom pertussis is suspected	Upper respiratory samples have a lower yield than sputum
Induced or expectorated sputum for PCR for *Pneumocystis jirovecii*	Children in whom pneumocystis pneumonia is suspected	May not distinguish colonizing from disease causing organisms
Acute and convalescent serum for respiratory pathogens and mycoplasma and chlamydia	Children with severe or complicated pneumonia	Clinical utility questionable, given need for comparison of acute and convalescent sera
Pleural fluid microscopy, culture, and pneumococcal antigen detection or PCR	If pleural effusion present	
Tracheal aspirate for bacterial culture; PCR for pneumocystis and viral pathogens	Intubated children	May not distinguish colonizing from disease causing organisms
Bronchoscopy, bronchoalveolar lavage, or lung biopsy for bacterial culture; PCR for pneumocystis and viral pathogens; culture and PCR for *M tuberculosis* (in epidemiological context)	Children with severe illness in whom no pathogen has been detected or those who have not responded to treatment	Invasive and requires specific expertise

Diagnosis pneumococcal disease

- Suspect serious pneumococcal disease, like meningitis or bloodstream infections, collect samples of cerebrospinal fluid or blood.
- Growing the bacteria in a laboratory helps identify the specific type of bacteria causing the infection.
- Possible use a urine test to help make a diagnosis of **pneumococcal pneumonia** in adults.

Diagnosis *Mycoplsama pneumoniae*

- CDC uses multiplex real-time polymerase chain reaction (PCR) as the primary laboratory procedure for *M. pneumoniae* identification.
- CDC does not use culture or serological testing as routine diagnostic methods. Specimens identified as positive for *M. pneumoniae* by previous lab testing and/or upon testing at CDC will be subsequently tested for macrolide susceptibility using molecular test methods.

  - A Dutch study found that *Mycoplasma pneumoniae* DNA was not detected any more often in children with any respiratory tract infection than in control children.
  - Two recent studies, a birth cohort in South Africa and a multicenter study in the US have reported similar findings. RSV, influenza virus, and *B pertussis* have been consistently and strongly associated with pneumonia in many studies.

There is considerable heterogeneity with regard to the causative role of many other viruses and bacteria detected in respiratory samples, which may relate to case definitions, sampling, and the inclusion of a relevant control group.

Antimicrobial resistance in *S. pneumoniae* and *M. pneumoniae*

ARTICLES | VOLUME 6, ISSUE 7, E744-E757, JULY 2018

Download Full Issue

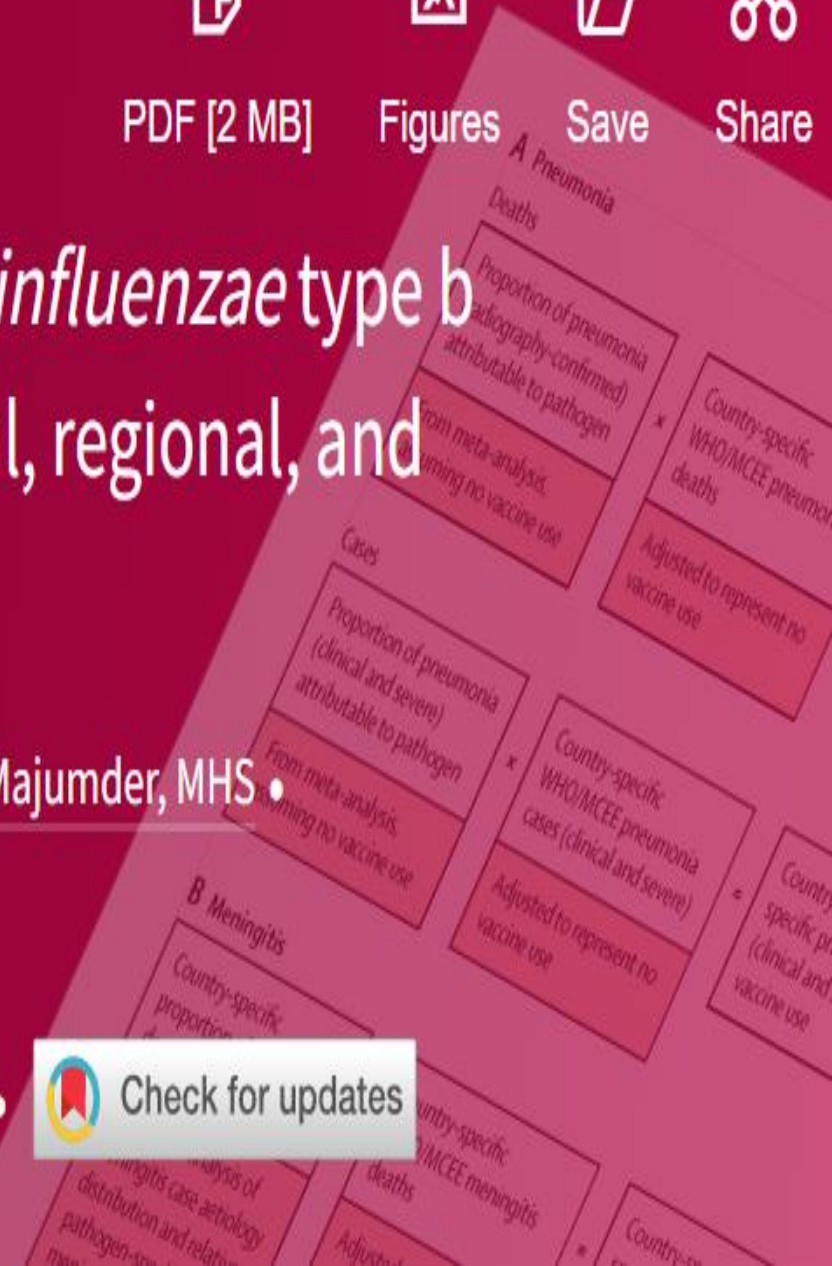
PDF [2 MB] Figures Save Share

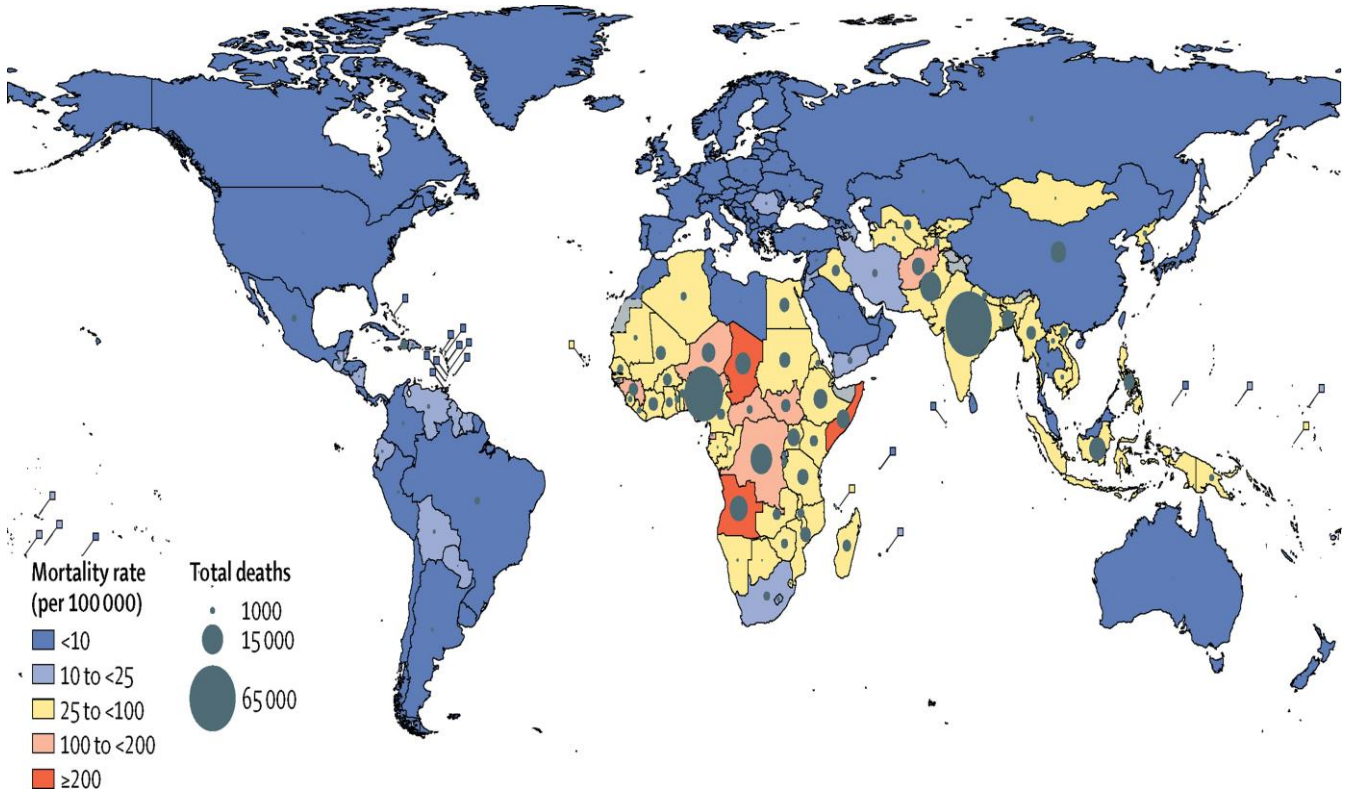
Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15

Brian Wahl, PhD • Prof Katherine L O'Brien, MD • Adena Greenbaum, MD • Anwesha Majumder, MHS • Li Liu, PhD • Yue Chu, MSPH • et al. Show all authors

Open Access • Published: July, 2018 • DOI: https://doi.org/10.1016/S2214-109X(18)30247-X •

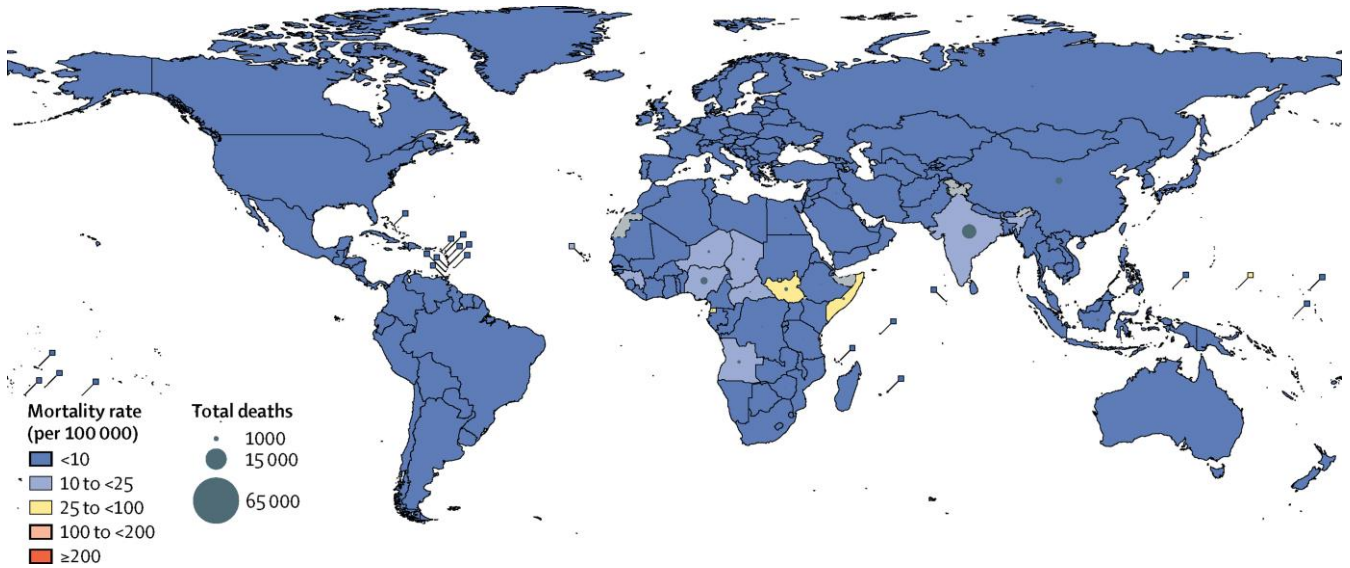
Check for updates





Country-specific mortality rates and deaths attributable to pneumococcus in 2015.

Mortality rates and deaths in children aged 1–59 months are HIV-negative deaths only. Mortality rates are deaths per 100 000 children aged 1–59 months. Pneumococcus=*Streptococcus pneumoniae*.



Country-specific mortality rates and deaths attributable to Hib in 2015.

ARTICLES | VOLUME 2, ISSUE 9, E450-E460, SEPTEMBER 2021

Antimicrobial resistance in paediatric *Streptococcus pneumoniae* isolates amid global implementation of pneumococcal conjugate vaccines: a systematic review and meta-regression analysis

Kristin Andrejko, BS • Buddhika Ratnasiri, BA • William P Hausdorff, PhD • Ramanan Laxminarayan, PhD • Joseph A Lewnard, PhD

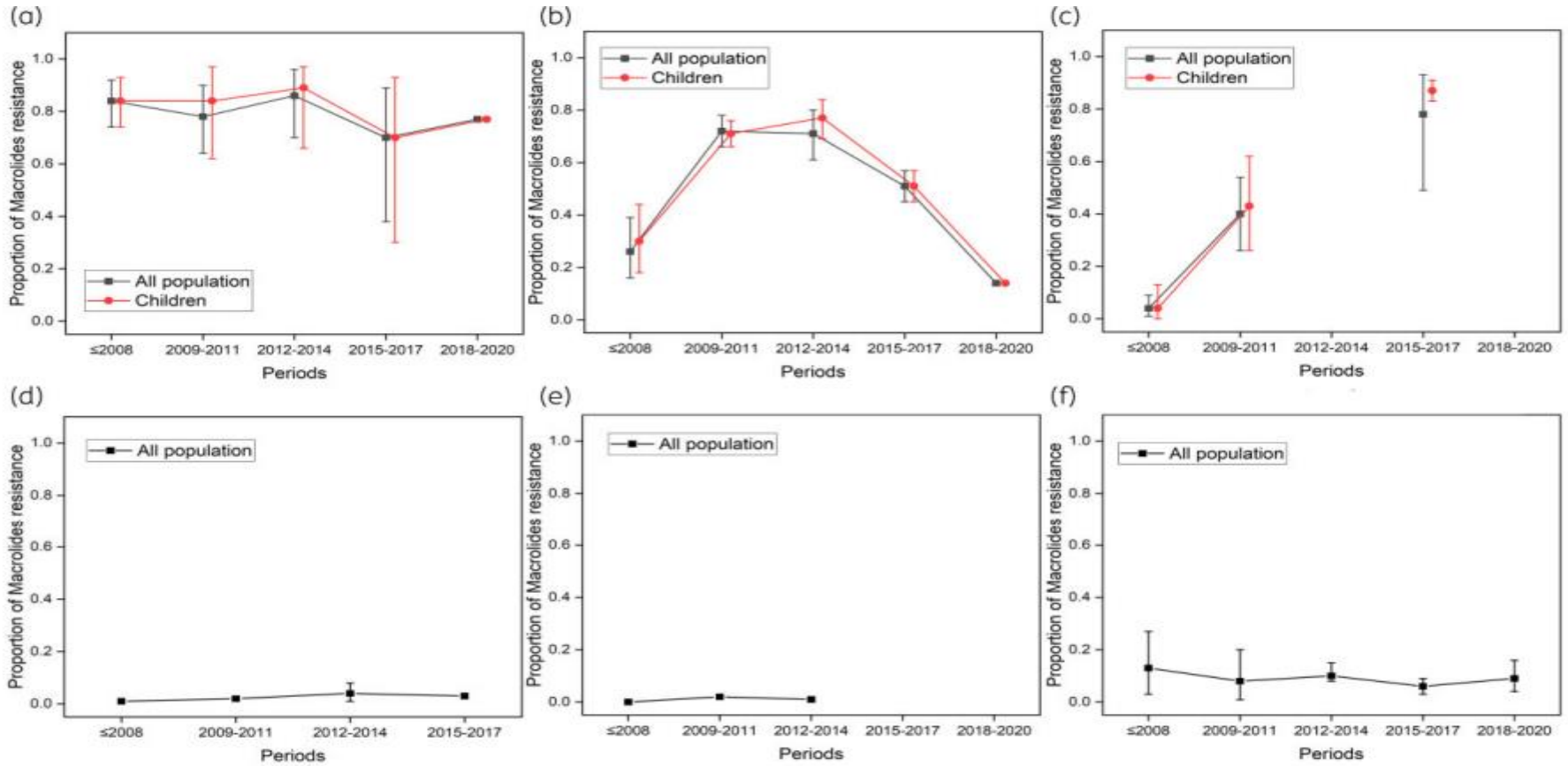


Figure 3. Prevalence change over time in (a) China, (b) Japan, (c) Korea, (d) Germany, (e) Slovenia and (f) America. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

- Circulating penicillin-resistant *S. pneumoniae* are much more heterogeneous.
- Internationally, penicillin and macrolide resistance were increasing at the turn of the 21st century.
- However, infection rates overall and with penicillin-resistant serotypes significantly decreased following the introduction of pneumococcal vaccines.
- Early U.S. studies noted an initial decrease in pneumococcal activity after the introduction of 7-valent vaccine (PCV7), with residual high resistance by serotypes not covered by PCV7. The 13-valent vaccine (PCV13) coverage of serotype 19A led to a decrease in one of the most pathogenic (and resistant) circulating serotypes, and since 2010, there has been an overall downward trend in non-susceptible invasive pneumococcal infections.
- Regional differences in circulating antibiotic-resistant *S. pneumoniae* remain; however, this resistance does not appear related to a dominant non-vaccine serotype.

Antibiotic resistance of *S. pneumonia* in Iran

- The 44 pneumococcal invasive isolates, 39 (89%) were isolated from children and 5 (11%) from adults.
  - All pneumococcal isolates were susceptible to linezolid but had varying resistance to trimethoprim-sulfamethoxazole (86%), erythromycin (73%), tetracycline (66%), clindamycin (43%), penicillin (16%), chloramphenicol (14%) and levofloxacin (2%).
  - All of the penicillin resistant isolates were multidrug resistant (MDR) and in addition to penicillin were resistant to tetracycline, erythromycin and trimethoprim-sulfamethoxazole.
  - The most common capsular types detected in 64% of the pneumococcal isolates was 6A/B, 19A, 15A, 23F.
  - The co resistance to erythromycin and clindamycin (the constitutive phenotype) was observed in 43% (19/44) of the isolates and resistance to erythromycin, but not to clindamycin (the M phenotype) was observed in 29.5% (13/44) of the isolates.

- Evaluating the level and type of *S. pneumoniae* resistance to macrolides in Iran2000–2017:
  - The results of the present study showed that resistance to macrolides is considerable in different parts of Iran.
  - Half of previous studies have reported prevalence rates of more than 50%.
  - Similar to other countries around the world, especially European countries, 23S rRNA methylation was the dominant mechanism in the development of macrolide resistance.
  - In addition, efflux-mediated resistance (independent or in combination with *ermB* gene) was considerable, as well.
- A study by Haghi Ashtiani et al., evaluating the trend of changes in resistance over 10 years, showed that levels of antibiotic resistance are increasing rapidly. For instance, resistance to macrolides, such as erythromycin, has increased from 25 to 65%.

J Antimicrob Chemother 2022; **77**: 2353–2363
https://doi.org/10.1093/jac/dkac170 Advance Access publication 9 June 2022

Journal of Antimicrobial Chemotherapy

Global prevalence of resistance to macrolides in *Mycoplasma pneumoniae*: a systematic review and meta-analysis

Guotuan Wang[1], Peng Wu[2], Rui Tang[3] and Weidong Zhang[1]*

[1]Department of pharmacy, Karamay central hospital of Xinjiang, Karamay, Xinjiang, China; [2]Department of emergency, Karamay central hospital of Xinjiang, Karamay, Xinjiang, China; [3]Department of pharmacy, West China hospital, Sichuan university, Chengdu, Sichuan, China

Table 1. Characteristics of included studies

	Macrolide resistance
Sampling years	2000–20
Geographical regions	
Asia	* 63% [56, 69], (8628/13 721)
Europe	3% [2, 7], (71/2285)
North America	8.6% [6, 11], (136/1795)
South America	0% (0/42)
Oceania	3.3% (1/30)
Annual data[a]	
≤2008	27% [15, 40], (491/2090)
2009–11	50% [39, 61], (3209/5630)
2012–14	51% [35, 68], (2551/4540)
2015–17	39% [24, 57], (1626/3545)
2018–20	23% [2, 54], (218/427)
Mutation (Total resistant sample detected for point mutation: 8257)	
A2063G	96% [94, 98], (7565)
A2063C	0.2% (17)
A2063T	5% [2, 10], (257)
A2064G	4% [2, 6], (213)
A2064C	0.01% (1)
C2617A	0.04% (4)
C2617G	0.13% (11)
C2617T	0.04% (3)
No mutation	2% (186)

The data are presented as mean prevalence [95% CI].
[a]Not all studies reported year-stratified prevalence data.

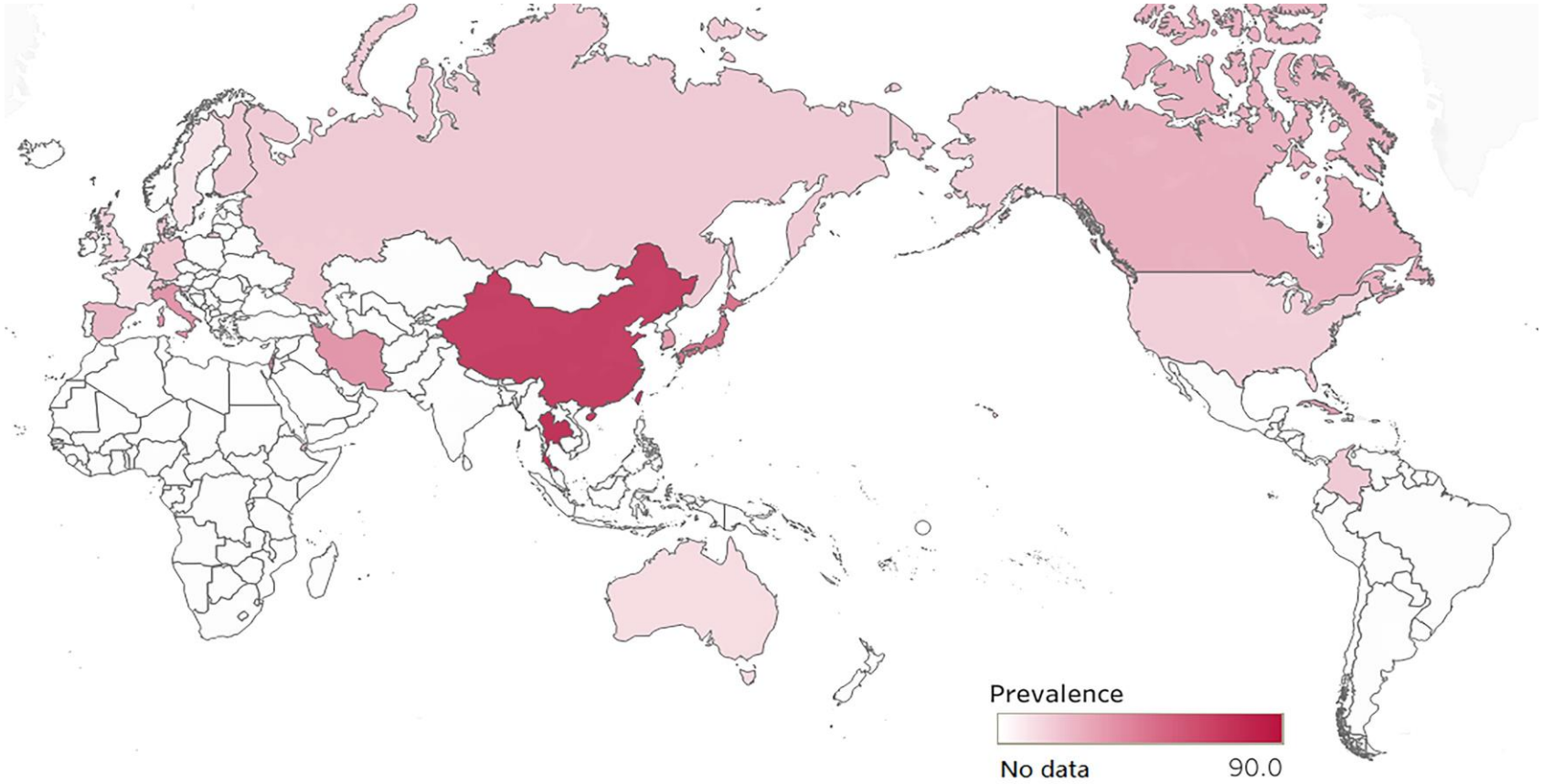


Figure 1. The mean prevalence map of macrolide resistance all over the world (from ≤2008 to 2020).

J Antimicrob Chemother, Volume 77, Issue 9, September 2022, Pages 2353–2363, https://doi.org/10.1093/jac/dkac170

MIC (mg/L) range of isolated *Mycoplasma pneumoniae*

Study	Mutation in 23S rRNA	ERY (MIC of reference strain)	CLR	AZM	TET	DOX	MIN	CIP	LVX	MXF	GAT	JOS
China												
Zhao F 2013	A2063G A2064G A2063T None (M129)*	128~>256 256~>256 32 0.008~0.016 (0.016)	64~>256 256~>256 16 <0.008~0.008 (<0.008)	2~64 4~32 0.064 <0.008~0.008 (<0.008)	0.032~0.5 0.125~0.25 0.25 0.016~0.5 (0.125)			0.125~2 0.5~1 0.5 0.008~1 (1)	0.125~2 0.25~1 0.25 0.008~1 (1)		0.016~0.125 0.016~0.125 0.064 0.008~0.125 (0.125)	
Xin D 2009	Not specified (FH)	0.01~512 (0.01)		0.001~256 (0.001)								0.01~64 (0.01)
Liu Y 2009	Not specified	≤0.007~>128 (≤0.007)	≤0.007~>128 (≤0.007)	≤0.007~>128 (≤0.007)	0.015~0.25 (0.06)	≤0.007~125 (0.03)	≤0.007~125 (0.06)	0.015~1 (0.25)	0.015~1 (0.25)	≤0.007–0.06 (0.06)		≤0.007~8 (≤0.007)

MIC ≤0.5 mg/L was considered as the breakpoint to discriminate erythromycin susceptible or resistant in CLSI guideline.
All *M. pneumoniae* with the **A2063G** mutation were resistant to **erythromycin** (32-1024 mg/L) and **clarithromycin** (32-256 mg/L), with some also resistant to **azithromycin** (≤0.007.64 mg/L);
The similar finding was suggested for the **A2064G** mutation (**erythromycin**: 64-256 mg/L; **clarithromycin**: 16-256 mg/L; **azithromycin**: 2-32 mg/L). Additionally, the **A2063T** mutation might not be associated with resistance to **azithromycin** (0.0642 mg/L).

- Two main challenges: a relatively high carriage in asymptomatic children, and a worldwide increase in macrolide-resistant *M. pneumoniae* (MRMP). This review focuses on the scientific and clinical implications of these crucial issues.

- Asia is the most common location for macrolide-resistant *M. pneumoniae*, and both erythromycin and clarithromycin are no longer effective in treating resistant pathogens.

- Azithromycin may be an option for treating susceptible *M. pneumoniae*, but its use should also be restricted to avoid building resistance via widespread use.


THANK YOU
FOR YOUR
ATTENTION