

# Section 1: Microbiology to molecular biology 'Germs to Genes'

Molecular Approaches to Clinical Microbiology in Africa 2024

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OXFORD

# Learning Outcomes

1. Overview of culture-based clinical microbiology.
2. The impact of molecular approaches.
3. Barriers to understanding molecular and genomic data.
4. How molecular data has affected our understanding of an exemplar disease, cholera.
5. 16S rRNA as an exemplar molecular approach, including an online exercise.

# Questions in clinical microbiology

evolution

emergence

epidemiology

diagnosis

Centuries+

decades

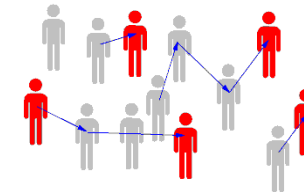
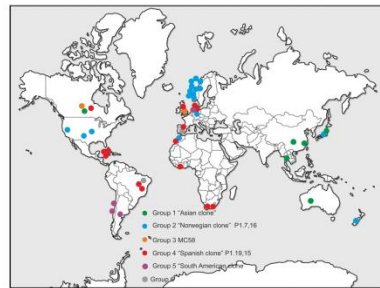
years

months

weeks

days

hours

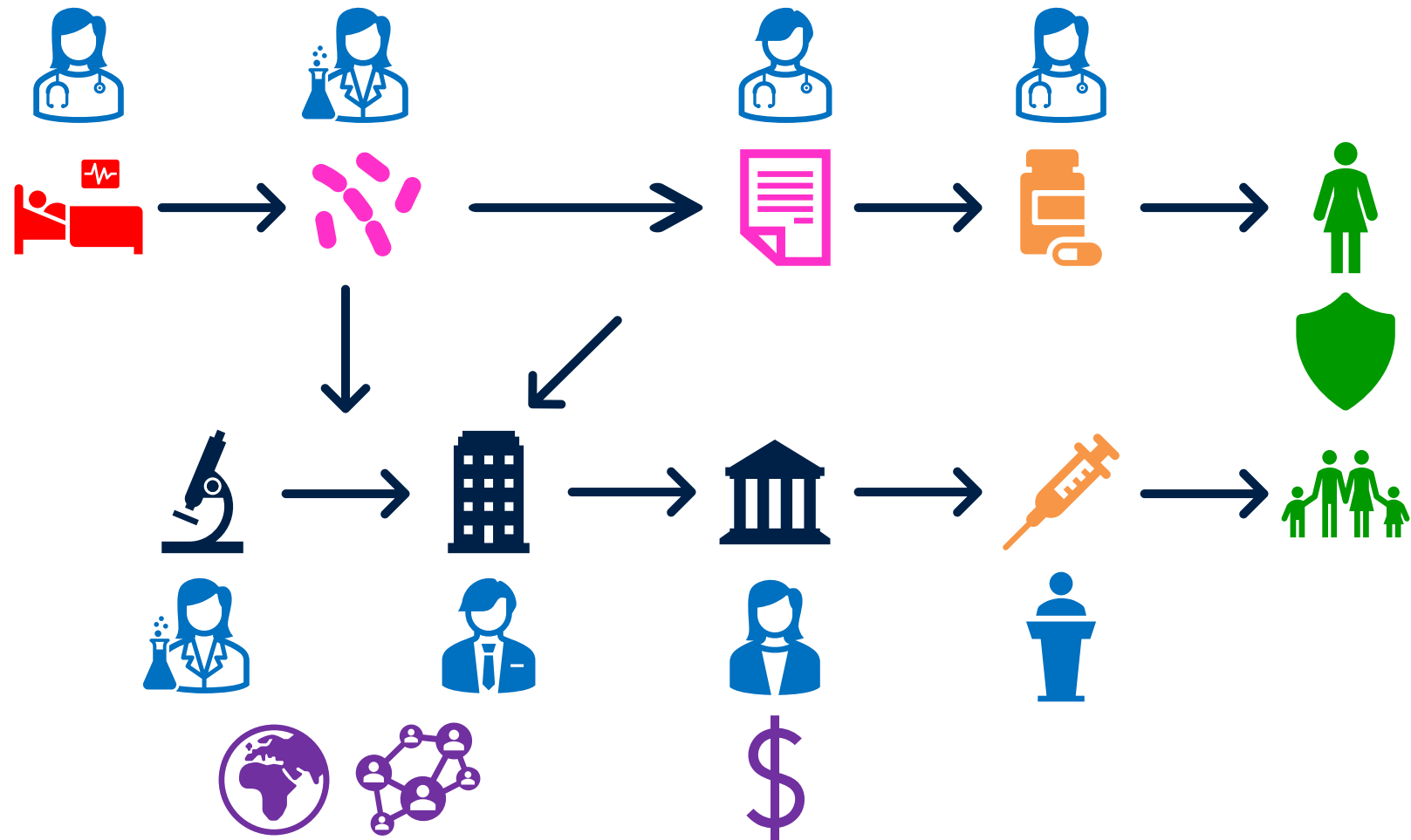


Low

Relative discrimination required

High

# Clinical and public health microbiology without molecular approaches

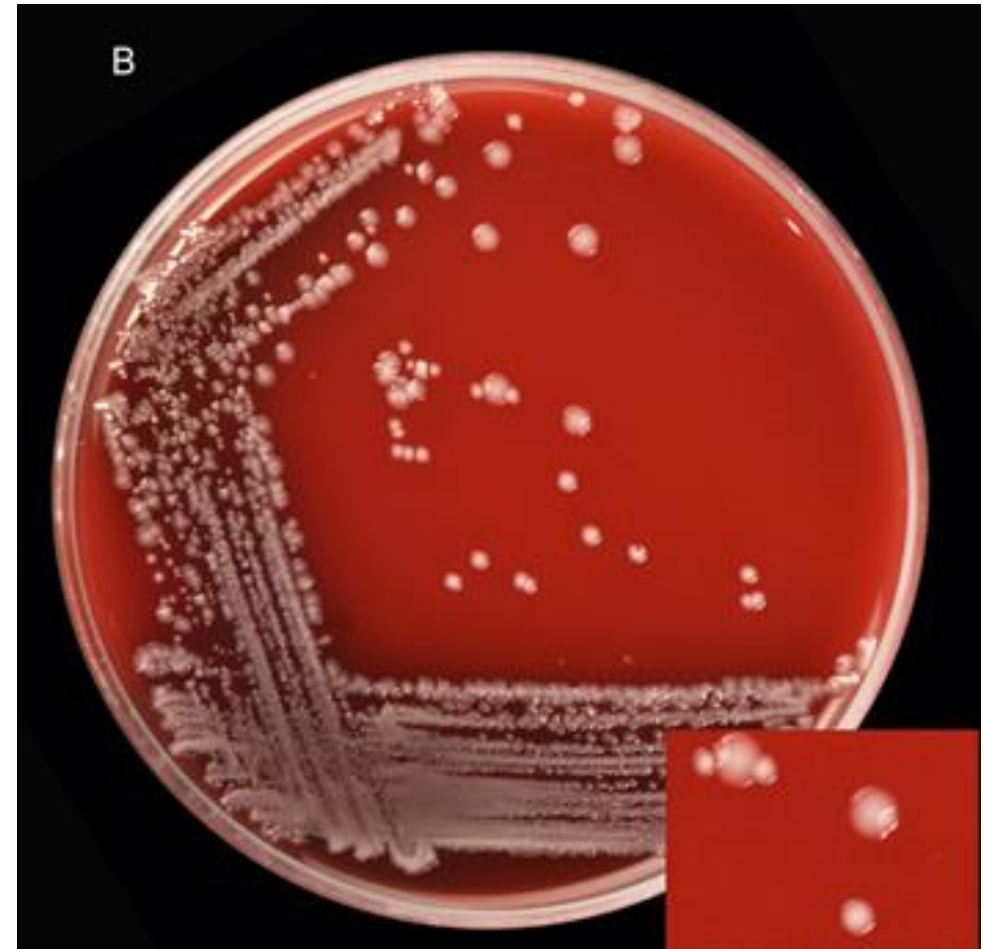


# Microbiology: pure culture

- The ability to isolate pathogens was foundational to microbiology.
  - Koch's postulates.
- In addition to diagnosis, this provides material for further characterisation.
  - Isolates remain widely used.

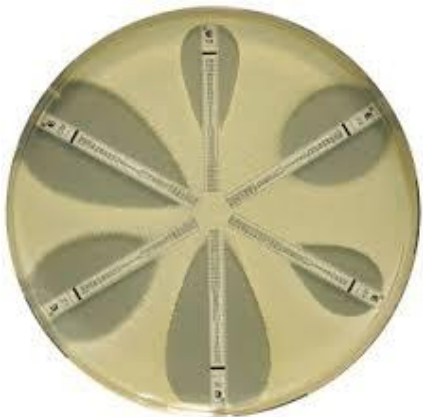
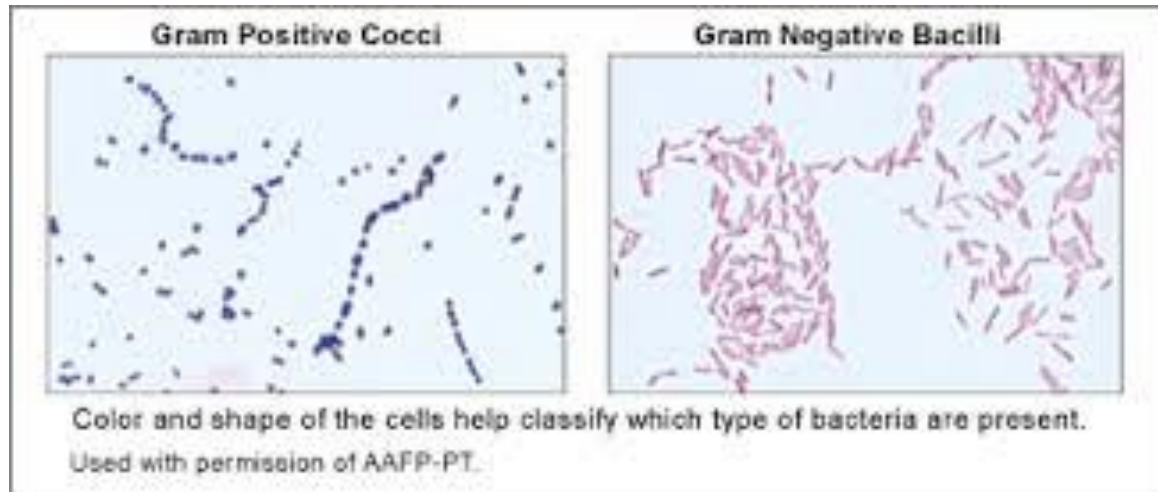
However;

- Culture requires sophisticated, often bespoke, reagents and equipment, and time;
- Many pathogens cannot be grown cultured:
  - lack of methods;
  - lack of suitable specimens.



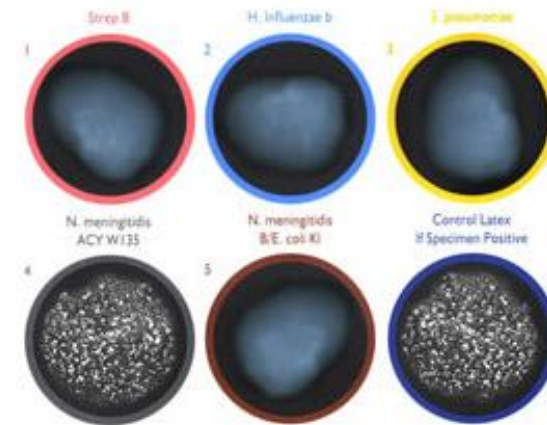
# Phenotypic characterisation

## Gram's Stain



Anti-microbial  
resistance  
testing

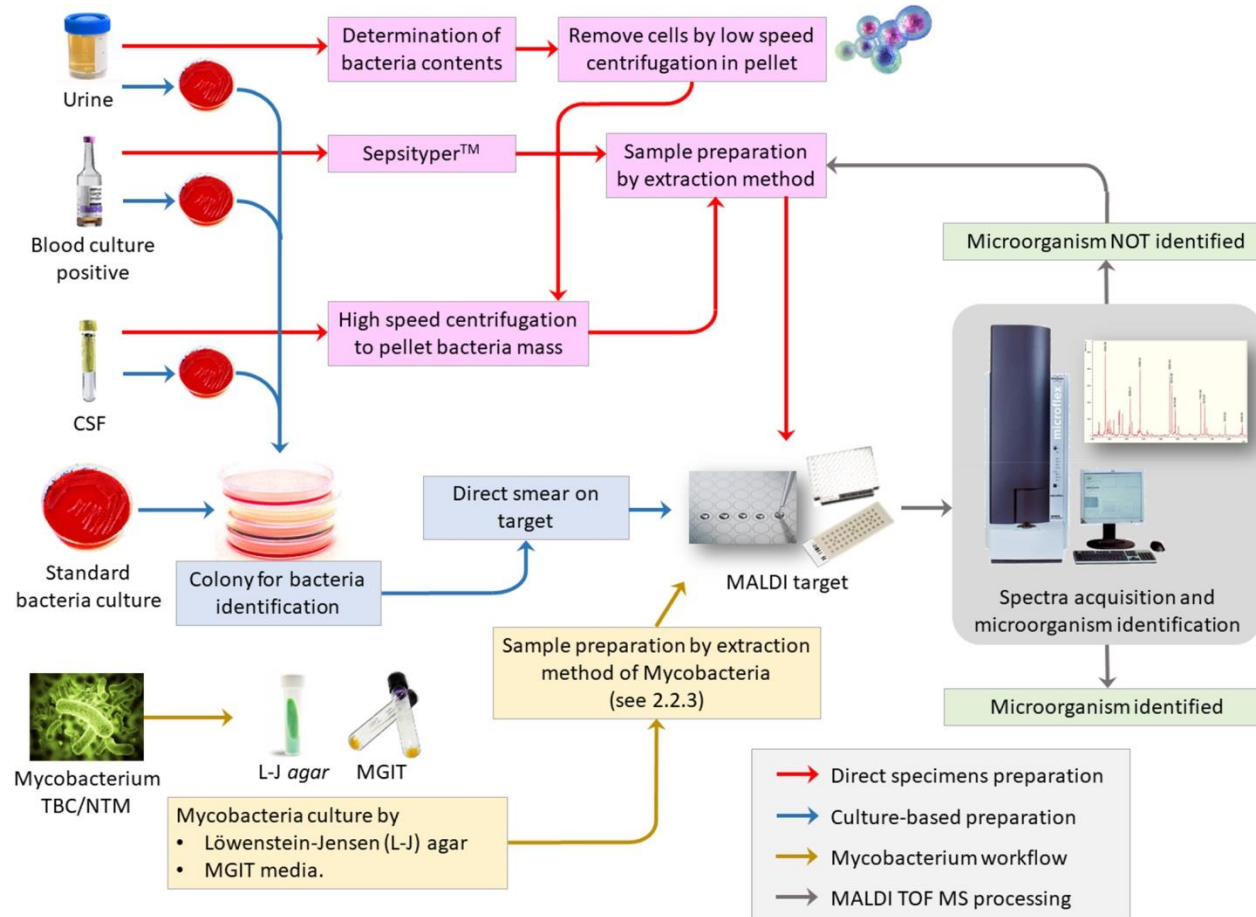
## Serology



Metabolic  
phenotyping  
For species  
identification



# MALDI-TOF phenotyping



Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry.

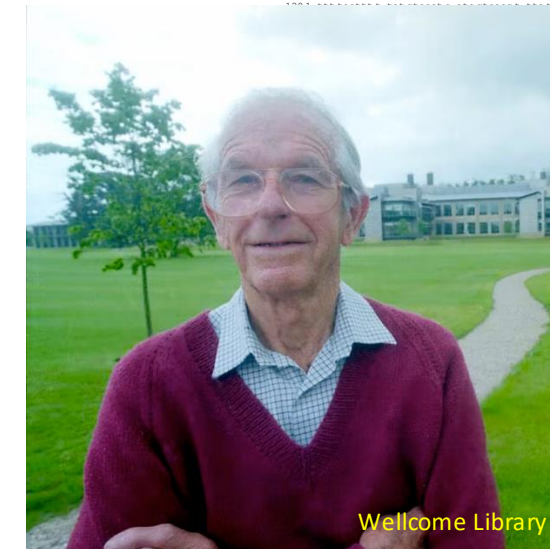
- Enables rapid and inexpensive (per sample) species identification
- Requires:
  - bacterial sample (usually an isolate);
  - the equipment;
  - a database linking spectra to bacterial species identification.
- Commercial systems are widely installed and used in clinical laboratories.

**Hou, T. Y., Chiang-Ni, C. & Teng, S. H. (2019).** Current status of MALDI-TOF mass spectrometry in clinical microbiology. *J Food Drug Anal.* **27**, 404-414.



# The molecular revolution and DNA sequences

- **Definitive:**
  - fundamental level of information;
  - any part of the genome can be accessed.
- **Reproducible:**
  - nucleotide sequences are either right or wrong and can be checked;
  - reverse mutations are (usually) rare.
- **Scalable:**
  - nucleotide sequencing technology can be conducted on one or many samples and on a few base pairs or a whole genome.
- **Manipulable:**
  - nucleotide sequences can be analysed with model-based methods.
- **Can be done from a PCR reaction or microbiome sample:**
  - an isolate is not necessarily required.



Wellcome Library

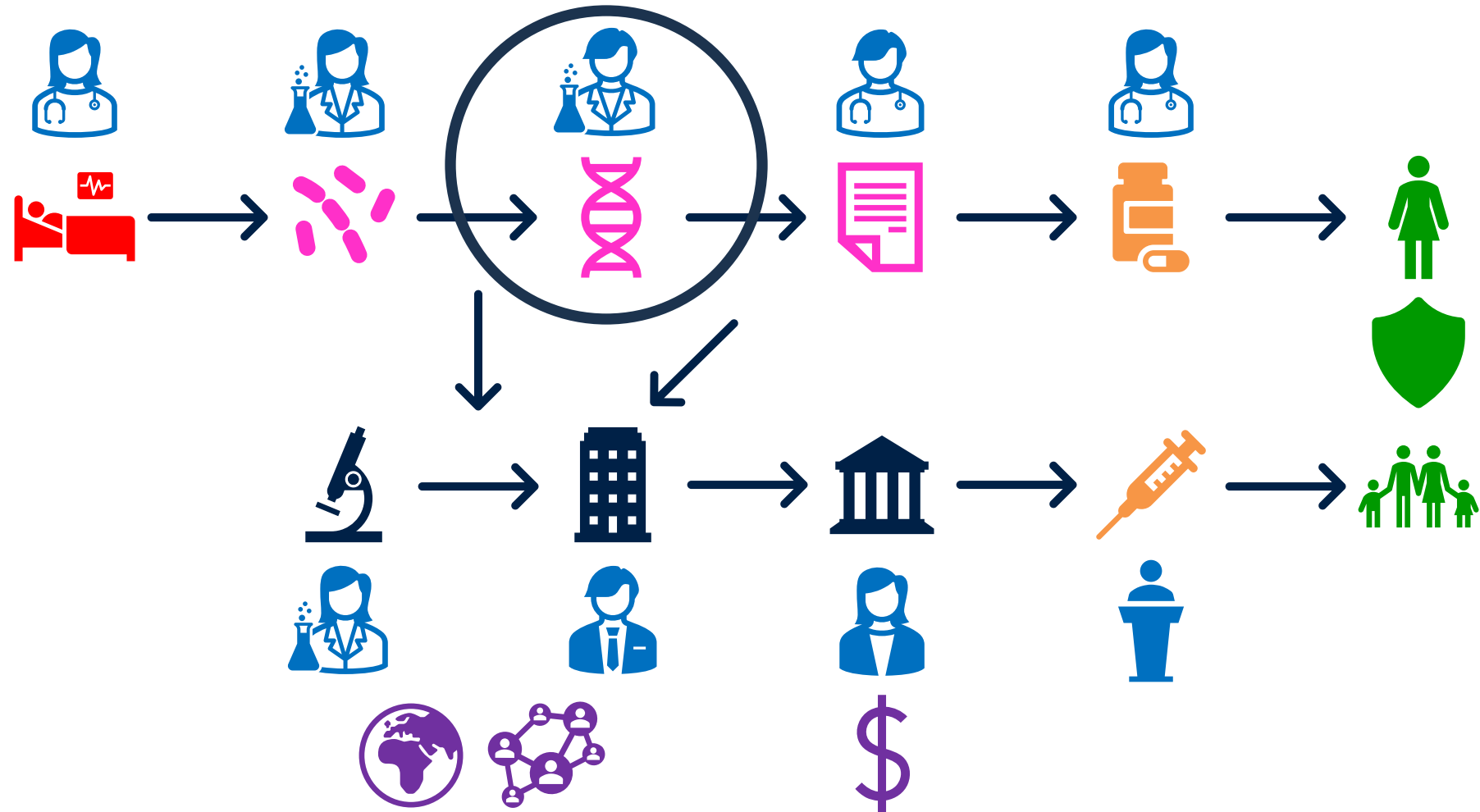
**Frederick Sanger (1918-2013)**

Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, C. A., Hutchison, C. A., Slocombe, P. M. & Smith, M. (1977). Nucleotide sequence of bacteriophage phi X174 DNA. *Nature* 265, 687-695.

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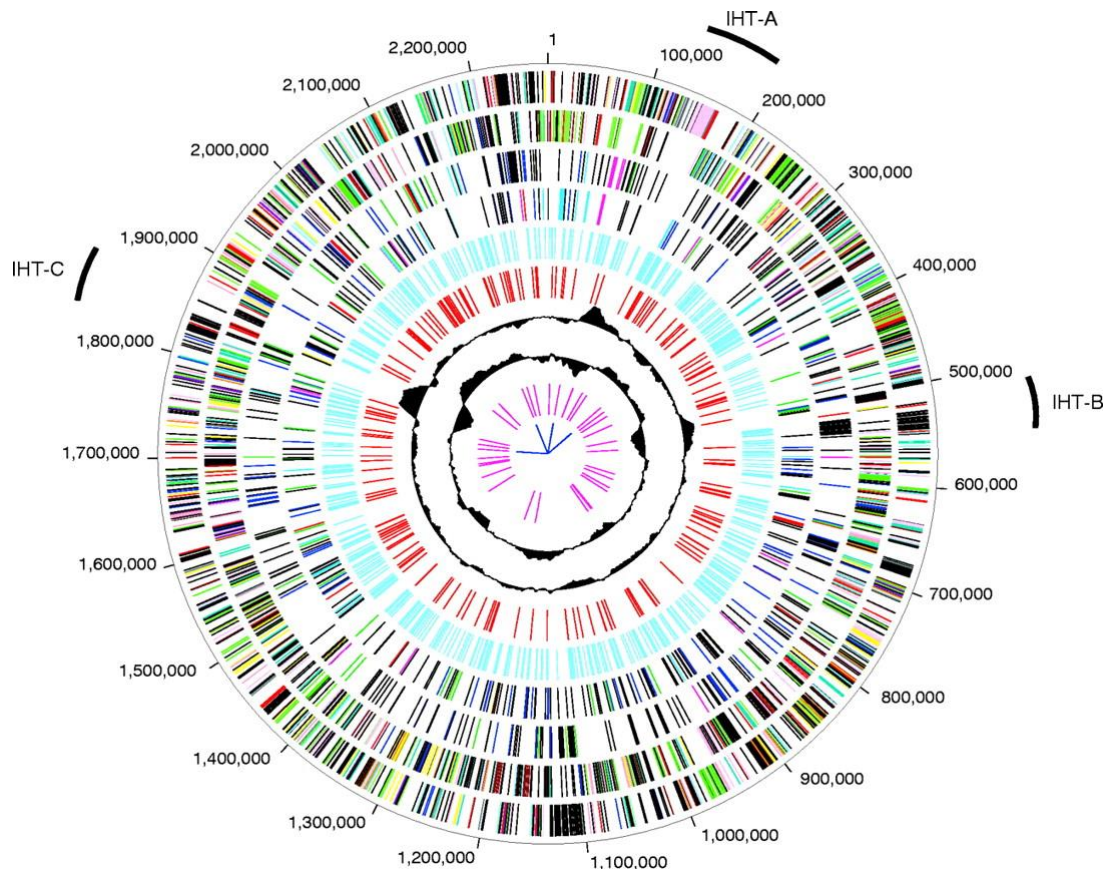
# Molecular approaches to clinical and public health microbiology



# The role of genetics and genomics

- Nucleotide sequencing facilitates specimen characterisation,
  - sequence-based typing, up to the level of whole genome sequences (WGSs).
- Sequences are definitive, reproducible, and comparable.
  - easily stored, transported, and manipulated with analysis algorithms;
- However, datasets are large and complex,
  - requiring interpretation for the practitioner.

# Translating molecular and genomic data



## Barriers:

- unfamiliarity;
- complexity, apparent and real;
- applicability.

## Solutions:

- explaining & summarising;
- structuring & curating;
- interpreting & synthesising.

Tettelin, H., Saunders, N. J., Heidelberg, J., Jeffries, A. C., Nelson, K. E., Eisen, J. A., Ketchum, K. A., Hood, D. W., Peden, J. F., Dodson, R. J., Nelson, W. C., Gwinn, M. L., DeBoy, R., Peterson, J. D., Hickey, E. K., Haft, D. H., Salzberg, S. L., White, O., Fleischmann, R. D., Dougherty, B. A., Mason, T., Ciecko, A., Parksey, D. S., Blair, E., Cittone, H., Clark, E. B., Cotton, M. D., Utterback, T. R., Khouri, H., Qin, H., Vamathevan, J., Gill, J., Scarlato, V., Massignani, V., Pizza, M., Grandi, G., Sun, L., Smith, H. O., Fraser, C. M., Moxon, E. R., Rappuoli, R. & Venter, J. C. (2000). Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. *Science* **287**, 1809-1815.

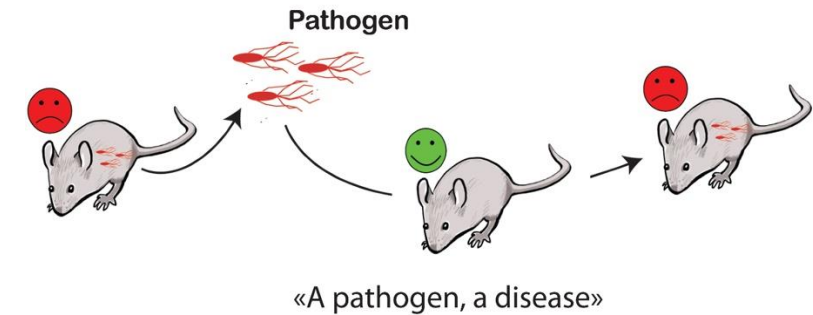
# Impacts of molecular data on clinical microbiology principles and practice

# Koch's Postulates revisited

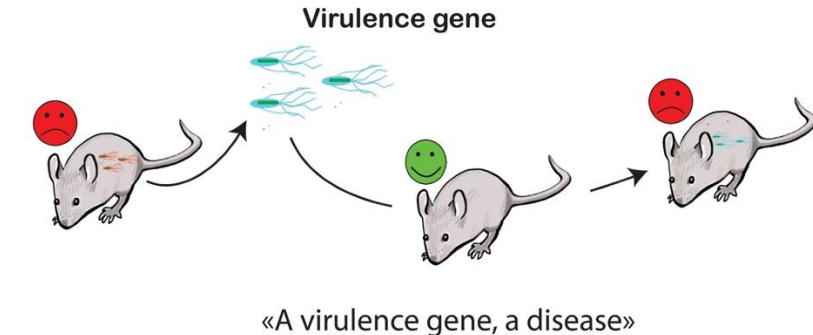
- Koch's postulates original formulation is, the:
  - microbe is found in all cases of disease and is absent in its absence;
  - microbe can be isolated and grown in pure culture;
  - cultured microbe can cause disease in a healthy host;
  - microbe can be re-isolated and cultured from this host.
- These ideas have been revisited in the light of knowledge on molecular pathology and the microbiome.
  - 'Molecular Koch's Postulates';
  - 'Ecological Koch's Postulates'.

**Vonaesch, P., Anderson, M. & Sansonetti, P. J. (2018).** Pathogens, microbiome and the host: emergence of the ecological Koch's postulates. *FEMS Microbiol Rev.* **42**, 273-292.

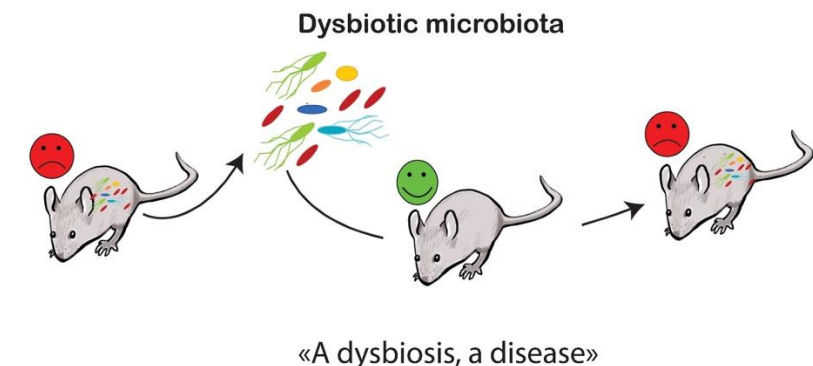
## Koch's postulate



## Molecular Koch's postulate





## Ecological Koch's postulate





# Case study: Cholera



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**Climate change** News feature 8 April 2024

## Why is cholera killing thousands in southern Africa?


'If we were doing enough, we would not have seen such a massive cholera outbreak.'



**Freddie Clayton**  
British journalist who covers climate, the environment, and public health



**Kennedy Phiri**  
A Zambian journalist reporting on environmental and gender issues, and the co-founder of Media Network Action on Climate Change.



Margaret Courtney Clarke

Southern Africa's informal settlements often lack adequate water, sanitation, and hygiene infrastructure, like this informal settlement in Windhoek, Namibia.

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<https://www.thenewhumanitarian.org/news-feature/2024/04/08/why-cholera-killing-thousands-southern-africa>



# Cholera: Koch's postulates

- Pacini 1854 - presence of 'comma shaped' bacteria in stools.
- Snow 1854 - cholera is water borne.
- Koch 1883 - isolation of *Vibrio cholerae*.
- Gradual introduction of improved sanitation in Europe & North America.

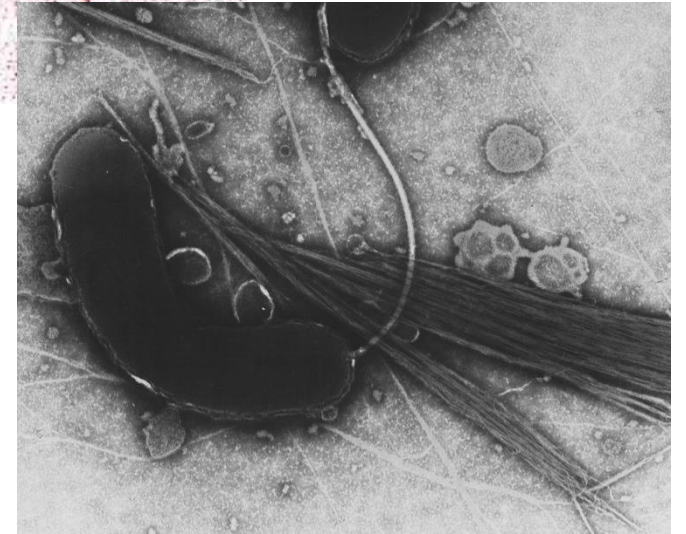
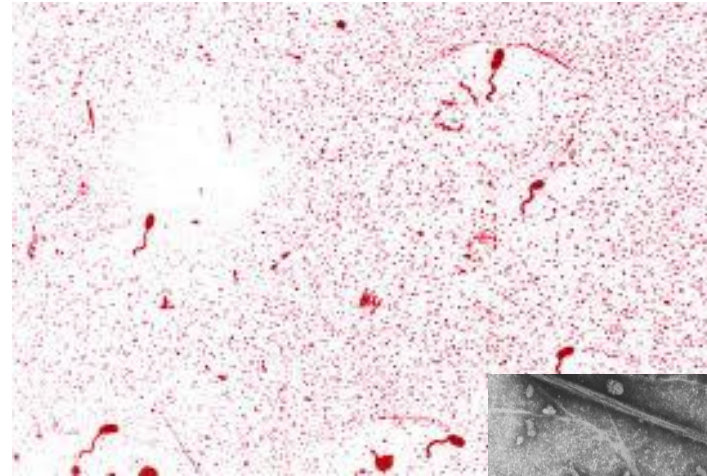


Robert Koch (third from the right) on a cholera research expedition in Egypt in 1884, one year after he identified *V. cholerae*, the microbe responsible for cholera.

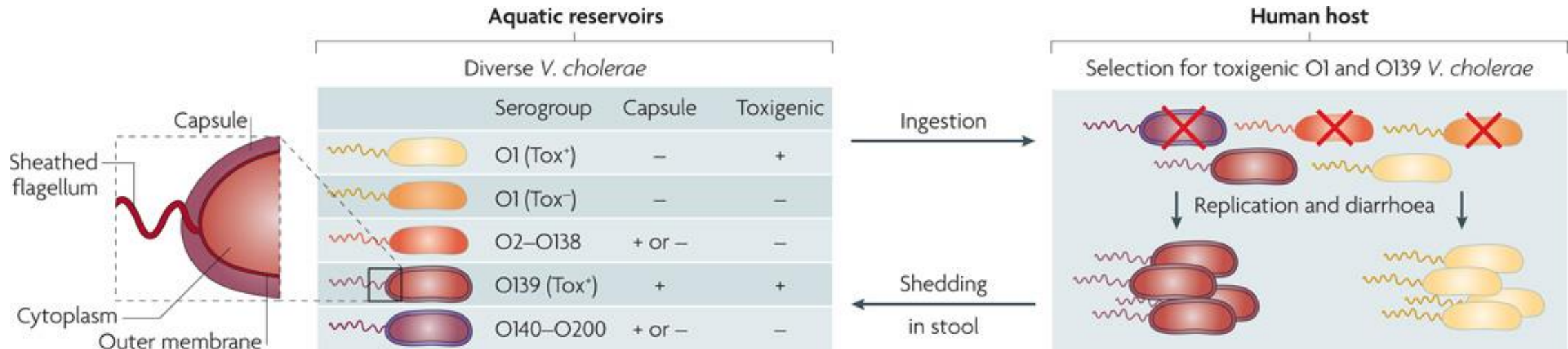
Hamlin, C. (2009). *Cholera: the biography*. Oxford, UK: Oxford University Press.

# Cholera: the bacterium, *Vibrio cholerae*

- A Gram-negative bacterium, commonly found in brackish estuarine water.
- Differentiated serologically on the basis of the O antigen of its LPS.
- Cholera toxin-producing strains of the O1 and O139 serogroups cause the great majority of disease.
- All pathogenic *V. cholerae* express cholera toxin (Ctx) and toxin co-regulated pilus (Tcp).



# Cholera: the genes, encoding capsule and toxin

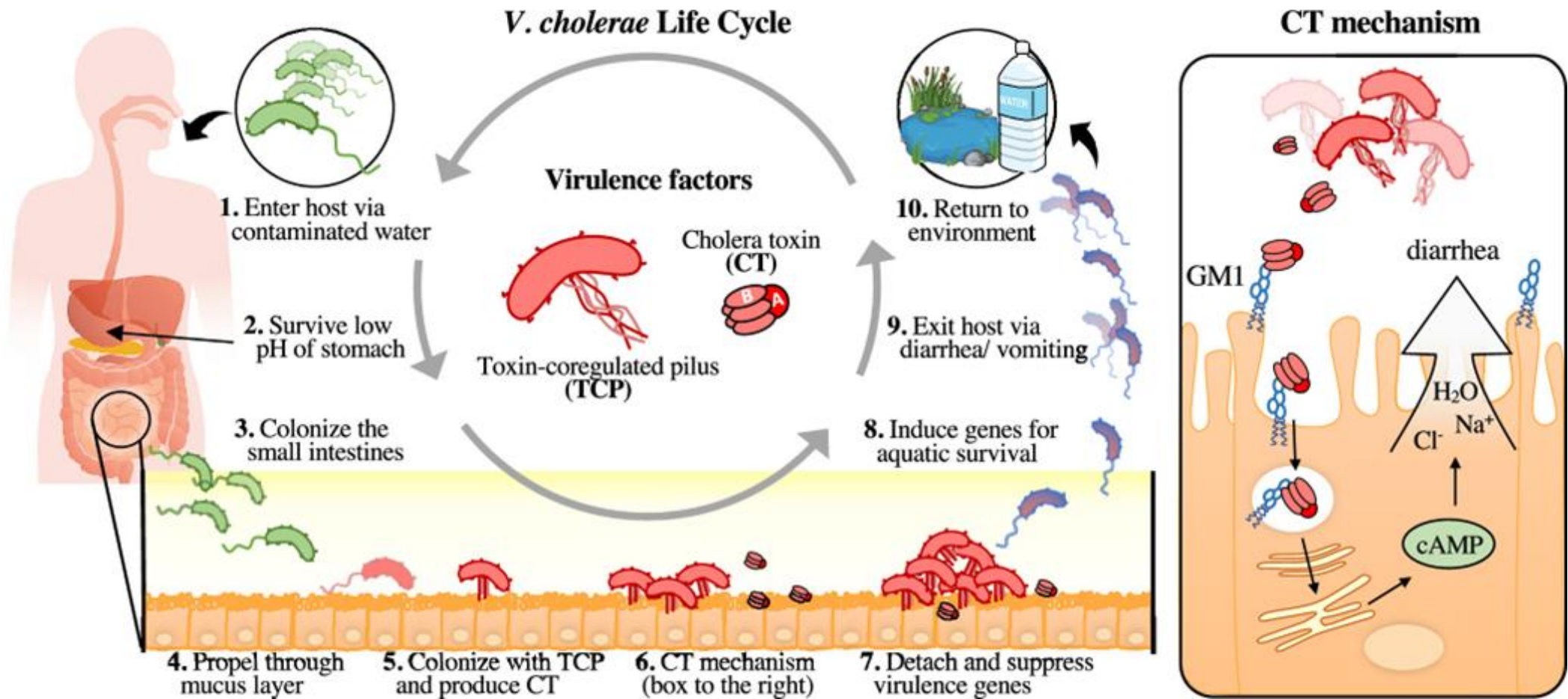


- More than 200 serogroups (O1- O200) of *Vibrio cholerae* exist.
- Only a subset of O1 and O139 are toxigenic (Tox<sup>+</sup>) and capable of causing cholera when ingested.
- The *tcp* gene is located on a pathogenicity island, the *ctxA* and *ctxB* genes on a filamentous phage.
- These variants are selected for in the host, Tox<sup>-</sup> are selected against

**Nelson E.J. et al** (2009). Cholera transmission: the host, pathogen and bacteriophage dynamic. *Nature reviews* **7**, 693-702



# Cholera: the dysbiosis

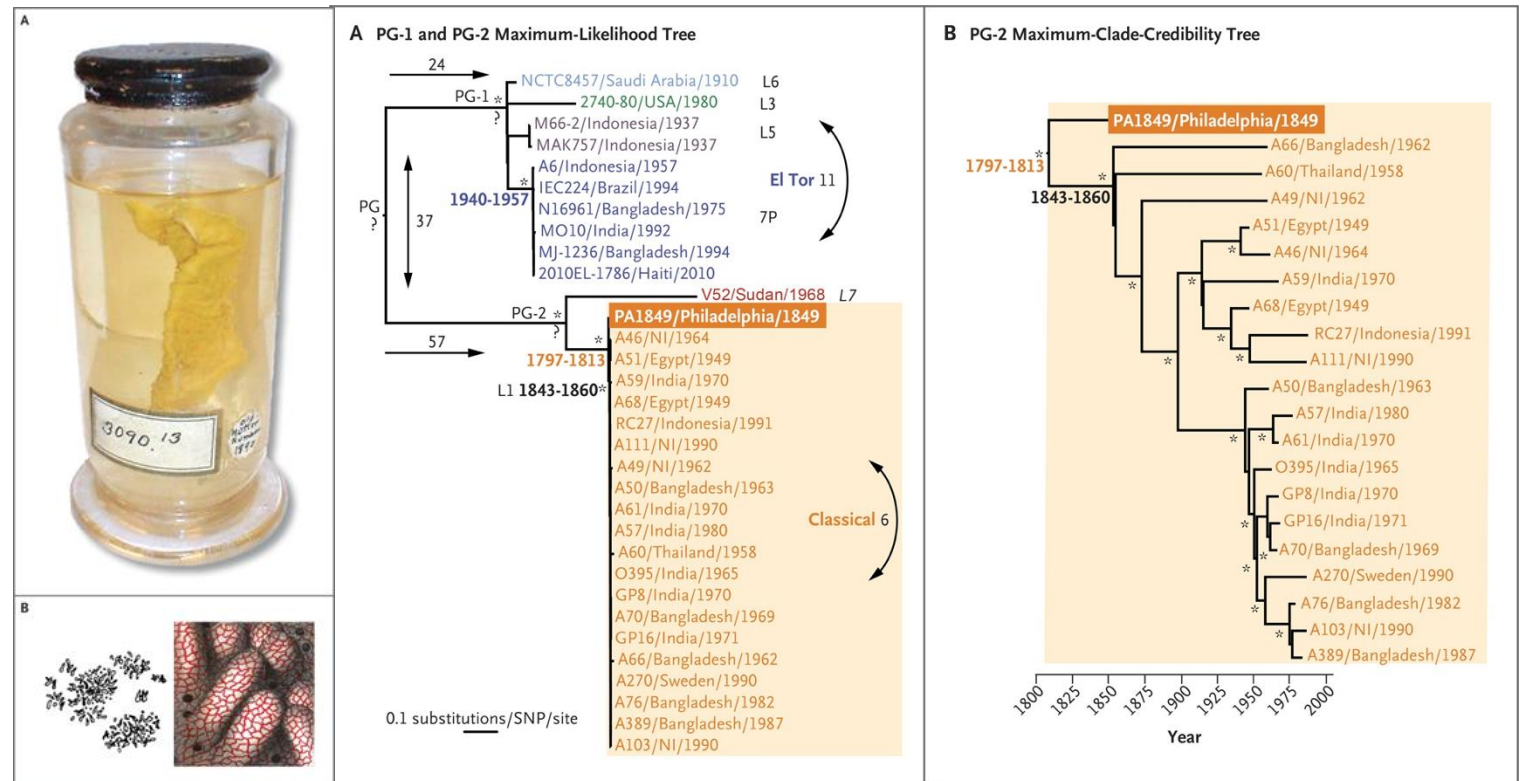


Chac, D., Dunmire, C. N., Singh, J. & Weil, A. A. (2021). Update on Environmental and Host Factors Impacting the Risk of *Vibrio cholerae* Infection. *ACS Infect Dis.* **7**, 1010-1019

# Cholera: the power of genomics,

## 2<sup>nd</sup> Pandemic strain, Philadelphia, 1849

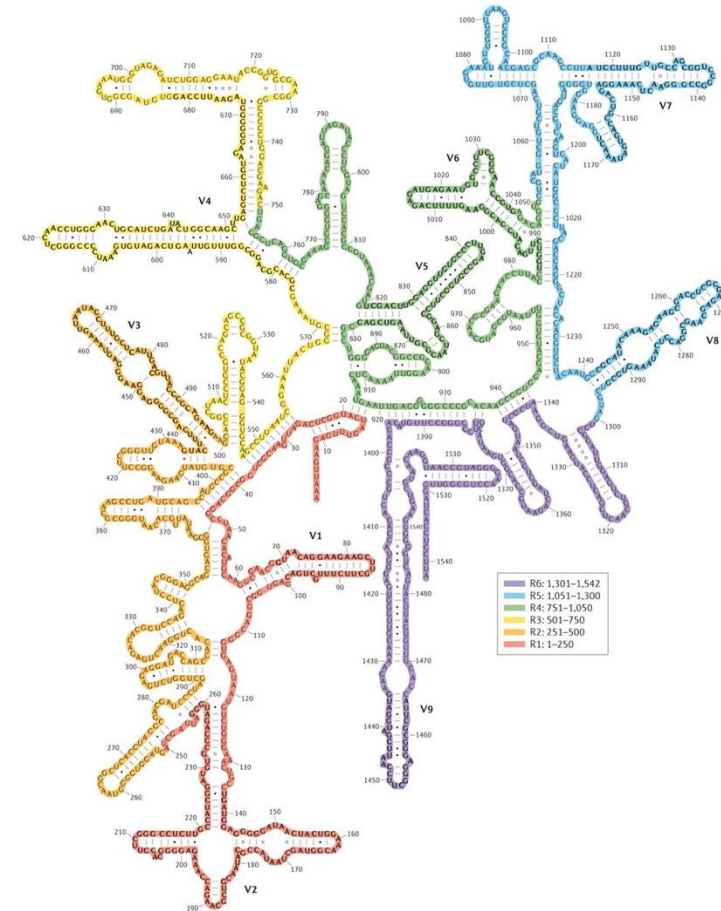
- A whole genome sequence was reconstructed
  - from preserved intestinal material from a cholera victim.
- The *V. cholerae* strain was O1 biotype,
  - 95-7% similarity to 'Classical' O395 genome.
- One or more tandem *ctx* phage sequences,
  - VPI-1 and VPI-2 present.



Devault, A. M., Golding, G. B., Waglechner, N., Enk, J. M., Kuch, M., Tien, J. H., Shi, M., Fisman, D. N., Dhody, A. N., Forrest, S., Bos, K. I., Earn, D. J., Holmes, E. C. & Poinar, H. N. (2014). Second-pandemic strain of *Vibrio cholerae* from the Philadelphia cholera outbreak of 1849. *N Engl J Med.* **370**, 334-340

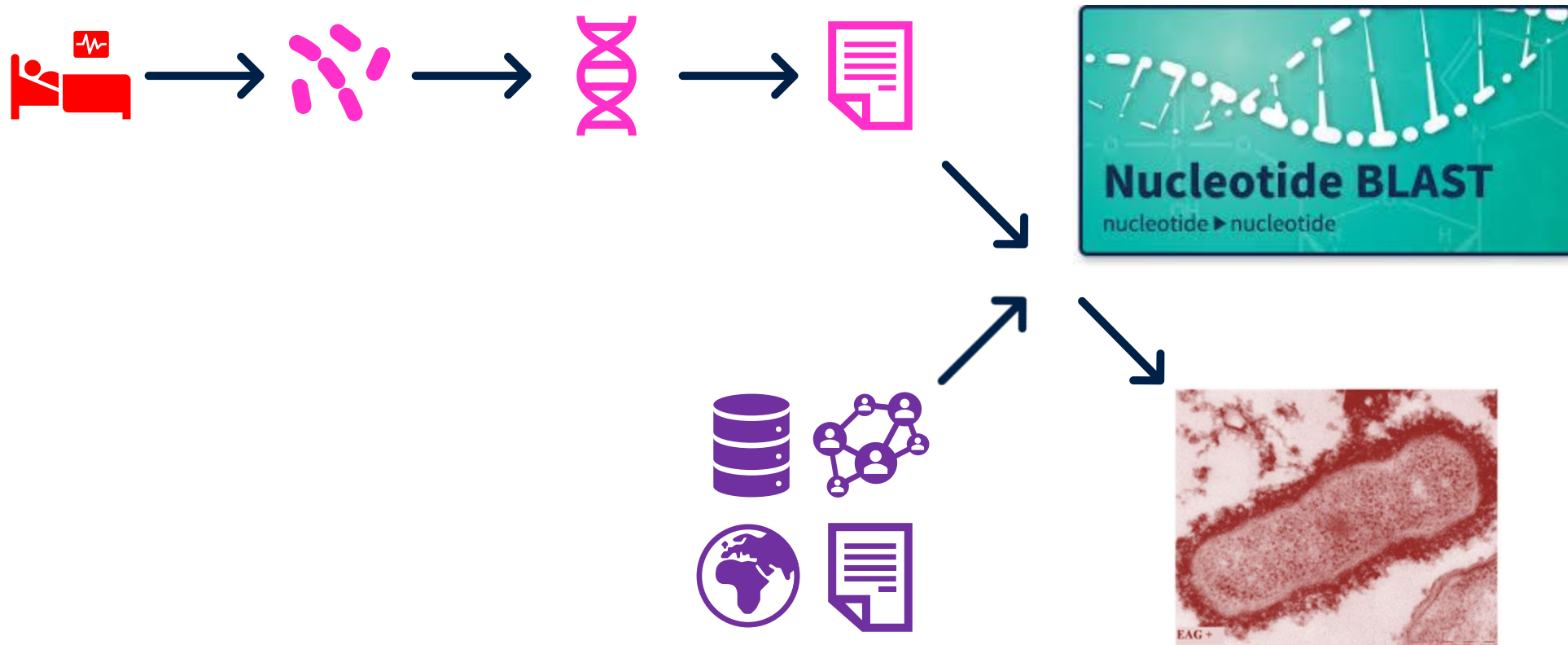
# Single gene case study: 16S rRNA gene analysis

- 16S rRNA is a functionally essential structural component of the ribosome, highly conserved.
- Varies among organisms, although most variation is at the genus level.
- 16S rRNA gene, ~1.5Kbp, with:
  - universal, conserved regions, which can be used to generate primers;
  - nine variable regions which can be used as signatures to determine genus/species.



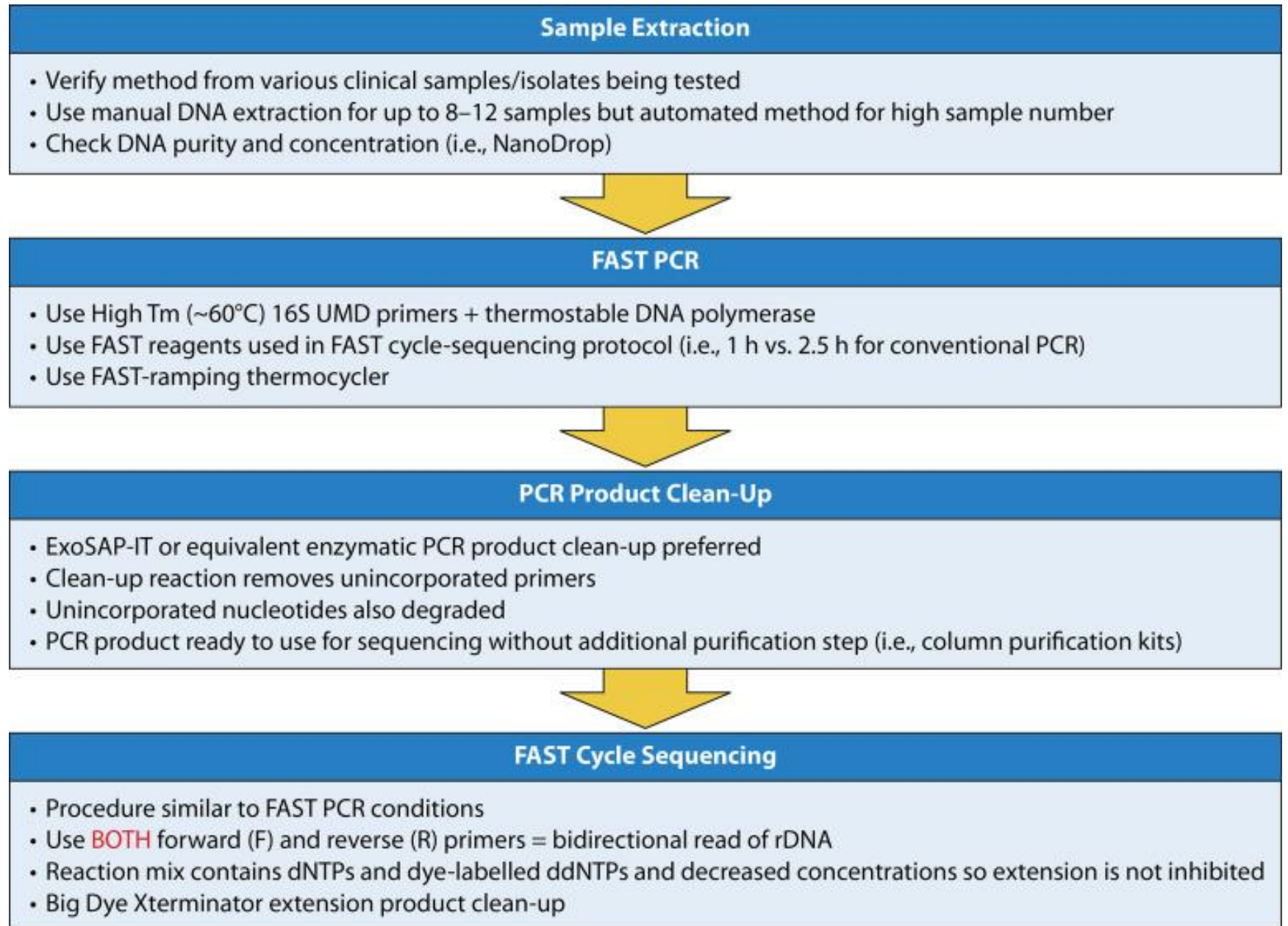


# 16S rRNA process overview



# 16S rRNA sequencing in the clinic

Church, D. L., Cerutti,  
L., Gurtler, A., Griener,  
T., Zelazny, A. & Emler,  
S. (2020). Performance  
and Application of 16S  
rRNA Gene Cycle  
Sequencing for Routine  
Identification of  
Bacteria in the Clinical  
Microbiology  
Laboratory. *Clin  
Microbiol Rev.* **33**,  
e00053-1



Exercise, searching an NCBI database with 16SrRNA  
sequences

# 16s rRNA BLAST search online (1)

- Navigate to NCBI BLAST home page:
  - <https://blast.ncbi.nlm.nih.gov/Blast.cgi>

- Click on:



- Under 'Enter Query sequence' paste in a test sequence (Test 1-3)
  - [https://github.com/WCSCourses/Molecular\\_Approaches\\_Clinical\\_Microbiology\\_2024/blob/main/course\\_data/bioinformatics/16S/16S\\_samples.fas](https://github.com/WCSCourses/Molecular_Approaches_Clinical_Microbiology_2024/blob/main/course_data/bioinformatics/16S/16S_samples.fas)
  - <https://tinyurl.com/228hpuev>
- Under 'Choose Search Set' click on 'rRNA/ITS databases'
- Check box 'Show results in a new window'. Click on

**BLAST**




**Important update**  
The core nucleotide database (**core\_nt**) is now the default nucleotide BLAST database. [Learn more about core\\_nt.](#)

Standard Nucleotide BLAST

BLASTN programs search nucleotide databases using a nucleotide query. more...

[Reset page](#) [Bookmark](#)

Enter Query Sequence


Enter accession number(s), gi(s), or FASTA sequence(s) 

```
>Test_1
AATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACA
CATGCAAG
TCGAACGGTAACAGGAAGAAGCTTGCTCTTTGCTGACGAGTGGCGGACGG
```


Query subrange 


From

To

Or, upload file  No file selected. 


Job Title

Enter a descriptive title for your BLAST search 

☐ Align two or more sequences 


Choose Search Set

Database ☐ Standard databases (nr etc.): ☒ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus ☐ Experimental databases

16S ribosomal RNA sequences (Bacteria and Archaea) 


[Targeted Loci Project Information](#)

Organism Optional  ☐ exclude [Add organism](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown 

Exclude Optional ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to Optional ☐ Sequences from type material

Entrez Query Optional  [YouTube](#) [Create custom database](#)  
Enter an Entrez query to limit search 

Program Selection

Optimize for ☒ Highly similar sequences (megablast)

☐ More dissimilar sequences (discontiguous megablast)

☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm 

BLAST

Search database 16S ribosomal RNA sequences (Bacteria and Archaea) using Megablast (Optimize for highly similar sequences)

☒ Show results in a new window

Note: Parameter values that differ from the default are highlighted in yellow and marked with  sign

+ Algorithm parameters

# What bacterium is it?

- Look at the results of the search (scroll down the page as necessary).
- What is the likely organism that the 16S rRNA sequence came from?

Sample	Candidate Bacterium
Test 1	<i>Escherichia/Shigella</i> ?
Test 2	<i>Mycobacterium</i> ?
Test 3	<i>Vibrio cholerae</i> ?



# Encapsulated Theme: applications of molecular techniques

Molecular Approaches to Clinical Microbiology in Africa 2024

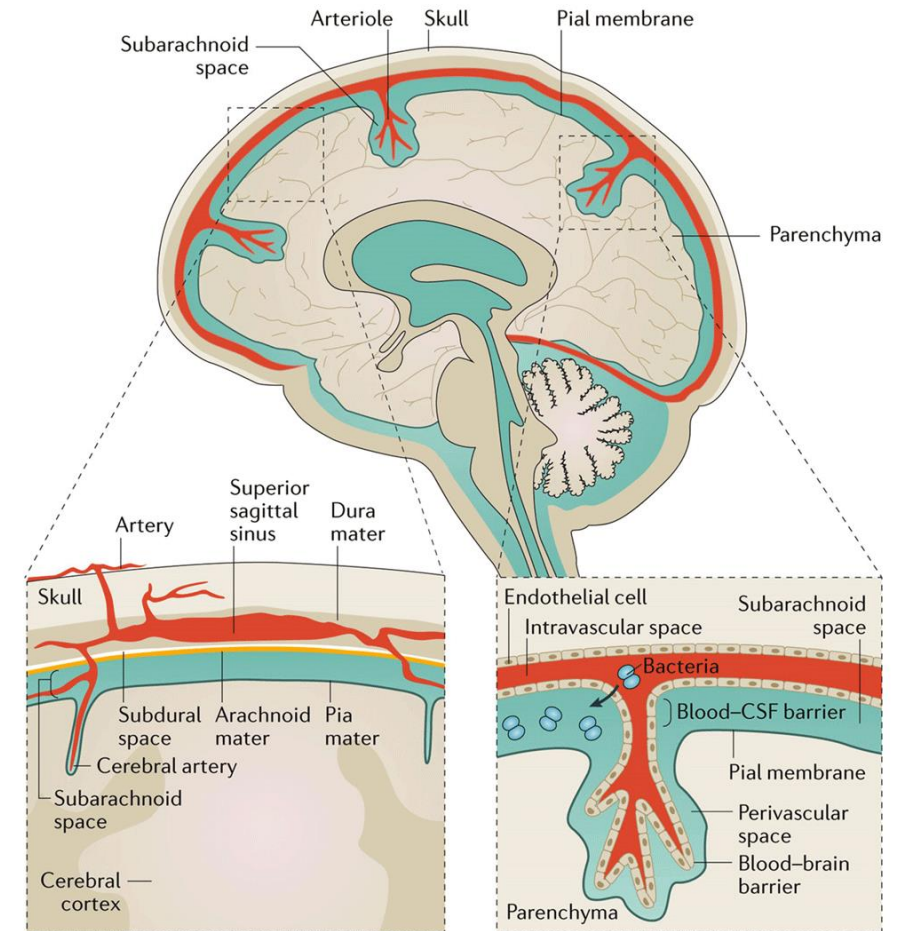
Martin Maiden, Keith Jolley



UNIVERSITY OF  
OXFORD

# Meningitis

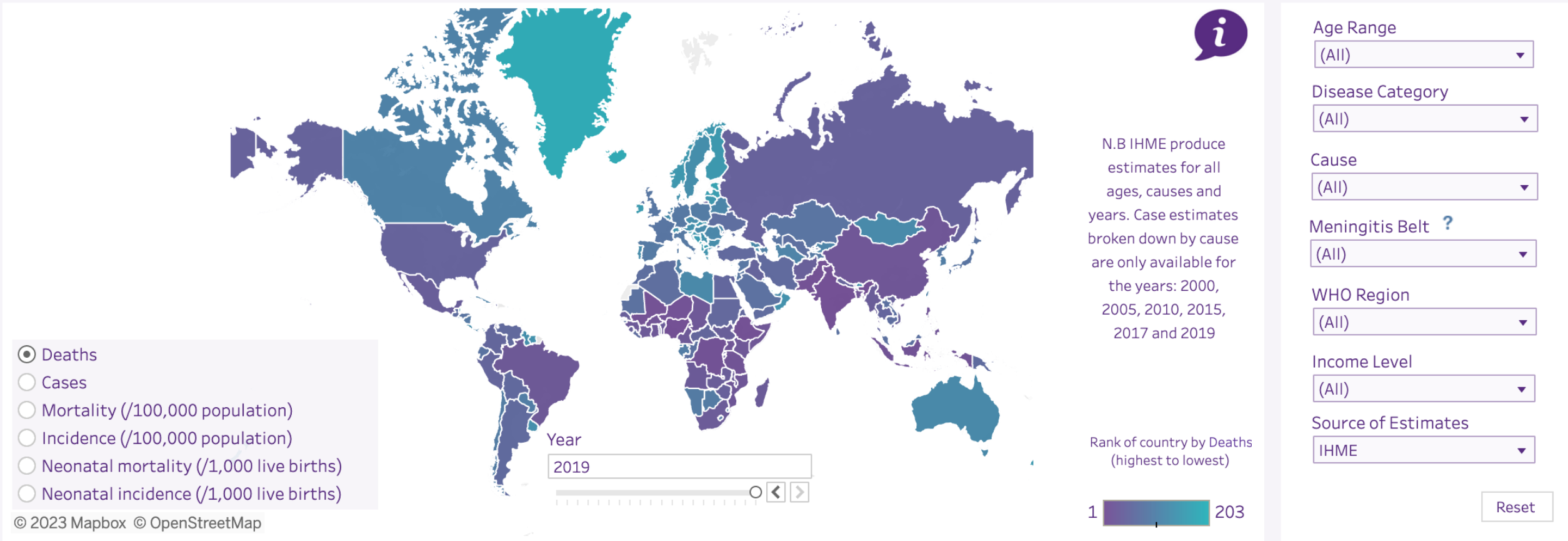
- Meningitis:
  - Inflammation of the meninges (tissues around the brain).
- Caused by invasion of the meninges by a pathogen:
  - Bacterial, viral, fungal, parasite;
  - Can be accompanied by other pathologies e.g. septicaemia (blood poisoning).
- Severe and frequently fatal especially when caused by bacteria:
  - Survivors frequently suffer sequelae e.g. digit or limb loss, brain damage, deafness.
- Diagnosis difficult
  - but essential for swift appropriate treatment.



**Rodrigues, C. M. C. & Maiden, M. C. J. (2018).** A world without bacterial meningitis: how genomic epidemiology can inform vaccination strategy. *F1000Res* **7**, 401.



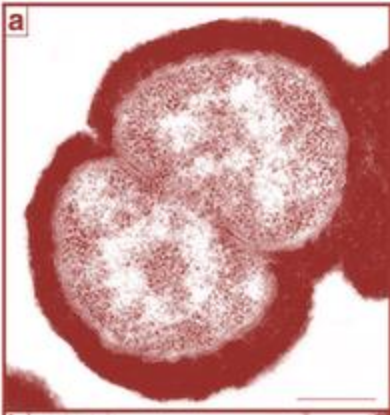
# Meningitis and Neonatal Sepsis



Estimated Total Deaths 462,452

Estimated Total Cases 8,427,054

# Meningitis causing organisms

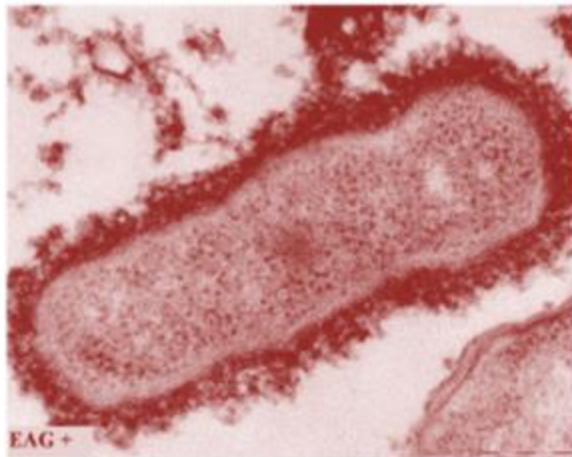
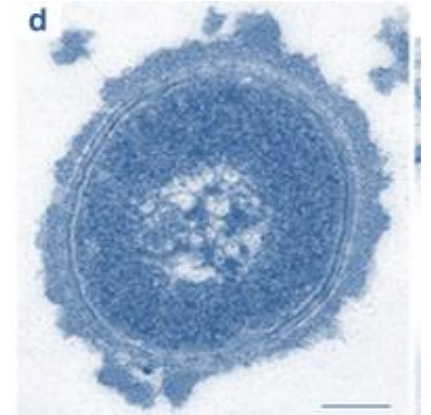


*Neisseria meningitidis*  
Meningococcus  
12 capsular serogroups  
Genome: 2.27Mbp

**Ganesh, K. et al.** (2017) Molecular characterization of invasive capsule null *Neisseria meningitidis* in South Africa. *BMC Microbiol.* **17**(1), 40.

*Streptococcus pneumoniae*  
Pneumococcus  
>100 capsular serotypes  
Genome: 2.04Mbp

**Ndlangisa, K.M., et al.** (2016) Two cases of serotypeable and non-serotypeable variants of *Streptococcus pneumoniae* detected simultaneously during invasive disease. *BMC Microbiol.* **16**, 126.

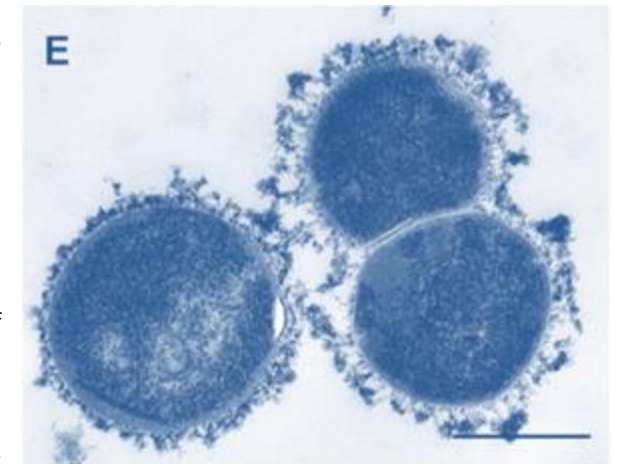


*Haemophilus influenzae*  
'Hib'  
6 capsular serotypes  
Genome: 1.83Mbp

**Schouls L., et al.** (2008) Two variants among *Haemophilus influenzae* serotype b strains with distinct *bcs4*, *hcsA* and *hcsB* genes display differences in expression of the polysaccharide capsule. *BMC Microbiol.* **8**, 35.

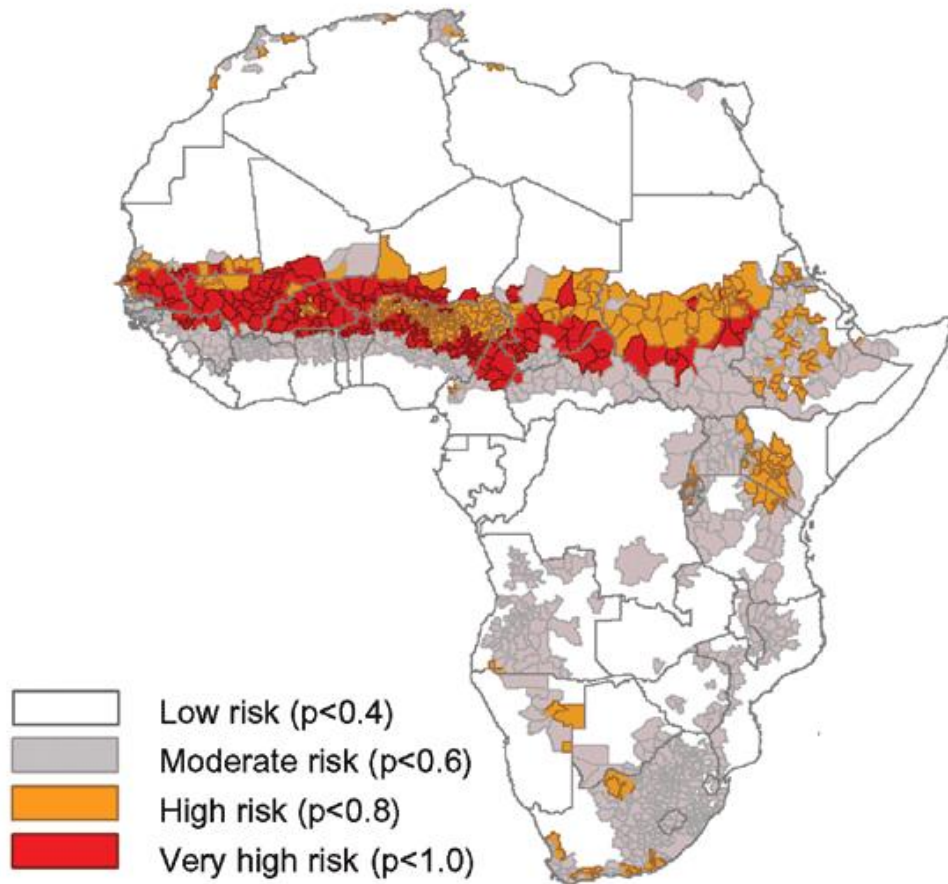
*Streptococcus agalactiae*  
Group B Strep. (GBS)  
10 capsular serotypes  
Genome: 2.16Mbp

**Lecours M.P., et al.** (2012) Sialylation of *Streptococcus suis* serotype 2 is essential for capsule expression but is not responsible for the main capsular epitope. *Microbes Infect.* **14**(11), 941-50.





# The meningitis belt



**Meningitis belt** extends from Ethiopia to Senegal.

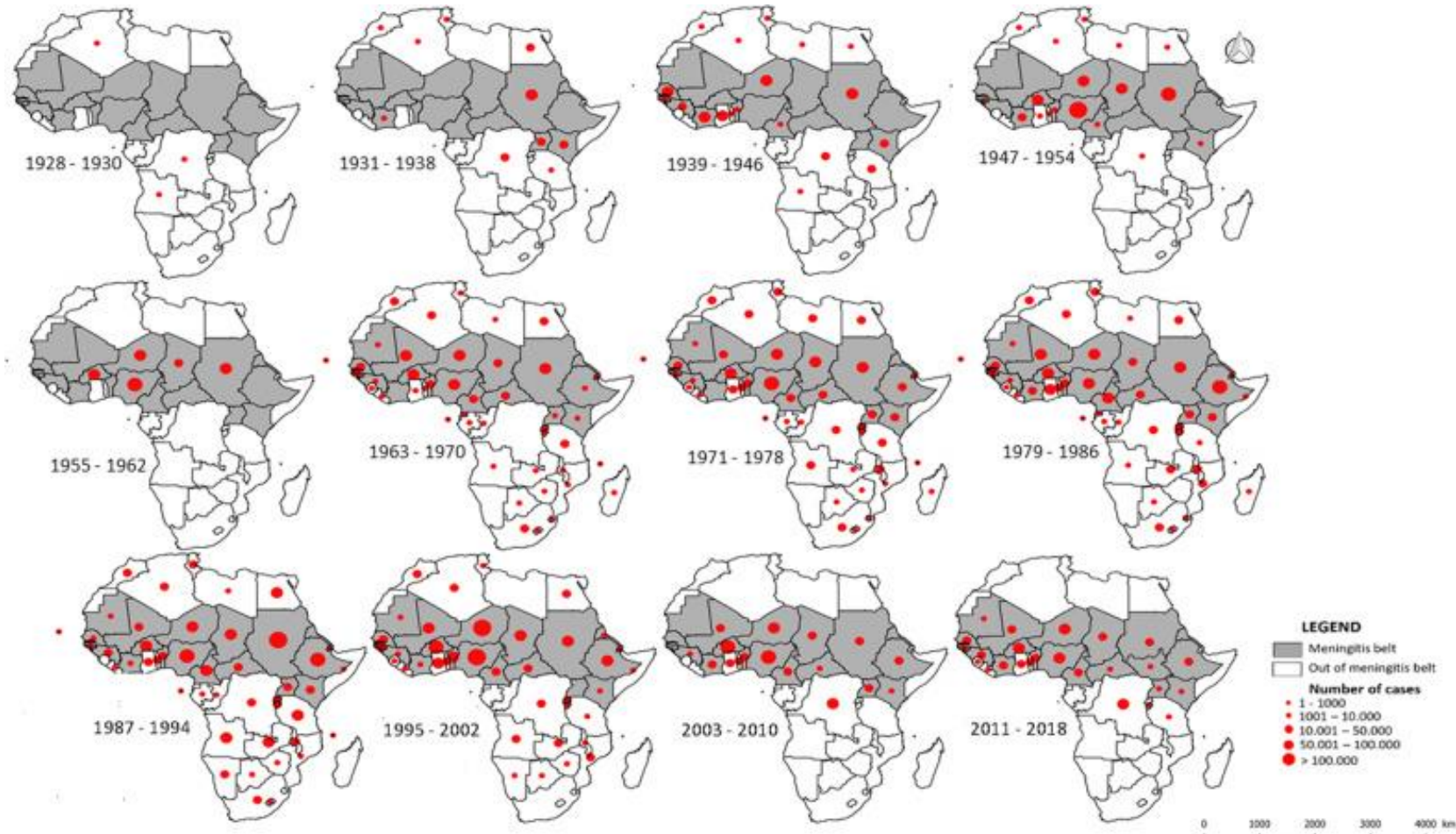
Sudan, Ethiopia, Chad, Niger, Northern Nigeria, Burkina Faso, and Mali are considered hyper-endemic.

- **1905**, first documented epidemic, Northern Nigeria.
- **1919-1924**, second cycle, >45,000 deaths, Northern Nigeria.
- **1935-1937**, third cycle, Nigeria, 6,456 deaths.
- 1951-60, 340,000 cases, 53,000 deaths.
- 1996-1997, 300,000 cases, 30,000 deaths

**Molesworth, A. M., Cuevas, L. E., Connor, S. J., Morse, A. P. & Thomson, M. C.** (2003). Environmental risk and meningitis epidemics in Africa. *Emerg Infect Dis* **9**, 1287-1293.

**Greenwood, B.** (1999). Manson Lecture. Meningococcal meningitis in Africa. *Trans R Soc Trop Med Hyg* **93**, 341-353

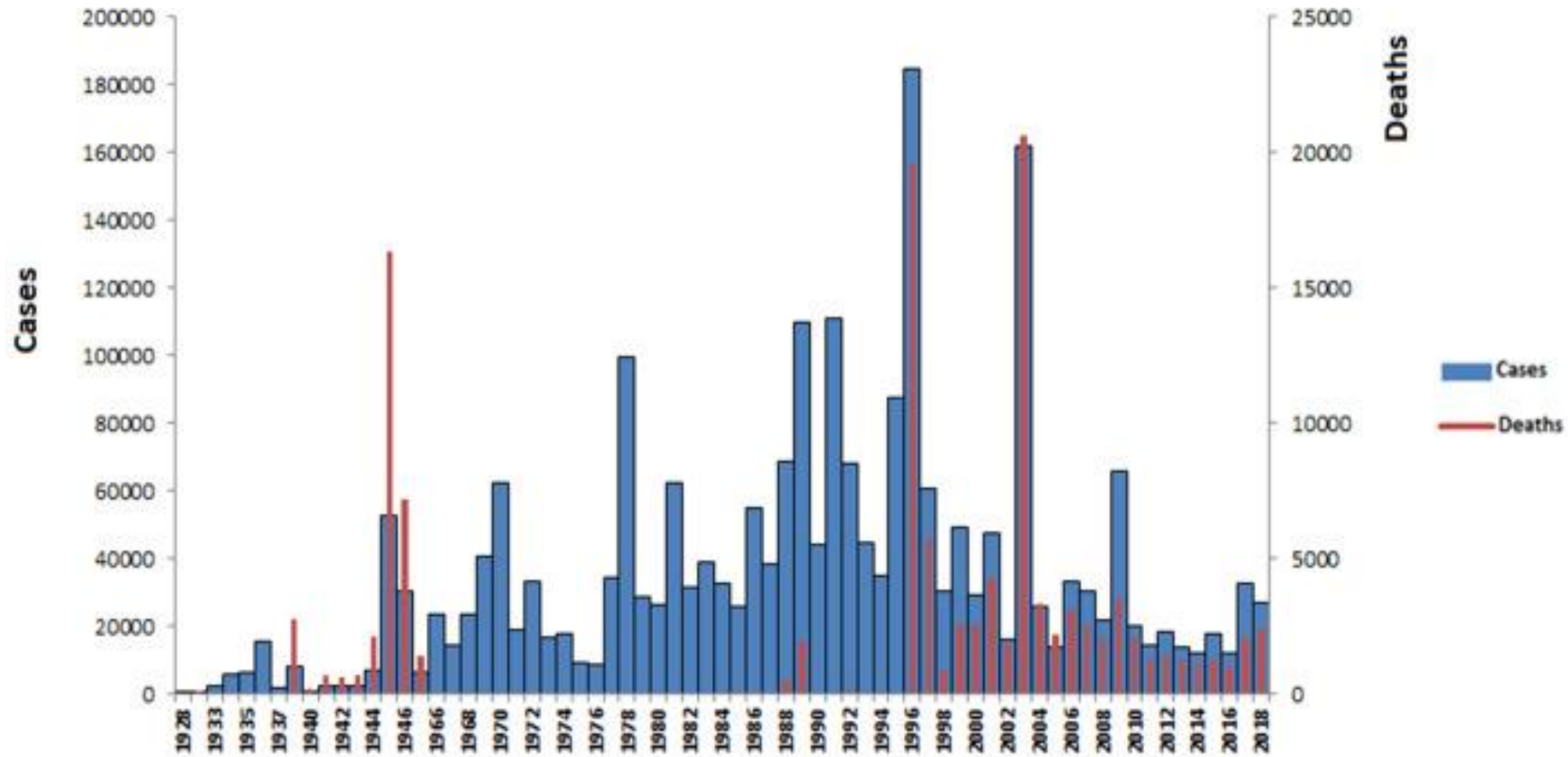
# Meningococcal disease in Africa



**Mazamay, S., Guegan, J. F., Diallo, N., Bompangue, D., Bokabo, E., Muyembe, J. J., Taty, N., Vita, T. P. & Broutin, H. (2021). An overview of bacterial meningitis epidemics in Africa from 1928 to 2018 with a focus on epidemics "outside-the-belt". *BMC Infect Dis.* **21**, 1027.**



# Meningococcal disease in Africa



**Mazamay, S., Guegan, J. F., Diallo, N., Bompangue, D., Bokabo, E., Muyembe, J. J., Taty, N., Vita, T. P. & Broutin, H. (2021).** An overview of bacterial meningitis epidemics in Africa from 1928 to 2018 with a focus on epidemics "outside-the-belt". *BMC Infect Dis.* **21**, 1027.

# MenAfriVac®: combatting epidemic meningococcal disease in Africa

- Meningitis Vaccine Project (MVP):
  - Gates funded, partnership with WHO and PATH;
  - Innovative development – Northern technology, southern manufacturer;
  - Low cost (less than \$1 per dose, for sustainable use);
- MenAfriVac®, a polysaccharide-protein conjugate vaccine, introduced 2010 in Burkina Faso;
  - Everyone aged 1-29 years immunised;
  - Immediate reduction in disease levels.



Diomande, F. V. K., Djingarey, M. H., Daugla, D. M., Novak, R. T., Kristiansen, P. A., Collard, J. M., Gamougam, K., Kandolo, D., Mbakuliyemo, N., Mayer, L., Stuart, J., Clark, T., Tevi-Benissan, C., Perea, W. A., Preziosi, M. P., LaForce, F. M., Caugant, D., Messonier, N., Walker, O. & Greenwood, B. (2015). Public Health Impact After the Introduction of PsA-TT: The First 4 Years. *Clinical Infectious Diseases* 61, S467-S472.



Global

Regions ▾



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Health Topics ▾

Countries ▾

Newsroom ▾

Emergencies ▾

Data ▾

About WHO ▾

Home / Initiatives / Defeating Meningitis by 2030



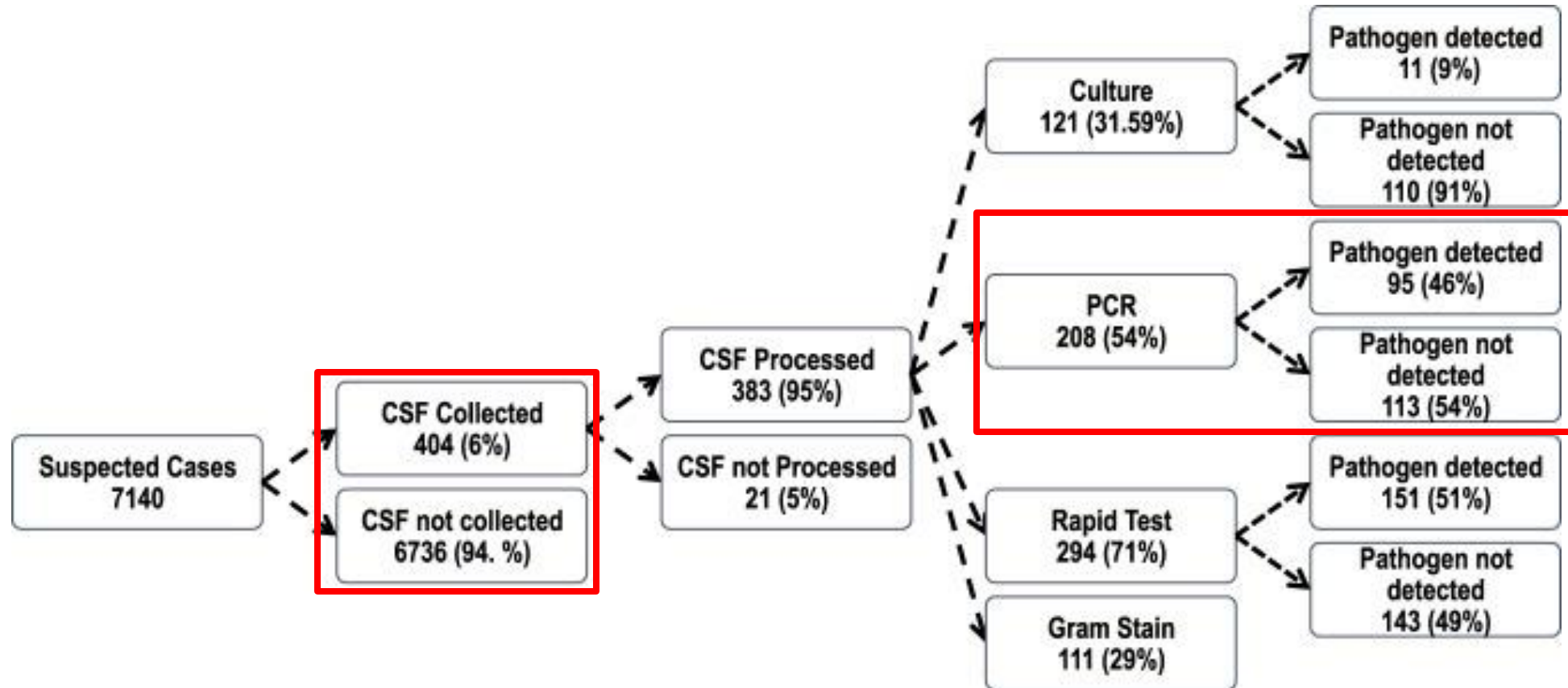
<https://www.who.int/initiatives/defeating-meningitis-by-2030>

# Global road map priorities for response, care and prevention

- Achievement of high immunization coverage, development of new affordable vaccines, and improved prevention strategies and outbreak response;
  - Speedy diagnosis and optimal treatment for patients;
  - Good data to guide prevention and control efforts;
- 
- Care and support for those affected, focusing on early recognition and improved access to care and support for after-effects; and
  - Advocacy and engagement, to ensure high awareness of meningitis, accountability for national plans, and affirmation of the right to prevention, care and after-care services.

<https://www.who.int/news-room/events/detail/2024/04/26/default-calendar/the-first-high-level-meeting-to-defeat-meningitis---institut-pasteur-paris--france>

# Increasing importance of molecular diagnosis and isolate characterisation in Africa



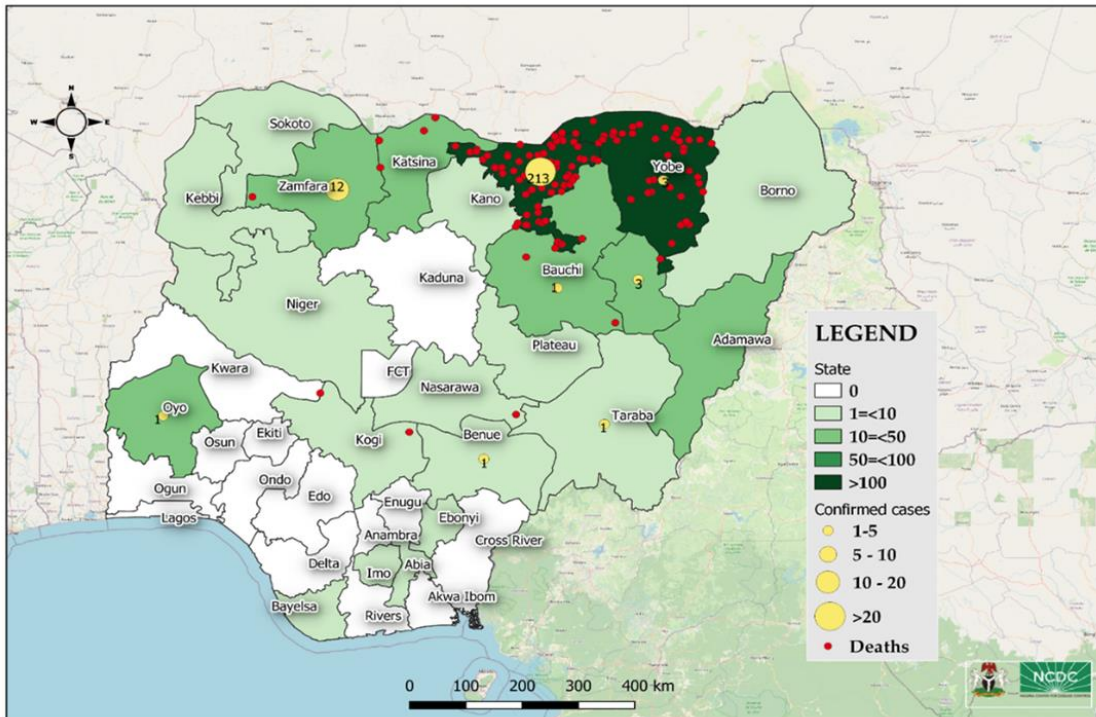
Kwambana-Adams, B. A., Amaza, R. C., Okoi, C., Rabi, M., Worwui, A., Foster-Nyarko, E., Ebruke, B., Sesay, A. K., Senghore, M., Umar, A. S., Usman, R., Atiku, A., Abdullahi, G., Buhari, Y., Sani, R., Bako, H. U., Abdullahi, B., Yarima, A. I., Sikiru, B., Moses, A. O., Popoola, M. O., Ekeng, E., Olayinka, A., Mba, N., Kankia, A., Mamadu, I. N., Okudo, I., Stephen, M., Ronveaux, O., Busuttil, J., Mwenda, J. M., Abdulaziz, M., Gummi, S. A., Adedeji, A., Bitu, A., Omar, L., Djingarey, M. H., Alemu, W., D'Alessandro, U., Ihekweazu, C. & Antonio, M. (2018). Meningococcus serogroup C clonal complex ST-10217 outbreak in Zamfara State, Northern Nigeria. *Scientific reports*. 8, 14194.



# Meningitis in Nigeria 2022-2024

**1686 suspected cases 1 October 2022 - 16 April 2023**


<https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON454>



**5 in 1 meningitis vaccine introduced**

<https://www.bbc.co.uk/programmes/w3ct5t8d>

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## Nigeria rolls out world's first 5-in-1 meningitis vaccine

World first 5-in-1 meningitis vaccine rolled out in Nigeria; How Brazil is coping with long Covid; Using patches of grafted skin to detect donated organ rejection

Available now  
⌚ 26 minutes

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Sun 21 Apr 2024  
02:32  
Local time  
BBC WORLD SERVICE

**More episodes**

**PREVIOUS**  
How we hope

**NEXT**  
Is turbulence injuring more and more flyers?

See all episodes from Health Check

Ashinze, P., Mafua, N. & Obafemi, E.(2024). Nigeria rolls out novel meningitis vaccine. *Lancet*. **403**, 2373.



Exercise, searching an NCBI database with *Neisseria*  
16S rRNA sequences

# 16s rRNA BLAST search online (2)

- Navigate to NCBI BLAST home page:
  - <https://blast.ncbi.nlm.nih.gov/Blast.cgi>


- Click on:



- Under 'Enter Query sequence' paste in a test sequence (Test 4-6)
  - [https://github.com/WCSCourses/Molecular\\_Approaches\\_Clinical\\_Microbiology\\_2024/blob/main/course\\_data/bioinformatics/16S/16S\\_samples.fas](https://github.com/WCSCourses/Molecular_Approaches_Clinical_Microbiology_2024/blob/main/course_data/bioinformatics/16S/16S_samples.fas)
  - <https://tinyurl.com/228hpuev>
- Under 'Choose Search Set' click on 'rRNA/ITS databases'
- Check box 'Show results in a new window'. Click on

**BLAST**



 **Important update**  
The core nucleotide database (**core\_nt**) is now the default nucleotide BLAST database. [Learn more about core\\_nt.](#)


Standard Nucleotide BLAST

blastn   blastp   blastx   tblastn   tblastx

BLASTN programs search nucleotide databases using a nucleotide query, more...

[Reset page](#) [Bookmark](#)

Enter Query Sequence


Enter accession number(s), gi(s), or FASTA sequence(s) 

```
>Test_1
AATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACA
CATGCAAG
TCGAACGGTAACAGGAAGAAGCTTGCTCTTTGCTGACGAGTGGCGGACGG
```

Query subrange 


From

To

Or, upload file  No file selected. 


Job Title

Enter a descriptive title for your BLAST search 

☐ Align two or more sequences 


Choose Search Set

Database ☐ Standard databases (nr etc.): ☒ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus ☐ Experimental databases

16S ribosomal RNA sequences (Bacteria and Archaea) 

[Targeted Loci Project Information](#)


Organism Optional  ☐ exclude [Add organism](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown 

Exclude Optional ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to Optional ☐ Sequences from type material

Entrez Query Optional  [YouTube](#) [Create custom database](#)

Enter an Entrez query to limit search 

Program Selection

Optimize for ☒ Highly similar sequences (megablast)

☐ More dissimilar sequences (discontinuous megablast)

☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm 

**BLAST** Search database 16S ribosomal RNA sequences (Bacteria and Archaea) using Megablast (Optimize for highly similar sequences)

☒ Show results in a new window

Note: Parameter values that differ from the default are highlighted in yellow and marked with  sign

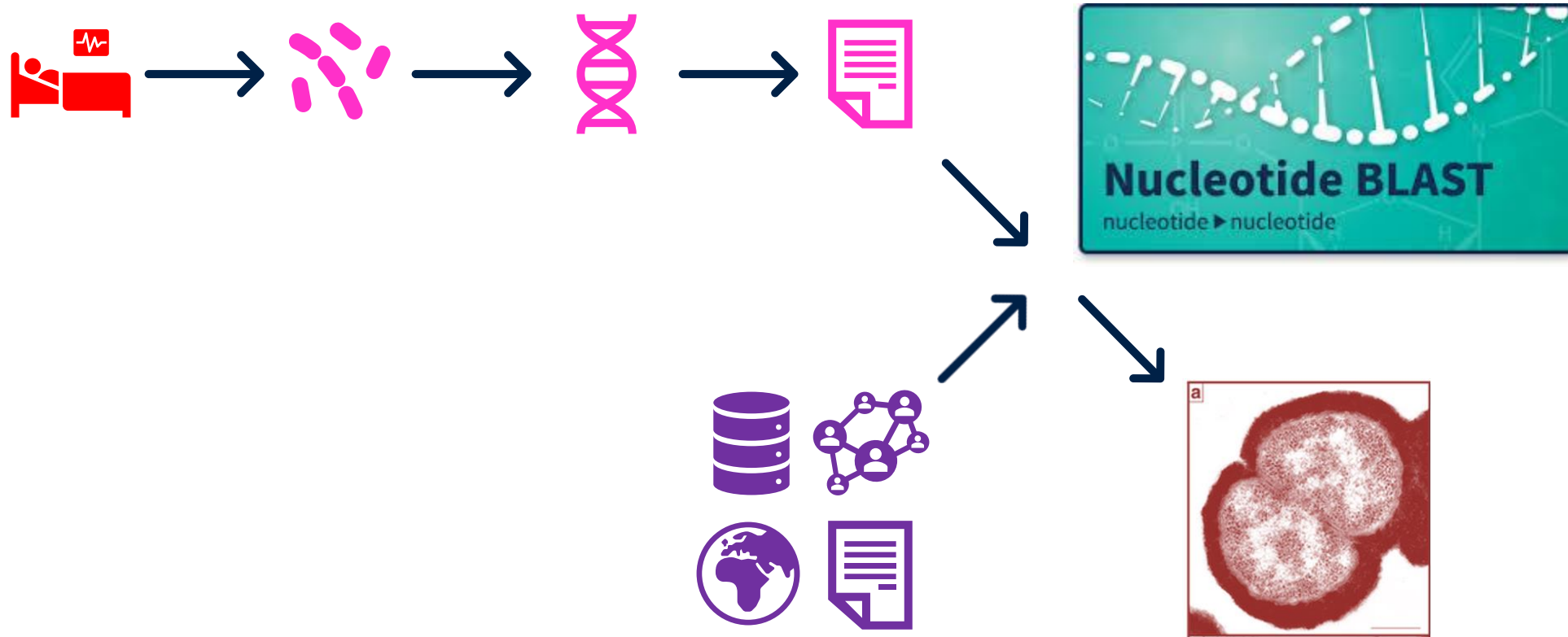
+ Algorithm parameters

# What bacterium is it?

- Look at the results of the search (scroll down the page as necessary).
- What is the likely organism that the 16S rRNA sequence came from?

Sample	Candidate Bacterium
Test 4	<i>Neisseria mucosa</i>
Test 5	<i>Neisseria gonorrhoeae</i>
Test 6	<i>Neisseria meningitidis</i>

# 16S rRNA summary





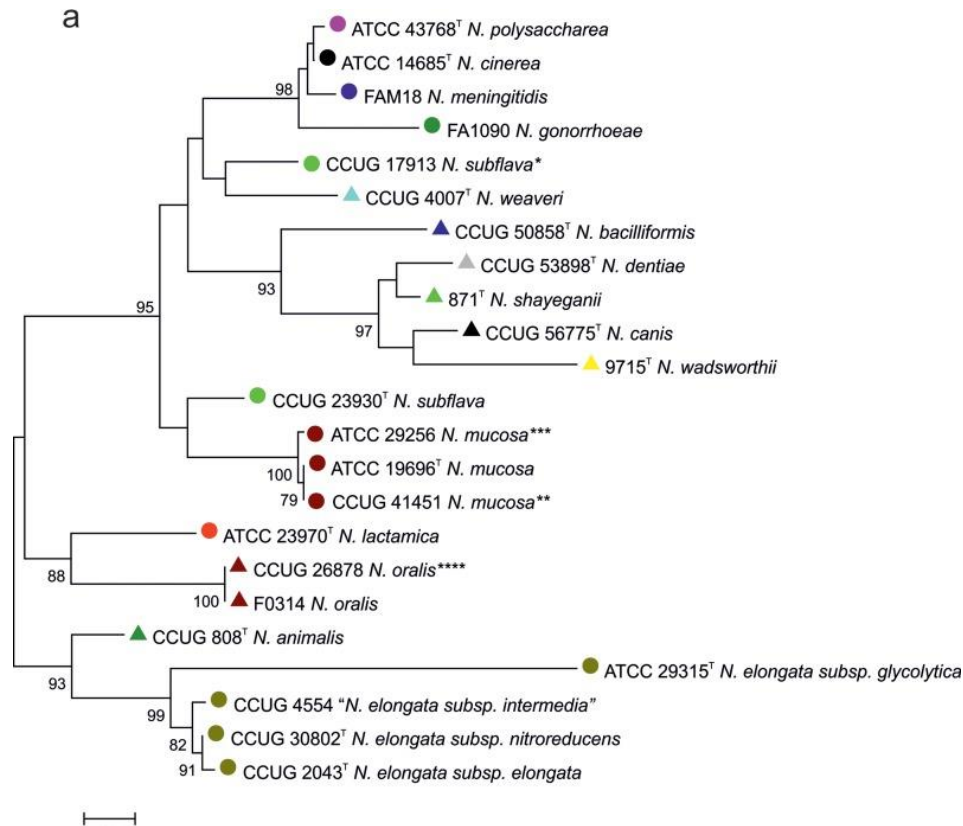
# Case study: *rplF* assay

- **Problem:** rapid cost effective determination of *Neisseria* species from 1000s of isolates obtained in the MenAfriCar surveys.
  - 16s rRNA sequencing too cumbersome and insufficient discrimination or resolution.
- **Solution:** identify a short sequenceable gene fragment (~400bp in length) diagnostic for species.
- **Implementation:** Phylogeny of *Neisseria* species generated and compared to phylogenies of individual genes
  - A fragment of *rplF* gene was congruent with clusters in the phylogeny of all genes and used for the assay.

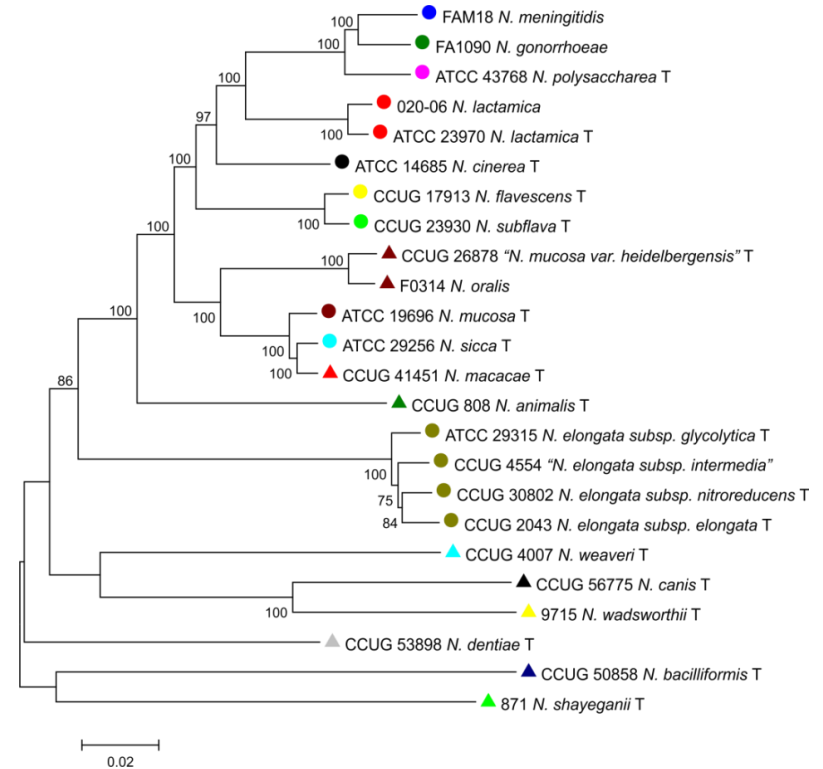
**Bennett, J. S., Watkins, E. R., Jolley, K. A., Harrison, O. B. & Maiden, M. C. (2014).** Identifying *Neisseria* species using the 50S ribosomal protein L6 (*rplF*) gene. *J Clin Microbiol* **52**, 1375-1381.

# Comparison of 16s rRNA sequencing and *rplF* sequencing

## 16s rRNA gene fragment phylogeny (single rRNA encoding gene)



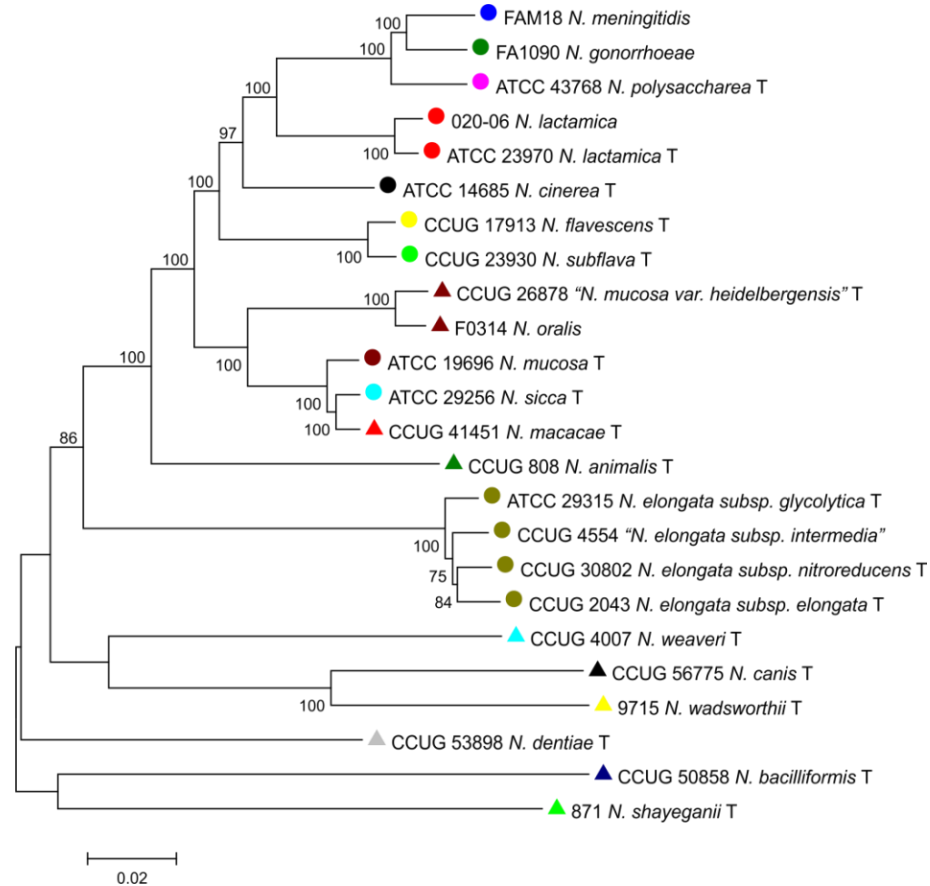
## rMLST phylogeny (53 ribosomal protein encoding genes)



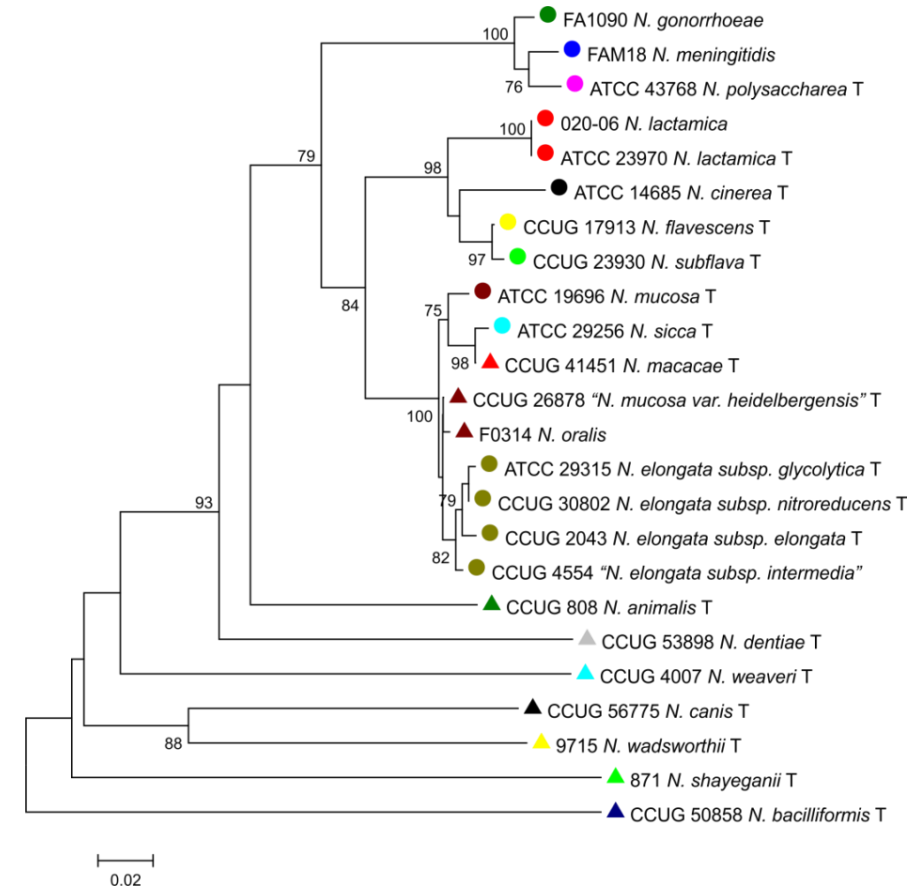
Bennett, J. S., Watkins, E. R., Jolley, K. A., Harrison, O. B. & Maiden, M. C. (2014). Identifying *Neisseria* species using the 50S ribosomal protein L6 (*rplF*) gene. *J Clin Microbiol* 52, 1375-1381.

# Rapid species assignment: *rplF* sequence

rMLST phylogeny (53 genes)

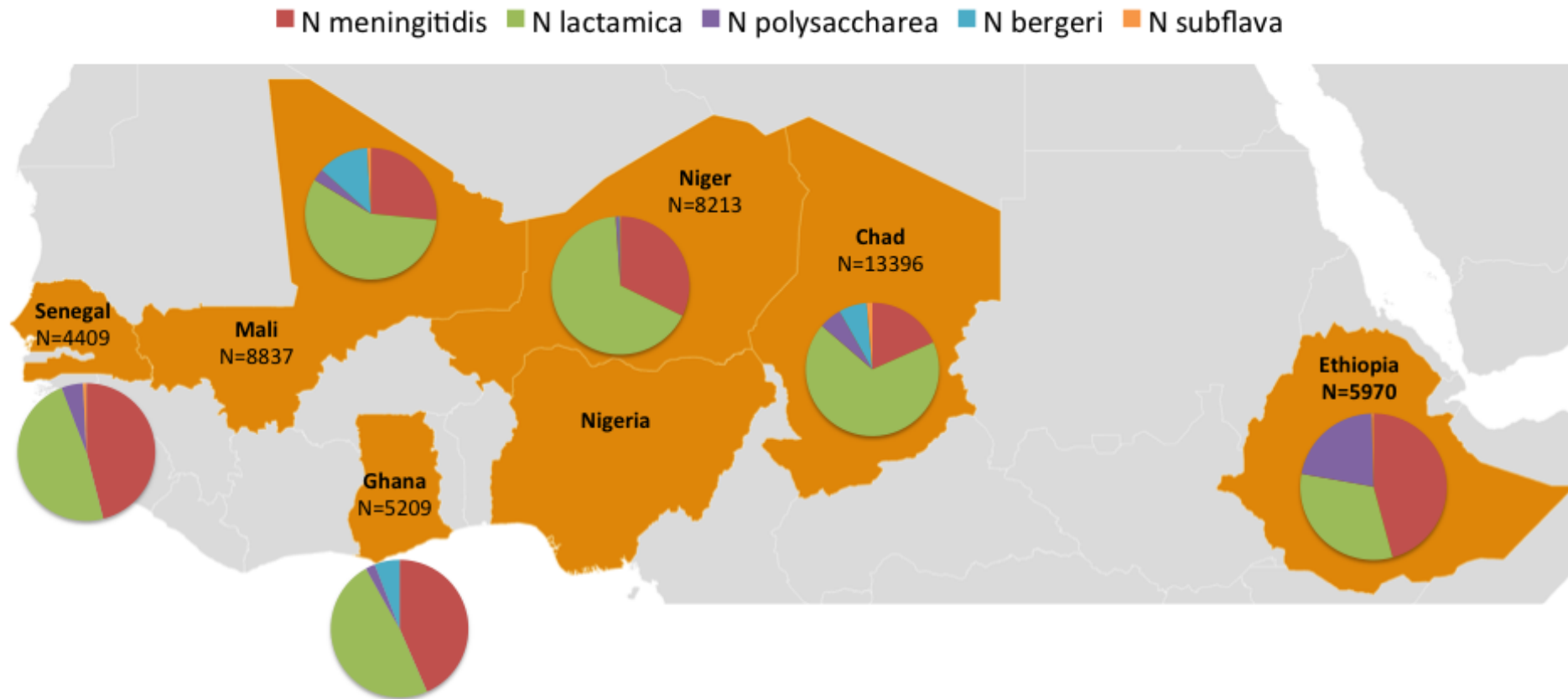


*rplF* fragment (413bp) phylogeny



Bennett, J. S., Watkins, E. R., Jolley, K. A., Harrison, O. B. & Maiden, M. C. (2014). Identifying *Neisseria* species using the 50S ribosomal protein L6 (*rplF*) gene. *J Clin Microbiol* 52, 1375-1381.

# *Neisseria* species distribution



Diallo, K., *et al.*, Greenwood, B. M. & Maiden, M. C. (2016). Pharyngeal carriage of *Neisseria* species in the African meningitis belt. *J Infect* **72**, 667-677.

