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Genomic surveillance of antimicrobial resistance in *Shigella sonnei* in Belgium 2013-2019

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EPIET & EUPHEM

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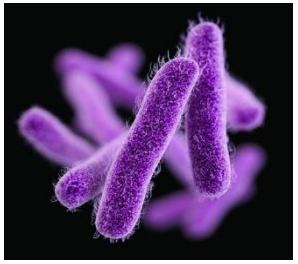
PAHO Webinar on Molecular Epidemiology, 28th September 2021

Objectives of this webinar

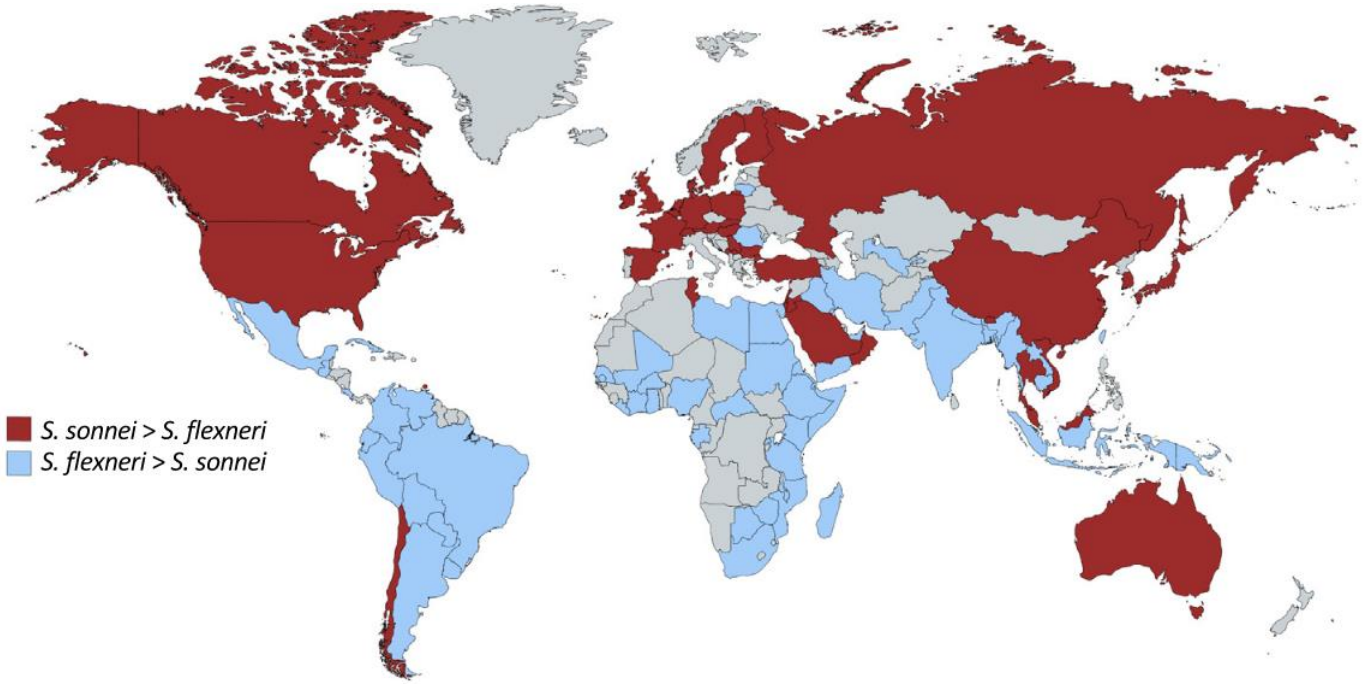
- Give an example of the use of WGS for national surveillance of bacterial pathogens
- Illustrate bioinformatic tools for analysis of WGS data:
  - Detection of AMR markers
  - Assignment of hierarchical genotypes
  - Visualization and annotation of phylogenetic trees
- Discuss advantages and limitations of WGS for surveillance

Shigella species and epidemiology

- *Shigella* are Gram-negative pathogenic enterobacteria
- Four species: *S. flexneri*, *S. sonnei*, *S. dysenteriae*, and *S. boydii*



Source: CDC, USA



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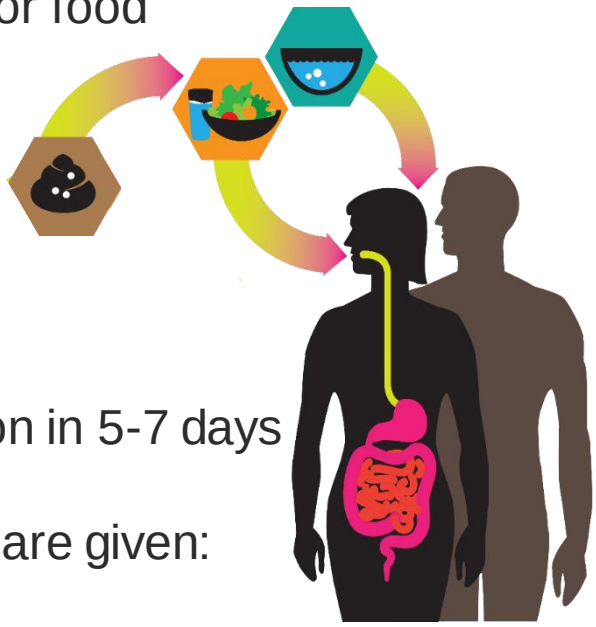
V. Torraca, K. Holt, S.Mostowy, 2020

Shigella transmission and disease

Fecal-Oral transmission through contaminated water or food



www.waterpathogens.org

→ Causing severe dysentery (diarrhea, fever, and stomach cramps)

- Most cases resolve without medication in 5-7 days
- For severe cases first-line antibiotics are given:
  - Ciprofloxacin (Cip, adults)
  - Azithromycin (Azm, children)

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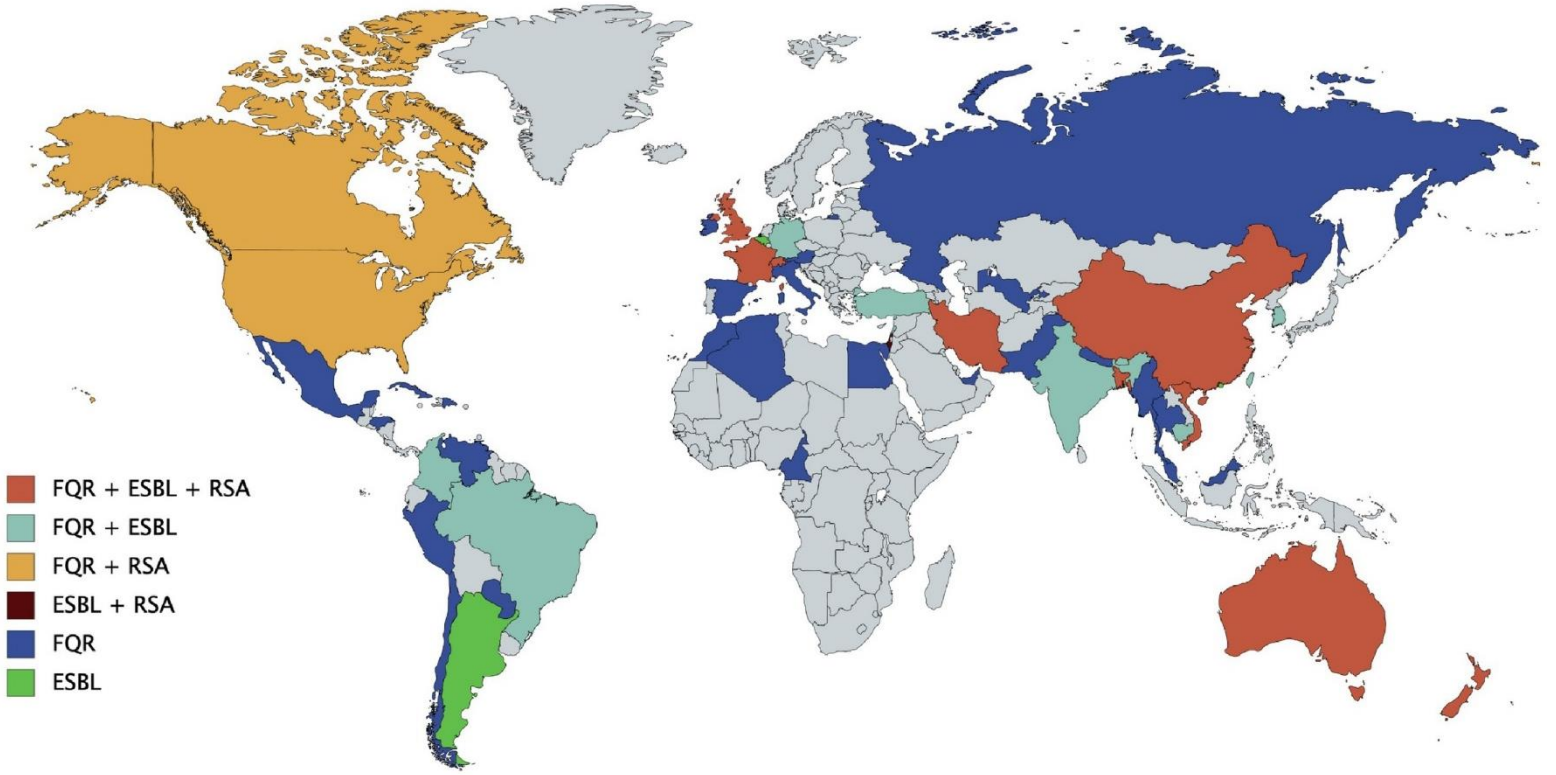
Global emergence of resistant *S. sonnei* strains



Trends in Microbiology

ESBL = extended-spectrum β-lactamase-producing (i.e., resistant to third-generation cephalosporins or carbapenem)
FQR = fluoroquinolone resistance (incl. Cip)
RSA = reduced sensitivity to azithromycin

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V. Torraca, K. Holt, S.Mostowy, 2020

AMR in *Shigella* is associated with men who have sex with men (MSM)

- England (Baker *et al.,* 2015, Mitchell *et al.,* 2019, Bardsley *et al.,* 2020)
- Taiwan (Chiou *et al.,* 2016)
- Canada (Gaudreau *et al.,* 2011, Yousfi *et al.,* 2019)
- Switzerland (Hinic *et al.,* 2018)
- Australia (Ingle *et al.,* 2019 & 2020)

CDC info sheet on Shigellosis among MSM

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Surveillance of *Shigellosis* in Belgium

~400 *Shigellosis* cases per year in Belgium 2013–2018 (Ventola *et al.*, 2019)

- 72 % caused by *Shigella sonnei (S. sonnei)*

Clinical laboratories

submit isolates and patient information (voluntary)

National Reference Centre for *Salmonella* and *Shigella* at Sciensano

- Typing of isolated strains to identify species and subtypes
- Determination of antibiotic resistance by broth microdilution

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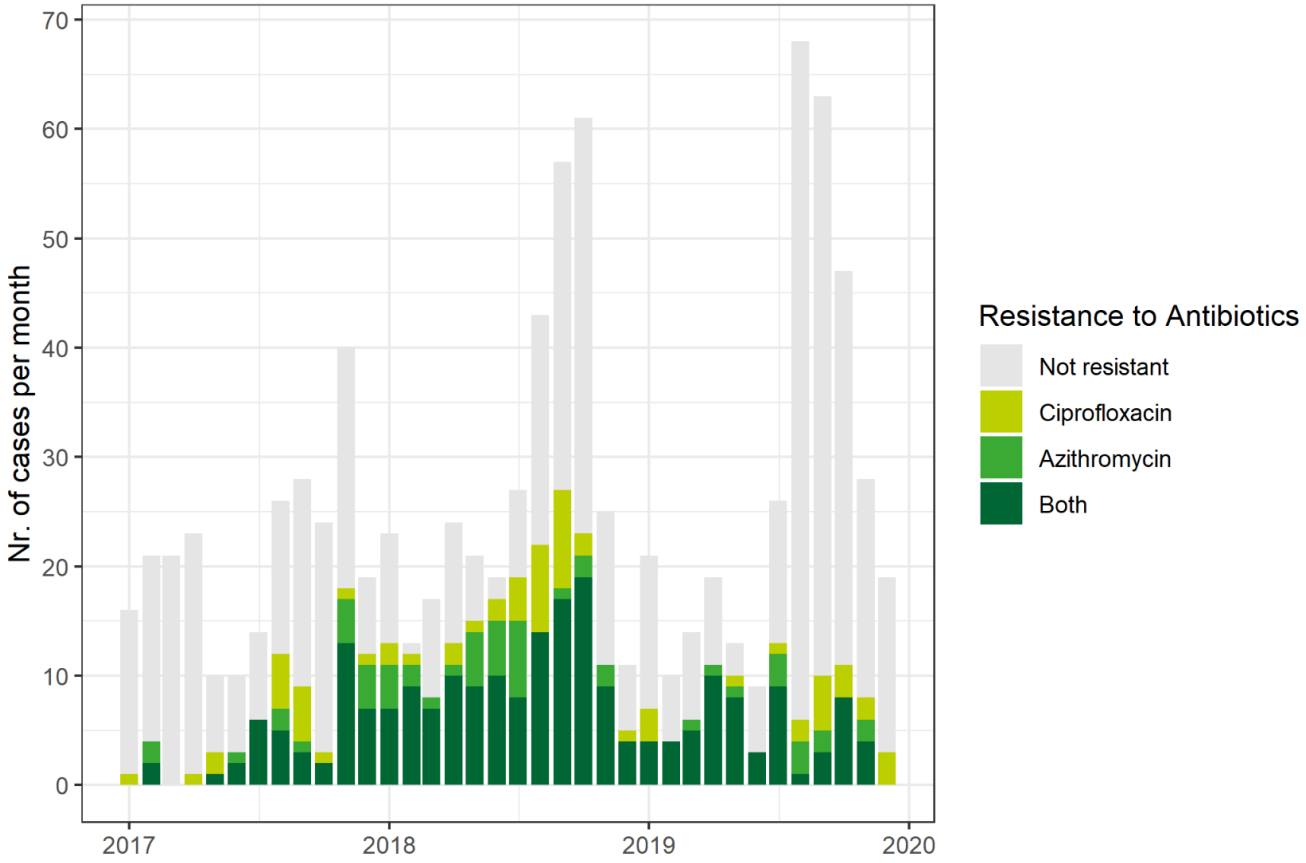
Epidemiology of *Shigellosis* in Belgium



37% of Belgian isolates were resistant to first-line antibiotics

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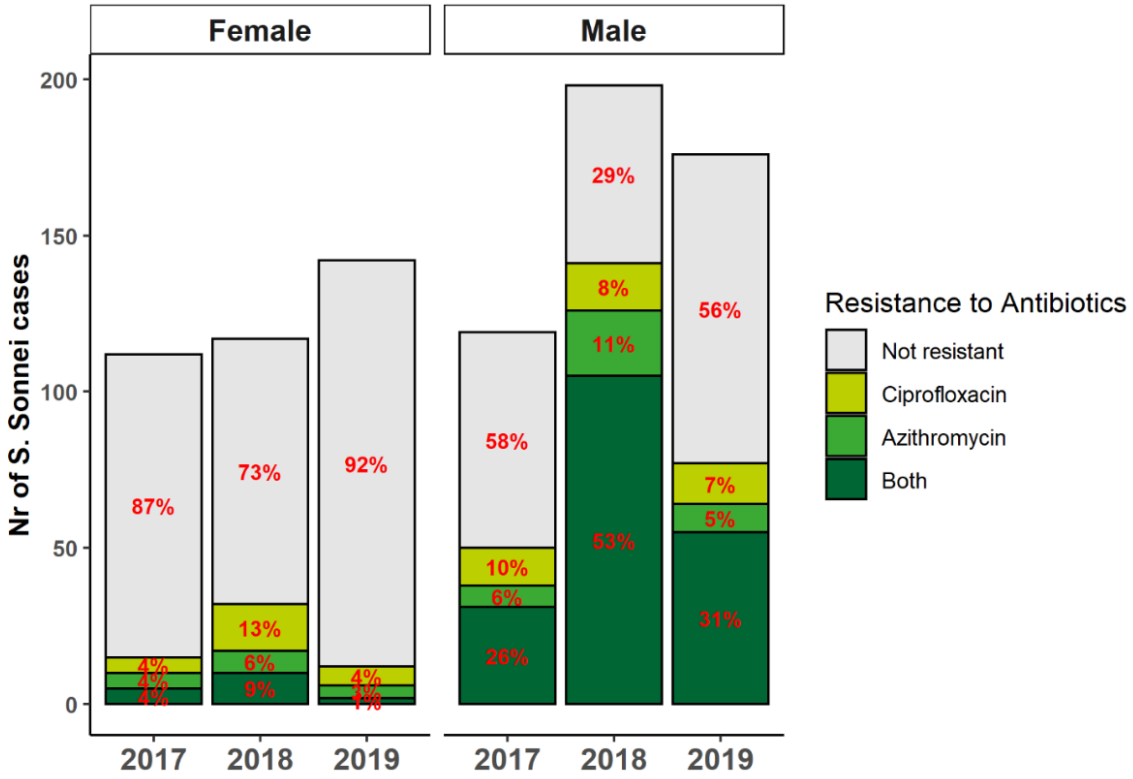
Epidemiology of *Shigellosis* in Belgium



Men have an increased risk to carry a strain with resistance as compared to women

Fischer *et al., in revision, JAC*

Why incorporate WGS in *Shigella* surveillance in Belgium?

- Difficulty to differentiate and track strains for public health surveillance or outbreak investigations using classical typing methods due to the limited genomic diversity of *S. sonnei*

- Detection of AMR in Belgium is currently limited to laboratory diagnostics
  - Testing for reduced sensitivity to Azithromycin (RSA) was only implemented in Belgium in 2017

- Difficulty to know the origins or identify import events of a strain
  - Travel history is only available for 10 % of cases in Belgium

Objective

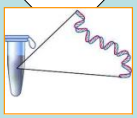
To describe clusters of multi-drug resistant *S. sonnei* in Belgium, through the use of whole genome sequencing (WGS), and to identify associated risk groups.

Methods

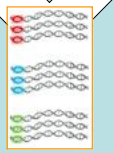
Sample selection:

- 372 Belgian *S. sonnei* isolates from 2013-2019
- 192 *S. sonnei* isolates from public databases (Latin-America, Asia, Europe, MSM-association)

Sample processing:



Shigella isolates were cultured overnight in BHI broth (BD) at 37°C
DNA was extracted using MgC Bacterial DNA Kit™



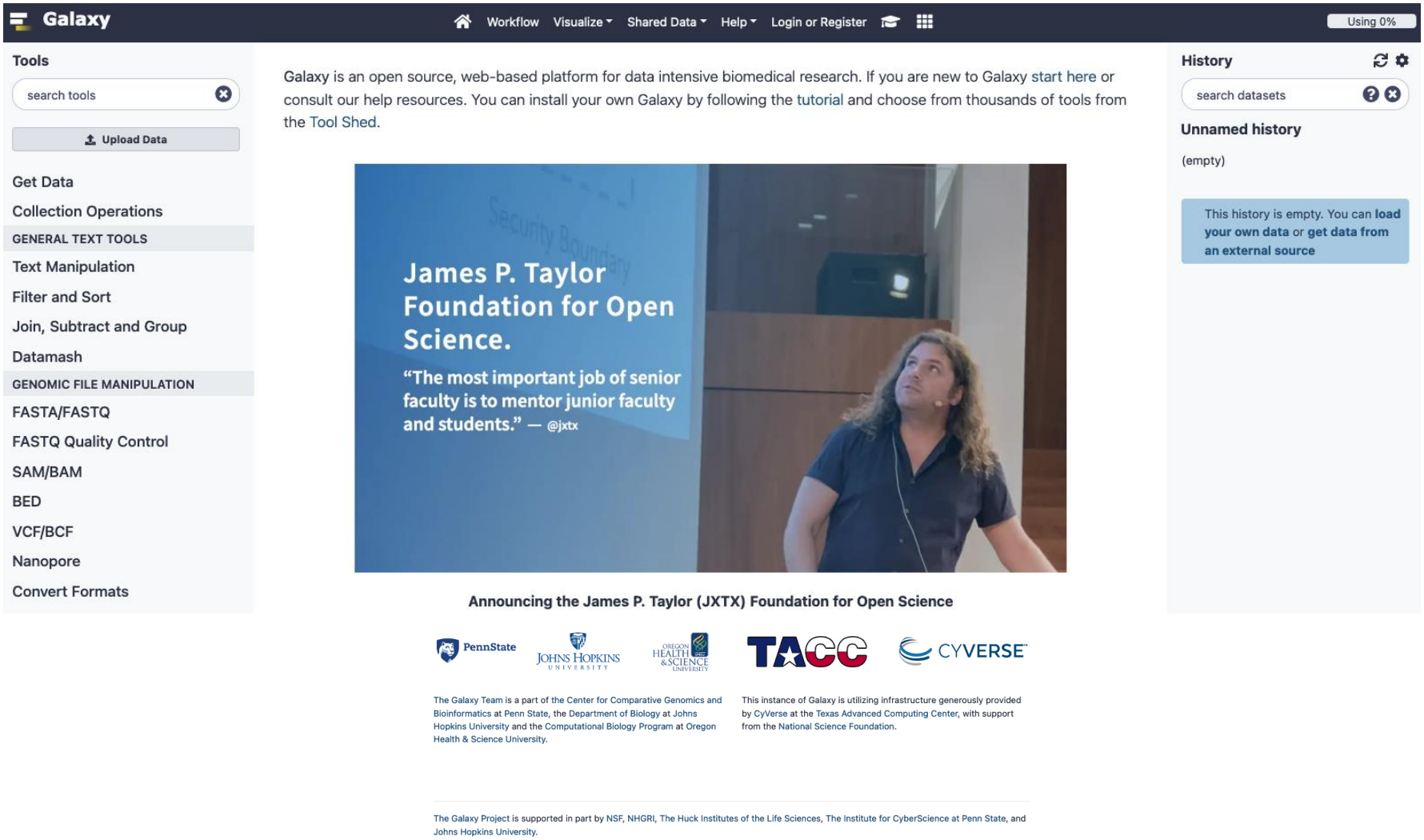
Library prep was performed with Nextera XT DNA sample preparation kit

Sequencing of 250-bp paired-end reads on Illumina MiSeq

Using Qiagen CLC Genomic Workbench 20.0.2:
Reads were mapped to *S. sonnei* Ss046 reference genome
Single-nucleotide variants were identified
Neighbor Joining trees were constructed

Methods

Bioinformatics Platform: https://usegalaxy.org/

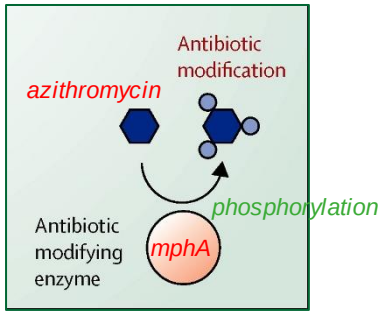


Methods: Bioinformatic detection of AMR markers

ResFinder: presence of AMR genes (Bortolaia *et al.* 2020)

→ macrolide resistance genes (*mphA*, *ermB*)

→ resistance to azithromycin

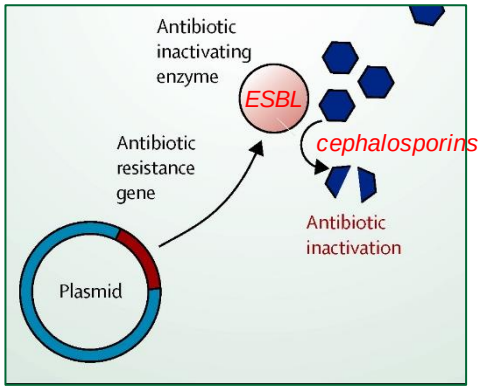


PlasmidFinder: detection of plasmid replicons (Carattoli *et al.* 2014)

→ Inc type plasmids

→ carry extended-spectrum-beta lactamases (ESBL genes)

→ resistance to third generation cephalosporins

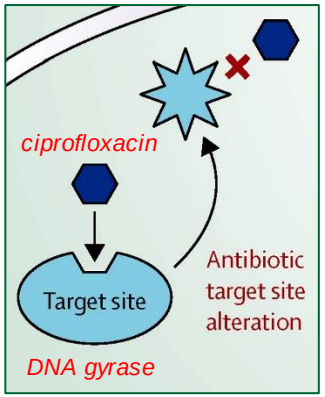


PointFinder: detection of chromosomal mutations (Zankari *et al.* 2017)

→ quinolone resistance determining region (QRDR) of bacterial DNA gyrase

→ *gyrA* (S83L), *gyrA* (D87G), *gyrA* (D87Y) and *parC* (S80I)

→ resistance to ciprofloxacin



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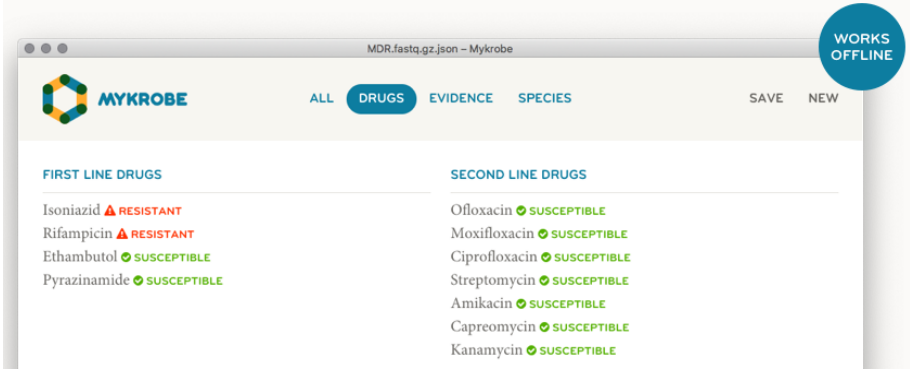
Adapted from Sherrard *et al.* 2014

Methods: Assignment of hierarchical genotypes



Hunt M, Bradley P, Lapierre SG et al. Antibiotic resistance prediction for Mycobacterium tuberculosis from genome sequence data with Mykrobe. Wellcome Open Res 2019

→ **https://www.mykrobe.com/**

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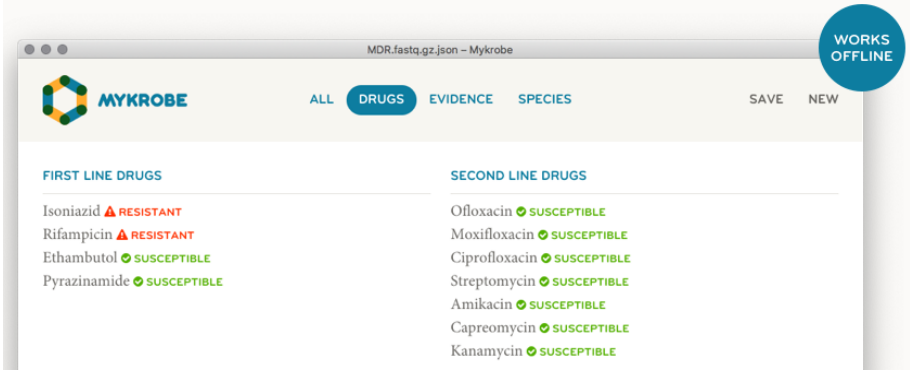
Methods: Assignment of hierarchical genotypes



Hunt M, Bradley P, Lapierre SG et al. Antibiotic resistance prediction for Mycobacterium tuberculosis from genome sequence data with Mykrobe. Wellcome Open Res 2019

→ **https://www.mykrobe.com/**

Integrated hierarchical SNV-based genotyping scheme for *S. sonnei* (Hawkey *et al.* 2021)

→ **https://github.com/katholt/sonneityping**

New genotyping framework		Previously defined as		Description
Genotype	Name	Name	Study	
Lineage II				
2.8	Korea II	Korea II	Holt 2012	Associated with Korea
2.9, 2.10, 2.11	Latin America II	South America II	Holt 2012	Associated with Latin America
		LA sublineage IIa & IIb	Baker 2017	
Lineage III				
3.4	Latin America III	South America III	Holt 2012	Associated with Latin America
		LA sublineage IIIa & IIIb	Baker 2017	
3.6	Central Asia III	Central Asia IIIa	Holt 2012	Associated with Central Asia
3.6.1	CipR parent	-	This study	Subclade from which ciprofloxacin-resistant sublineage emerged
3.6.1.1	CipR	Ciprofloxacin-resistant Pop2	The 2015 The 2019	Ciprofloxacin-resistant triple mutation sublineage

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Methods: Visualization and annotation of the phylogenetic tree

ggtree: visualization and annotation of phylogenetic trees

release version 1.14.6 | devel version 1.15.5 | download 42242/total | download 1656/month

The `ggtree` package extending the *ggplot2* package. It based on grammar of graphics and takes all the good parts of *ggplot2*. *ggtree* is designed for not only viewing phylogenetic tree but also displaying annotation data on the tree. *ggtree* is released within the Bioconductor project and the source code is hosted on GitHub.

Authors

Guangchuang Yu, School of Basic Medical Sciences, Southern Medical University.

https://yulab-smu.top/treedata-book/

https://github.com/YuLab-SMU/ggtree

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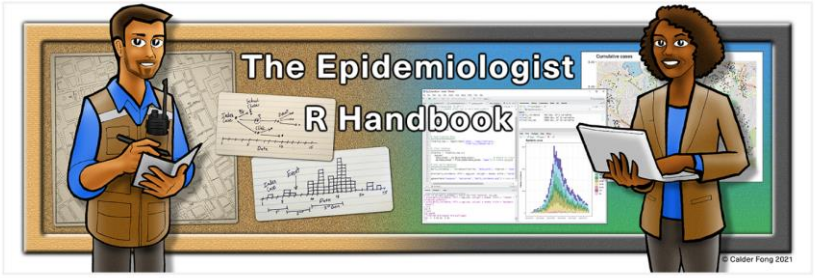
Yu *et al.* 2017

Resources and R Code for phylogenetic analysis



https://epirhandbook.com/

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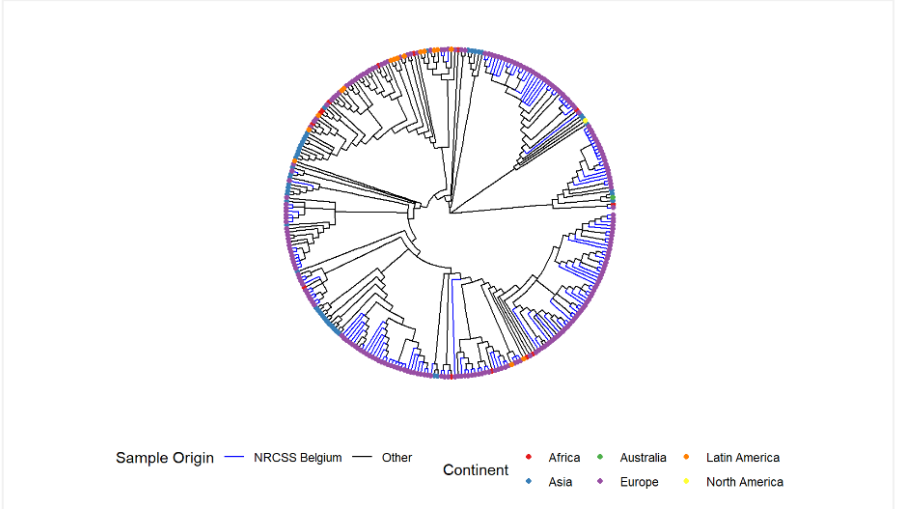
38 Phylogenetic trees

38.1 Overview

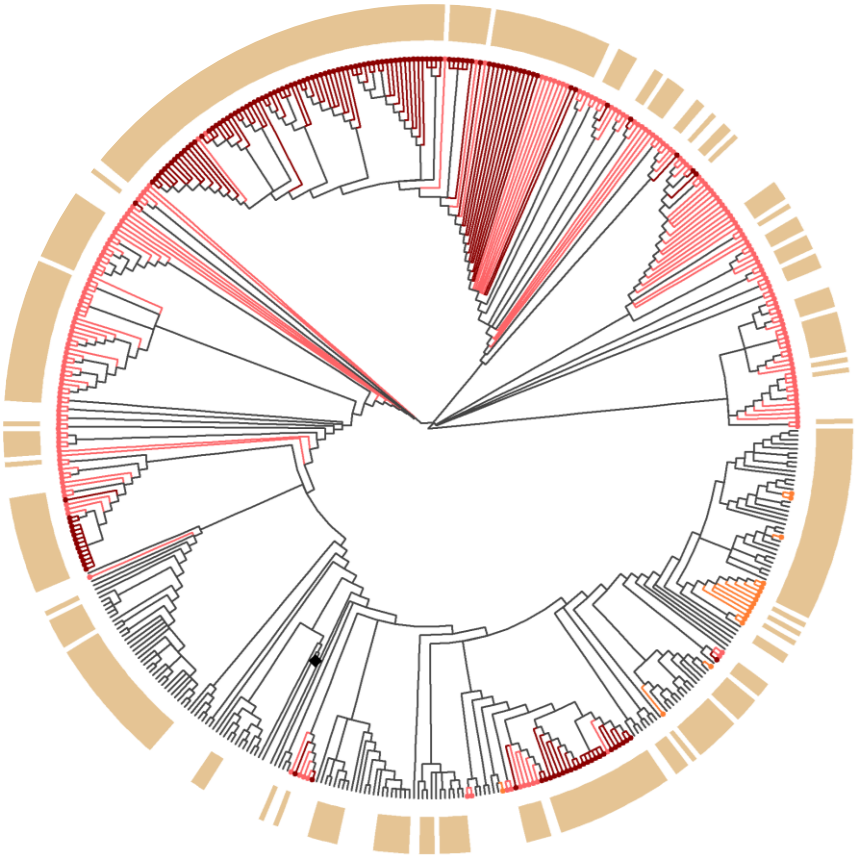
Phylogenetic trees are used to visualize and describe the relatedness and evolution of organisms based on the sequence of their genetic code.

They can be constructed from genetic sequences using distance-based methods (such as neighbor-joining method) or character-based methods (such as maximum likelihood and Bayesian Markov Chain Monte Carlo method). Next-generation sequencing (NGS) has become more affordable and is becoming more widely used in public health to describe pathogens causing infectious diseases. Portable sequencing devices decrease the turn around time and hold promises to make data available for the support of outbreak investigation in real-time. NGS data can be used to identify the origin or source of an outbreak strain and its propagation, as well as determine presence of antimicrobial resistance genes. To visualize the genetic relatedness between samples a phylogenetic tree is constructed.

In this page we will learn how to use the **ggtree** package, which allows for combined visualization of phylogenetic trees with additional sample data in form of a dataframe. This will enable us to observe patterns and improve understanding of the outbreak dynamic.



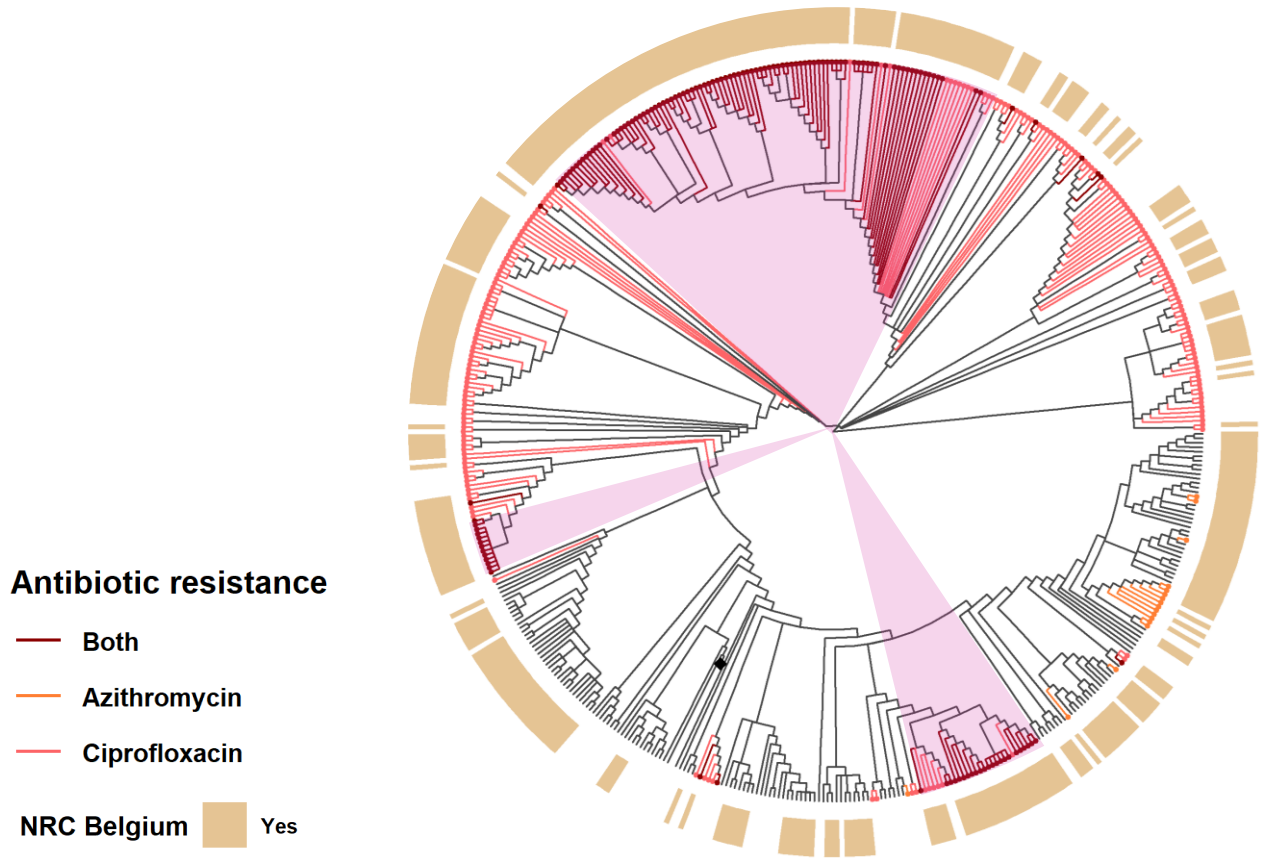
Results: Phylogenetic analysis reveals several distinct clusters with double resistance to first-line antibiotics



Visualized with ggtree(), Yu *et al.* 2017

Fischer *et al., in revision, JAC*

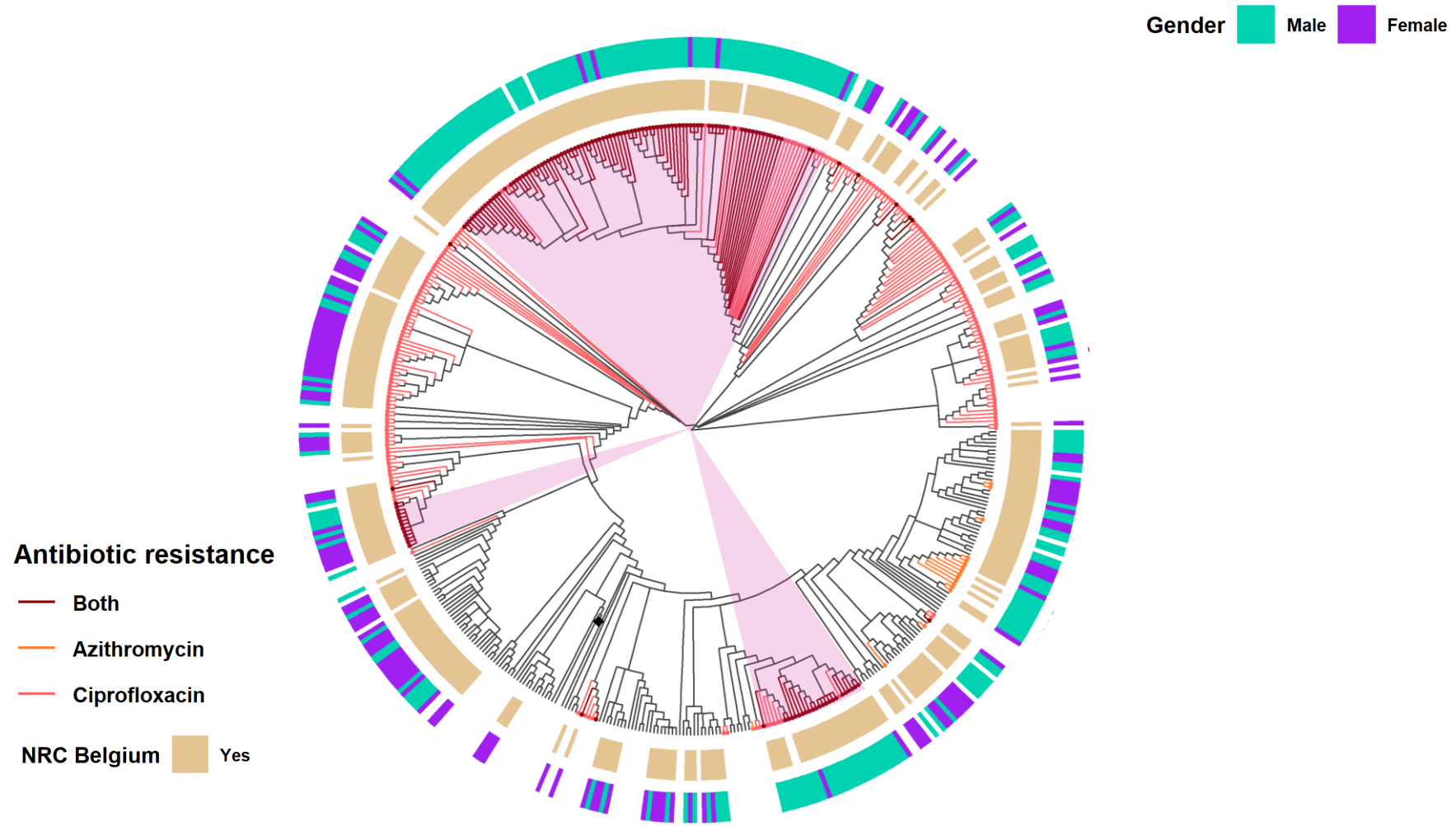
Results: Phylogenetic analysis reveals several distinct clusters with double resistance to first-line antibiotics



Visualized with ggtree(), Yu *et al.* 2017

Fischer *et al., in revision, JAC*

Results: Resistant clusters are predominantly comprised of male cases

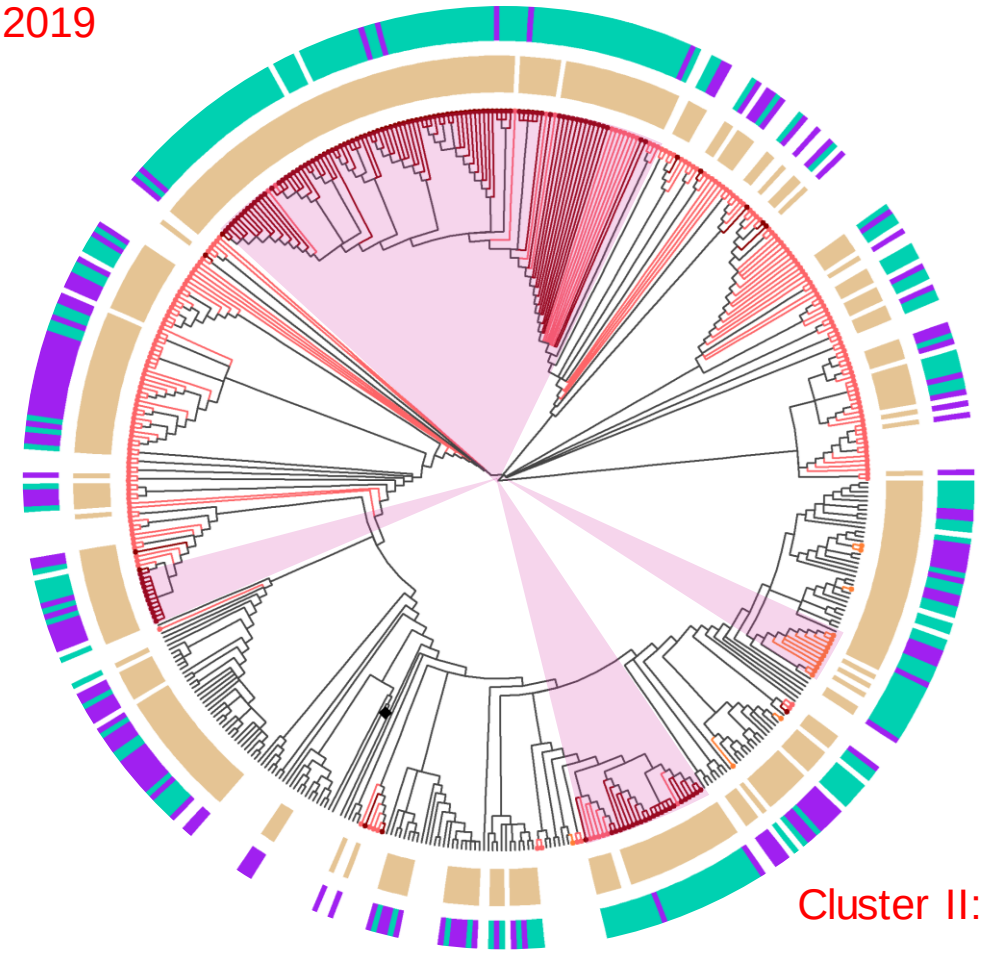


Visualized with ggtree(), Yu *et al.* 2017

Fischer *et al., in revision, JAC*

Results: Resistant clusters are predominantly comprised of male cases

Cluster I: 102 cases, 94% ♂
2015-2019



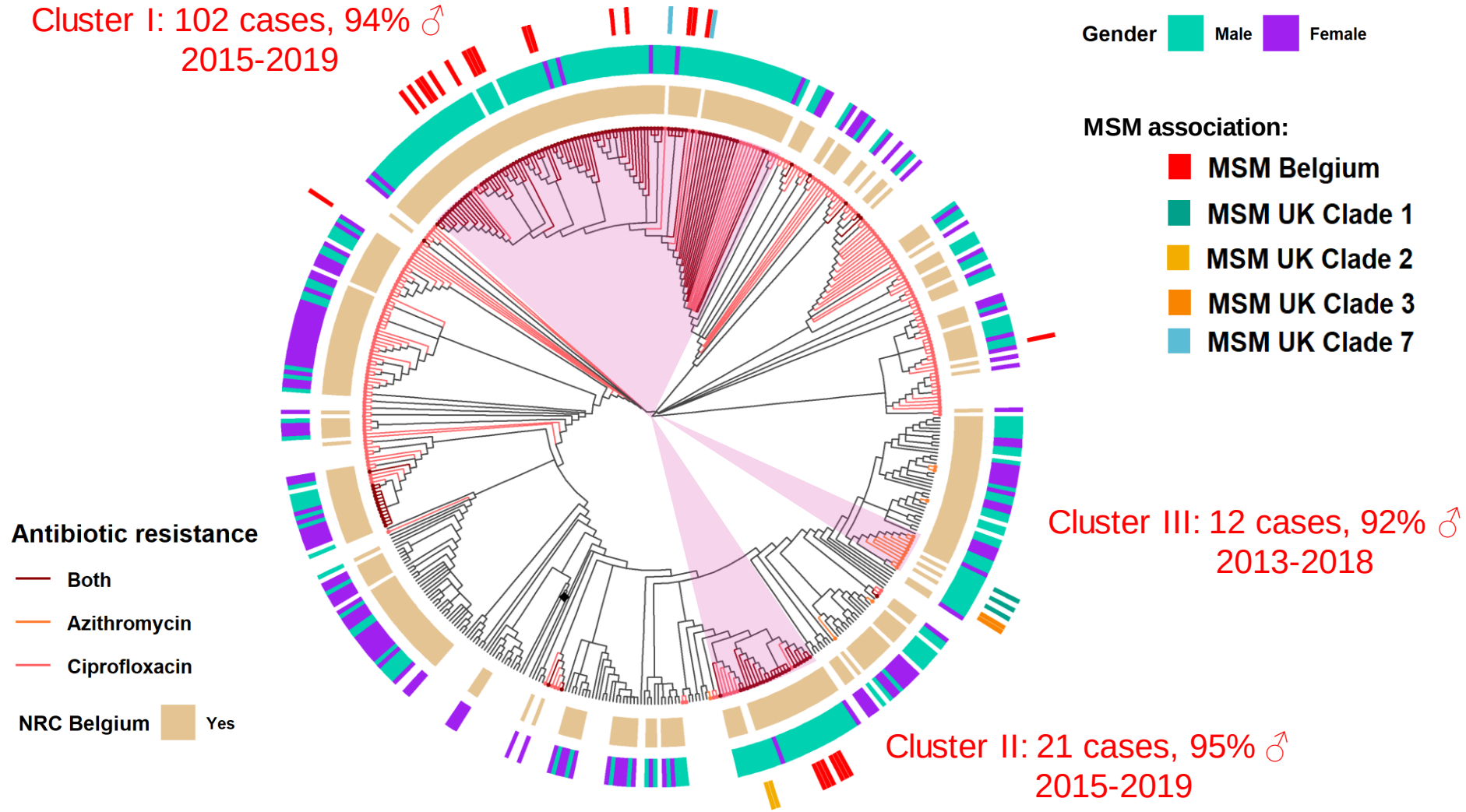
Cluster III: 12 cases, 92% ♂
2013-2018

Cluster II: 21 cases, 95% ♂
2015-2019

Visualized with ggtree(), Yu *et al.* 2017

Fischer *et al., in revision, JAC*

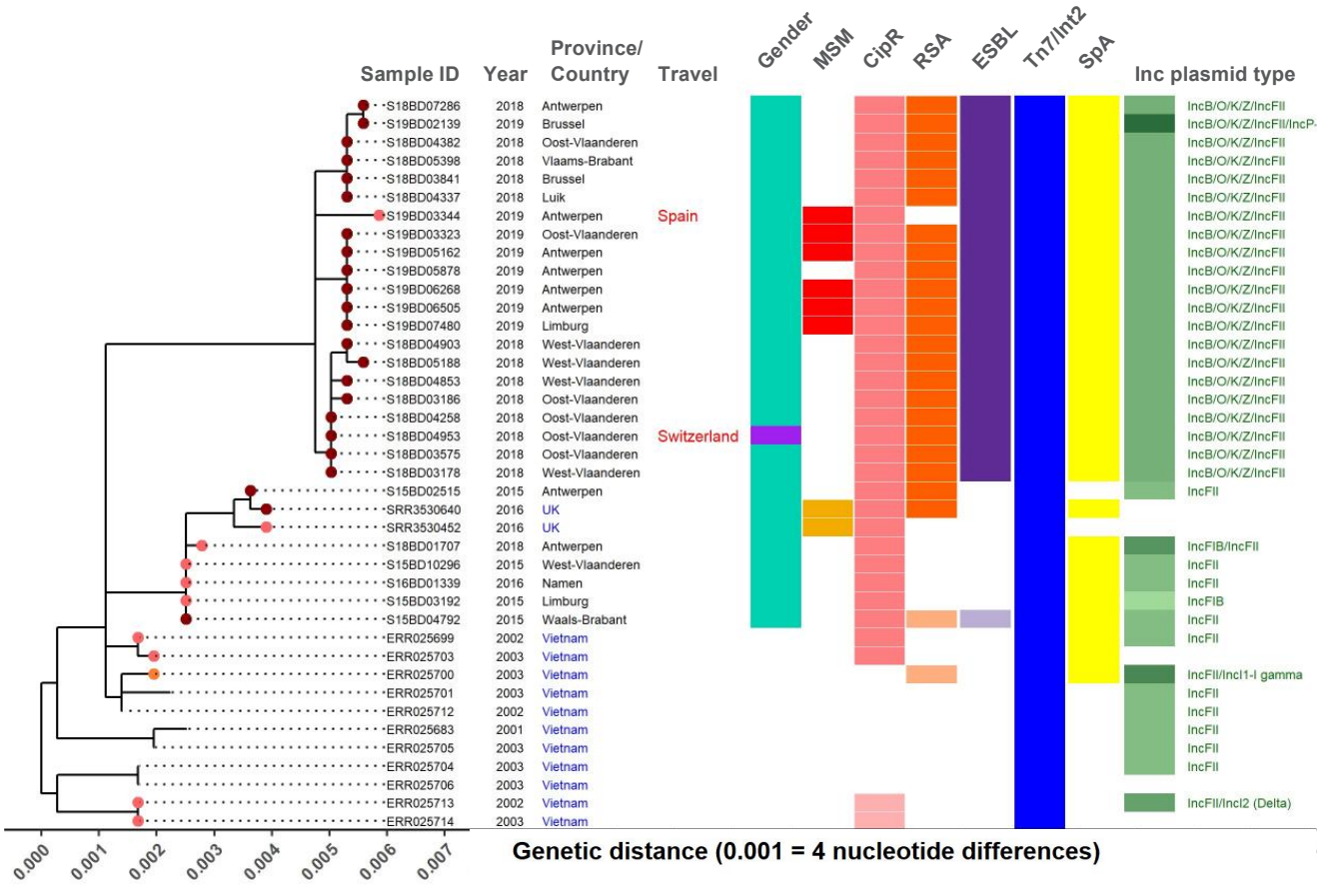
Results: Resistant clusters are associated with men who have sex with men (MSM)



Visualized with ggtree(), Yu *et al.* 2017

Fischer *et al., in revision, JAC*

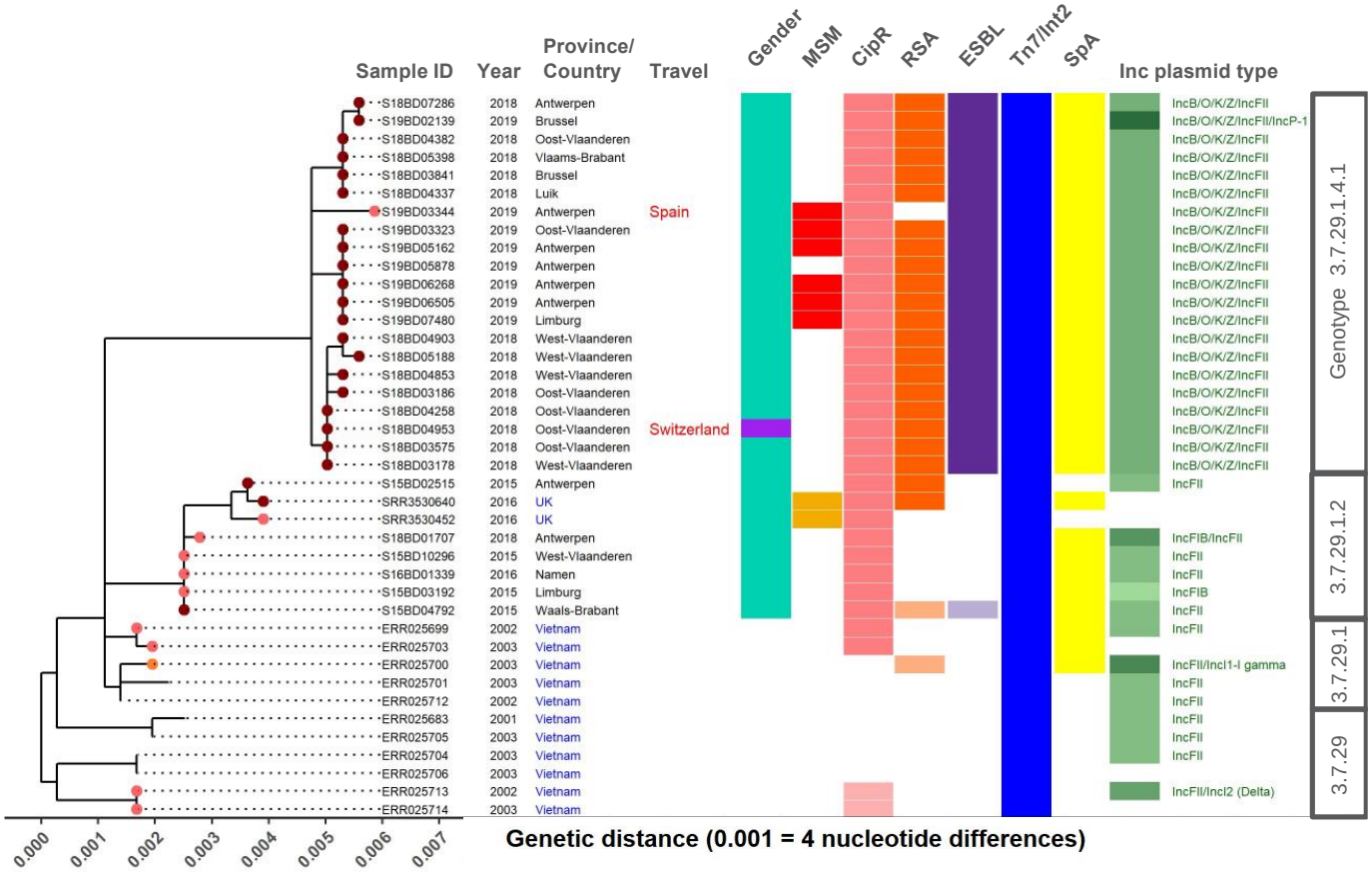
Results: Detailed analysis of Cluster II revealed evolution of multi-drug resistance from Vietnamese strains



Visualized with ggtree(), Yu *et al.* 2017

Fischer *et al., in revision, JAC*

Results: Genotype assignment with Mykobe revealed two internationally circulating MSM-related clusters in Belgium



Outbreaks in MSM in Australia 2016-2019 (Ingle *et al.*, 2019 & 2020)

Circulation among MSM in the UK (Bardsley *et al.* 2020)

Antibiotic resistance: RSA & CipR; RSA; CipR

Gender: Male; Female

MSM UK Clade 2

AMR markers:

QRDR mutations: *gyrA* (S83L); *gyrA* (D87G)

Macrolide resistance genes: *mph(A)*; *mph(A)* & *erm(B)*

Chromosomal resistance island: Tn7/Int2

ESBL genes: *bla*CTX-M-27; *bla*DHA-1

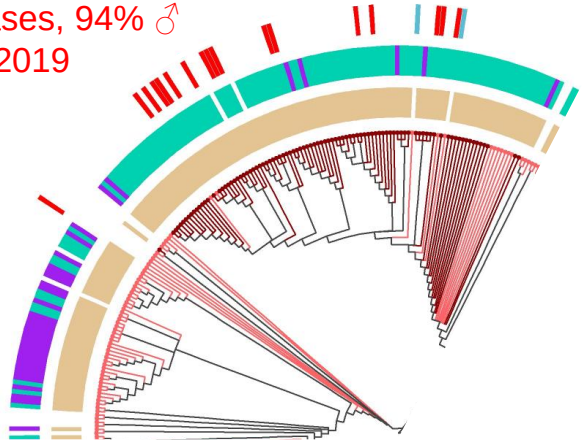
Plasmids: SpA; Other Inc type plasmids

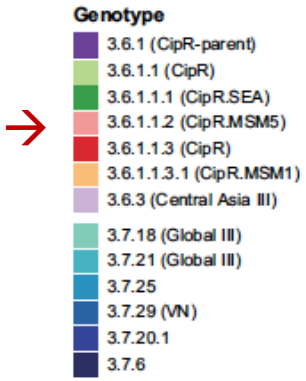
Visualized with ggtree(), Yu *et al.* 2017

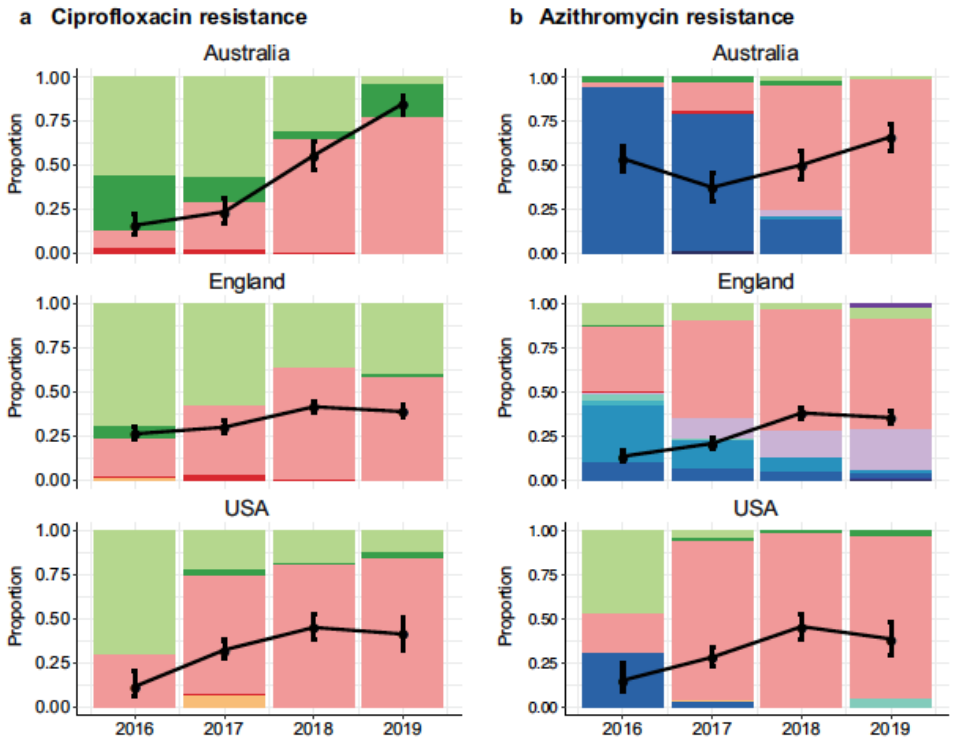
Fischer *et al., in revision, JAC*

Belgian cluster 1 was of genotype 3.6.1.1.2, linked to increased resistance in ciprofloxacin and azithromycin in Australia, England and the USA

Cluster I: 102 cases, 94% ♂
2015-2019







Hawkey *et al.* 2021

Conclusions

- More than 1/3 of all *S. sonnei* strains isolated in Belgium 2017-2019 harbor resistance to at least one important first-line antibiotic
- Men have a higher risk to carry strains with antibiotic resistance
- WGS identified several clusters of multi-drug resistant *S. sonnei* strains, which are continuously circulating in the MSM-community in Belgium and are of the same genotype as MSM-related isolates from other parts of the world

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Fischer *et al., in revision, JAC*

Recommendations

Increased Surveillance:

- Reporting and strain collection across all three regions in Belgium
- Inclusion of whole genome sequencing in routine surveillance
- Enhanced contact tracing of multi-resistant cases of *Shigellosis*

Case Management:

- Include AMR testing and improve antimicrobial stewardship

Assessment of the epidemiological situation in MSM in Belgium:

- Data collection on sexual orientation
- Further case control studies

Prevention and education campaigns:

- Raising awareness of *Shigellosis* as a sexually transmittable infection

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Fischer *et al., in revision, JAC*

WGS for surveillance can be useful to:

- Monitor the circulation of strains on a national as well as international level
- Detect a variety of AMR markers – independent of laboratory constraints
- Generate hypothesis about origins of strains and history of acquisition of AMR

Open-source tools, code and data sharing are needed to bridge current gaps in surveillance and build global capacity for WGS and data analysis

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Limitations and considerations for WGS

- Proper sample collection, transport and processing is necessary to obtain sufficient genetic material of high quality
- Unique identifiers and proper labelling are needed to link samples to patient data
- Voluntary isolate submission may introduce bias due to non-exhaustive sample collection
- Missing isolates and data from countries without capacity for WGS may introduce bias in global dissemination of strains

THANK YOU FOR YOUR ATTENTION

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