

# **Computational Practical 11**

## **National Genomic Surveillance of Antimicrobial Resistance**

### **11.1 Extensively Drug-Resistant *Salmonella enterica* Serovar Typhi in Pakistan**

#### **11.1.1 Introduction**

Typhoid fever is a bacterial infection caused by the bacterium *Salmonella enterica* serovar Typhi. The bacteria spreads via contaminated food and water, particularly in communities with poor sanitation, and is highly contagious. Symptoms include fever, stomach pain, headache and constipation or diarrhoea. If left untreated, the infection can be fatal. Vaccination, access to clean water, and improved sanitation are effective means to prevent typhoid.

Typhoid can be treated with antibiotics, although antibiotic-resistant *Salmonella* Typhi (*S. Typhi*) strains have become increasingly prevalent. Historically, typhoid has been treated with ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol (Crump *et al.* 2015). *S. Typhi* strains that are resistant to all these three first-line antibiotics are labelled as multidrug resistant (MDR). Resistance to fluoroquinolones has also been reported in regions where MDR infections are treated with these second-line antibiotics. Ceftriaxone, a third generation cephalosporin, and azithromycin, a macrolide, are reserved to treat multidrug-resistant infections.

In November 2016, an unusually high number of ceftriaxone-resistant cases were identified in the province of Sindh, Pakistan, primarily from the cities of Hyderabad and Karachi. The provincial public health authorities in Pakistan launched an investigation into possible sources and control measures including an emergency vaccination campaign were put in place. Around the same time, scientists at Public Health England identified a strain of typhoid with the same high levels of resistance from an individual in the United Kingdom returning from Pakistan.

The cases were resistant to five antibiotics in total: chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, and third-generation cephalosporins. Although MDR, quinolone-resistant and sporadic ceftriaxone resistance cases had been reported before in Pakistan, this was the first time such high level of drug resistance was seen in Typhoid cases. The investigators labelled these cases as extensively drug-resistant (XDR). The doctors were left with few antibiotics available to effectively treat the infection. Scientists at the Wellcome Sanger Institute in Cambridge were approached by scientists at Aga Khan University with a request for genetic analysis of samples (Klemm *et al.* 2018).

#### **11.1.2 Available data**

Whole-genome sequencing was carried out on 87 of the XDR *S. Typhi* strains isolated in Sindh, Pakistan, over a 6-month period between November 2016 and March 2017. Twelve contemporaneous ceftriaxone-susceptible isolates collected from the same region were also sequenced for context. The authors found that all XDR isolates and

11 out of 12 of the contextual (ceftriaxone-sensitive) isolates belonged to the same clade (4.3.1/H58 clade). They constructed a maximum-likelihood phylogenetic tree with the XDR and contextual strains from Pakistan and previously published *S. Typhi* genomes from the same clade (4.3.1/H58 clade). We will make use of this phylogenetic tree along with metadata on the country of origin, year of isolation and antibiotic resistance genetic determinants to determine the origin of these XDR *S. Typhi* cases.

### 11.1.3 Creating a MicroReact project

Start by opening up a new window in Firefox and typing <https://Microreact.org> in the address bar. Click on “Upload” as shown in Figure 10.1 to create a new project.

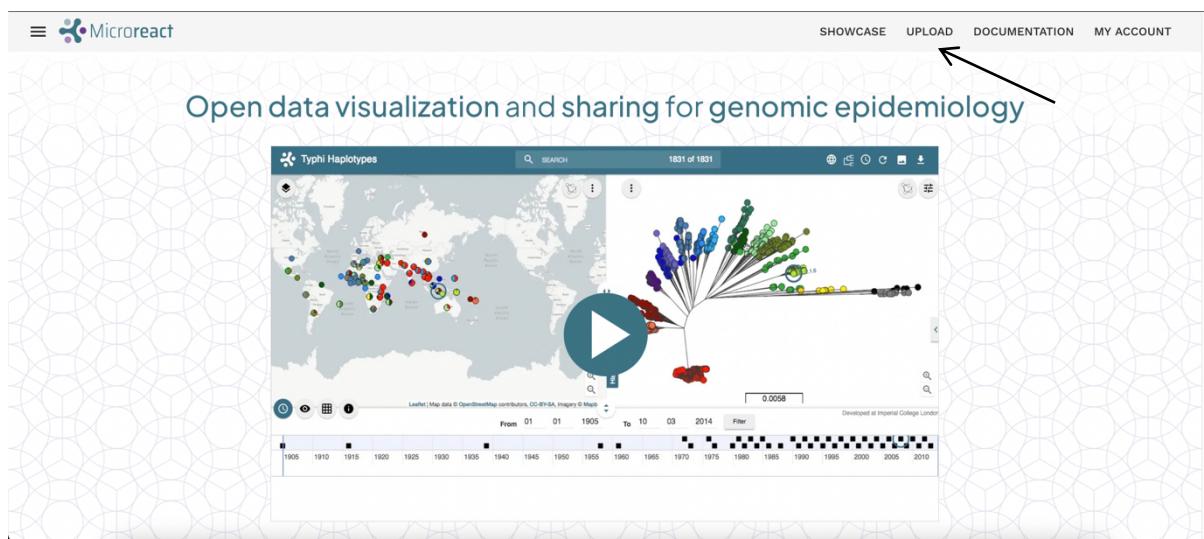


Figure 11.1 Microreact home page

Drag and drop files “Klemm2018\_metadata.csv” and “Klemm2018\_tree.nwk” from your file browser onto your Internet browser (Figure 11.2).

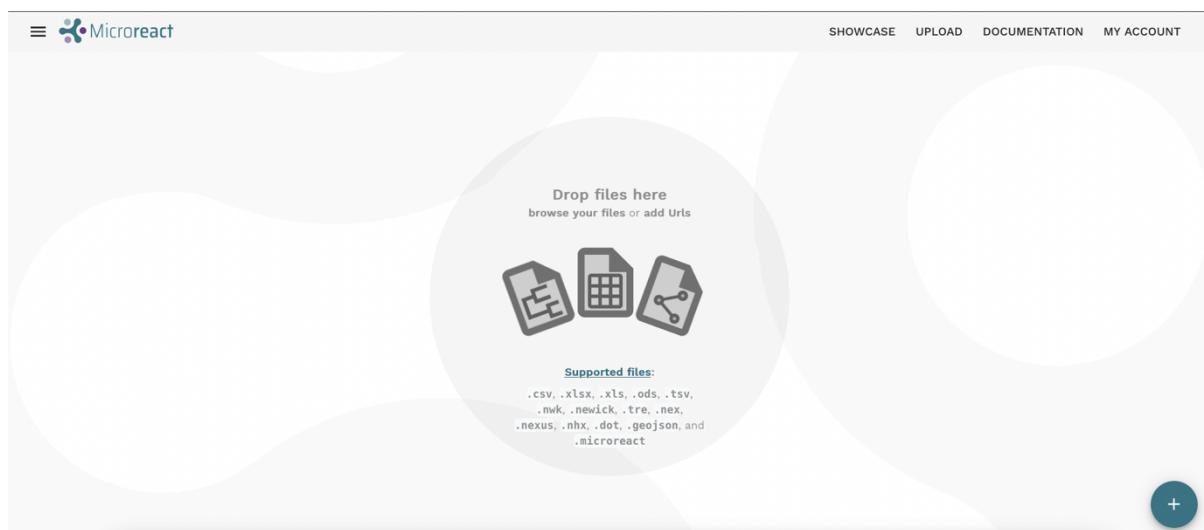
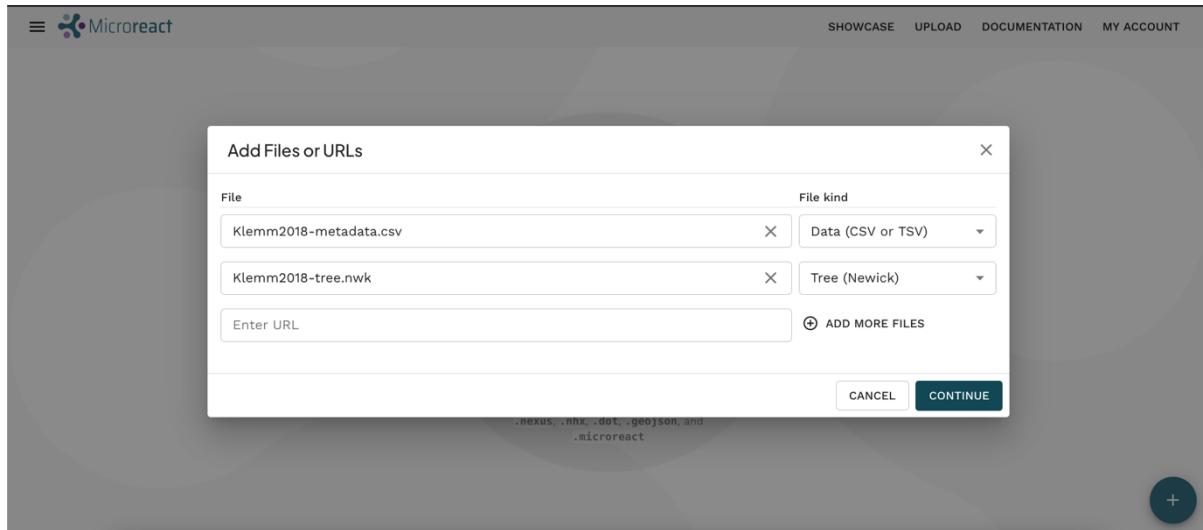


Figure 11.2 Microreact upload page

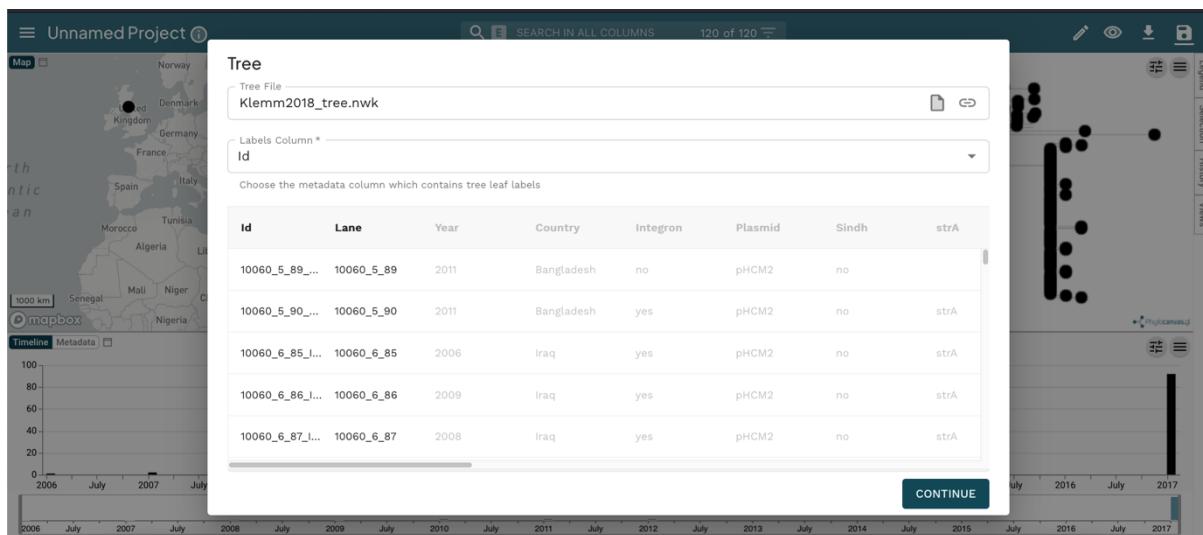
Once the tree and metadata files are loaded you will be directed to a new window where files will be automatically detected as Data (CSV or TSV) file

(Klemm2018\_metadata.csv) and Tree (Newick) file (Klemm2018\_tree.nwk). In this new window click on ‘Continue’ (Figure 11.3).



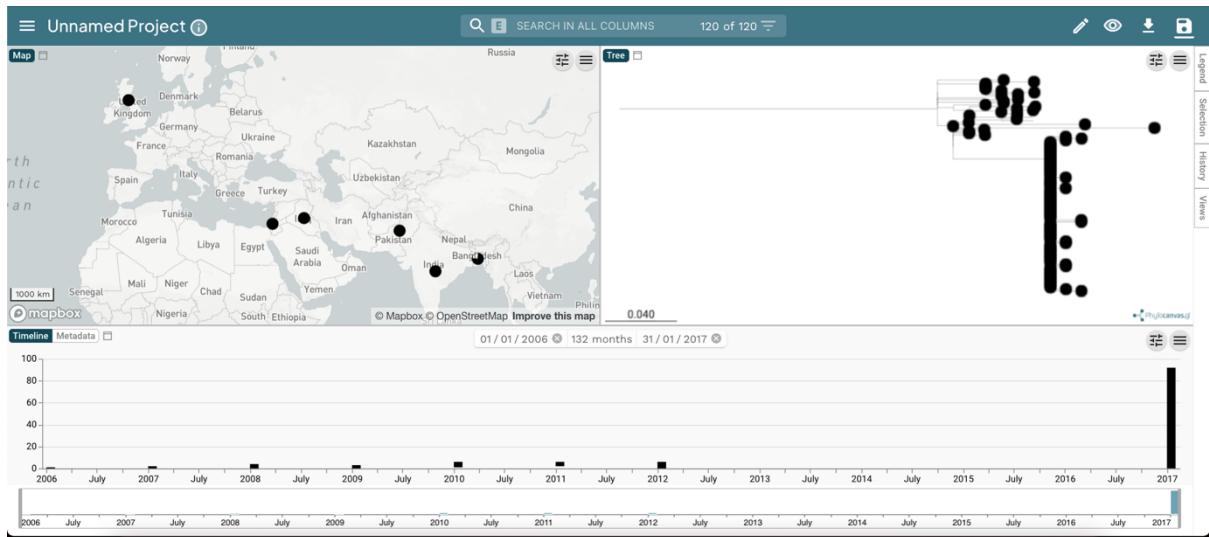
**Figure 11.3 Adding files to create a Microreact project**

In the next window (Figure 11.4), make sure column ‘Id’ is selected under ‘Labels Column’ and then click on ‘Continue’.



**Figure 11.4 Data table window in Microreact**

Once these forms are completed your data will be utilized to create a Microreact project. You should now have a view similar to the one in Figure 11.5. You should see a map view, tree view and metadata view. You can use click-drag-zoom to navigate both the tree and the map.

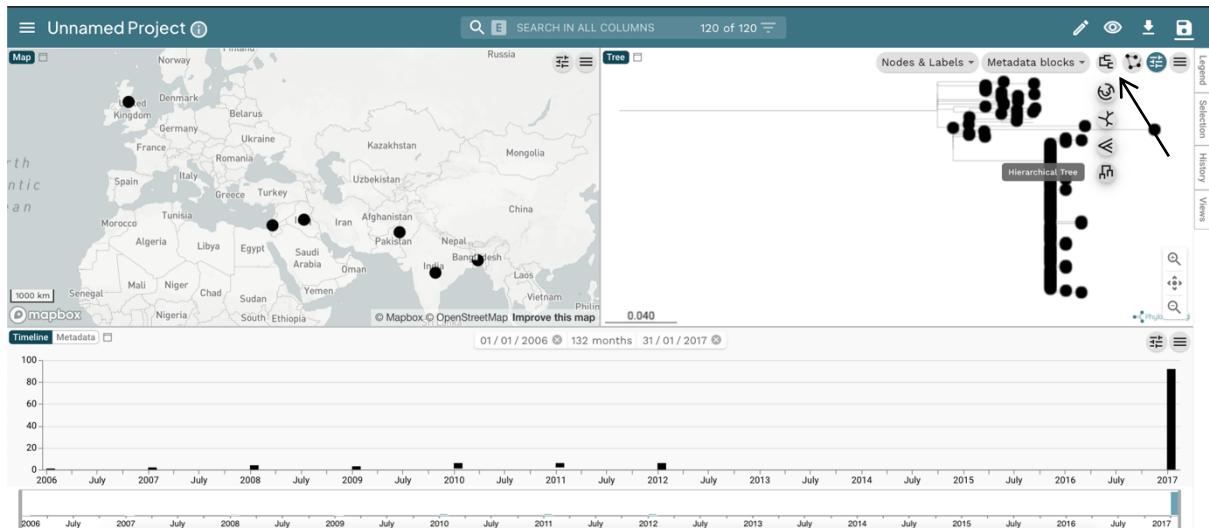


**Figure 11.5 Klemm2018 microreact project**

#### 11.1.4 Identifying the origin and spread of extensively drug-resistant *S. typhi* in Pakistan

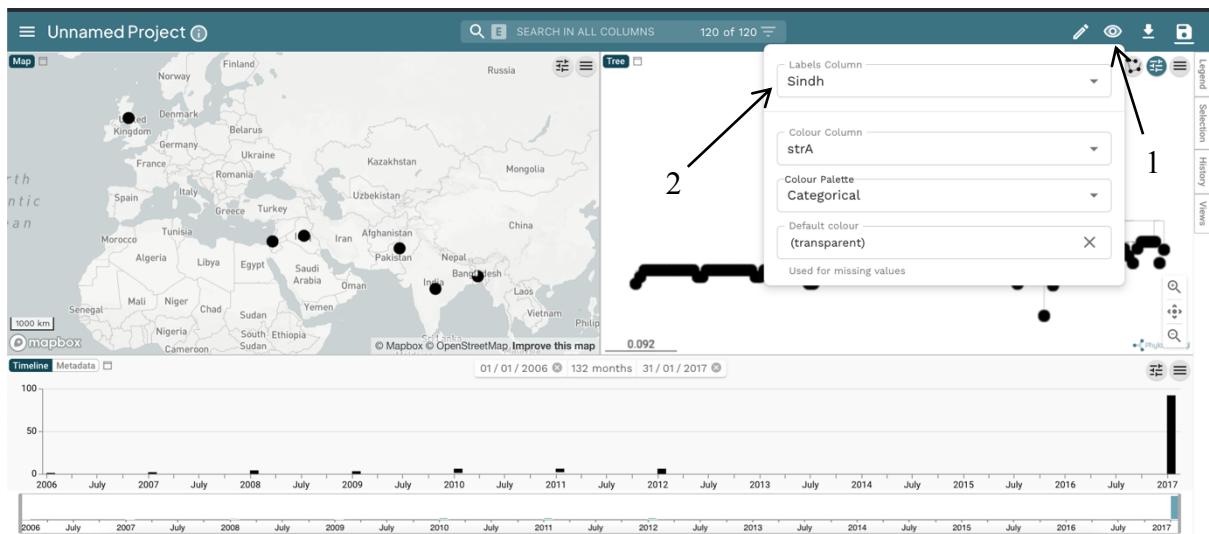
We will use the phylogeny and the map to identify the origin of the XDR *S. typhi* cases in Pakistan.

Click on the configuration button (arrow in Figure 11.6) and under ‘Tree Type’ select ‘Hierarchical’ .



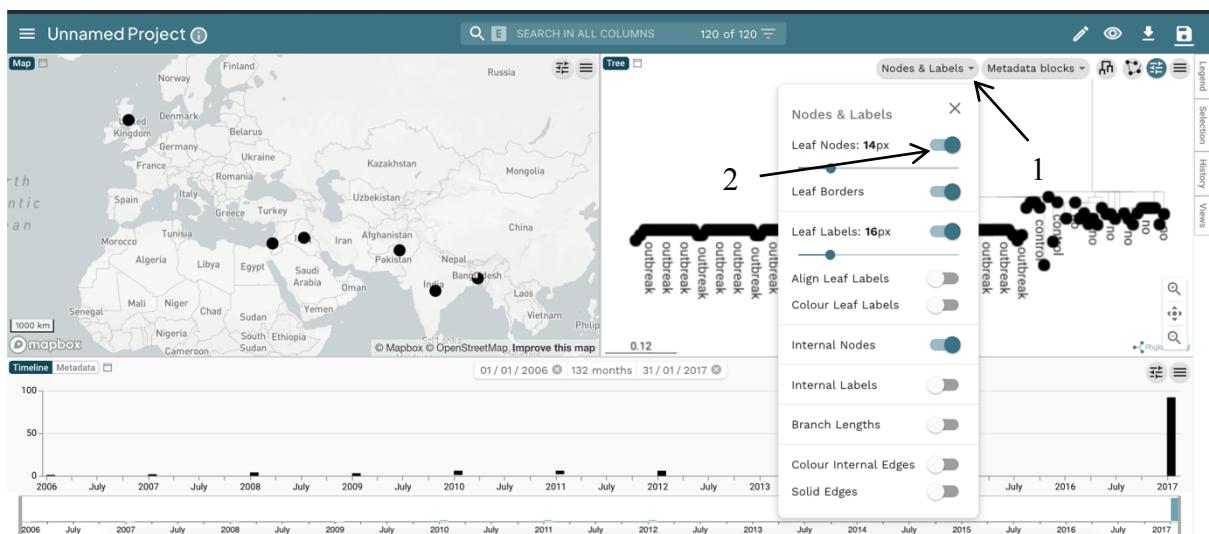
**Figure 11.6 Changing phylogenetic tree layout to hierarchical**

Now click on the ‘Labels, Colors and Shapes’ icon on the top-right corner of the window (arrow 1 in Figure 11.7). Here, in the drop-down list under ‘Labels Column’, select ‘Sindh’ (arrow 2) to label samples based on whether they belong to the Sindh outbreak or not.



**Figure 11.7 Tip nodes are labelled by Sindh outbreak label**

Under ‘Nodes & Labels’ (arrow 1 in Figure 11.8), make sure you click on the ‘Leaf Nodes’ toggle button to display tip labels (arrow 2). Reduce the size of labels if necessary.



**Figure 11.8 Displaying tip labels on the phylogenetic tree**

Open the Timeline panel (arrow 1 in Figure 11.8), set the ‘From’ date to the 01/01/2016 (arrow 2), set the ‘To’ date to 31/12/2017 (arrow 3) to highlight samples collected during 2016 and 2017 on the tree and on the map.

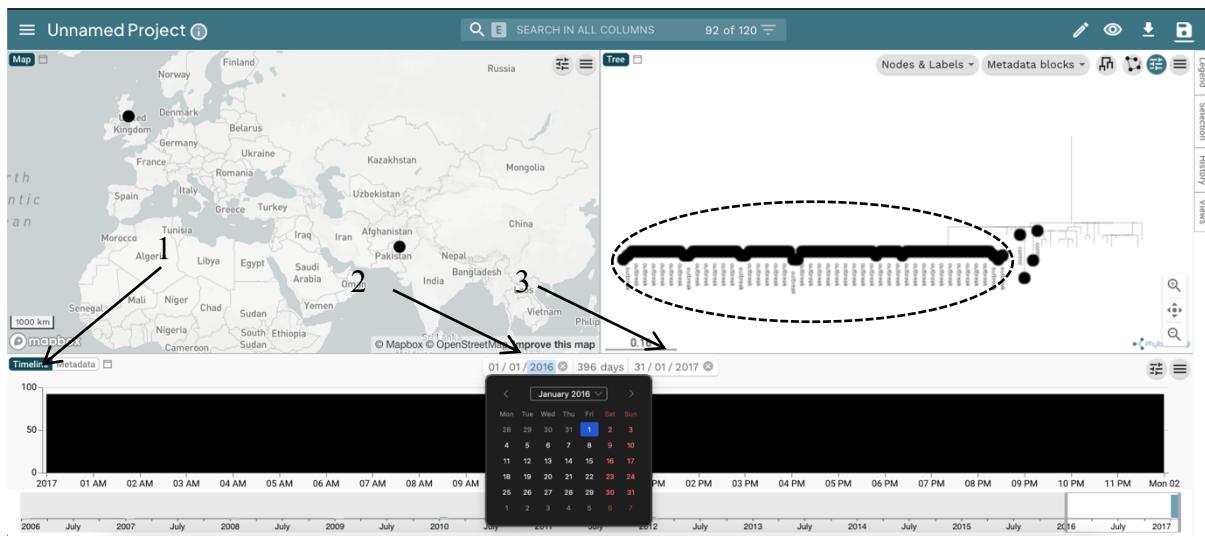


Figure 11.8 Phylogenetic and geographical location of *S. typhi* cases identified in 2017

You should notice that most of samples collected during 2016/2017 originated from Pakistan (with the exception a one sample isolated in the UK) and are clustered in the same monophyletic clade in the tree with very short branches (circled in Figure 11.8). This lack of genetic diversity and highly clonal nature of most of 2016/2017 samples are indicative of a single point source outbreak. The ‘Sindh’ label indicate whether samples belong to the Sindh province outbreak, whether they are non-outbreak samples (labelled as ‘control’) from the same province or whether they originated from other geographical regions (labelled as ‘no’).

The authors used whole-genome sequencing to build a phylogeny of all the *S. typhi* strains but also to investigate the genetic determinants of antibiotic resistance in this outbreak clone and in the rest of samples.

You can select AMR labels under ‘Colour Column’ (arrows 1 and 2 in Figure 11.9) to identify samples with and without antibiotic resistant genes/mutations shown as black and white circles on the tips of the tree, respectively.

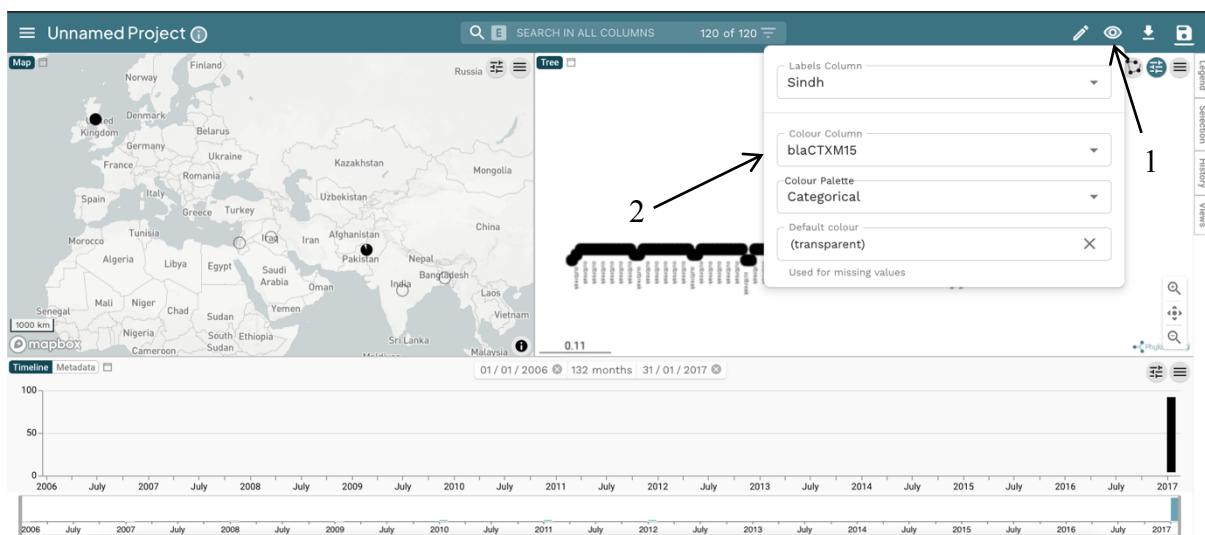


Figure 11.9 Presence of AMR genes on the tree

You will notice that almost all samples in the tree (including Sindh outbreak strains) harbour *catA1* gene (which confers resistance to chloramphenicol), *blaTEM-1* (ampicillin), *dfrA7* (trimethoprim), *sul1* and *sul2* (sulfamethoxazole), and *strA* and *strB* (streptomycin). All these genes are present in the same composite transposon antimicrobial resistance cassette integrated into the chromosome. The label ‘Integron’ (arrow 3 in Figure ) indicates whether samples have this AMR cassette integrated or not. The stability of this ARM cassette (i.e. it is present in almost all samples from the same phylogenetic lineage and rarely lost) is characteristic of chromosomally-integrated transposons which, once integrated in the chromosome, are inherited vertically. All samples have the S83F mutation in *gyrA* which mediates resistance to fluoroquinolones.

The Sindh outbreak clone (circled in Figure 11.10) carries two additional AMR genes: *blaCTX-M-15* which confers resistance to ceftriaxone (a third-generation cephalosporin) and *qnrS* which mediates resistance to ciprofloxacin, both carried on the same IncY plasmid. You can see what samples carry these two genes by colour-coding by ‘blaCTXM15’ and ‘*qnrS*’, respectively, and what type of plasmid they carry by selecting the ‘Plasmid’ option (Figure 11.10).

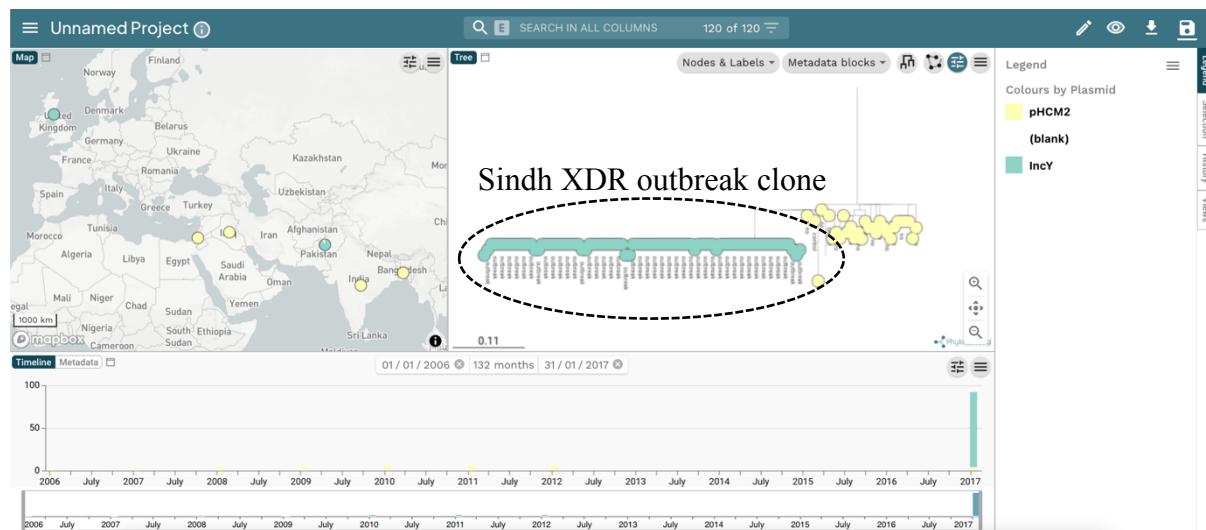
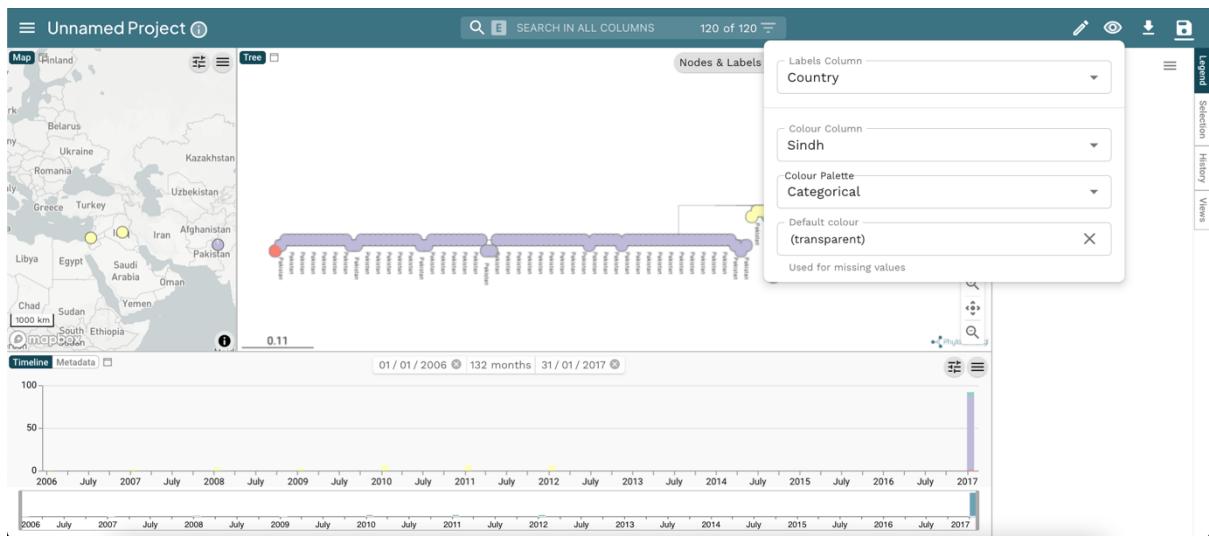


Figure 11.10 The Sindh XDR outbreak clone carries an IncY plasmid with additional AMR genes

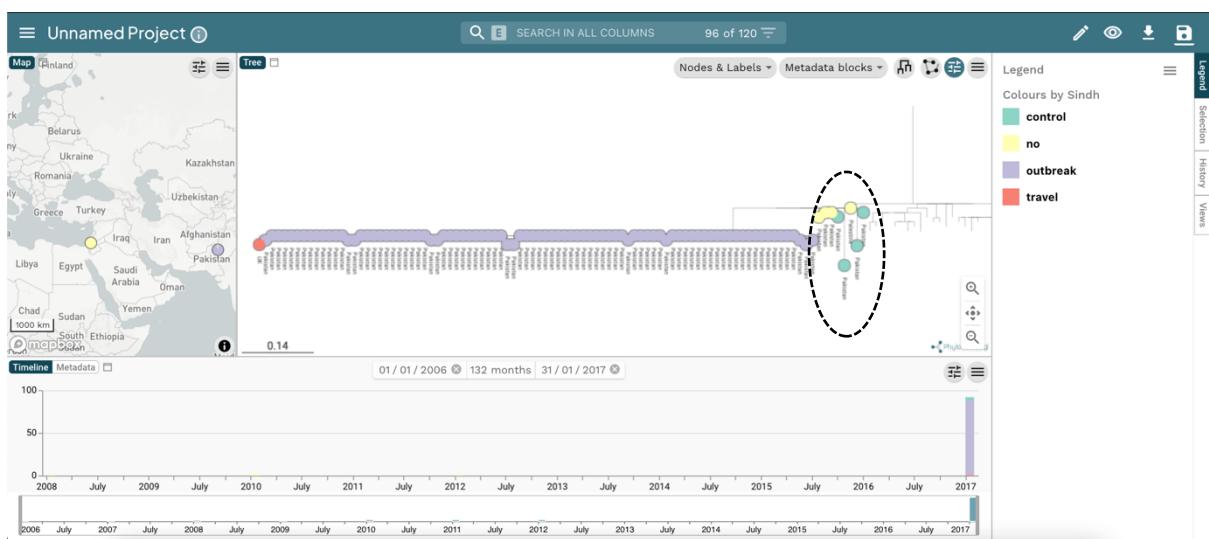
We will now investigate what is the most plausible origin of the Sindh XDR outbreak clone.

First, click on the ‘Labels, Colors and Shapes’ icon and select ‘Country’ under ‘Labels Column’, and ‘Sindh’ under ‘Colour Column’ to show the country of isolation of all samples on the tree, and colour tip nodes based on outbreak status (Figure 11.11).

Second, identify the genetically closest strains to the outbreak clone (circled in Figure 11.12). You will notice that, except for one sample isolated from Palestine in 2008, these strains were sampled in Pakistan between 2010 and 2017. The fact that the most genetically related strains to the Sindh XDR outbreak clone were also isolated in the same country earlier in time, suggest the outbreak clone derived from an endemic Pakistan clone as opposed to being imported from a different country.



**Figure 11.11 Labelling sample by country and colour-coding by outbreak status**



**Figure 11.12 Identifying the origin of the Sindh XDR outbreak clone**