

# Computational Group Activity: Local Genomic Surveillance of Antimicrobial Resistance

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## Introduction

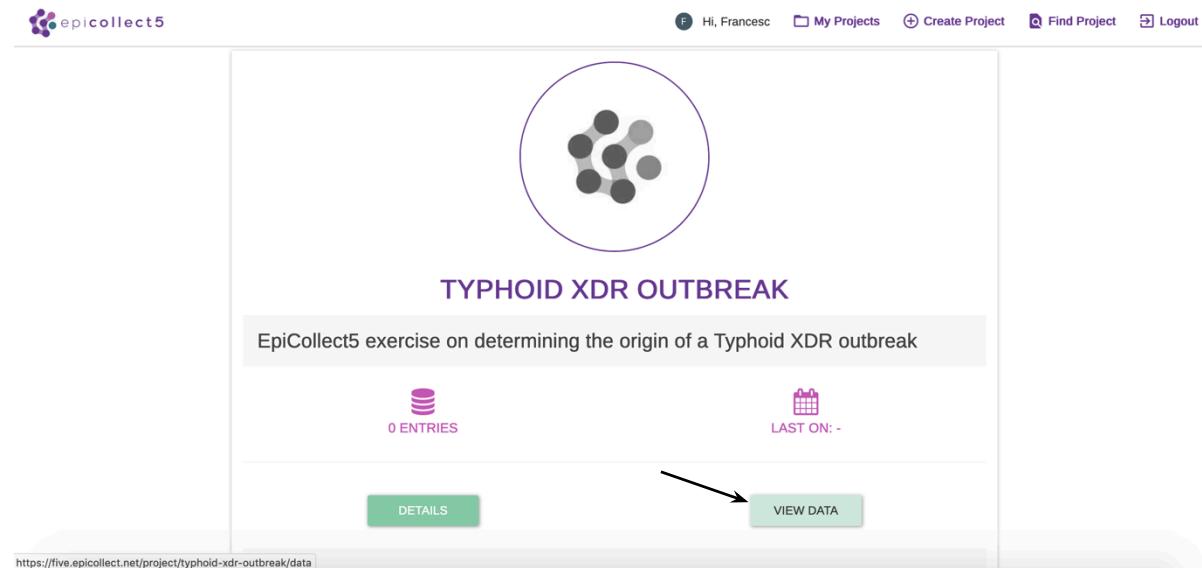
In this practical we will investigate an extensively drug-resistant (XDR) Typhoid outbreak on campus. We will integrate the epidemiological and antibiotic susceptibility data collected with EpiCollect with a phylogenetic tree visualised on MicroReact to contextualise the local outbreak in relation to other Typhoid isolates collected from other South African cities and countries.

## Download collected data from Epicollect

Visit the following URL to visualise the data collected by all participants:

<https://five.epicollect.net/project/typhoid-xdr-outbreak>

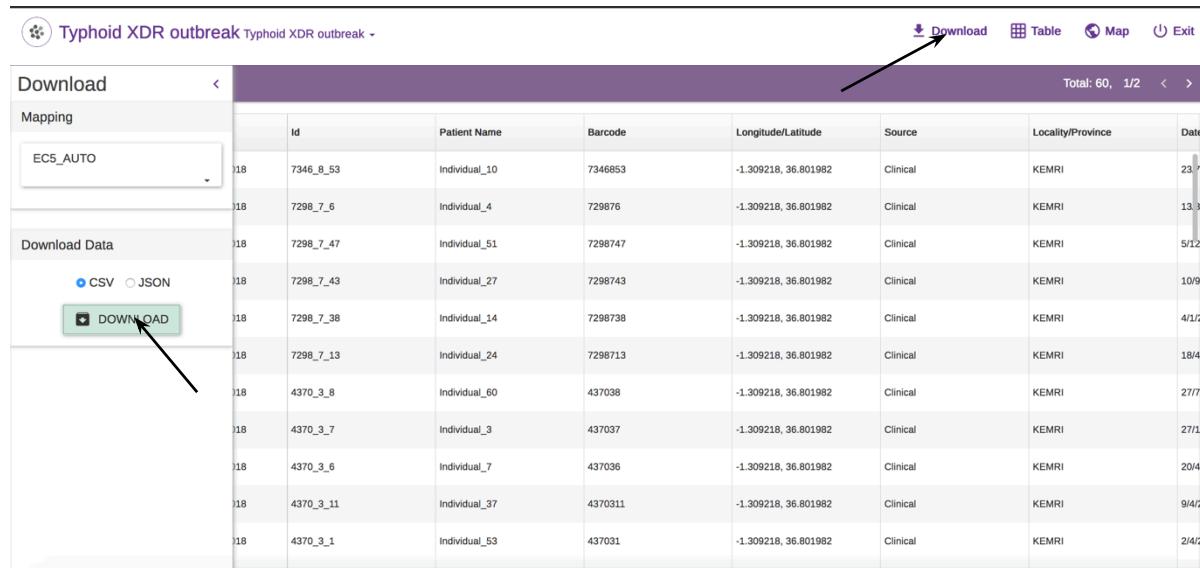
Login using the provided credentials to access the project main page (Figure 1). Click on ‘VIEW DATA’ to access all entries in this project.



https://five.epicollect.net/project/typhoid-xdr-outbreak

**Figure 1** Typhoid XDR Outbreak EpiCollect page

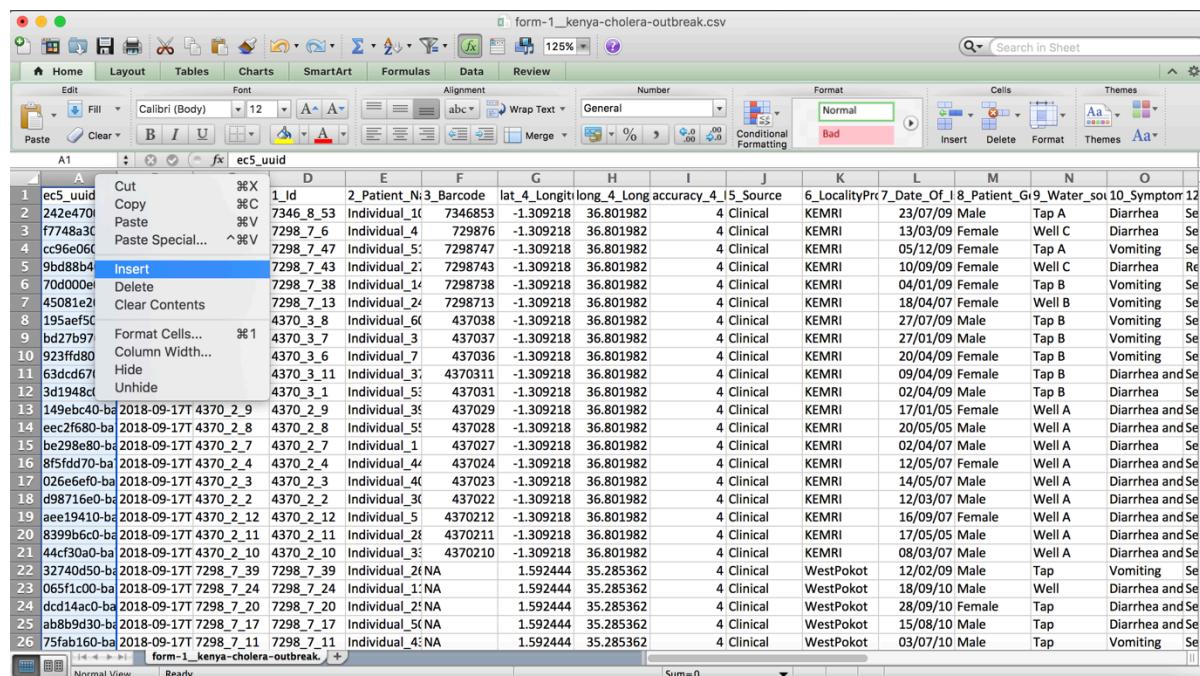
Click on ‘Download’ to download all entries as a CSV file and rename the downloaded file as ‘Klemm2018\_metadata.johannesburg.epicollect.csv’.



	Id	Patient Name	Barcode	Longitude/Latitude	Source	Locality/Province	Date
18	7346_8_53	Individual_10	7346853	-1.309218, 36.801982	Clinical	KEMRI	23/7/18
18	7298_7_6	Individual_4	729876	-1.309218, 36.801982	Clinical	KEMRI	13/3/18
18	7298_7_47	Individual_51	7298747	-1.309218, 36.801982	Clinical	KEMRI	5/12
18	7298_7_43	Individual_27	7298743	-1.309218, 36.801982	Clinical	KEMRI	10/9
18	7298_7_38	Individual_14	7298738	-1.309218, 36.801982	Clinical	KEMRI	4/1/2
18	7298_7_13	Individual_24	7298713	-1.309218, 36.801982	Clinical	KEMRI	18/4
18	4370_3_8	Individual_60	437038	-1.309218, 36.801982	Clinical	KEMRI	27/7
18	4370_3_7	Individual_3	437037	-1.309218, 36.801982	Clinical	KEMRI	27/1
18	4370_3_6	Individual_7	437036	-1.309218, 36.801982	Clinical	KEMRI	20/4
18	4370_3_11	Individual_37	4370311	-1.309218, 36.801982	Clinical	KEMRI	9/4/2
18	4370_3_1	Individual_53	437031	-1.309218, 36.801982	Clinical	KEMRI	2/4/2

Figure 2 Download EpiCollect entries

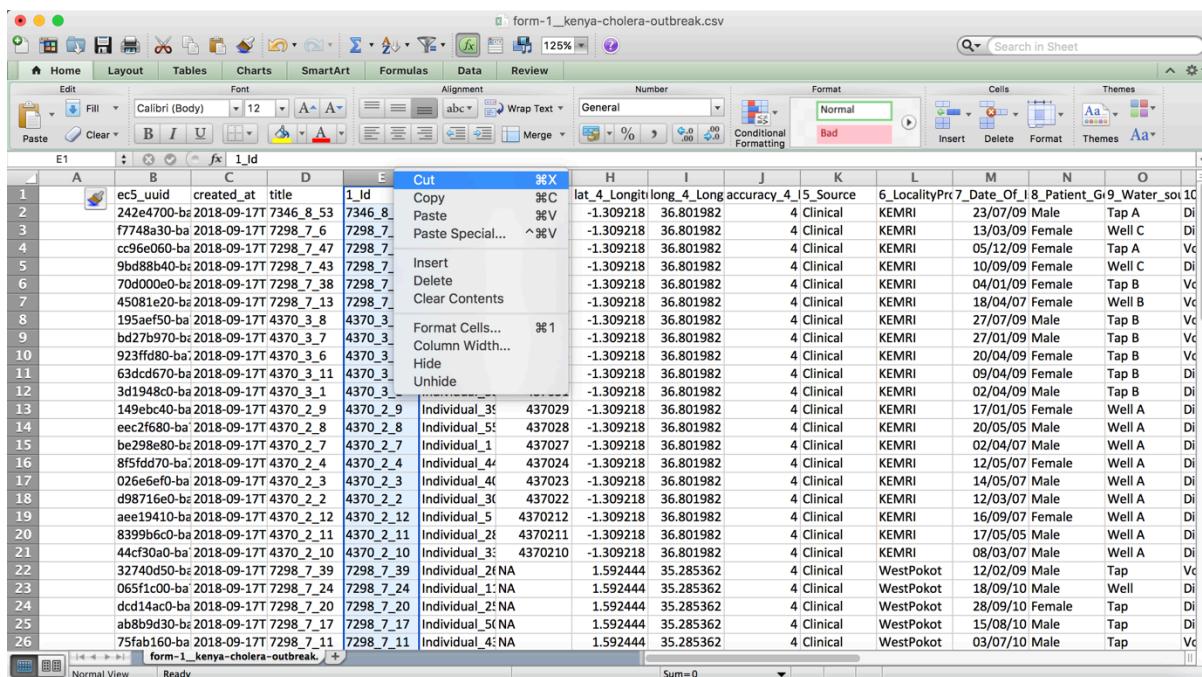
Unzip the file ‘klemm2018\_tree.johannesburg.epicollect.nwk’ and open it with Excel. We will need to edit this file first to be able to read with MicroReact. Insert a new column on the left-hand side of the Excel spreadsheet. Right-click on the first column and select ‘Insert’ as shown in Figure 3.



ec5_uuid	1_Id	2_Patient_Nr	3_Barcode	lat_4_Longit/long_4_Lon accuracy_4_15_Source	6_LocalityPrx_7_Date_Of_I_8_Patient_G9_Water_so10_Symptom_12
1_ec5_uuid	7346_8_53	Individual_10	7346853	-1.309218, 36.801982	4 Clinical KEMRI 23/07/09 Male Tap A Diarrhea Se
2_4246470	7298_7_6	Individual_4	729876	-1.309218, 36.801982	4 Clinical KEMRI 13/03/09 Female Well C Diarrhea Se
3_f77483c	7298_7_47	Individual_51	7298747	-1.309218, 36.801982	4 Clinical KEMRI 05/12/09 Female Tap A Vomiting Se
4_cc96e06	7298_7_43	Individual_27	7298743	-1.309218, 36.801982	4 Clinical KEMRI 10/09/09 Female Well C Diarrhea Re
5_9bd88b4	7298_7_38	Individual_14	7298738	-1.309218, 36.801982	4 Clinical KEMRI 04/01/09 Female Tap B Vomiting Se
6_70d000e	7298_7_13	Individual_3	7298713	-1.309218, 36.801982	4 Clinical KEMRI 18/04/09 Female Well B Vomiting Se
7_45081e2	4370_3_8	Individual_60	437038	-1.309218, 36.801982	4 Clinical KEMRI 27/07/09 Male Tap B Vomiting Se
8_b98716e0	4370_3_7	Individual_3	437037	-1.309218, 36.801982	4 Clinical KEMRI 27/02/09 Male Tap B Vomiting Se
9_bd27b97	4370_3_6	Individual_7	437036	-1.309218, 36.801982	4 Clinical KEMRI 20/04/09 Female Tap B Vomiting Se
10_923ffd80	4370_3_11	Individual_3	4370311	-1.309218, 36.801982	4 Clinical KEMRI 09/04/09 Female Tap B Diarrhea and Se
11_63ddc671	4370_3_1	Individual_5	437031	-1.309218, 36.801982	4 Clinical KEMRI 02/04/09 Male Tap B Diarrhea Se
12_3d1948cd	4370_2_9	Individual_35	437029	-1.309218, 36.801982	4 Clinical KEMRI 17/01/05 Female Well A Diarrhea and Se
13_149ebc40-ba	4370_2_8	Individual_28	437028	-1.309218, 36.801982	4 Clinical KEMRI 20/05/05 Male Well A Diarrhea and Se
14_eec2f680-ba	4370_2_7	Individual_27	437027	-1.309218, 36.801982	4 Clinical KEMRI 02/04/07 Male Well A Diarrhea Se
15_be298e80-ba	4370_2_4	Individual_44	437024	-1.309218, 36.801982	4 Clinical KEMRI 12/05/07 Female Well A Diarrhea and Se
16_8f5fdd70-ba	4370_2_3	Individual_40	437023	-1.309218, 36.801982	4 Clinical KEMRI 14/05/07 Male Well A Diarrhea and Se
17_d266eef0-ba	4370_2_2	Individual_30	437022	-1.309218, 36.801982	4 Clinical KEMRI 12/03/07 Male Well A Diarrhea and Se
18_d98716e0-ba	4370_2_12	Individual_5	4370212	-1.309218, 36.801982	4 Clinical KEMRI 16/09/07 Female Well A Diarrhea and Se
19_aee19410-ba	4370_2_11	Individual_28	4370211	-1.309218, 36.801982	4 Clinical KEMRI 17/05/05 Male Well A Diarrhea and Se
20_8399b6c0-ba	4370_2_10	Individual_33	4370210	-1.309218, 36.801982	4 Clinical KEMRI 08/03/07 Male Well A Diarrhea and Se
21_44cf30a0-ba	7298_7_39	Individual_20NA	1.592444	35.285362	4 Clinical WestPokot 12/02/09 Male Tap Vomiting Se
22_32740d50-ba	7298_7_24	Individual_11NA	1.592444	35.285362	4 Clinical WestPokot 18/09/10 Male Well Diarrhea and Se
23_065f1c00-ba	7298_7_20	Individual_21NA	1.592444	35.285362	4 Clinical WestPokot 28/09/10 Female Tap Diarrhea and Se
24_dcd14ac0-ba	7298_7_17	Individual_5CNA	1.592444	35.285362	4 Clinical WestPokot 15/08/10 Male Tap Diarrhea and Se
25_ab889d30-ba	7298_7_11	Individual_4NA	1.592444	35.285362	4 Clinical WestPokot 03/07/10 Male Tap Vomiting Se

Figure 3 Editing EpiCollect entries using Excel

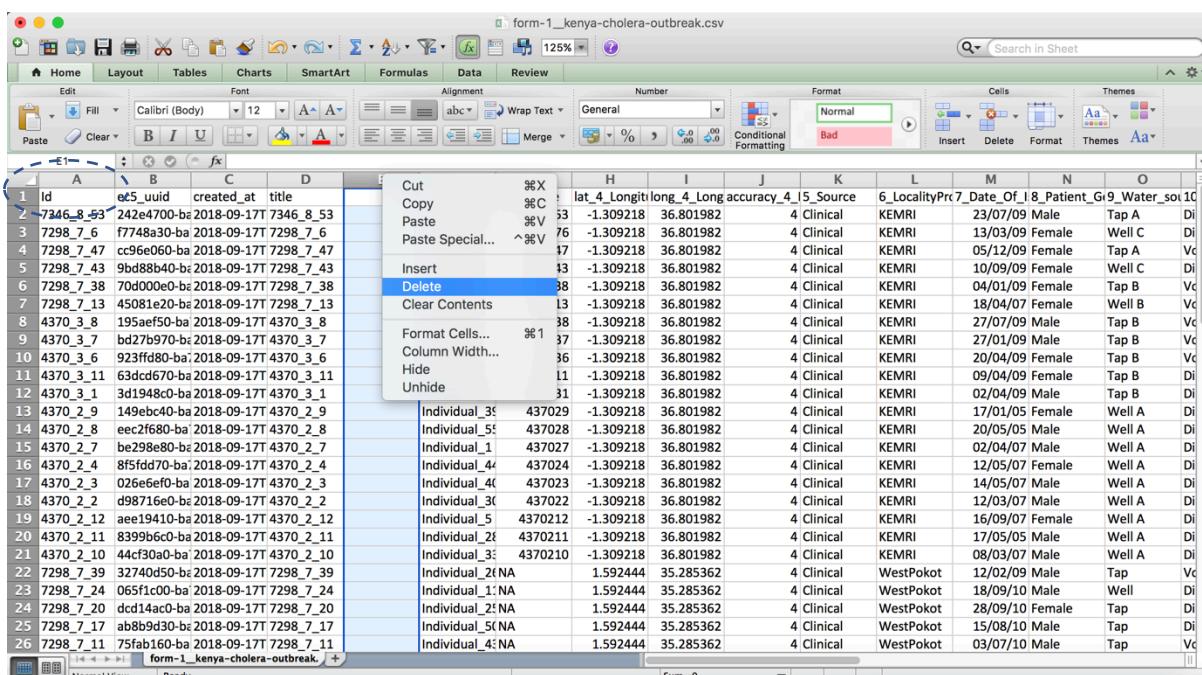
Cut the column ‘1\_Id’ and paste it on the newly inserted column as shown in Figure 4.



E1	A	B	C	D	E	H	I	J	K	L	M	N	O		
1	ec5_uuid	created_at	title	1_Id	Cut	%C	lat_4_Longit	long_4_Latitud	accuracy_4_15_Source	6_LocalityPr	7_Date_Of_I	8_Patient_G	9_Water_soi		
2	242e4700-ba	2018-09-17T	7346_8_53	7346_8	Copy	%C	36.801982	4 Clinical	KEMRI	23/07/09	Male	Tap A	Di		
3	f7748a30-ba	2018-09-17T	7298_7_6	7298_7	Paste	%V	-1.309218	36.801982	4 Clinical	KEMRI	13/03/09	Female	Well C	Di	
4	cc56e060-ba	2018-09-17T	7298_7_47	7298_7	Paste Special...	^%V	-1.309218	36.801982	4 Clinical	KEMRI	05/12/09	Female	Tap A	Vc	
5	9b68840-ba	2018-09-17T	7298_7_43	7298_7	Insert		-1.309218	36.801982	4 Clinical	KEMRI	10/09/09	Female	Well C	Di	
6	70d000e0-ba	2018-09-17T	7298_7_38	7298_7	Delete		-1.309218	36.801982	4 Clinical	KEMRI	04/01/09	Female	Tap B	Vc	
7	45081e20-ba	2018-09-17T	7298_7_13	7298_7	Clear Contents		-1.309218	36.801982	4 Clinical	KEMRI	18/04/07	Female	Well B	Vc	
8	195afe50-ba	2018-09-17T	4370_3_8	4370_3	Format Cells...	^%1	-1.309218	36.801982	4 Clinical	KEMRI	27/07/09	Male	Tap B	Vc	
9	bd27b970-ba	2018-09-17T	4370_3_7	4370_3	Column Width...		-1.309218	36.801982	4 Clinical	KEMRI	27/01/09	Male	Tap B	Vc	
10	923fd80-ba	2018-09-17T	4370_3_6	4370_3	Hide		-1.309218	36.801982	4 Clinical	KEMRI	20/04/09	Female	Tap B	Vc	
11	63ddc670-ba	2018-09-17T	4370_3_11	4370_3	Unhide		-1.309218	36.801982	4 Clinical	KEMRI	09/04/09	Female	Tap B	Di	
12	3d1948c0-ba	2018-09-17T	4370_3_1	4370_3			-1.309218	36.801982	4 Clinical	KEMRI	02/04/09	Male	Tap B	Di	
13	149ebc40-ba	2018-09-17T	4370_2_9	4370_2			Individual_35	437029	36.801982	4 Clinical	KEMRI	17/01/05	Female	Well A	Di
14	ee2f680-ba	2018-09-17T	4370_2_8	4370_2			Individual_55	437028	36.801982	4 Clinical	KEMRI	20/05/05	Male	Well A	Di
15	be298e80-ba	2018-09-17T	4370_2_7	4370_2			Individual_1	437027	36.801982	4 Clinical	KEMRI	02/04/07	Male	Well A	Di
16	8f5fd70-ba	2018-09-17T	4370_2_4	4370_2			Individual_44	437024	36.801982	4 Clinical	KEMRI	12/05/07	Female	Well A	Di
17	026e6ef0-ba	2018-09-17T	4370_2_3	4370_2			Individual_40	437023	36.801982	4 Clinical	KEMRI	14/05/07	Male	Well A	Di
18	d987160-ba	2018-09-17T	4370_2_2	4370_2			Individual_30	437022	36.801982	4 Clinical	KEMRI	12/03/07	Male	Well A	Di
19	aae19410-ba	2018-09-17T	4370_2_12	4370_2			Individual_5	4370212	36.801982	4 Clinical	KEMRI	16/09/07	Female	Well A	Di
20	8399b6c0-ba	2018-09-17T	4370_2_11	4370_2			Individual_25	4370211	36.801982	4 Clinical	KEMRI	17/05/05	Male	Well A	Di
21	44cf30a0-ba	2018-09-17T	4370_2_10	4370_2			Individual_30	4370210	36.801982	4 Clinical	KEMRI	08/03/07	Male	Well A	Di
22	32740d50-ba	2018-09-17T	7298_7_39	7298_7			Individual_2NA	1.592444	35.285362	4 Clinical	WestPokot	12/02/09	Male	Tap	Vc
23	065f1c00-ba	2018-09-17T	7298_7_24	7298_7			Individual_1NA	1.592444	35.285362	4 Clinical	WestPokot	18/09/10	Male	Well	Di
24	ddc14ac0-ba	2018-09-17T	7298_7_20	7298_7			Individual_2NA	1.592444	35.285362	4 Clinical	WestPokot	28/09/10	Female	Tap	Di
25	ab8b9d30-ba	2018-09-17T	7298_7_17	7298_7			Individual_5NA	1.592444	35.285362	4 Clinical	WestPokot	15/08/10	Male	Tap	Di
26	75fab160-ba	2018-09-17T	7298_7_11	7298_7			Individual_4NA	1.592444	35.285362	4 Clinical	WestPokot	03/07/10	Male	Tap	Vc

Figure 4 Editing EpiCollect entries using Excel

Change the name of the first column to ‘Id’ and delete the empty column (Figure 5).



E1	A	B	C	D	E	H	I	J	K	L	M	N	O		
1	Id	ec5_uuid	created_at	title	Cut	%C	lat_4_Longit	long_4_Latitud	accuracy_4_15_Source	6_LocalityPr	7_Date_Of_I	8_Patient_G	9_Water_soi		
2	7346_8_53	242e4700-ba	2018-09-17T	7346_8_53	Copy	%C	36.801982	4 Clinical	KEMRI	23/07/09	Male	Tap A	Di		
3	7298_7_6	f7748a30-ba	2018-09-17T	7298_7_6	Paste	%V	-1.309218	36.801982	4 Clinical	KEMRI	13/03/09	Female	Well C	Di	
4	7298_7_47	cc56e060-ba	2018-09-17T	7298_7_47	Paste Special...	^%V	-1.309218	36.801982	4 Clinical	KEMRI	05/12/09	Female	Tap A	Vc	
5	7298_7_43	9b68840-ba	2018-09-17T	7298_7_43	Delete		-1.309218	36.801982	4 Clinical	KEMRI	10/09/09	Female	Well C	Di	
6	7298_7_38	70d000e0-ba	2018-09-17T	7298_7_38	Clear Contents		-1.309218	36.801982	4 Clinical	KEMRI	04/01/09	Female	Tap B	Vc	
7	7298_7_13	45081e20-ba	2018-09-17T	7298_7_13	Format Cells...	^%1	-1.309218	36.801982	4 Clinical	KEMRI	18/04/07	Female	Well B	Vc	
8	4370_3_8	195afe50-ba	2018-09-17T	4370_3_8	Column Width...		-1.309218	36.801982	4 Clinical	KEMRI	27/07/09	Male	Tap B	Vc	
9	4370_3_7	bd27b970-ba	2018-09-17T	4370_3_7	Hide		-1.309218	36.801982	4 Clinical	KEMRI	27/01/09	Male	Tap B	Vc	
10	4370_3_6	923fd80-ba	2018-09-17T	4370_3_6	Unhide		-1.309218	36.801982	4 Clinical	KEMRI	20/04/09	Female	Tap B	Di	
11	4370_3_11	63ddc670-ba	2018-09-17T	4370_3_11			Individual_35	437029	36.801982	4 Clinical	KEMRI	09/04/09	Female	Tap B	Di
12	4370_3_1	3d1948c0-ba	2018-09-17T	4370_3_1			Individual_55	437028	36.801982	4 Clinical	KEMRI	02/04/09	Male	Tap B	Di
13	4370_2_9	149ebc40-ba	2018-09-17T	4370_2_9			Individual_1	437027	36.801982	4 Clinical	KEMRI	17/01/05	Female	Well A	Di
14	4370_2_8	ee2f680-ba	2018-09-17T	4370_2_8			Individual_44	437024	36.801982	4 Clinical	KEMRI	20/05/05	Male	Well A	Di
15	4370_2_7	be298e80-ba	2018-09-17T	4370_2_7			Individual_40	437023	36.801982	4 Clinical	KEMRI	02/04/07	Male	Well A	Di
16	4370_2_4	8f5fd70-ba	2018-09-17T	4370_2_4			Individual_30	437022	36.801982	4 Clinical	KEMRI	12/05/07	Female	Well A	Di
17	4370_2_3	026e6ef0-ba	2018-09-17T	4370_2_3			Individual_25	437021	36.801982	4 Clinical	KEMRI	14/05/07	Male	Well A	Di
18	4370_2_2	d987160-ba	2018-09-17T	4370_2_2			Individual_20	437021	36.801982	4 Clinical	KEMRI	12/03/07	Male	Well A	Di
19	4370_2_12	aae19410-ba	2018-09-17T	4370_2_12			Individual_5	4370212	36.801982	4 Clinical	KEMRI	16/09/07	Female	Well A	Di
20	4370_2_11	8399b6c0-ba	2018-09-17T	4370_2_11			Individual_25	4370211	36.801982	4 Clinical	KEMRI	17/05/05	Male	Well A	Di
21	4370_2_10	44cf30a0-ba	2018-09-17T	4370_2_10			Individual_3	4370210	36.801982	4 Clinical	KEMRI	08/03/07	Male	Well A	Di
22	7298_7_39	32740d50-ba	2018-09-17T	7298_7_39			Individual_2NA	1.592444	35.285362	4 Clinical	WestPokot	12/02/09	Male	Tap	Vc
23	7298_7_24	065f1c00-ba	2018-09-17T	7298_7_24			Individual_1NA	1.592444	35.285362	4 Clinical	WestPokot	18/09/10	Male	Well	Di
24	7298_7_20	ddc14ac0-ba	2018-09-17T	7298_7_20			Individual_2NA	1.592444	35.285362	4 Clinical	WestPokot	28/09/10	Female	Tap	Di
25	7298_7_17	ab8b9d30-ba	2018-09-17T	7298_7_17			Individual_5NA	1.592444	35.285362	4 Clinical	WestPokot	15/08/10	Male	Tap	Di
26	7298_7_11	75fab160-ba	2018-09-17T	7298_7_11			Individual_4NA	1.592444	35.285362	4 Clinical	WestPokot	03/07/10	Male	Tap	Vc

Figure 5 Editing EpiCollect entries using Excel

Change the name of column ‘lat\_4\_LongitudeLatitude’ to ‘Latitude’ and ‘long\_4\_LongitudeLatitude’ to ‘Longitude’ (Figure 6). Save your changes and close.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Id	ec5_uuid	created_at	title	2_Patient_N;3_Barcod	Latitude	Longitude	accuracy_4	I5_Source	6_LocalityPrc	7_Date_Of_I8_Patient_G;9_Water_sot	10_Symptom	12	
2	7346_8_53	242e4700-ba	2018-09-17T	7346_8_53	Individual_1t	7346853	-1.309218	36.801982	4 Clinical	KEMRI	23/07/09	Male	Tap A	Diarrhea Se
3	7298_7_6	f7748a30-ba	2018-09-17T	7298_7_6	Individual_4	729876	-1.309218	36.801982	4 Clinical	KEMRI	13/03/09	Female	Well C	Diarrhea Se
4	7298_7_47	cc96e060-ba	2018-09-17T	7298_7_47	Individual_5t	7298747	-1.309218	36.801982	4 Clinical	KEMRI	05/12/09	Female	Tap A	Vomiting Se
5	7298_7_43	9bd8b840-ba	2018-09-17T	7298_7_43	Individual					MRI	10/09/09	Female	Well C	Diarrhea Re
6	7298_7_38	70d000e0-bc	2018-09-17T	7298_7_38	Individual					MRI	04/01/09	Female	Tap B	Vomiting Se
7	7298_7_13	45081e20-ba	2018-09-17T	7298_7_13	Individual					MRI	18/04/07	Female	Well B	Vomiting Se
8	4370_3_8	195aef50-ba	2018-09-17T	4370_3_8	Individual					MRI	27/07/09	Male	Tap B	Vomiting Se
9	4370_3_7	bd27b970-bc	2018-09-17T	4370_3_7	Individual					MRI	27/01/09	Male	Tap B	Vomiting Se
10	4370_3_6	923fd80-ba	2018-09-17T	4370_3_6	Individual					MRI	20/04/09	Female	Tap B	Vomiting Se
11	4370_3_11	63dc6d70-ba	2018-09-17T	4370_3_11	Individual					MRI	09/04/09	Female	Tap B	Diarrhea and Se
12	4370_3_1	3d1948c0-ba	2018-09-17T	4370_3_1	Individual					MRI	02/04/09	Male	Tap B	Diarrhea Se
13	4370_2_9	149ebc40-ba	2018-09-17T	4370_2_9	Individual					MRI	17/01/05	Female	Well A	Diarrhea and Se
14	4370_2_8	ee2c7680-ba	2018-09-17T	4370_2_8	Individual					MRI	20/05/05	Male	Well A	Diarrhea and Se
15	4370_2_7	be298e80-ba	2018-09-17T	4370_2_7	Individual_1	437027	-1.309218	36.801982	4 Clinical	KEMRI	02/04/07	Male	Well A	Diarrhea Se
16	4370_2_4	8f5ffd70-ba	2018-09-17T	4370_2_4	Individual_4	437024	-1.309218	36.801982	4 Clinical	KEMRI	12/05/07	Female	Well A	Diarrhea and Se
17	4370_2_3	026e6ef0-ba	2018-09-17T	4370_2_3	Individual_4C	437023	-1.309218	36.801982	4 Clinical	KEMRI	14/05/07	Male	Well A	Diarrhea and Se
18	4370_2_2	d98716e0-ba	2018-09-17T	4370_2_2	Individual_3C	437022	-1.309218	36.801982	4 Clinical	KEMRI	12/03/07	Male	Well A	Diarrhea and Se
19	4370_2_12	ace19410-ba	2018-09-17T	4370_2_12	Individual_5	4370212	-1.309218	36.801982	4 Clinical	KEMRI	16/09/07	Female	Well A	Diarrhea and Se
20	4370_2_11	8399b6c0-ba	2018-09-17T	4370_2_11	Individual_28	4370211	-1.309218	36.801982	4 Clinical	KEMRI	17/05/05	Male	Well A	Diarrhea and Se
21	4370_2_10	44cf30a0-ba	2018-09-17T	4370_2_10	Individual_3:	4370210	-1.309218	36.801982	4 Clinical	KEMRI	08/03/07	Male	Well A	Diarrhea and Se
22	7298_7_39	32740d50-bc	2018-09-17T	7298_7_39	Individual_2tNA		1.592444	35.285362	4 Clinical	WestPokot	12/02/09	Male	Tap	Vomiting Se
23	7298_7_24	065f1c00-ba	2018-09-17T	7298_7_24	Individual_1:NA		1.592444	35.285362	4 Clinical	WestPokot	18/09/10	Male	Well	Diarrhea and Se
24	7298_7_20	ddcd14ac0-ba	2018-09-17T	7298_7_20	Individual_2:NA		1.592444	35.285362	4 Clinical	WestPokot	28/09/10	Female	Tap	Diarrhea and Se
25	7298_7_17	ab889d30-bc	2018-09-17T	7298_7_17	Individual_5tNA		1.592444	35.285362	4 Clinical	WestPokot	15/08/10	Male	Tap	Diarrhea and Se
26	7298_7_11	75fab160-ba	2018-09-17T	7298_7_11	Individual_4:NA		1.592444	35.285362	4 Clinical	WestPokot	03/07/10	Male	Tap	Vomiting Se

Figure 6 Editing EpiCollect entries using Excel

## Data visualization with Microreact

Start by opening a new window in Firefox and typing <https://microreact.org/> in the address bar. Click on “Upload” and browse for the phylogenetic tree (klemm2018\_tree.johannesburg.epicollect.nwk) and metadata files (klemm2018\_metadata.johannesburg.epicollect.csv) to create a new MicroReact project (Figure 7).

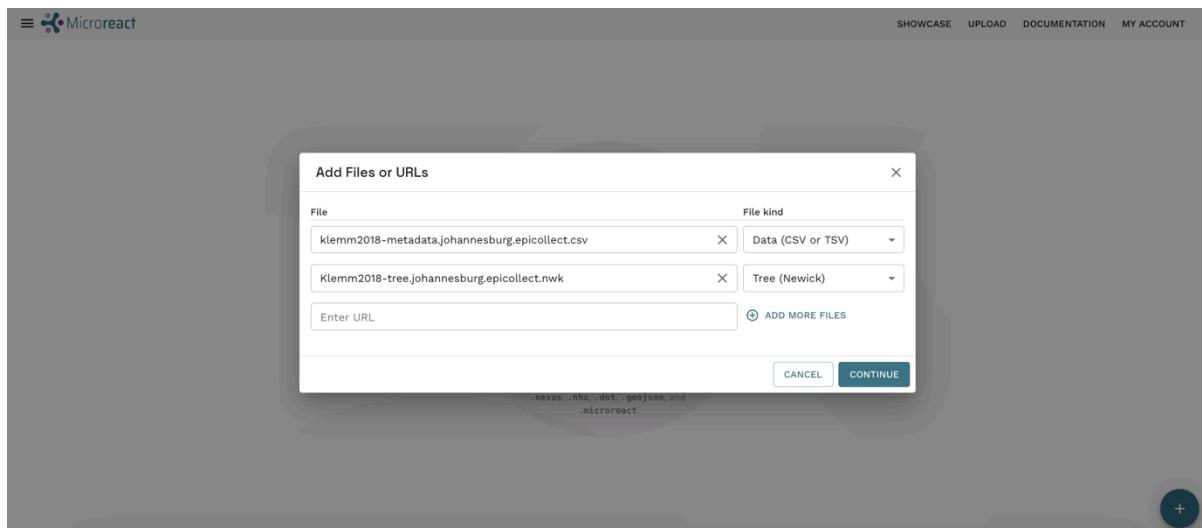
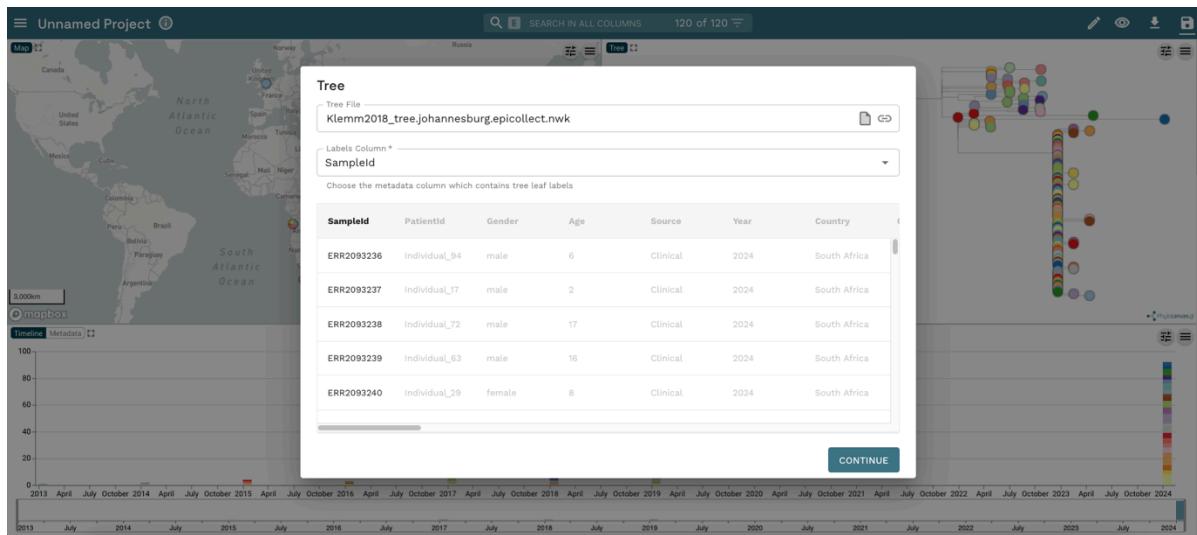


Figure 7 Editing EpiCollect entries using Excel.

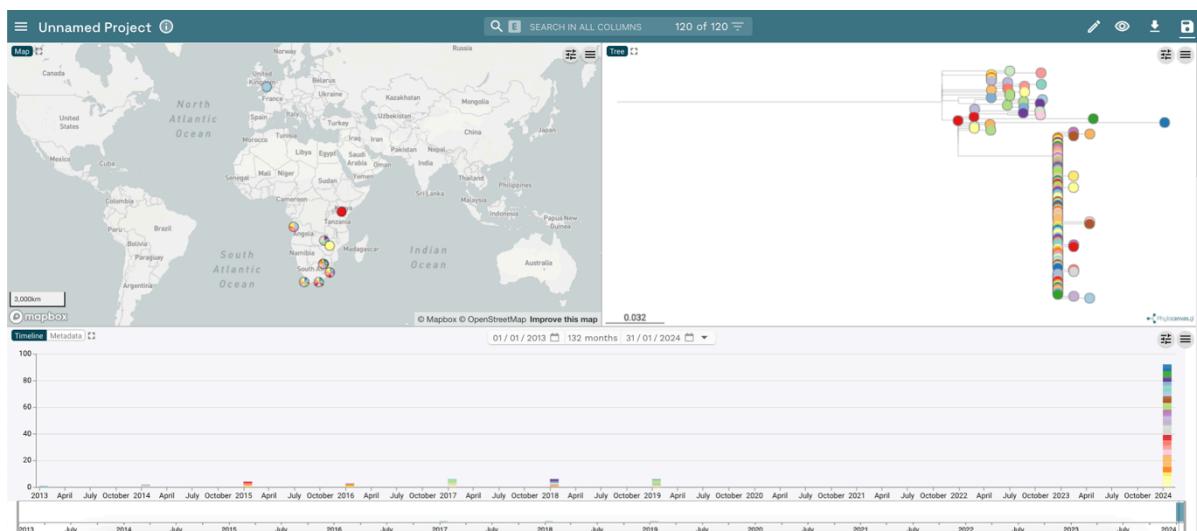
Once the tree and metadata files are loaded you will be directed to a new window where files will be automatically detected as Data (CSV or TSV) file (klemm2018\_metadata.johannesburg.epicollect.csv) and Tree (Newick) file (klemm2018\_tree.johannesburg.epicollect.nwk). In this new window click on

'Continue'. In the next window, make sure the column with samples' "ERR" codes is selected as the 'Labels Column' (Figure 8) and then click on 'Continue'.



**Figure 8 Data Table window in Microreact**

Once the form is completed your data will be utilized to create a MicroReact project. You should now have a view like the one shown in Figure 9.

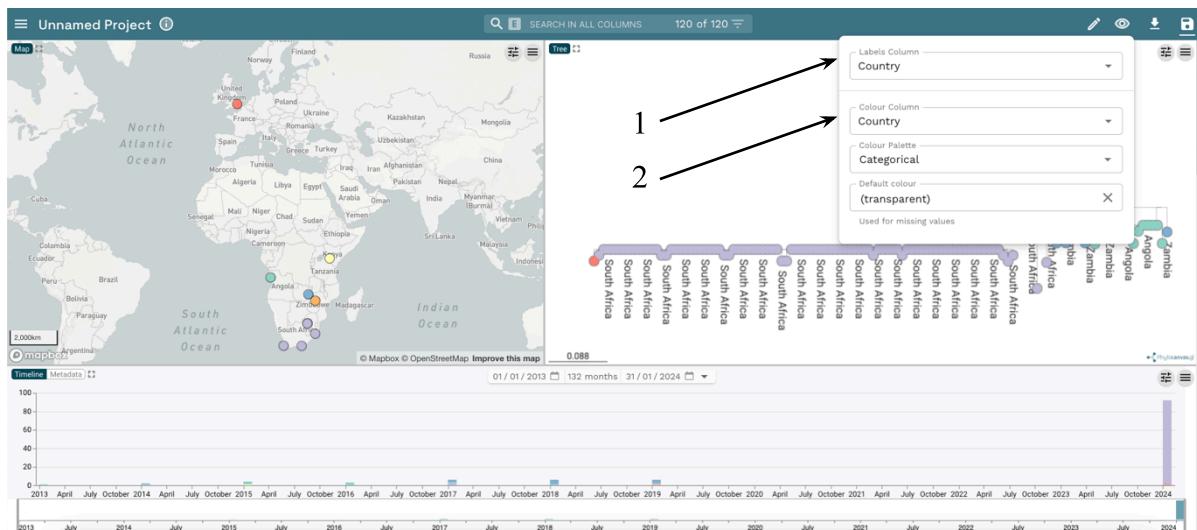


**Figure 9 Klemm2018 Johannesburg MicroReact project**

We will use the phylogeny and the map to identify the origin of the XDR *S. Typhi* cases in Johannesburg. We can reorient the tree horizontally to make it easier to view. Click the control panel symbol  then click the tree-view control button  then select the 'Hierarchical tree' button (the last option at the bottom). By default, the tree tips should be coloured by PatientId (as in Figure 9).

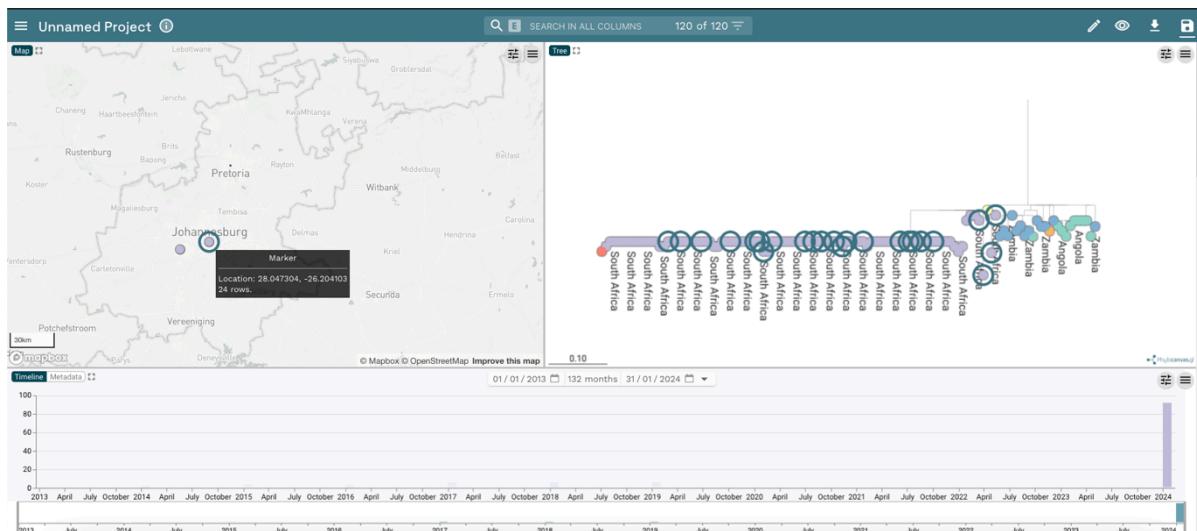
Click on the 'Labels, Colours, and Shapes'  icon and select 'Country' under the lists 'Labels Column' and 'Colour Column' (arrows 1 and 2 in Figure 10). The map view shows the colours assigned to each country. You can also click the 'Legend' tab

(top right) in the Microreact window to see a legend. It should be easy to see that most of the isolates from South Africa (shown in purple) cluster together, separately from strains from other countries.



**Figure 10 Colouring and labelling isolates on the tree**

Zoom in on the map and click on the isolates from Johannesburg to visualise where they are placed in the phylogenetic tree (Figure 11). Based on their location on the tree, is there evidence of a single-source outbreak or multiple circulating clones?



**Figure 11 Phylogenetic location of Johannesburg S. typhi isolates in the context of other African isolates.**

Click on the ‘Show controls’ button (arrow 1 in Figure 12) to then click the ‘Nodes & Labels’ menu (above the tree) and toggle the ‘Leaf Labels’ button (arrow 2) to switch on labelling (arrow 2). Change the size to 10px (using the sliding scale) to make it easier to see all the labels in one view.

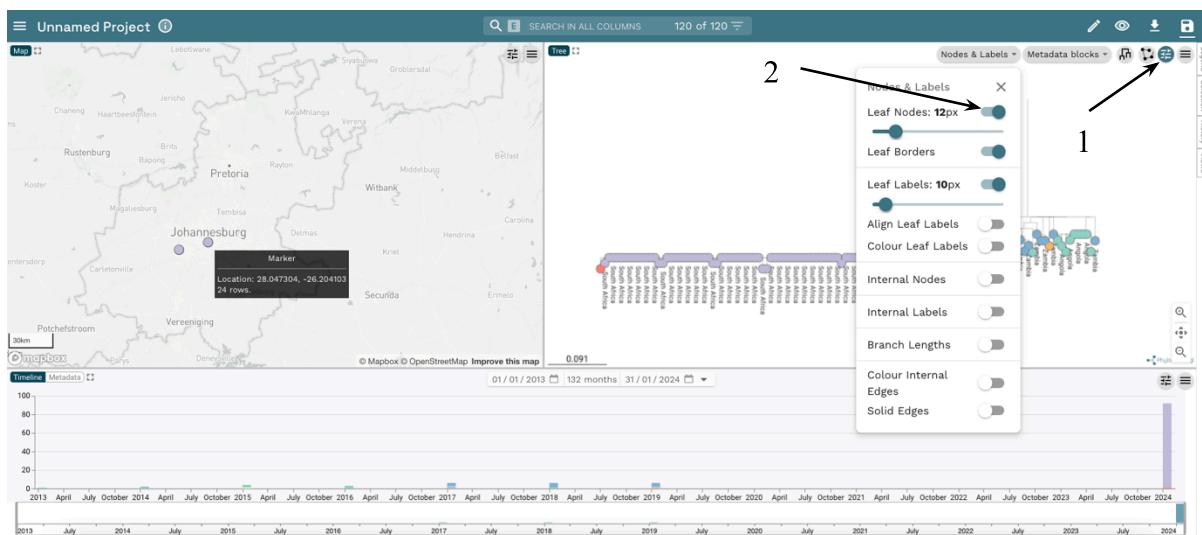


Figure 12 Keeping leaf labels visible on the tree.

#### 14.4 Identifying the origin and spread of extensively drug-resistant *S. typhi* in South Africa

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

Open the Timeline panel (arrow 1 in Figure 13), move the ‘From’ date and ‘To’ date bars to 01/01/2024 (circle 2) to highlight samples collected during 2024 on the tree and map.

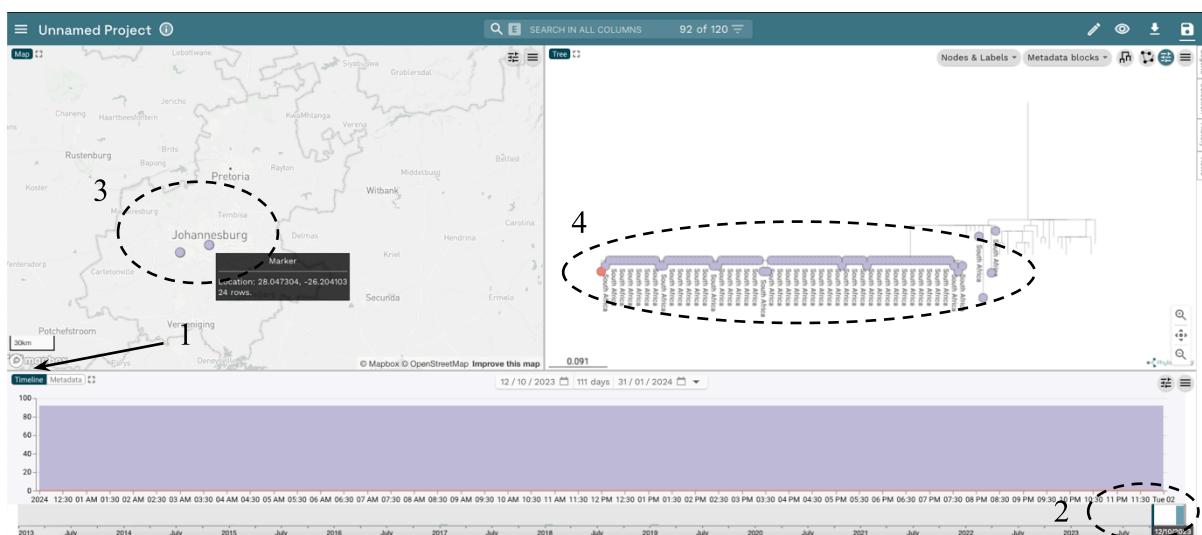
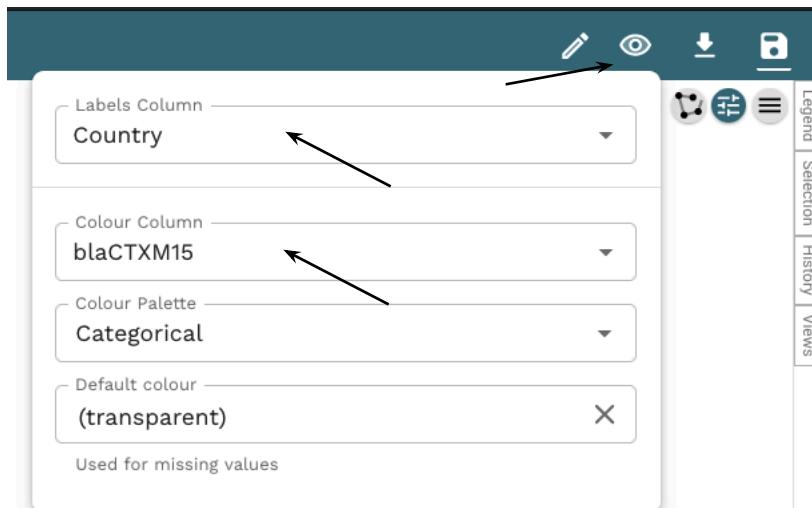


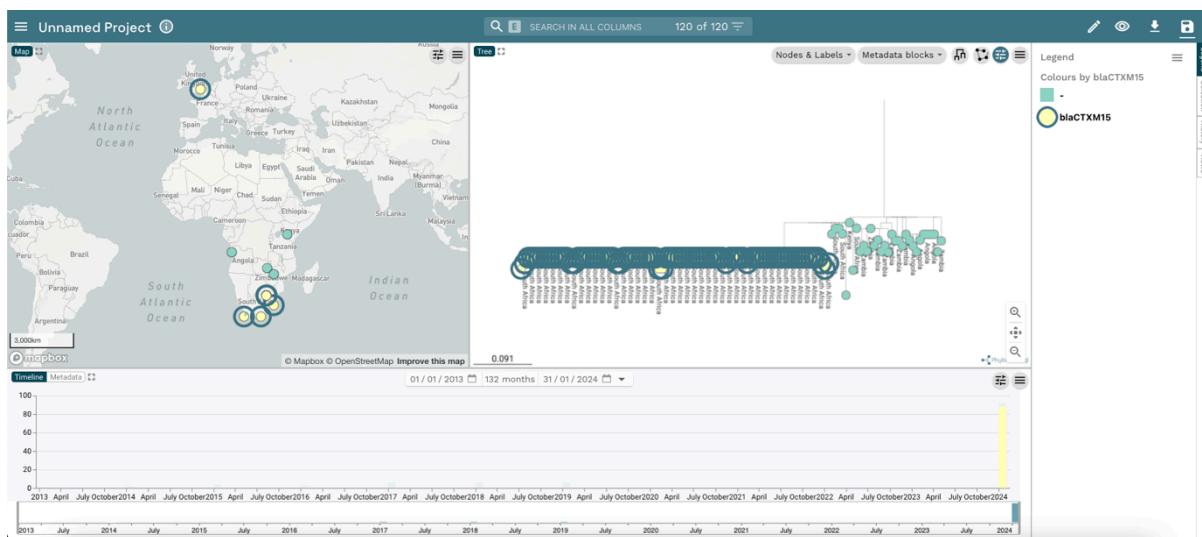
Figure 13 Phylogenetic and geographical location of *S. typhi* cases identified in 2024.

You should notice that most of samples collected during 2024 originated from South Africa (circle 3 in Figure 14.13), 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 (circle 4). This lack of genetic diversity and highly clonal nature of most of 2024 samples are indicative of a single point source outbreak.

One option is to colour the tips of the tree by a specific AMR gene of interest. Click the eye symbol top right and change ‘Colour Column’ to a resistance gene – for example, the blaCTXM15 gene, which is responsible for the phenotype of resistance to third generation cephalosporins such as ceftriaxone.



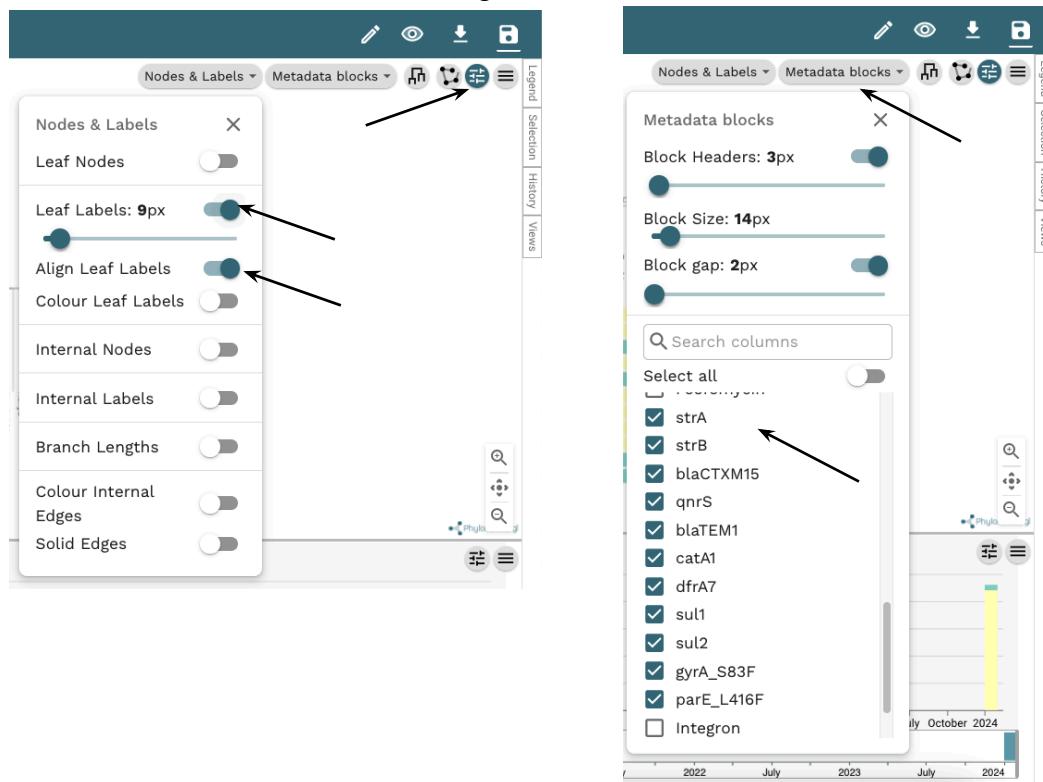
You can explore the distribution of other AMR determinants, such as the *qnrS* gene (plasmid-borne fluoroquinolone resistance), *catA1* gene (which confers resistance to chloramphenicol), *blaTEM-1* (ampicillin), *dfrA7* (trimethoprim), *sul1* and *sul2* (sulfamethoxazole), and *strA* and *strB* (streptomycin). Which ones are specific to the outbreak and which are common in other strains?



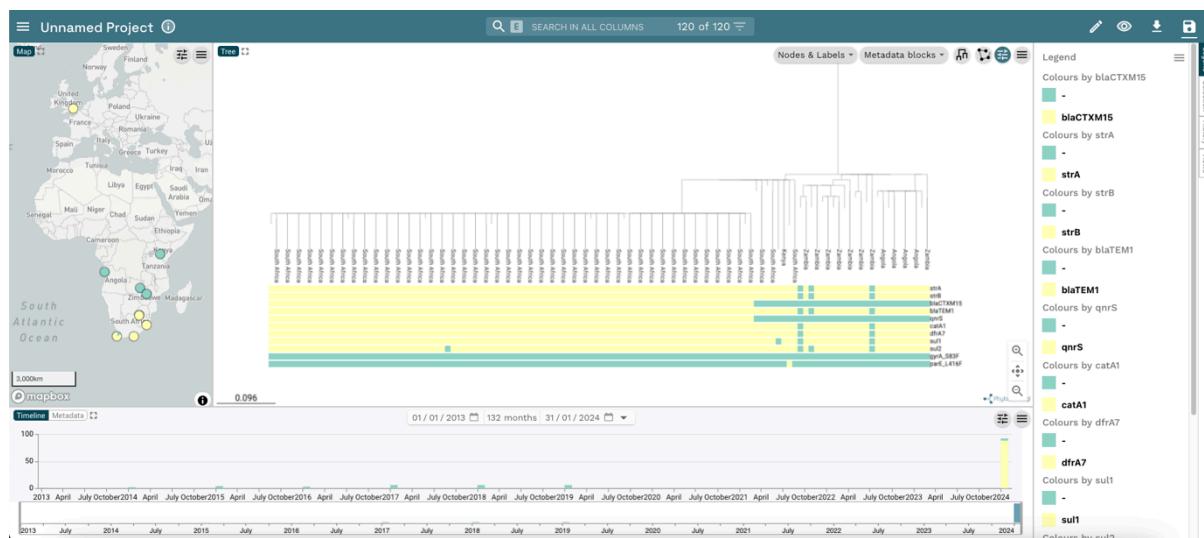
**Figure 14** Displaying the tree with a resistance determinant overlaid

It is also possible to view multiple variables against the tree simultaneously. First, click on the ‘Show controls’ button  and under ‘Nodes & Labels’ menu to toggle

both the ‘Leaf Labels’ and ‘Align Leaf Labels’ buttons  to switch on and make sure leaf labels are visible and aligned.



Next, click the ‘Metadata blocks’ menu button and check all the boxes that relate to resistance determinants (from blaCTXM15 down to parE\_L416F).



**Figure 15 AMR determinants displayed as metadata blocks.**

You will notice that almost all samples in the tree (including XDR outbreak strains) harbour *catA1* gene (chloramphenicol resistance), *blaTEM-1* (ampicillin), *dfrA7* (trimethoprim), *sul1* and *sul2* (sulfamethoxazole), and *strA* and *strB* (streptomycin).

All these genes are present in the same composite AMR transposon that is integrated into the chromosome.

The label ‘Integron’ indicates whether samples have this AMR transposon integrated or not. The stability of this AMR 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 into the chromosome, are inherited vertically. All samples also have the S83F mutation in *gyrA* which increases the minimum inhibitory concentration of fluoroquinolones.

The XDR outbreak clone 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 the presence of the plasmid either by adding ‘Plasmid’ to the list of variables shown in the ‘Metadata blocks’, or by setting the ‘Colour Column’ to ‘Plasmid’ (click on the eye symbol to get this menu).

Now let’s consider what is the most plausible country of origin of the XDR outbreak clone. First, set the ‘Colour Column’ back to ‘Country’ (click on the eye symbol to get this menu), to show the country of isolation of all samples on the tree. Second, identify the genetically closest strains to the outbreak clone (circled in Figure 16). You will notice that, except for one sample isolated from Kenya, these strains were sampled in South Africa between 2017 and 2024. The fact that the most genetically related strains to the XDR outbreak clone were also isolated in the same country earlier in time, suggest the outbreak clone derived from an endemic South African clone as opposed to being imported from a different country.



Figure 16 Identifying the origin of the *S. typhi* XDR outbreak clone.