
DTU

Simulated exercise on CPO outbreak – *Acinetobacter baumannii*

EURGen-RefLabCap
Virtual multidisciplinary training workshop
January-February 2024

Jette S. Kjeldgaard & Faisal Khan
(jetk@food.dtu.dk – fakh@food.dtu.dk)

Agenda for today (Jan 22, 2024)

- **10:00-10:15 Welcome and Introduction (Jette)**
  - Purpose of exercise
  - Introduction to typing methods; short review of species ID, AMR, cgMLST, MLST related to *A. baumannii*
- **10:15-10:30 Information about exercise (Faisal)**
  - Exercise setup, data, tasks, and question surveys

- **10:30 -10:55 Epilinx (Henrik Hasman, SSI)**
  - Visualization of patient metadata to investigate nosocomial transmission and outbreaks
  - Short coffee break

- **11:00- 11:25 Beta-lactam resistance in *Acinetobacter baumannii* (Valeria Bortolaia, SSI)**

- **11:30 – Summary and time for questions**

Simulated exercises - background

- Series of multidisciplinary training workshops 2022-2024
  - Sept/Oct 2022 – introduction to SNP analysis and cgMLST for cluster analysis (WS1)
  - May 2023 – Simulated exercise on outbreak analysis (*Klebsiella pneumoniae*; WS1)
  - Sept 2023– Simulated exercise on outbreak analysis (*Pseudomonas aeruginosa*; WS2)

  - **Jan 2024: Simulated exercises on outbreak analysis (*Acinetobacter baumannii;* WS2)**

  - Autumn 2024: Simulated exercises on outbreak analysis (WS1)

WS1: CCRE/ *E. coli* and *Klebsiella* spp.

WS2: CPO/ *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

https://www.cdc.gov/pulsenet/pathogens/protocol-images.html#wgs

Purpose of the workshop

- To build capacity to work with outbreak investigations
  - background information about bacterial subtyping and cluster analysis
  - Web-based bioinformatics tools to get started on bacterial phylogenetics and outbreak detection

- To work with larger sets of sequencing data and metadata and analyse outputs from typing and SNP analyses

- January 2024 iteration:
  - **More focus on epidemiological data and selection of isolates for sequencing**

The workshops build on the previous workshops

– video recordings of previous workshops are available

Brief introduction to *A. baumannii*

- *Acinetobacter baumannii* is a common cause of serious nosocomial infections
  - *A. baumannii* infections are almost exclusively nosocomial

- Multi-faced pathogen:
  - Some of the clinical manifestations of *A. baumannii* nosocomial infection are
    - pneumonia & lower respiratory tract infections
    - urinary tract infections,
    - wound infections; burn infections, skin and soft tissue infections
      - including necrotizing fasciitis;
    - bloodstream infections; meningitis; osteomyelitis; and endocarditis

- *A. baumannii* has an extraordinary genetic plasticity that results in a high capacity to acquire antimicrobial resistance traits and virulence factors

A. baumannii complex

- *Acinetobacter calcoaceticus-baumannii* complex (Acb-complex)
  - – *A. baumannii*
  - – *A. pittii*
  - – *A. nosocomialis*
  - – *A. calcoaceticus*

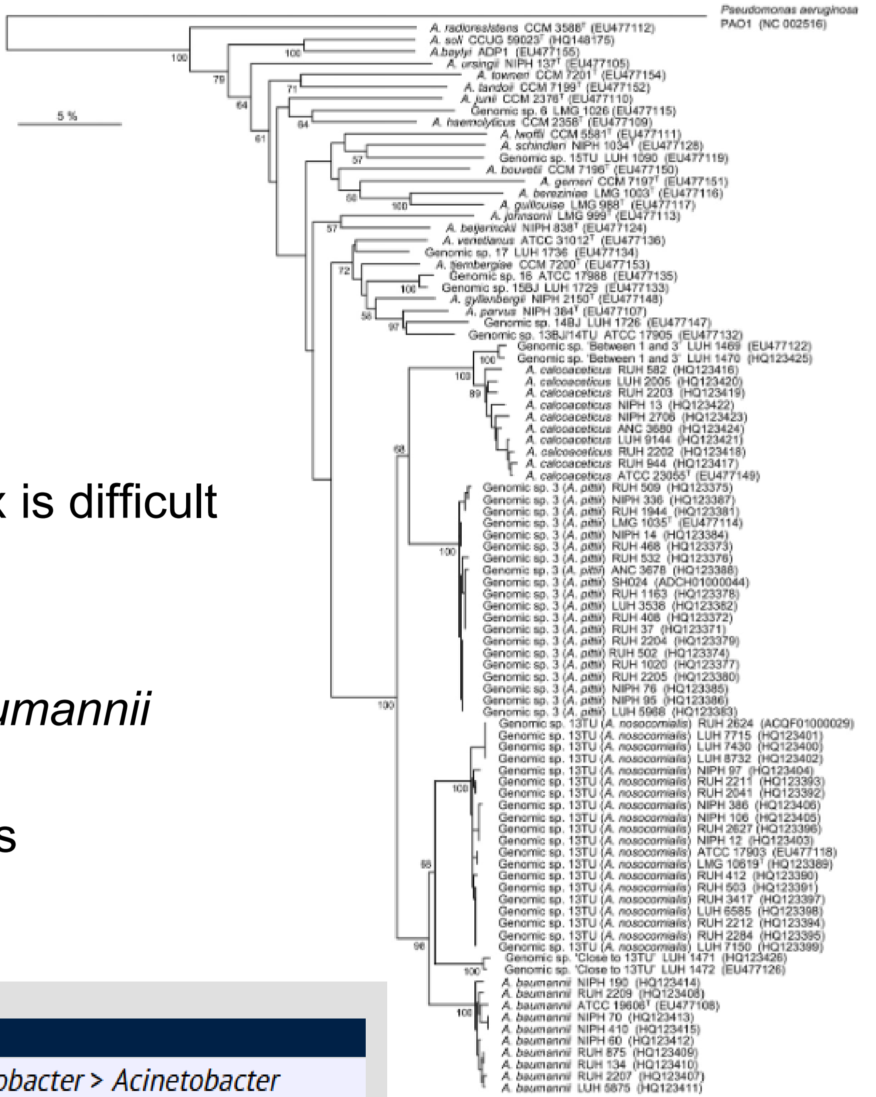
Later also added:

A. dijkshoorniae

A. seifertii

- Phenotypic differentiation between the species of the Acb-complex is difficult
  - – differ in antibiotic susceptibility and clinical outcomes
- MALDI-TOF MS is useful for the identification of *Acinetobacter baumannii* nosocomial outbreaks
- Genomic methods works well to differentiate *Acinetobacter* species
  - – (KmerFinder), rMLST:

Rank	Taxon	Support	Taxonomy
SPECIES	Acinetobacter baumannii	100%	*Pseudomonadota > Gammaproteobacteria > Moraxellales > Moraxellaceae > Acinetobacter > Acinetobacter baumannii*



https://doi.org/10.1016/j.resmic.2011.02.006 DOI: 10.1016/j.cmi.2016.11.020

A. baumannii subtyping by MLST and CC

PubMLST

https://pubmlst.org/organisms/acinetobacter-baumannii

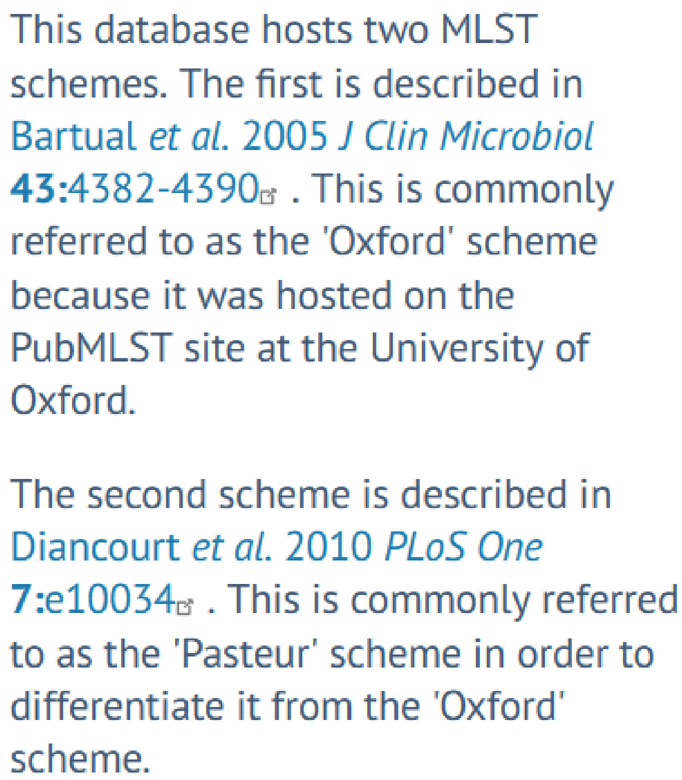
Subtyping by MLST

- The population structure of Acb has been studied using **two** MLST schemes
  - Bartual and coworkers (**Oxford scheme; 2005**)
  - Diancourt and coworkers (**Pasteur scheme; 2010**)

- Both are 7-gene MLST (covering 2895 and 2976 nucleotides)
- They have three genes in common (*cpn60*, *gltA*, and *recA*)
  - underlie two coexisting nomenclatures of sequence types and clonal complexes
  - complicates communication on *A. baumannii* genotypes

Division into clonal complexes

- International clones/clonal lineages



This database hosts two MLST schemes. The first is described in Bartual *et al.* 2005 *J Clin Microbiol* **43:**4382-4390. This is commonly referred to as the 'Oxford' scheme because it was hosted on the PubMLST site at the University of Oxford.

The second scheme is described in Diancourt *et al.* 2010 *PLoS One* **7:**e10034. This is commonly referred to as the 'Pasteur' scheme in order to differentiate it from the 'Oxford' scheme.

https://doi.org/10.3389

Which MLST scheme to use?

Recent comparative analysis: DOI:10.3389/fmicb.2019.00930

- The Pasteur scheme appears to be
  - less discriminant among closely related isolates
  - less affected by homologous recombination
  - more appropriate for precise strain classification in clonal groups

- The Oxford scheme has important issues:
  - *gdhB* paralogy
  - recombination and primers sequences
  - position of the genes on the genome

  - possibility of getting **two different ST's for same isolate**
  - the wrong calling of alleles at *gdhB2* locus has artifactually inflated the diversity recorded using the Oxford scheme

"Pasteur scheme is more appropriate for population biology and epidemiological studies of *A. baumannii* and related species, together with core genome MLST (cgMLST)"

Gaiarsa *et al.* 2019

A. baumannii species and subtyping for this exercise

- rMLST for species confirmation
- Two MLST schemes
  - Compare output of both
- cgMLST
- SNP analysis

Currently (as of Dec. 2023), due to a computer node breakdown, CGE tools are running on 25 % capacity

This affects also CSIPhylogeny in the coming weeks

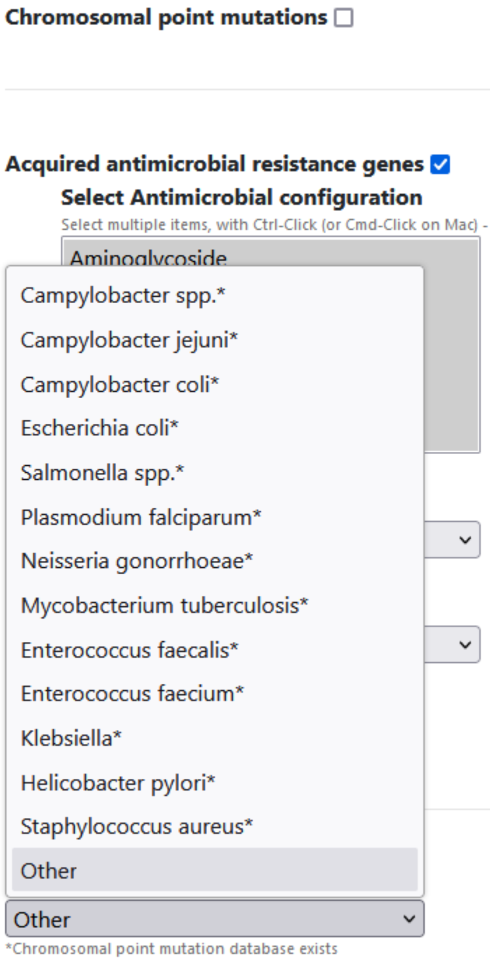
'New' ResFinder tool still running
http://genepi.food.dtu.dk/resfinder

- **Data from the above analyses will be provided for the exercise isolates**

Identification of resistance mechanisms

- ResFinder
  - – No direct database for *A. baumannii*
  - – > use *‘Other’*
  - – No PointFinder for *A. baumannii*
  - – > Use CARD-RGI, AMRFinderPlus or other tools and compare outputs

- Beta-lactam resistance more complicated
  - – You will hear more about resistance from Valeria later today



Chromosomal point mutations ☐

Acquired antimicrobial resistance genes ☑

Select Antimicrobial configuration

Select multiple items, with Ctrl-Click (or Cmd-Click on Mac) -

Other

*Chromosomal point mutation database exists

Examples of ResFinder vs. CARD outputs

ResFinder

Resistance gene	Phenotype
aac(3)-Ia	Gentamicin, Astromicin, Fortimicin
aadA1	Spectinomycin, Streptomycin
aph(3')-Ia	Kanamycin, Neomycin, Lividomycin, Paromomycin, Ribostamycin
aph(3'')-Ib	Streptomycin
aph(6)-Id	Streptomycin
blaADC-25	Unknown Beta-lactam
blaOXA-23	Imipenem, Meropenem
blaOXA-66	Unknown Beta-lactam
blaTEM-1D	Amoxicillin, Ampicillin, Cephalothin, Piperacillin, Ticarcillin
sul1	Sulfamethoxazole
sul2	Sulfamethoxazole
tet(B)	Doxycycline, Tetracycline, Minocycline

CARD

AAC(3)-Ia	aminoglycoside antibiotic
aadA	aminoglycoside antibiotic
ADC-30	cephalosporin
ANT(3'')-IIc	aminoglycoside antibiotic
APH(3')-Ia	aminoglycoside antibiotic
APH(3'')-Ib	aminoglycoside antibiotic
APH(6)-Id	aminoglycoside antibiotic
LpsB	peptide antibiotic
OXA-23	carbapenem, cephalosporin, penam
OXA-66	carbapenem, cephalosporin, penam
gyrA mut	fluoroquinolone antibiotic
parC mut	fluoroquinolone antibiotic
sul1	sulfonamide antibiotic
sul2	sulfonamide antibiotic
TEM-1	monobactam, cephalosporin, penam, penem
tet(B)	tetracycline antibiotic
tetR	tetracycline antibiotic

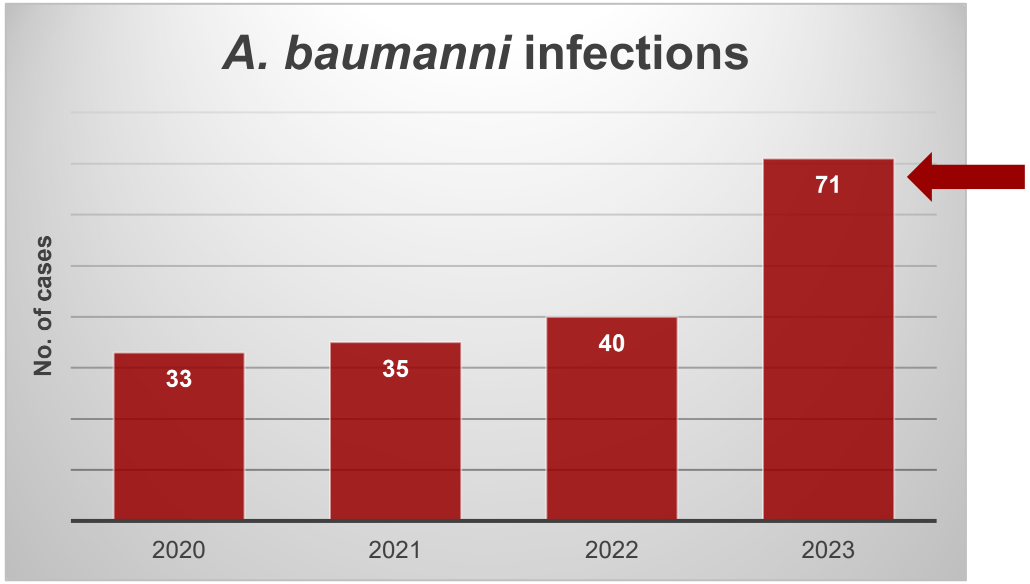
CARD

abeS	macrolide antibiotic, aminocoumarin antibiotic	antibiotic efflux
AbaF	phosphonic acid antibiotic	antibiotic efflux
AbaQ	fluoroquinolone antibiotic	antibiotic efflux
AmvA	macrolide antibiotic, disinfecting agents and antiseptics	antibiotic efflux
adeA	glycylcycline, tetracycline antibiotic	antibiotic efflux
adeF	fluoroquinolone antibiotic, tetracycline antibiotic	antibiotic efflux
adeG	fluoroquinolone antibiotic, tetracycline antibiotic	antibiotic efflux
adeH	fluoroquinolone antibiotic, tetracycline antibiotic	antibiotic efflux
adeI	macrolide antibiotic, fluoroquinolone antibiotic, lincosamide antibiotic, carbapenem, cephalosporin, tetracycline antibiotic, rifamycin antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic, penem	antibiotic efflux
adeJ	macrolide antibiotic, fluoroquinolone antibiotic, lincosamide antibiotic, carbapenem, cephalosporin, tetracycline antibiotic, rifamycin antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic, penem,	antibiotic efflux
adeK	macrolide antibiotic, fluoroquinolone antibiotic, lincosamide antibiotic, carbapenem, cephalosporin, tetracycline antibiotic, rifamycin antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic, penem	antibiotic efflux
adeL	fluoroquinolone antibiotic, tetracycline antibiotic	antibiotic efflux
adeR	glycylcycline, tetracycline antibiotic	antibiotic efflux
qacEdelta1	disinfecting agents and antiseptics	antibiotic efflux

Scenario

Location: Country M, Europe

- *“In 2023, an increasing number of Acinetobacter baumannii infections has been observed and referred to the National Reference Laboratory (NRL). The majority of these are caused by carbapenem resistant A. baumannii (CRAB). Several hospitals in different cities have asked for assistance to investigate the possibility of one or more outbreaks, and the NRL has urged hospitals to share epidemiological and patient data of the cases. The NRL also requested the hospitals to send the isolates to the NRL for reference testing and whole genome sequencing (WGS) for the retrospective investigation of possible outbreak(s)”.*



Fictitious

Scenario-Roles

Outbreak Management Team (OMT)

- Inter-hospital communication
- Patient health records
- Epidemiological and surveillance data (e.g. movements and contacts of cases)
- Laboratory data (e.g. whole genome sequencing and AST)

Participant's Role

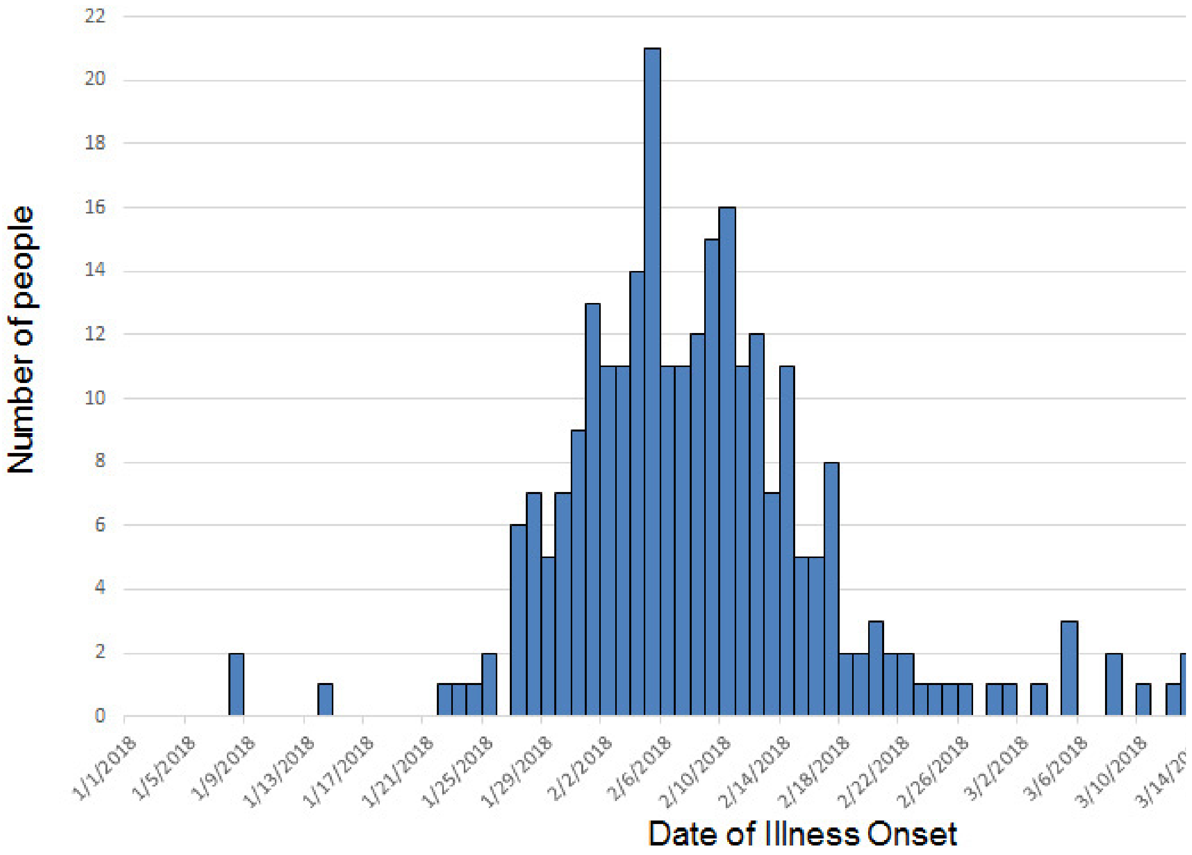
The exercise participants role is to support OMT in the analyses of epidemiology and laboratory data (including WGS data) to generate a hypothesis of the most likely exposure that has caused the outbreak.

Scenario - Tasks in the exercise

- Epidemiological, surveillance, and laboratory data from six hospitals will be used for retrospective outbreak investigation
  - Hospital A being the largest in the region

- Step 1: Investigate the epidemiological and surveillance data
  - Epidemiological curve
  - Confirm possible outbreak
  - Select isolates for whole genome sequencing

- Step 2: Sequence and cluster analysis of selected isolates
  - Confirm the existence of outbreak(s)
  - Identify clusters and possible source



An epidemic curve, also known as an epi curve, shows the number of illnesses in an outbreak over time.

Injects 1.1 to 1.3

22 Jan, 2024

-Introduction to the exercise/scenario

-Brief overview of AMR in *A. baumannii*

-Epilinx, tool for the descriptive analysis of epidemiological data

Inject 1.1 (Introduction)

The epidemiological, surveillance, and laboratory data of *A. baumannii* cases from 6 hospitals will be available.

Actions: Identify possible outbreak isolates

Inject 1.2

Survey 1

Actions: Submit answers by 26 January, 12:00 CET

Inject 1.3

Inject 2.1 and 2.2

26 Jan, 2024

WGS data from hospital A will be available, **the largest hospital in the country**

Actions: cluster analysis, MLST, Identify carbapenemase gene

Inject 2.1

Survey 2

Action: Submit answers by 05 February, 12:00 CET

Inject 2.2

Injects 3.1 to 3.2

31 Jan, 2024

WGS data from hospital B and C will be available

Actions: cluster analysis

Inject 3.1

WGS data from hospital D, E and F will be available

Actions: cluster analysis

Inject 3.2

Inject 4

06 Feb, 2024

-Conclusion of the Exercise

-Additional tools for *A. baumannii* WGS analysis

Questions

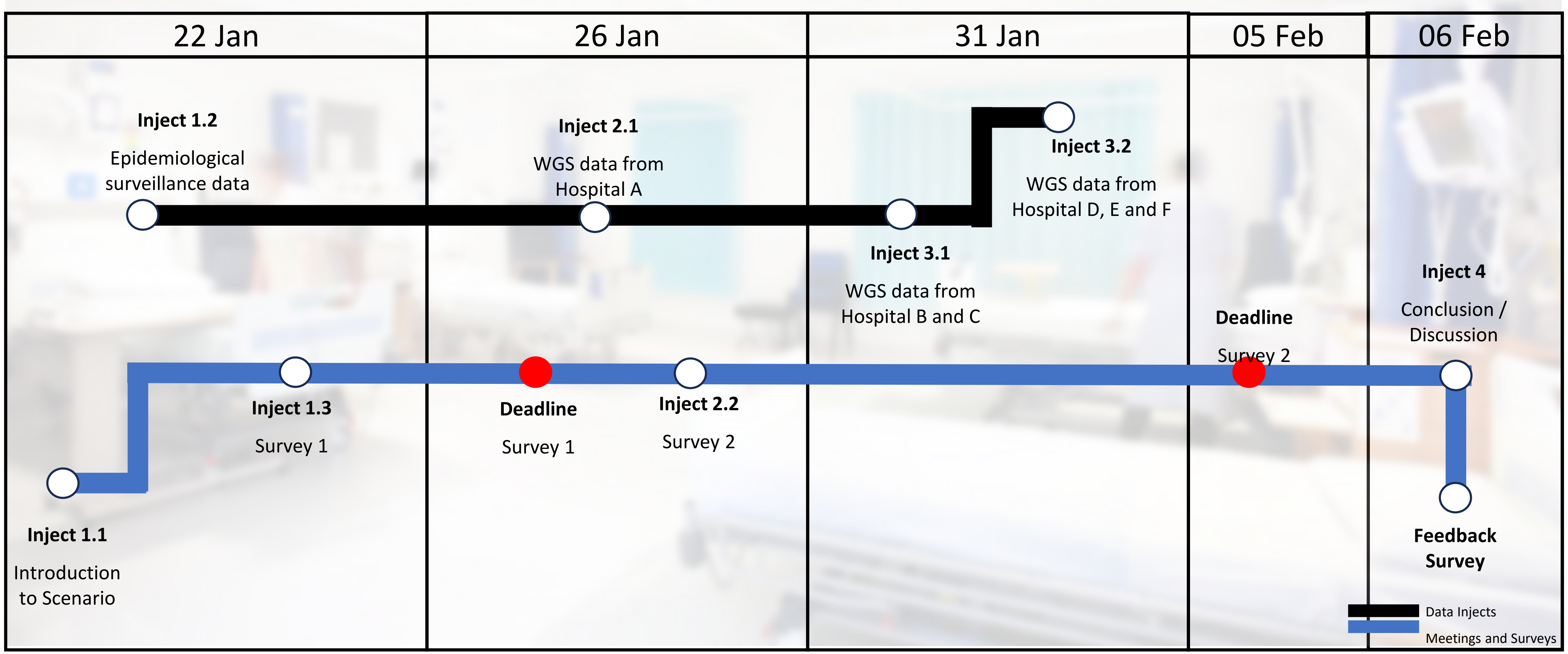
Inject 4

Survey questions

- Epidemiological curve
- MLST’s and cgMLST
- How many clusters?
- Part of a cluster?
  - How many SNPs difference within core-cluster?
- Possible source of the outbreak?
  - Local spread or travel related

Questions/Comments?

Exercise overview



22 Jan

Inject 1.1
Introduction to Scenario

Inject 1.2
Epidemiological surveillance data

Inject 1.3
Survey 1

26 Jan

Deadline
Survey 1

Inject 2.1
WGS data from Hospital A

Inject 2.2
Survey 2

31 Jan

Inject 3.1
WGS data from Hospital B and C

Inject 3.2
WGS data from Hospital D, E and F

05 Feb

Deadline
Survey 2

06 Feb

Inject 4
Conclusion / Discussion

Feedback Survey

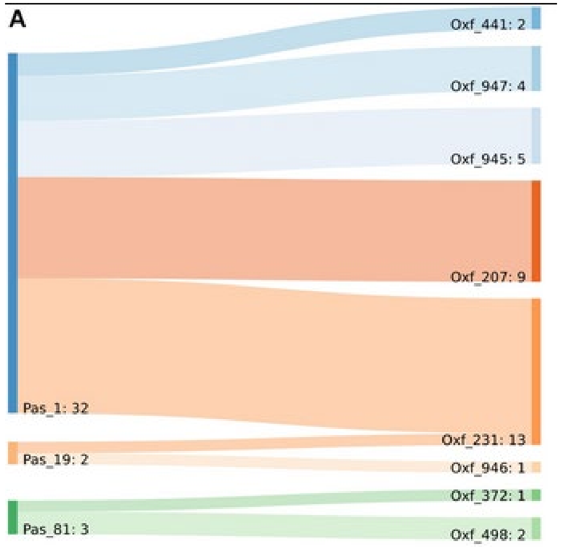
Data Injects
Meetings and Surveys

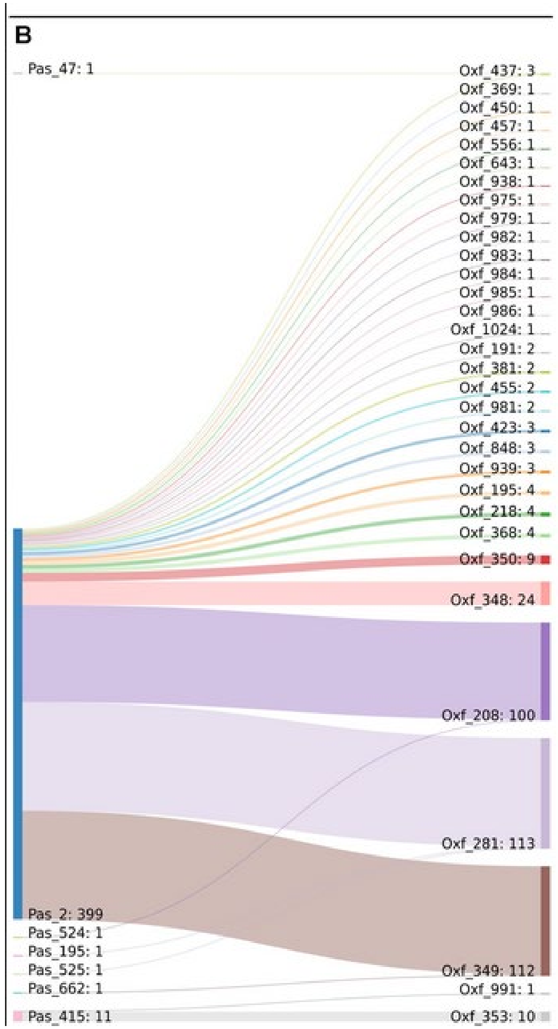
A. baumannii subtyping by MLST

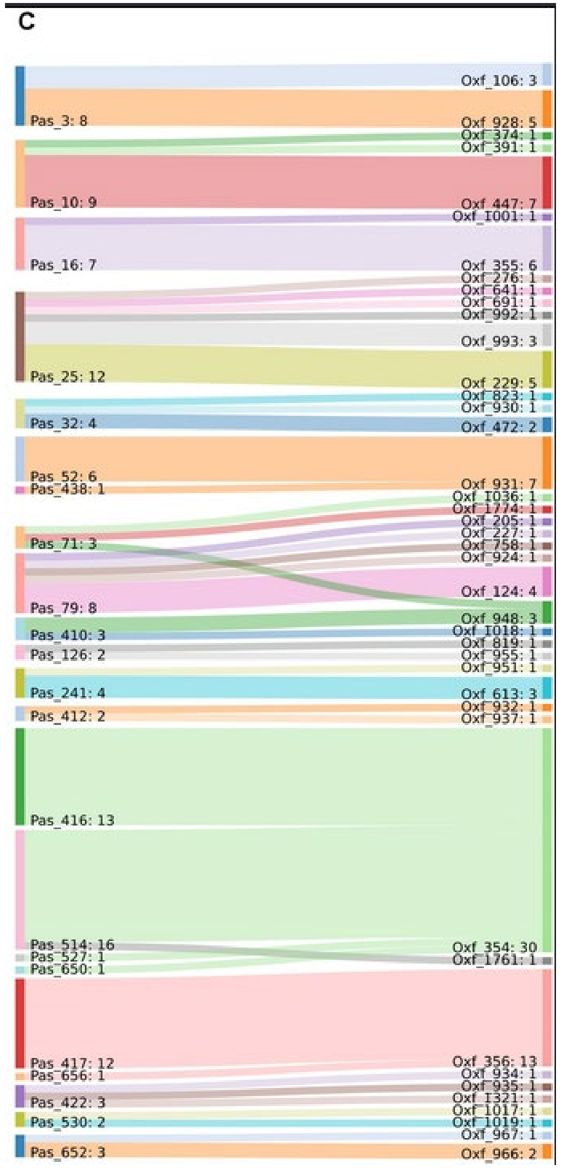
Correspondance between the two MLST schemes:

- Sankey diagram of the MLST classification of the 730 genomes in use, as performed with the Pasteur and Oxford schemes. Two-way corresponding STs were removed to improve image clarity. Captions show the corresponding STs belonging to **(A)** International Clone 1, **(B)** International Clone 2, and **(C)** all the other genomes

https://doi.org/10.3389







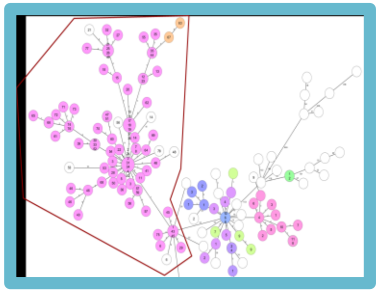
EpiLinx

A software to visualize patient networks for outbreak detection

Henrik Hasman
Senior scientist / Molecular microbiologist
Statens Serum Institut (SSI), Denmark

AM I A MOLECULAR EPIDEMIOLOGIST?

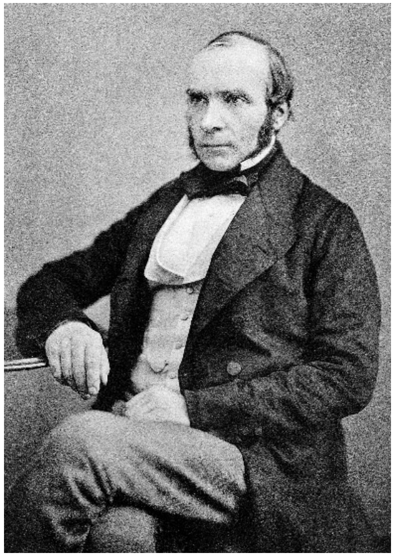
Epidemiology



Molecular typing

Evasive action

Central dogma of (hospital) epidemiology



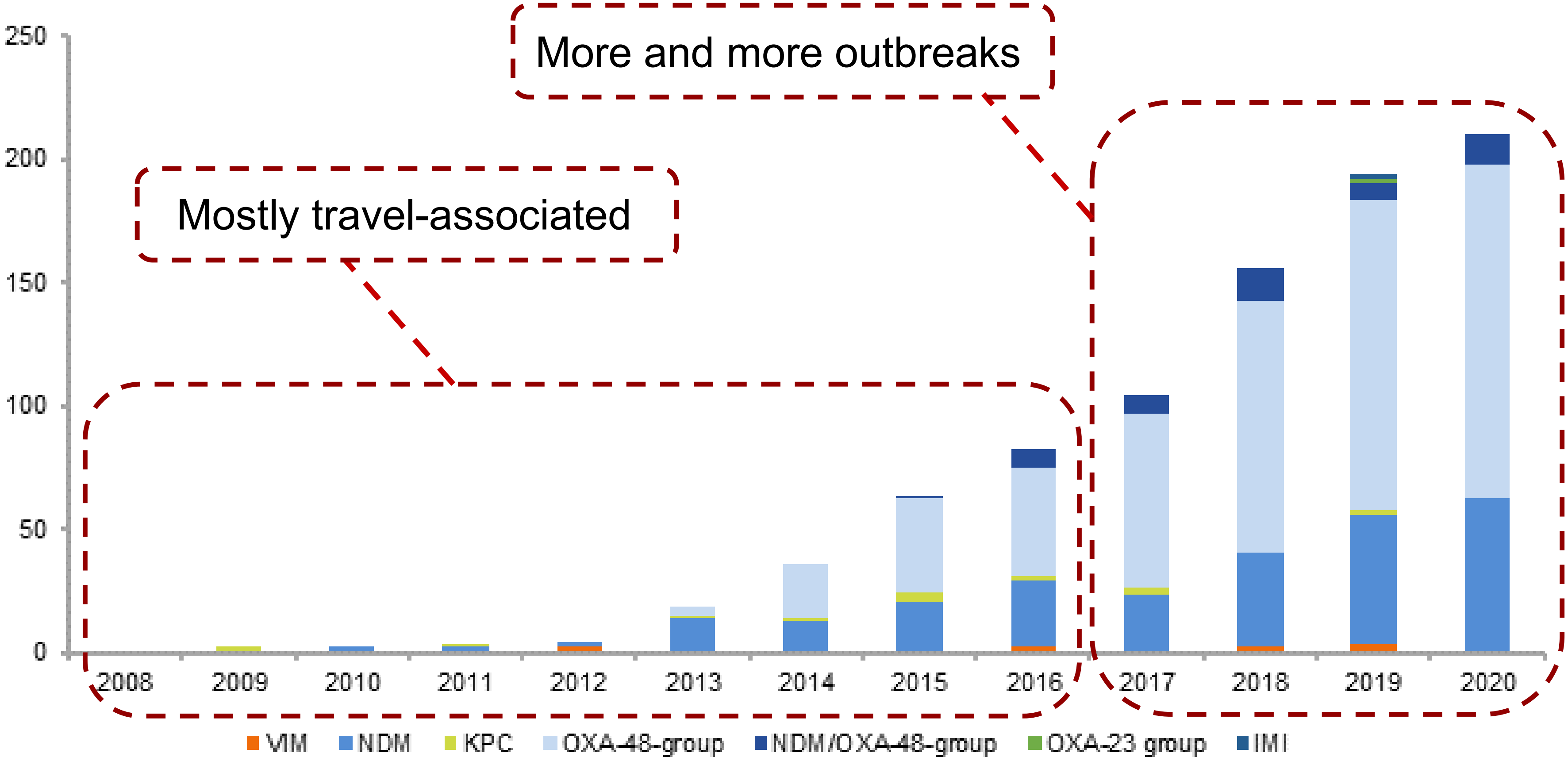
From genomes we get:

- Typing information about the bacteria
- But no information about the patient carrying this bacteria

The epidemiologist ask for:

- **D**isease & symptoms
- **H**ospitalization events
- **A**ge & gender
- **T**ravel history
- **A**dditional information…..

CPO* NATIONAL SURVEILLANCE IN DENMARK



All isolates are submitted to WGS (Illumina) and analyzed (Ridom SeqSphere+) to detect genomic (clonal) clusters across departments, hospitals and regions.

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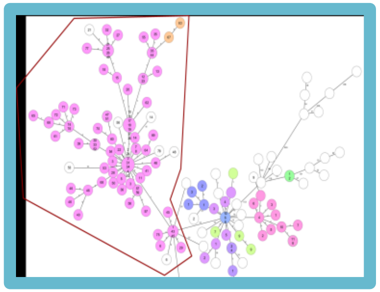
* Carbapenemase producing organisms

NOSOCOMIAL OUTBREAKS



Molecular typing

Epidemiology

Evasive action

Revised central dogma of (hospital) epidemiology

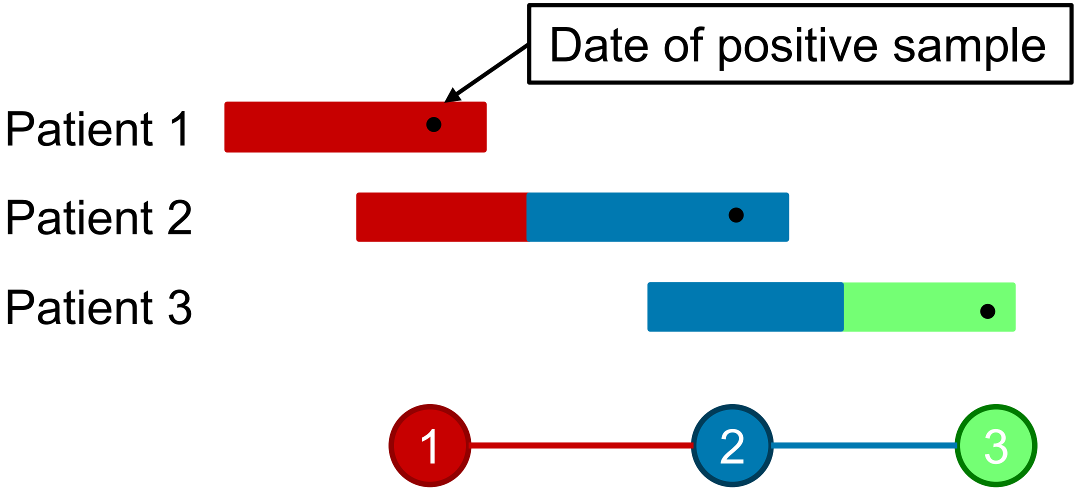
Direct chain of transmission

Patients have pairwise been at the same department on the same date

Indirect chain of transmission

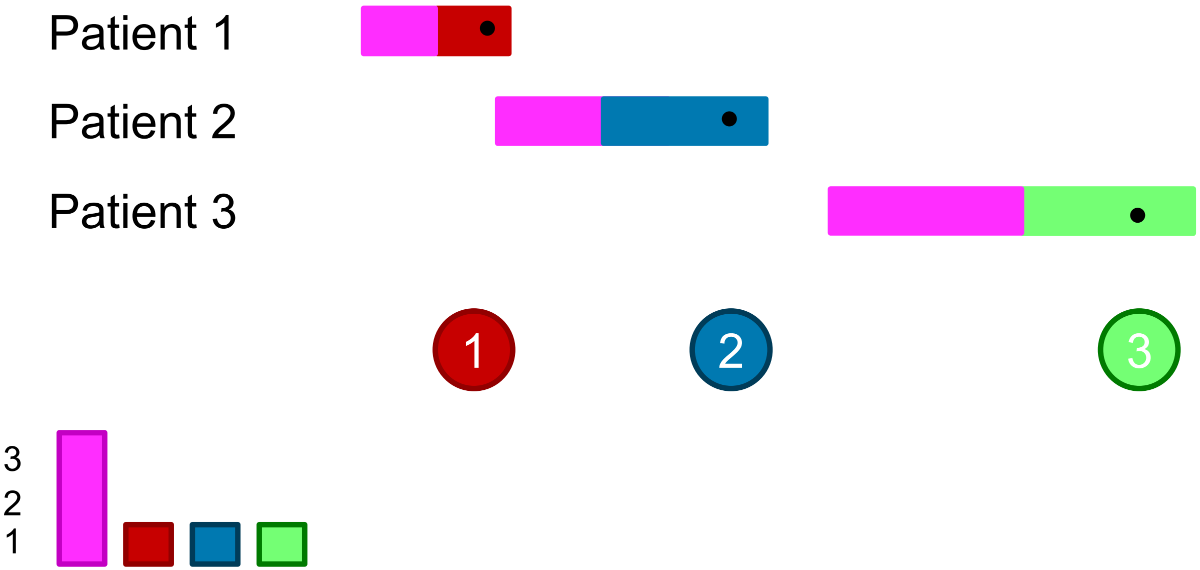
Patients have been at the same (single) department but not on the same date

DIRECT VS INDIRECT TRANSMISSION



Direct chain of transmission

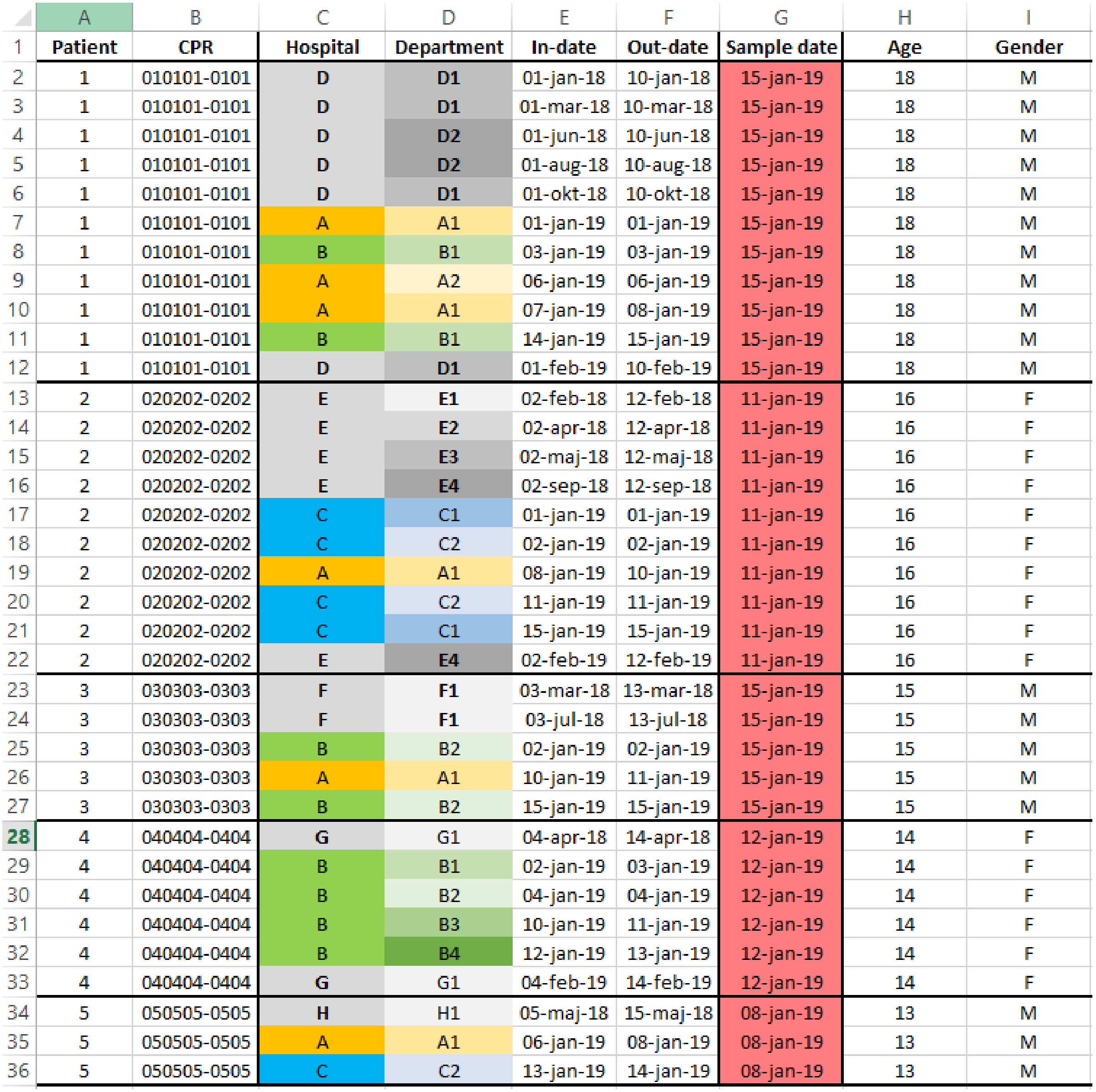
Patients have pairwise been at the same department on the same date



Indirect chain of transmission

Patients have been at the same (single) department but not on the same date

PATIENT INFORMATION INPUT TO EPILINX



	A	B	C	D	E	F	G	H	I
1	Patient	CPR	Hospital	Department	In-date	Out-date	Sample date	Age	Gender
2	1	010101-0101	D	D1	01-jan-18	10-jan-18	15-jan-19	18	M
3	1	010101-0101	D	D1	01-mar-18	10-mar-18	15-jan-19	18	M
4	1	010101-0101	D	D2	01-jun-18	10-jun-18	15-jan-19	18	M
5	1	010101-0101	D	D2	01-aug-18	10-aug-18	15-jan-19	18	M
6	1	010101-0101	D	D1	01-okt-18	10-okt-18	15-jan-19	18	M
7	1	010101-0101	A	A1	01-jan-19	01-jan-19	15-jan-19	18	M
8	1	010101-0101	B	B1	03-jan-19	03-jan-19	15-jan-19	18	M
9	1	010101-0101	A	A2	06-jan-19	06-jan-19	15-jan-19	18	M
10	1	010101-0101	A	A1	07-jan-19	08-jan-19	15-jan-19	18	M
11	1	010101-0101	B	B1	14-jan-19	15-jan-19	15-jan-19	18	M
12	1	010101-0101	D	D1	01-feb-19	10-feb-19	15-jan-19	18	M
13	2	020202-0202	E	E1	02-feb-18	12-feb-18	11-jan-19	16	F
14	2	020202-0202	E	E2	02-apr-18	12-apr-18	11-jan-19	16	F
15	2	020202-0202	E	E3	02-maj-18	12-maj-18	11-jan-19	16	F
16	2	020202-0202	E	E4	02-sep-18	12-sep-18	11-jan-19	16	F
17	2	020202-0202	C	C1	01-jan-19	01-jan-19	11-jan-19	16	F
18	2	020202-0202	C	C2	02-jan-19	02-jan-19	11-jan-19	16	F
19	2	020202-0202	A	A1	08-jan-19	10-jan-19	11-jan-19	16	F
20	2	020202-0202	C	C2	11-jan-19	11-jan-19	11-jan-19	16	F
21	2	020202-0202	C	C1	15-jan-19	15-jan-19	11-jan-19	16	F
22	2	020202-0202	E	E4	02-feb-19	12-feb-19	11-jan-19	16	F
23	3	030303-0303	F	F1	03-mar-18	13-mar-18	15-jan-19	15	M
24	3	030303-0303	F	F1	03-jul-18	13-jul-18	15-jan-19	15	M
25	3	030303-0303	B	B2	02-jan-19	02-jan-19	15-jan-19	15	M
26	3	030303-0303	A	A1	10-jan-19	11-jan-19	15-jan-19	15	M
27	3	030303-0303	B	B2	15-jan-19	15-jan-19	15-jan-19	15	M
28	4	040404-0404	G	G1	04-apr-18	14-apr-18	12-jan-19	14	F
29	4	040404-0404	B	B1	02-jan-19	03-jan-19	12-jan-19	14	F
30	4	040404-0404	B	B2	04-jan-19	04-jan-19	12-jan-19	14	F
31	4	040404-0404	B	B3	10-jan-19	11-jan-19	12-jan-19	14	F
32	4	040404-0404	B	B4	12-jan-19	13-jan-19	12-jan-19	14	F
33	4	040404-0404	G	G1	04-feb-19	14-feb-19	12-jan-19	14	F
34	5	050505-0505	H	H1	05-maj-18	15-maj-18	08-jan-19	13	M
35	5	050505-0505	A	A1	06-jan-19	08-jan-19	08-jan-19	13	M
36	5	050505-0505	C	C2	13-jan-19	14-jan-19	08-jan-19	13	M

Patient 1

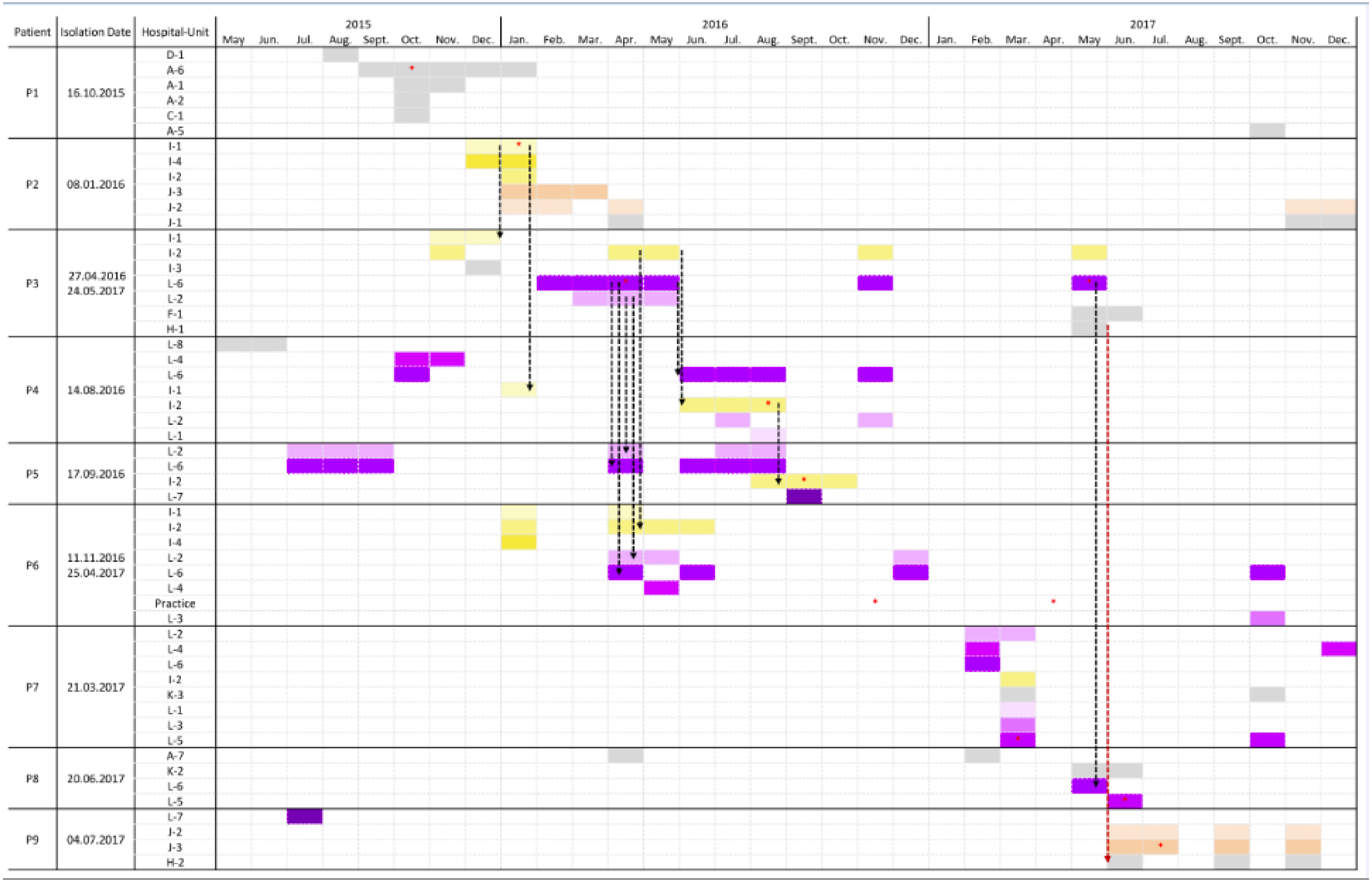
Patient 2

Patient 3

Patient 4

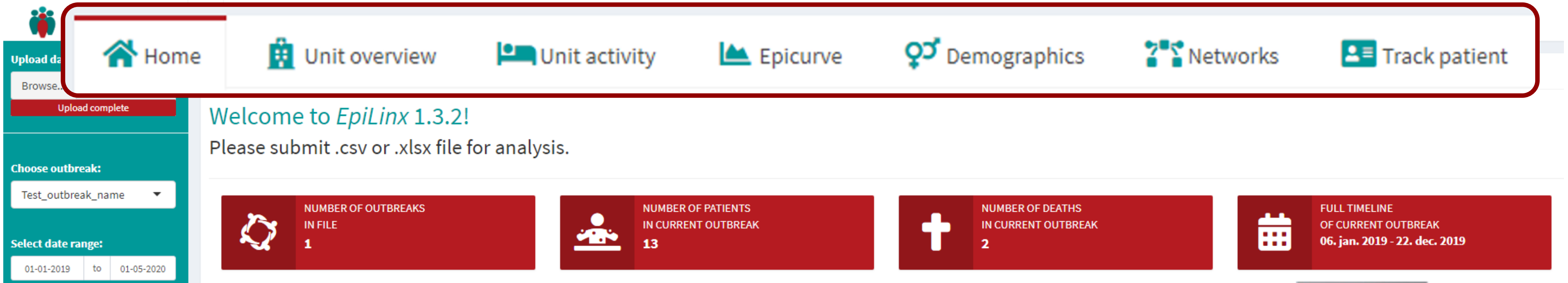
Patient 5

EPIDEMIOLOGICAL INVESTIGATIONS



Patient	Isolation Date	Hospital-Unit
P1	16.10.2015	D-1
		A-6
		A-1
		A-2
		C-1
		A-5
P2	08.01.2016	I-1
		I-4
		I-2
		J-3
		J-2
		J-1
P3	27.04.2016 24.05.2017	I-1
		I-2
		I-3
		L-6
		L-2
		F-1
		H-1
P4	14.08.2016	L-8
		L-4
		L-6
		I-1
		I-2
		L-2
		L-1
P5	17.09.2016	L-2
		L-6
		I-2
		L-7
P6	11.11.2016 25.04.2017	I-1
		I-2
		I-4
		L-2
		L-6
		L-4
		Practice
		L-3
P7	21.03.2017	L-2
		L-4
		L-6
		I-2
		K-3
		L-1
		L-3
		L-5
P8	20.06.2017	A-7
		K-2
		L-6
		L-5
P9	04.07.2017	L-7
		J-2
		J-3
		H-2

EPILINX – A TOOL FOR MAPPING PATIENT NETWORKS



Upload da

Upload complete

Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

Choose outbreak: Test_outbreak_name

Select date range: 01-01-2019 to 01-05-2020

Select Region(s): Alpha Beta Delta Epsilon

Select no. of days between admissions: 14

Refresh

NUMBER OF OUTBREAKS IN FILE **1**

NUMBER OF PATIENTS IN CURRENT OUTBREAK **13**

NUMBER OF DEATHS IN CURRENT OUTBREAK **2**

FULL TIMELINE OF CURRENT OUTBREAK **06. jan. 2019 - 22. dec. 2019**

https://nrar.shinyapps.io/epilinx_web/

Anna Emilie Henius

Statens Serum Institut

Generic “*base R*” software tool (“*Shiny*” tool package)

(Under development)

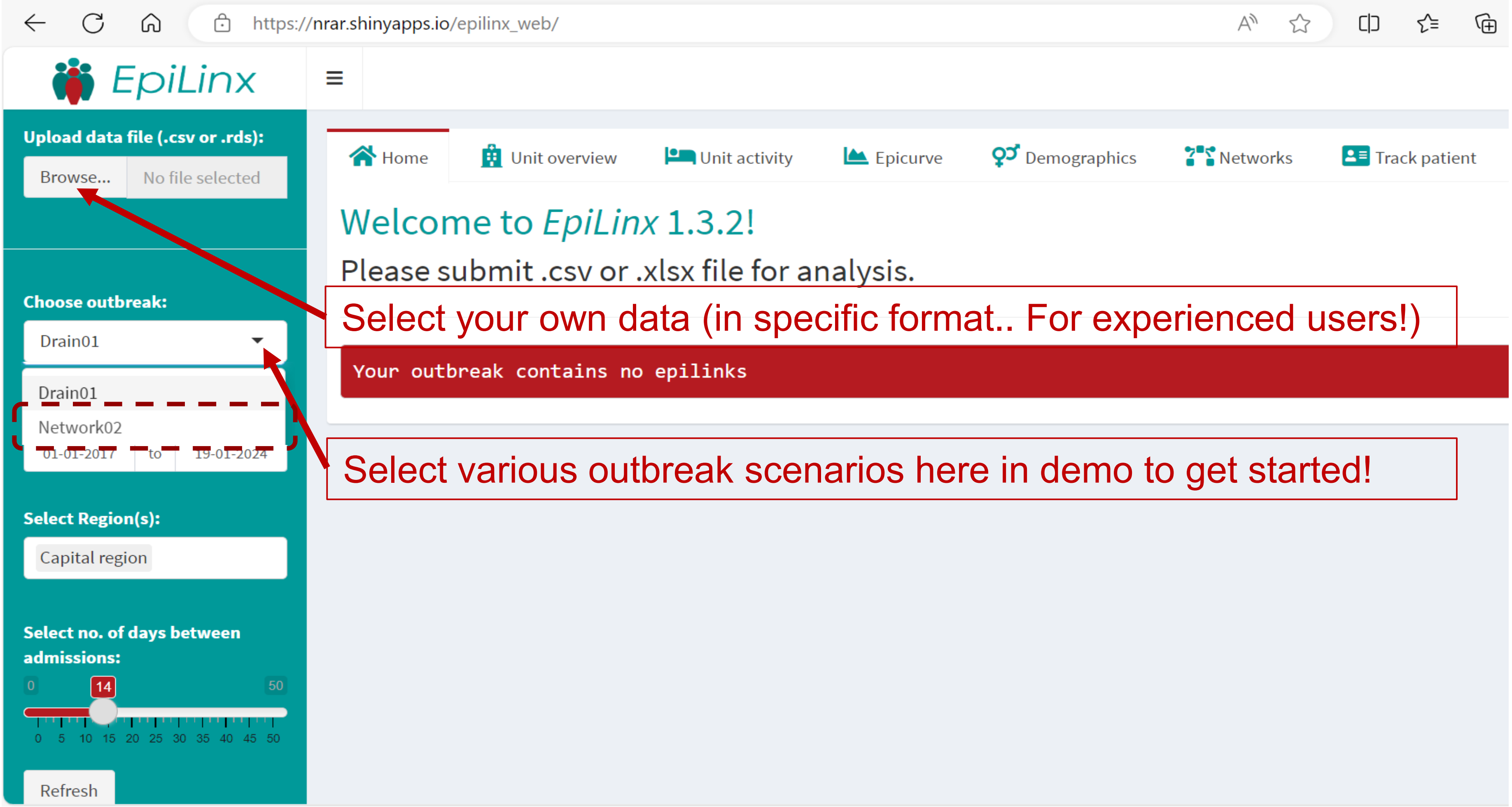
Input data:

- Patient hospitalization information (National or hospital)
- Other epidemiological data (age, gender, sample date & date of death)

Output:

- Graphical views and tables sorting complex patient and department data

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/



Upload data file (.csv or .rds): No file selected

Choose outbreak: Drain01

01-01-2017 to 19-01-2024

Select Region(s): Capital region

Select no. of days between admissions: 14

Welcome to *EpiLinx* 1.3.2!

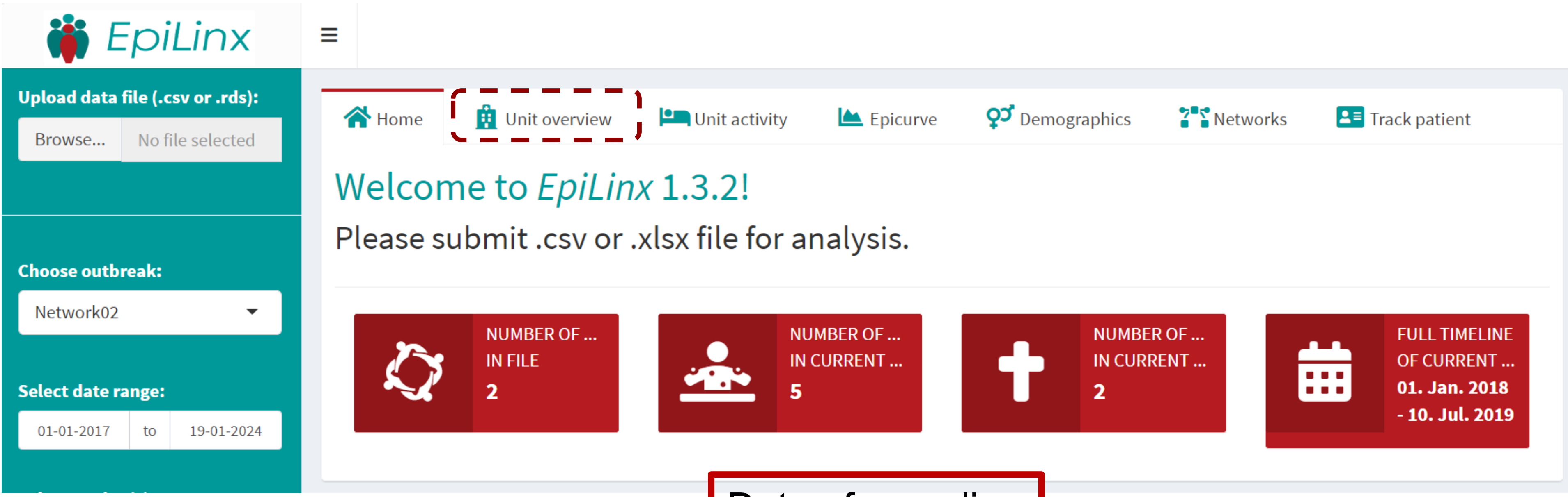
Please submit .csv or .xlsx file for analysis.

`Your outbreak contains no epilinks`

Select your own data (in specific format.. For experienced users!)

Select various outbreak scenarios here in demo to get started!

EXAMPLE #2 – DIRECT CONTACT



Upload data file (.csv or .rds):

Browse... No file selected

Choose outbreak:

Network02

Select date range:

01-01-2017 to 19-01-2024

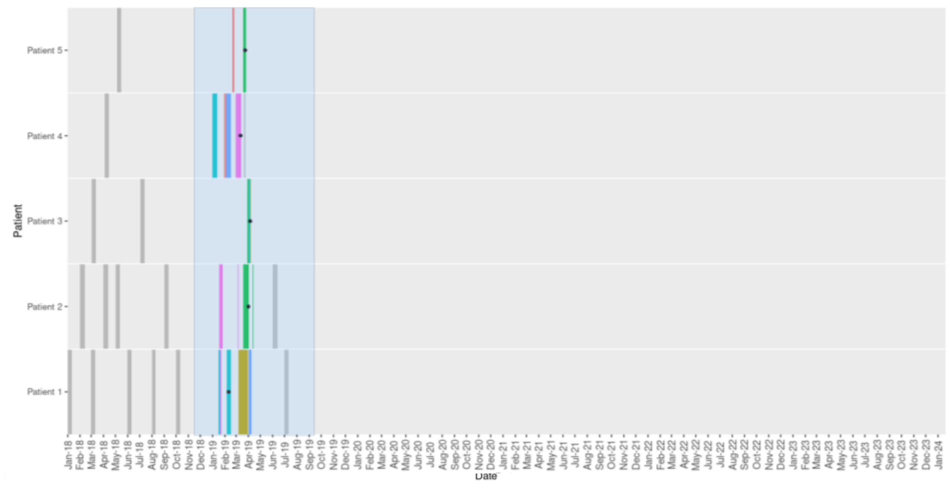
Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

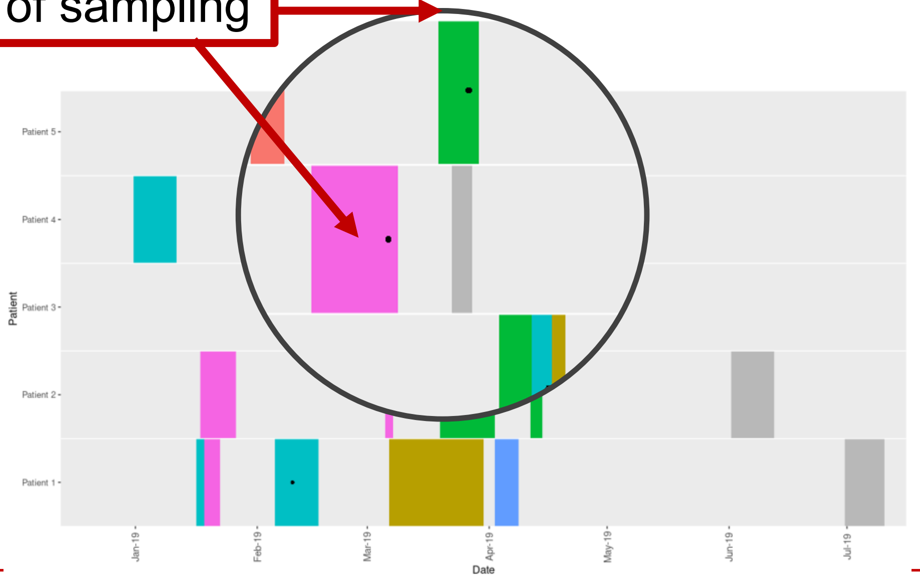
NUMBER OF ... IN FILE	2
NUMBER OF ... IN CURRENT ...	5
NUMBER OF ... IN CURRENT ...	2
FULL TIMELINE OF CURRENT ...	**01. Jan. 2018 - 10. Jul. 2019**

● Unit ○ Ward ○ Hospital

Mouse over datapoint in plot to show values of patient ID, date and unit respectively!



Date of sampling



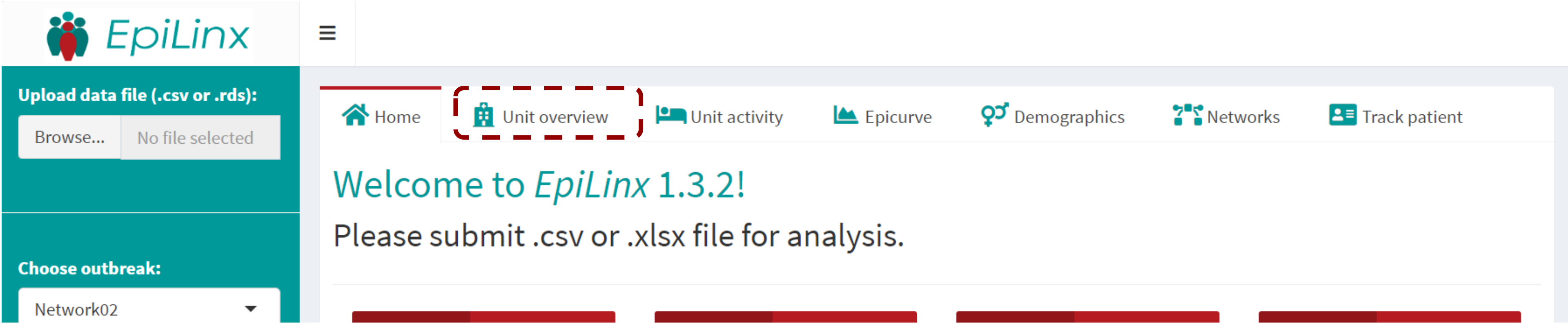
EXAMPLE #2 – DIRECT CONTACT

STATENS SERUM INSTITUT

Direct links = Same department + Same date

	Patient.1	Patient.2	unit	Start	End	Duration_days
	All	All	All	All	All	All
1	Patient 2	Patient 3	Hosp_B, Dept_B1	2019-03-29	2019-04-02	5
2	Patient 2	Patient 5	Hosp_B, Dept_B1	2019-03-20	2019-03-25	6
3	Patient 2	Patient 4	Hosp_I, Dept_I6	2019-03-06	2019-03-07	2
4	Patient 1	Patient 2	Hosp_I, Dept_I6	2019-01-19	2019-01-22	4

EXAMPLE #2 – DIRECT CONTACT



EpiLinx

Upload data file (.csv or .rds):

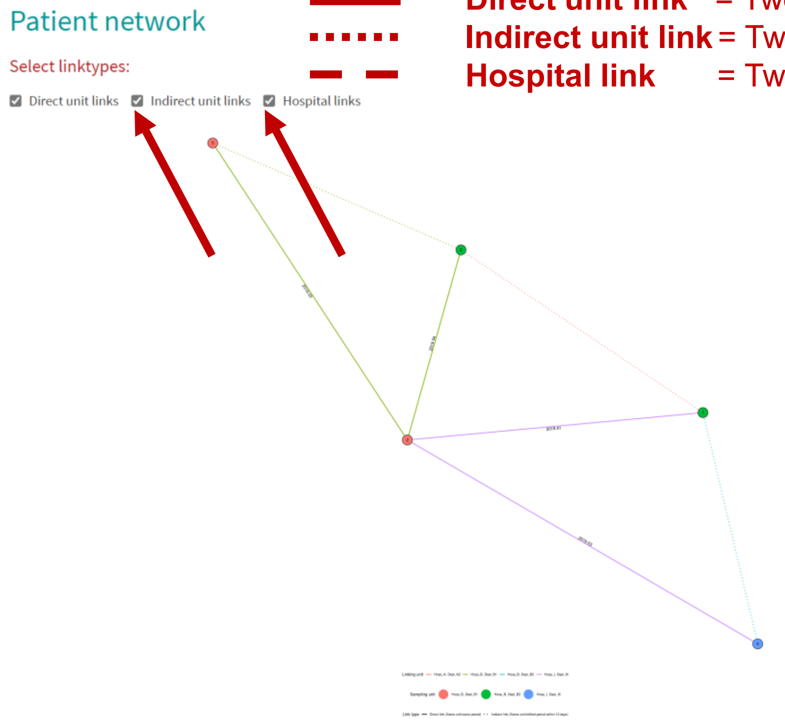
Browse... No file selected

Choose outbreak:

Network02

Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.



Patient network

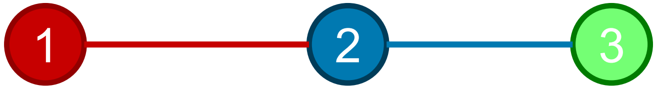
Select linktypes:

☑ Direct unit links ☑ Indirect unit links ☑ Hospital links

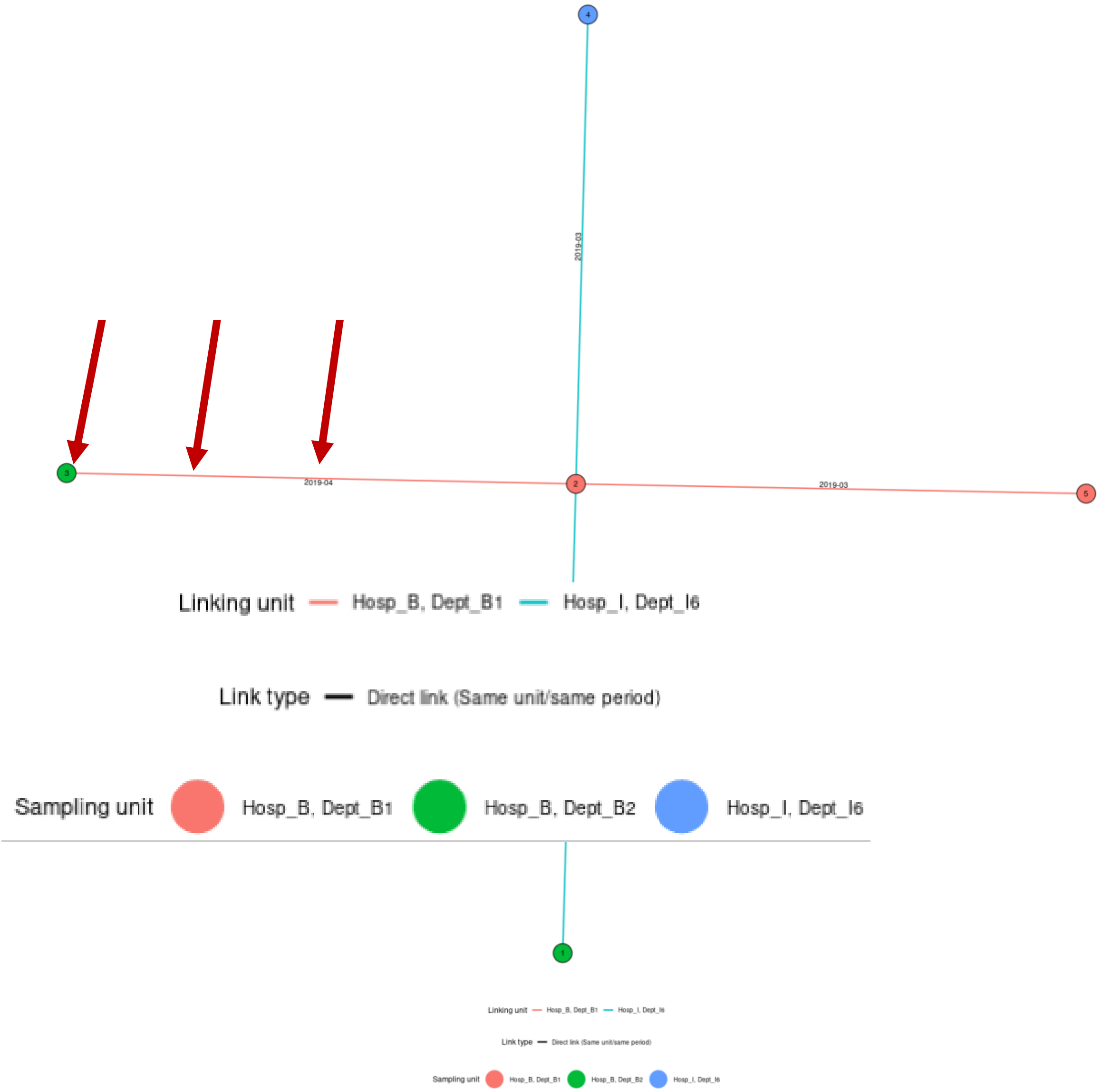
▬ **Direct unit link** = Two patients meet on the **same department** on the **same day**.

┅ **Indirect unit link** = Two patients have been on the **same department** but with **X days between stays**.

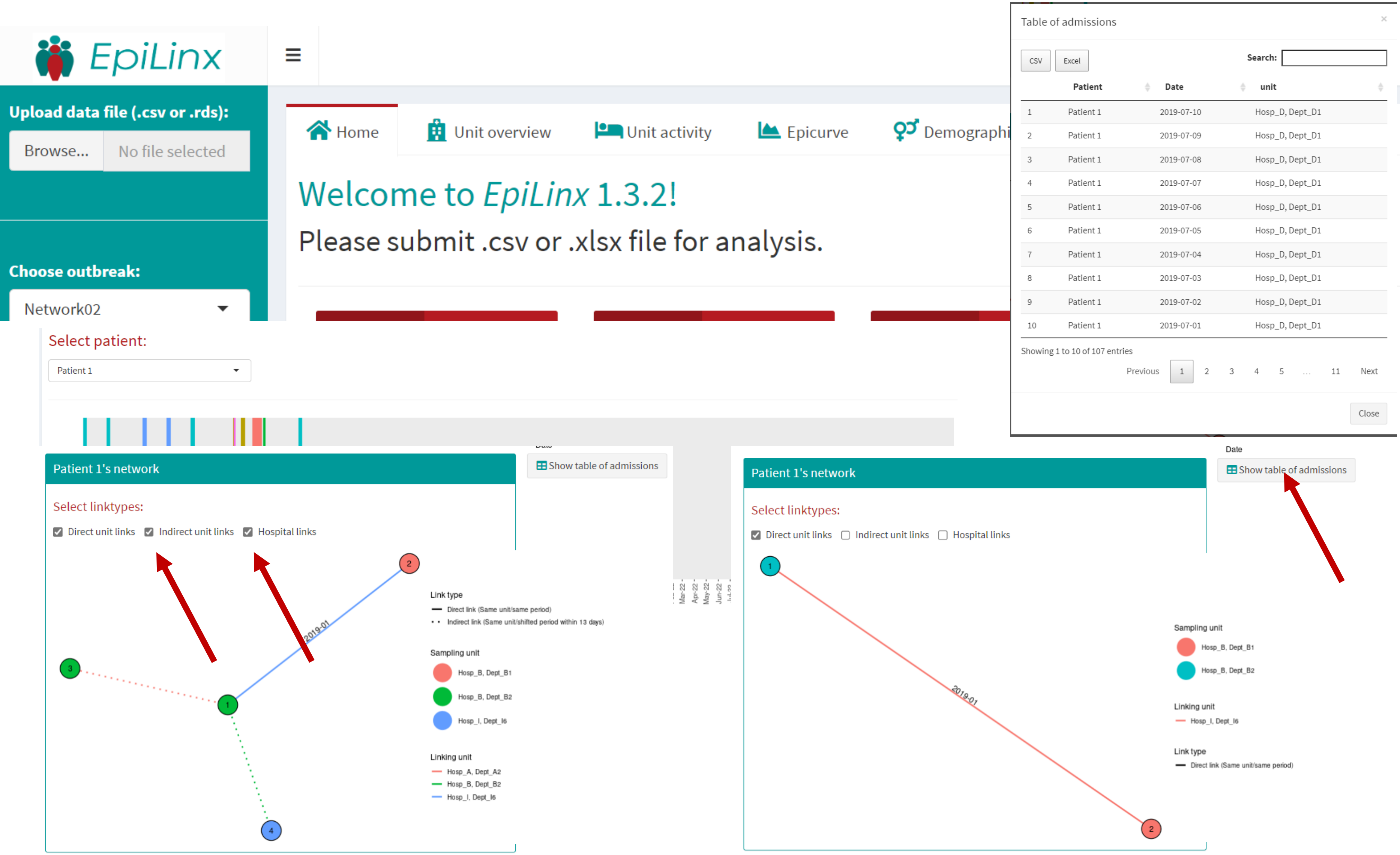
╍ **Hospital link** = Two patients have been on the **same hospital** on the **same date**.



EXAMPLE #2 – DIRECT CONTACT



EXAMPLE #2 – DIRECT CONTACT



Upload data file (.csv or .rds): No file selected

Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

Choose outbreak: Network02

Select patient: Patient 1

Patient 1's network

Select linktypes:

☑ Direct unit links ☑ Indirect unit links ☑ Hospital links

Patient 1's network

Select linktypes:

☑ Direct unit links ☐ Indirect unit links ☐ Hospital links

Table of admissions

CSV Excel Search:

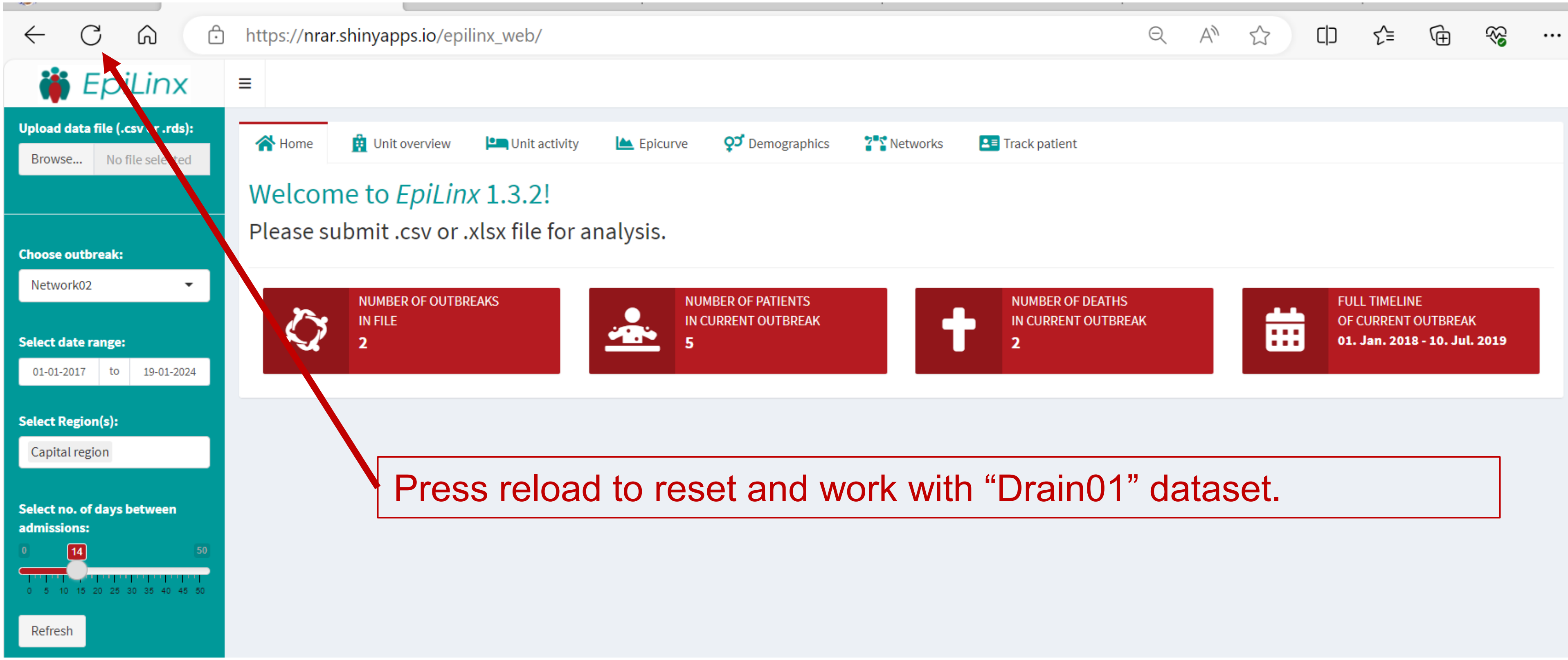
	Patient	Date	unit
1	Patient 1	2019-07-10	Hosp_D, Dept_D1
2	Patient 1	2019-07-09	Hosp_D, Dept_D1
3	Patient 1	2019-07-08	Hosp_D, Dept_D1
4	Patient 1	2019-07-07	Hosp_D, Dept_D1
5	Patient 1	2019-07-06	Hosp_D, Dept_D1
6	Patient 1	2019-07-05	Hosp_D, Dept_D1
7	Patient 1	2019-07-04	Hosp_D, Dept_D1
8	Patient 1	2019-07-03	Hosp_D, Dept_D1
9	Patient 1	2019-07-02	Hosp_D, Dept_D1
10	Patient 1	2019-07-01	Hosp_D, Dept_D1

Showing 1 to 10 of 107 entries

Previous 1 2 3 4 5 … 11 Next

Close

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/



Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

NUMBER OF OUTBREAKS IN FILE **2**

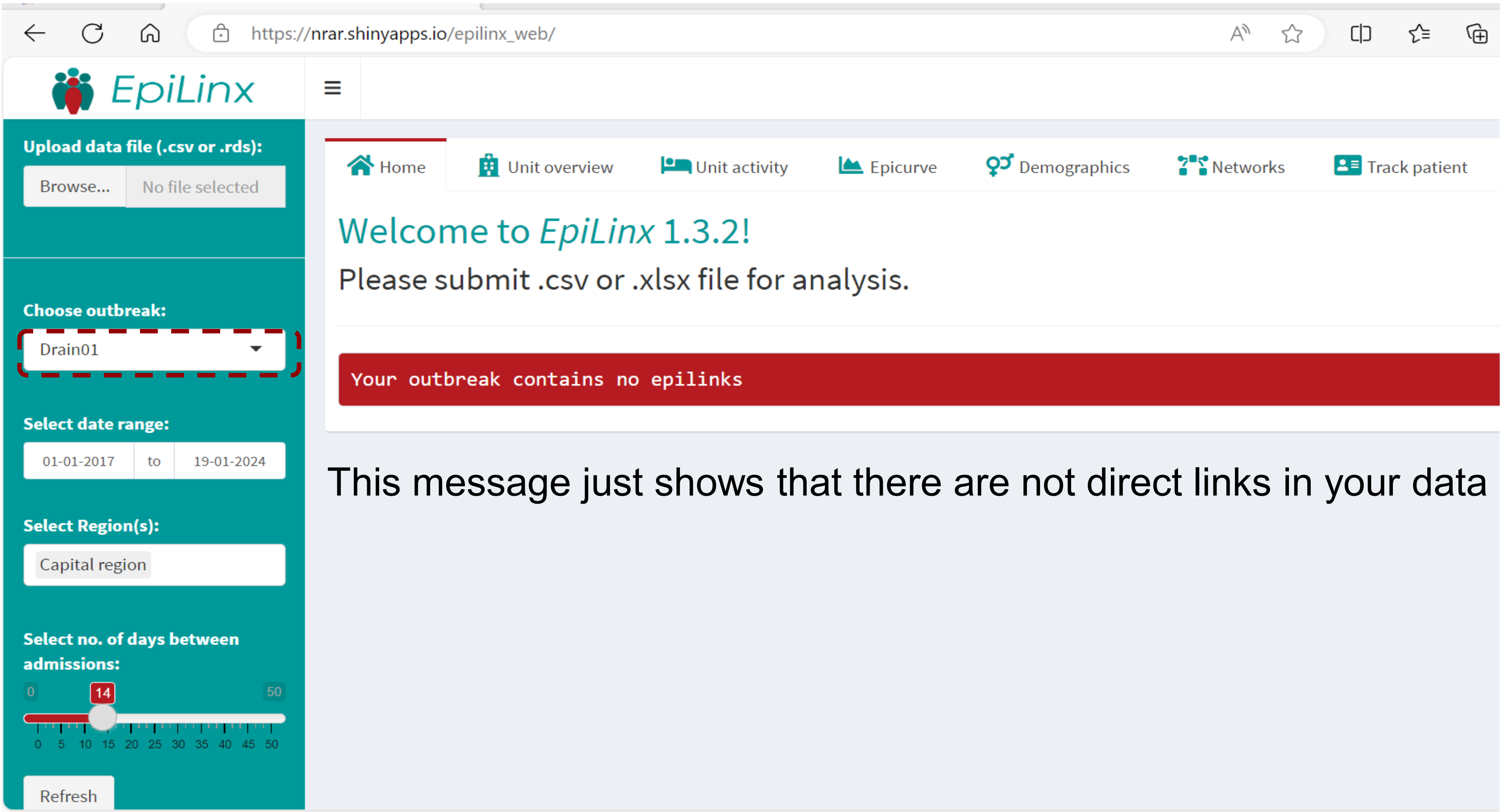
NUMBER OF PATIENTS IN CURRENT OUTBREAK **5**

NUMBER OF DEATHS IN CURRENT OUTBREAK **2**

FULL TIMELINE OF CURRENT OUTBREAK **01. Jan. 2018 - 10. Jul. 2019**

Press reload to reset and work with “Drain01” dataset.

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/



Upload data file (.csv or .rds): No file selected

Choose outbreak: Drain01

Select date range: 01-01-2017 to 19-01-2024

Select Region(s): Capital region

Select no. of days between admissions: 14

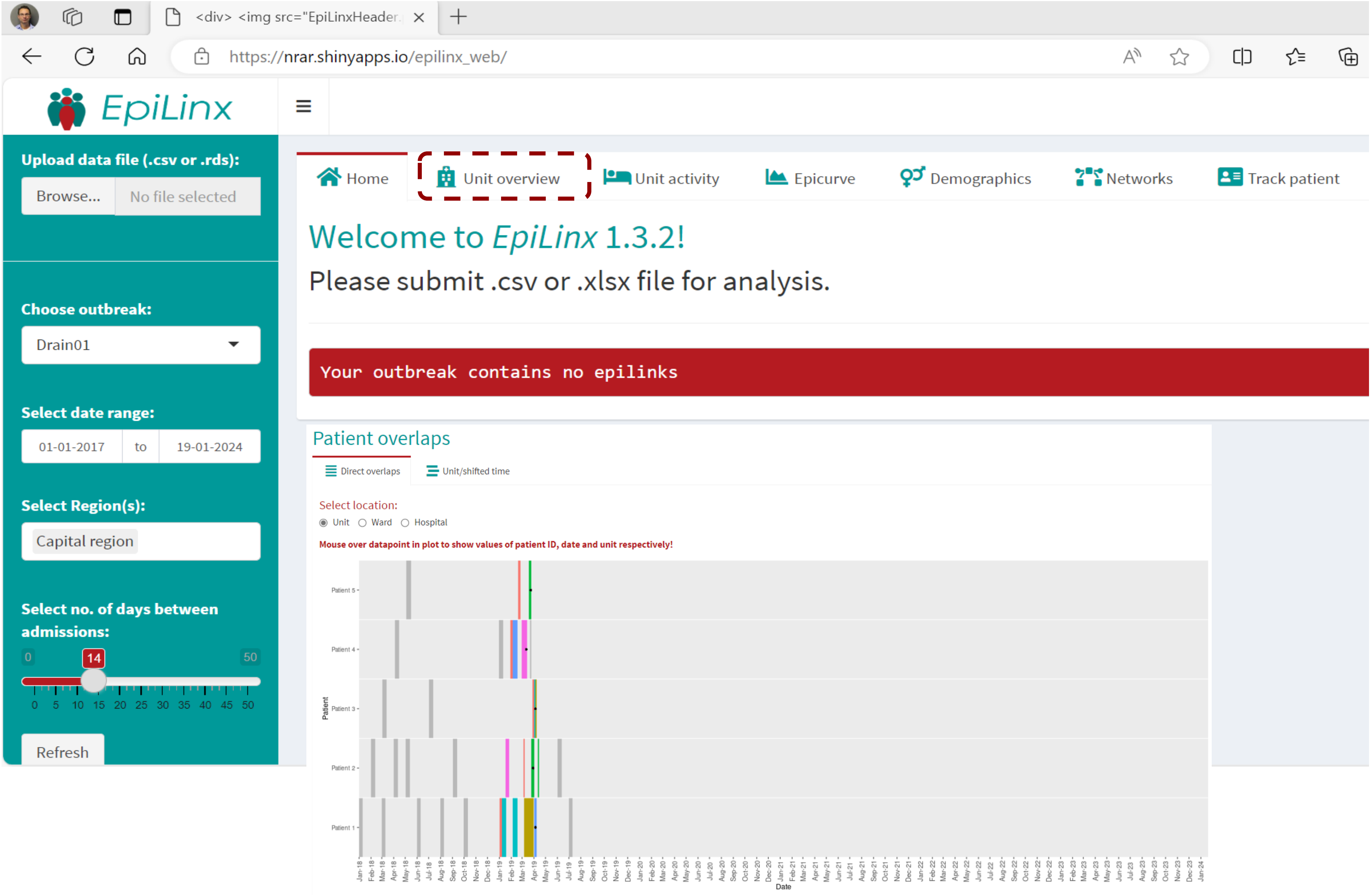
Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

```
Your outbreak contains no epilinks
```

This message just shows that there are not direct links in your data

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/



Upload data file (.csv or .rds):

Choose outbreak: Drain01

Select date range: 01-01-2017 to 19-01-2024

Select Region(s): Capital region

Select no. of days between admissions: 14 (0–50)

Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

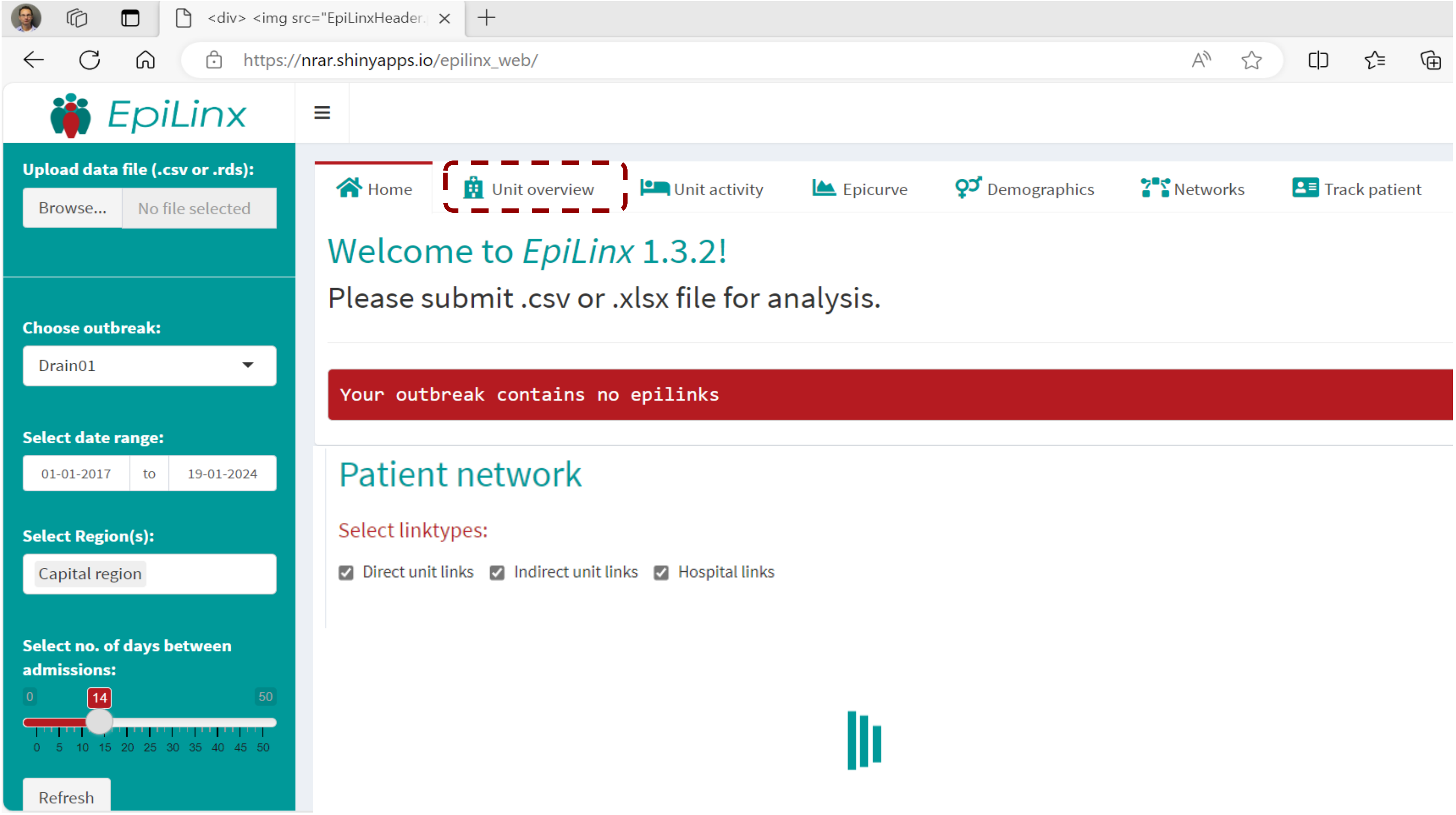
`Your outbreak contains no epilinks`

Patient overlaps

Select location:
● Unit ○ Ward ○ Hospital

Mouse over datapoint in plot to show values of patient ID, date and unit respectively!

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/



Upload data file (.csv or .rds): No file selected

Choose outbreak: Drain01

Select date range: 01-01-2017 to 19-01-2024

Select Region(s): Capital region

Select no. of days between admissions: 14 (0–50)

Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

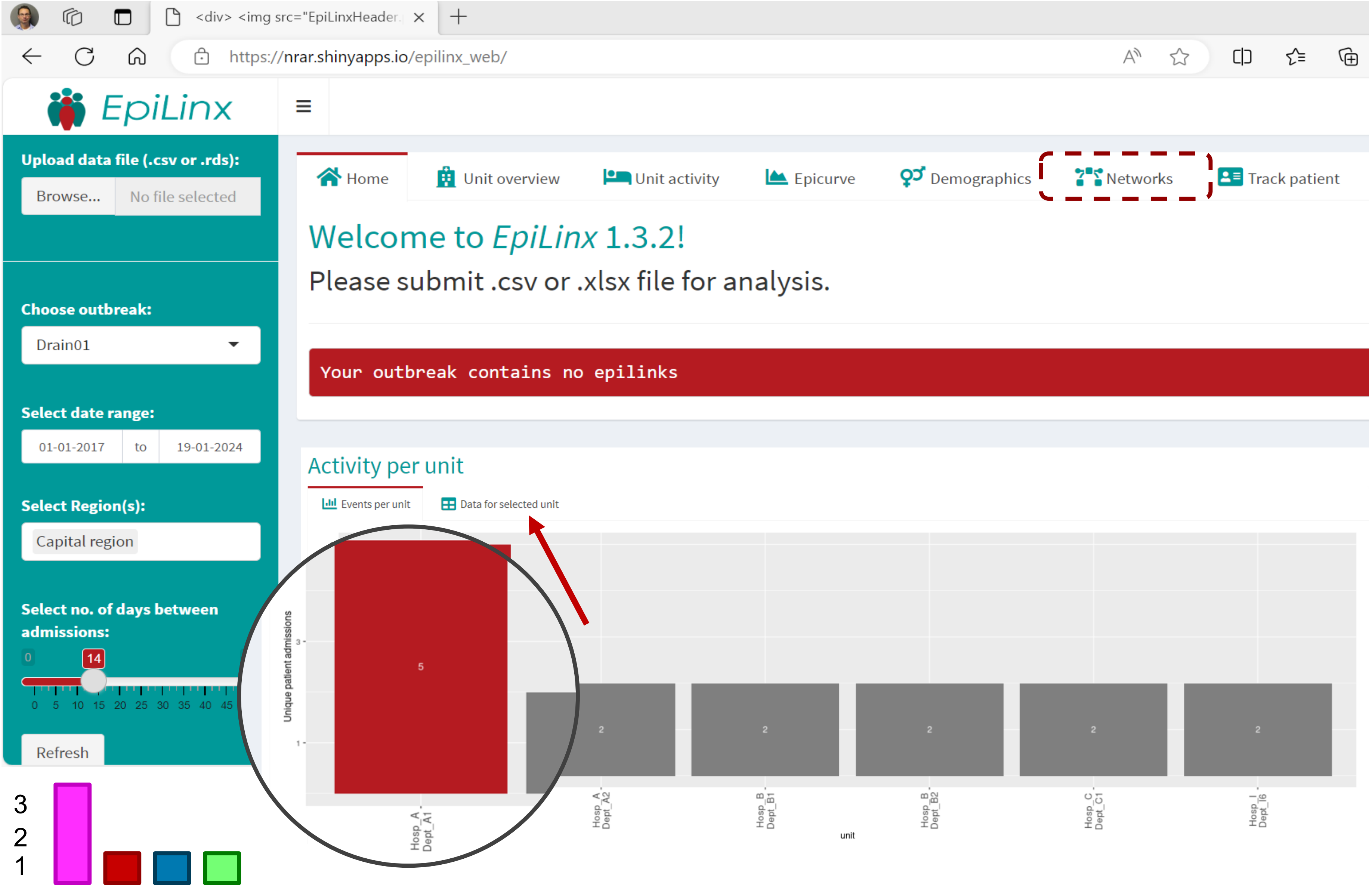
`Your outbreak contains no epilinks`

Patient network

Select linktypes:

☑ Direct unit links ☑ Indirect unit links ☑ Hospital links

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/



Upload data file (.csv or .rds):
No file selected

Choose outbreak:
Drain01

Select date range:
01-01-2017 to 19-01-2024

Select Region(s):
Capital region

Select no. of days between admissions:
14

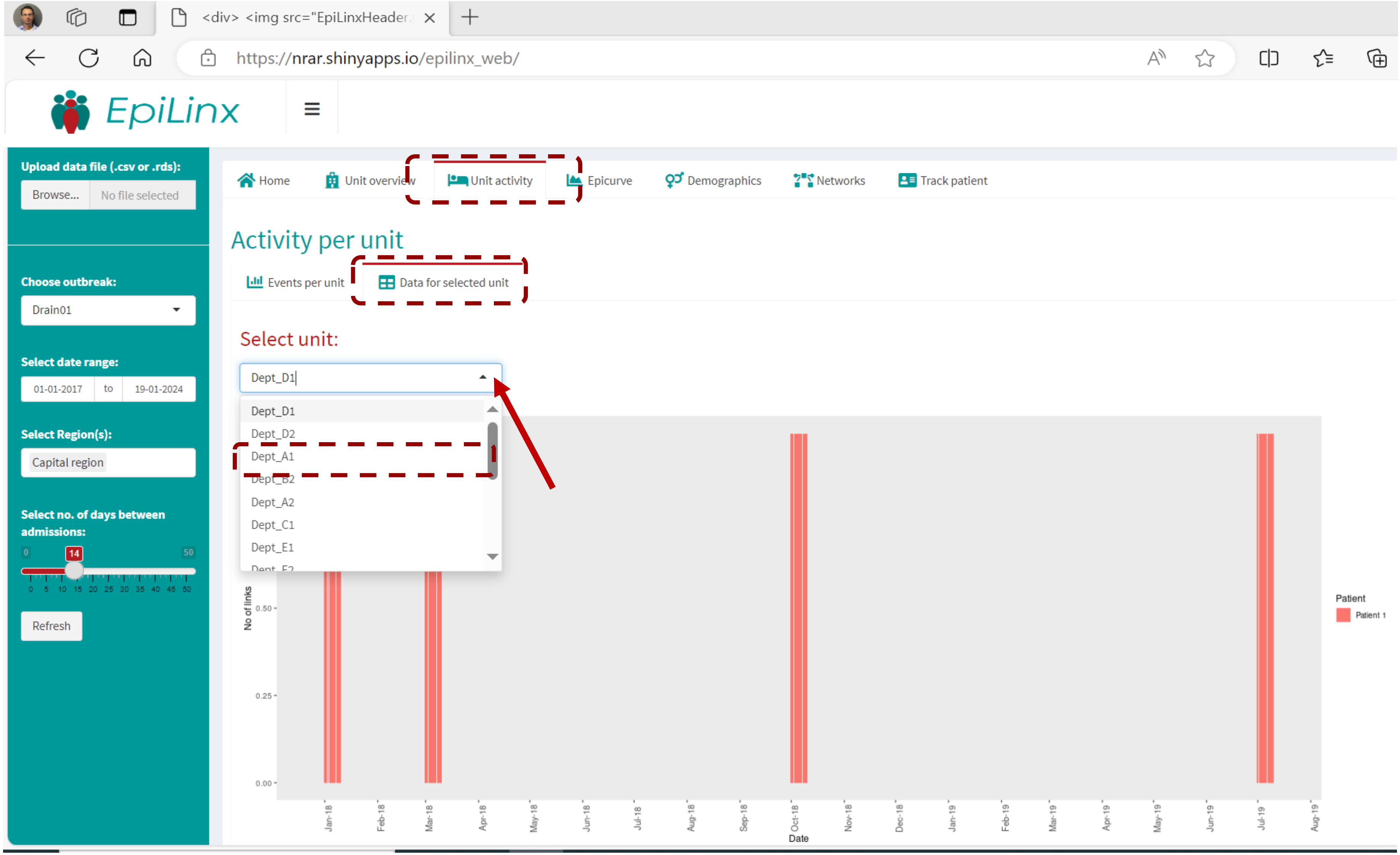
Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

`Your outbreak contains no epilinks`

Activity per unit

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/



Upload data file (.csv or .rds): No file selected

Choose outbreak: Drain01

Select date range: 01-01-2017 to 19-01-2024

Select Region(s): Capital region

Select no. of days between admissions: 14

Activity per unit

Select unit: Dept_D1

HTTPS://NRAR.SHINYAPPS.IO/EPILINX_WEB/

Activity per unit

Select unit:

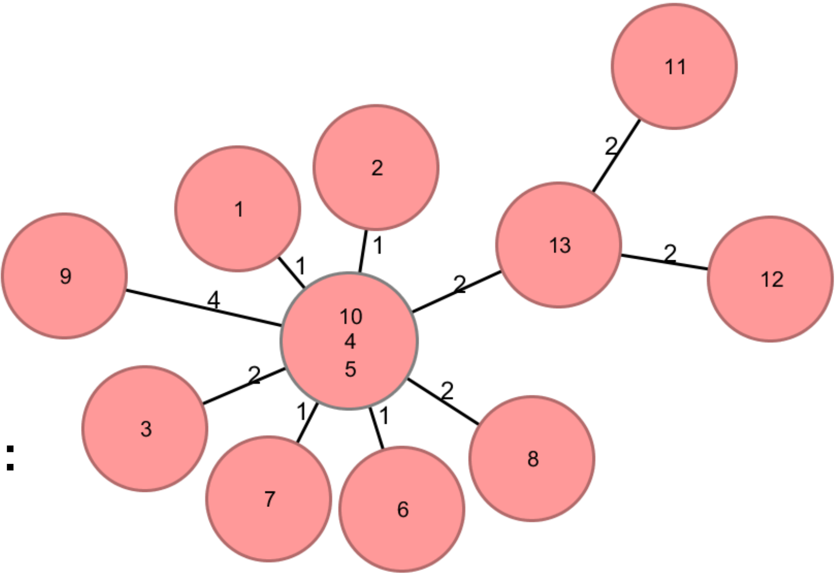
OUTBREAK EXAMPLE – A. BAUMANNII

The molecular microbial data:

- WGS-based data (cgMLST)
- 13 patients with the same cgMLST type
- 0-5 alleles difference between isolates
- A maximum of 8 alleles in total

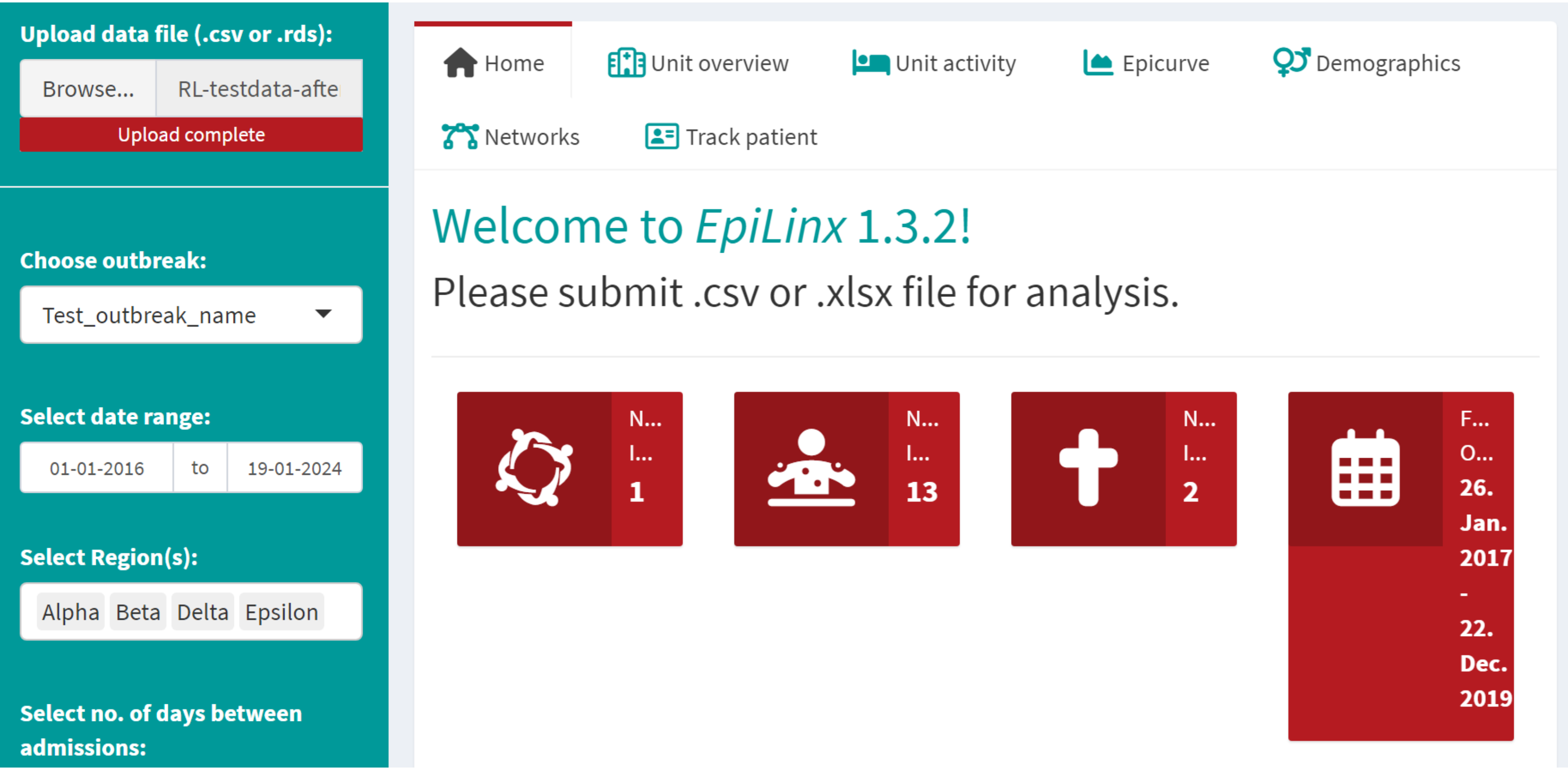
The epidemiological data of the 13 patients:

- Hospitalization events
- Date of the positive sample
- Date of death (if applicable)



cgMLST

EPILINX – REAL-LIFE DATA



Upload data file (.csv or .rds):

Browse... RL-testdata-afte

Upload complete

Choose outbreak:

Test_outbreak_name

Select date range:

01-01-2016 to 19-01-2024

Select Region(s):

Alpha Beta Delta Epsilon

Select no. of days between admissions:

Welcome to *EpiLinx* 1.3.2!

Please submit .csv or .xlsx file for analysis.

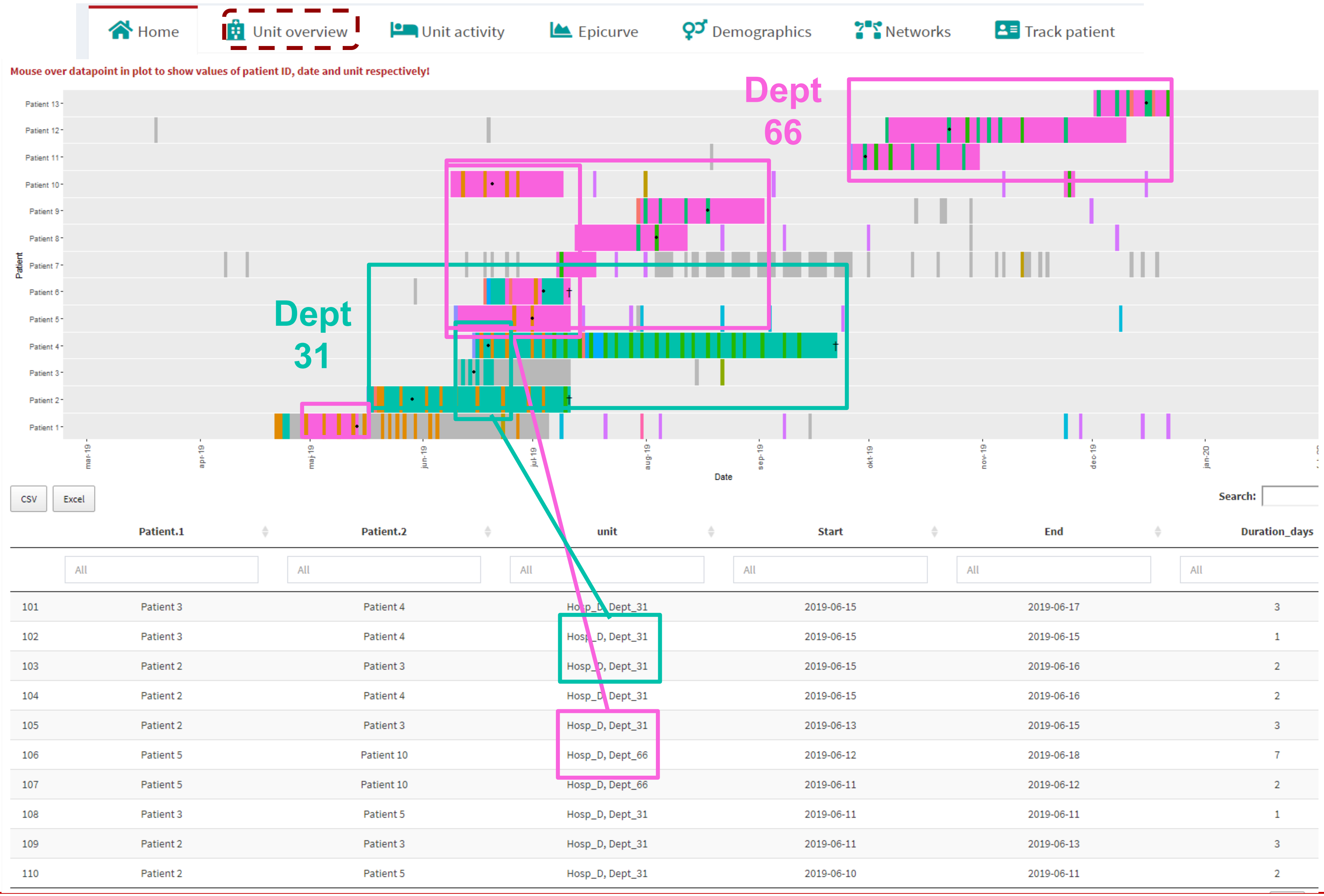
N... I... 1

N... I... 13

N... I... 2

F... O... 26. Jan. 2017 - 22. Dec. 2019

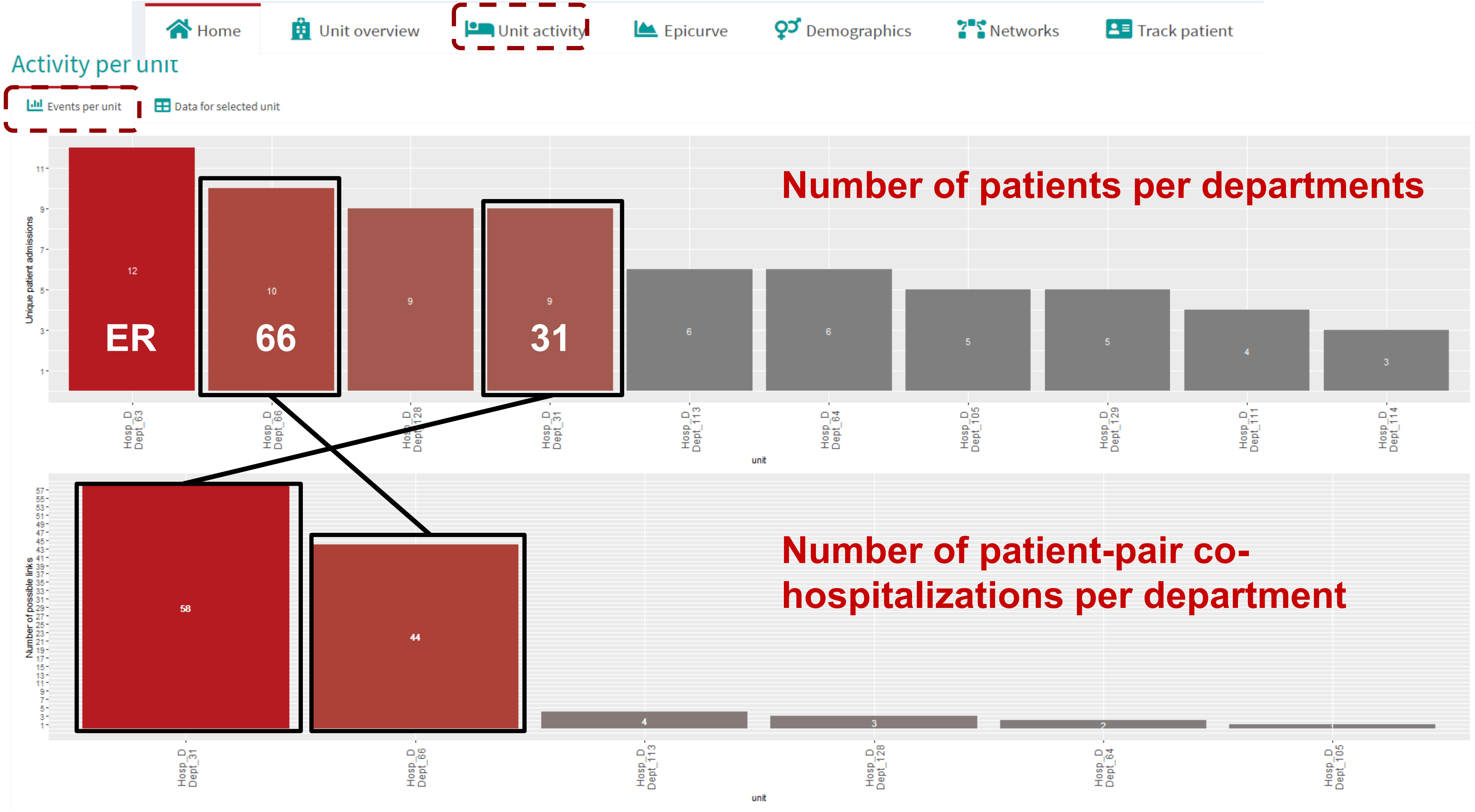
EPILINX – OVERVIEW TAB



Mouse over datapoint in plot to show values of patient ID, date and unit respectively!

	Patient.1	Patient.2	unit	Start	End	Duration_days
	All	All	All	All	All	All
101	Patient 3	Patient 4	Hosp_D, Dept_31	2019-06-15	2019-06-17	3
102	Patient 3	Patient 4	Hosp_D, Dept_31	2019-06-15	2019-06-15	1
103	Patient 2	Patient 3	Hosp_D, Dept_31	2019-06-15	2019-06-16	2
104	Patient 2	Patient 4	Hosp_D, Dept_31	2019-06-15	2019-06-16	2
105	Patient 2	Patient 3	Hosp_D, Dept_31	2019-06-13	2019-06-15	3
106	Patient 5	Patient 10	Hosp_D, Dept_66	2019-06-12	2019-06-18	7
107	Patient 5	Patient 10	Hosp_D, Dept_66	2019-06-11	2019-06-12	2
108	Patient 3	Patient 5	Hosp_D, Dept_31	2019-06-11	2019-06-11	1
109	Patient 2	Patient 3	Hosp_D, Dept_31	2019-06-11	2019-06-13	3
110	Patient 2	Patient 5	Hosp_D, Dept_31	2019-06-10	2019-06-11	2

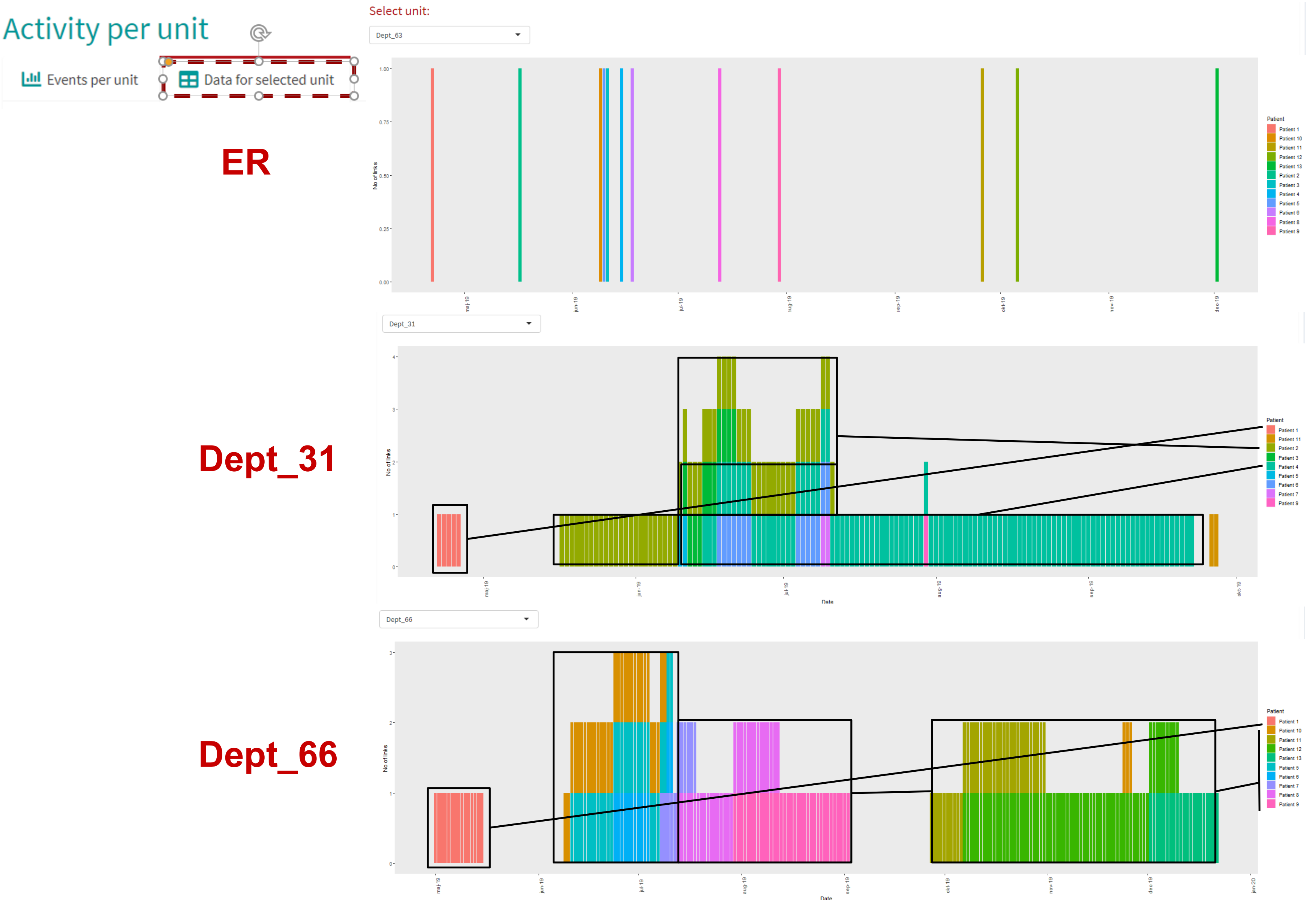
ACTIVITY PER DEPARTMENT



Number of patients per departments

Number of patient-pair co-hospitalizations per department

PATIENT VISITS PER DEPARTMENT



ER

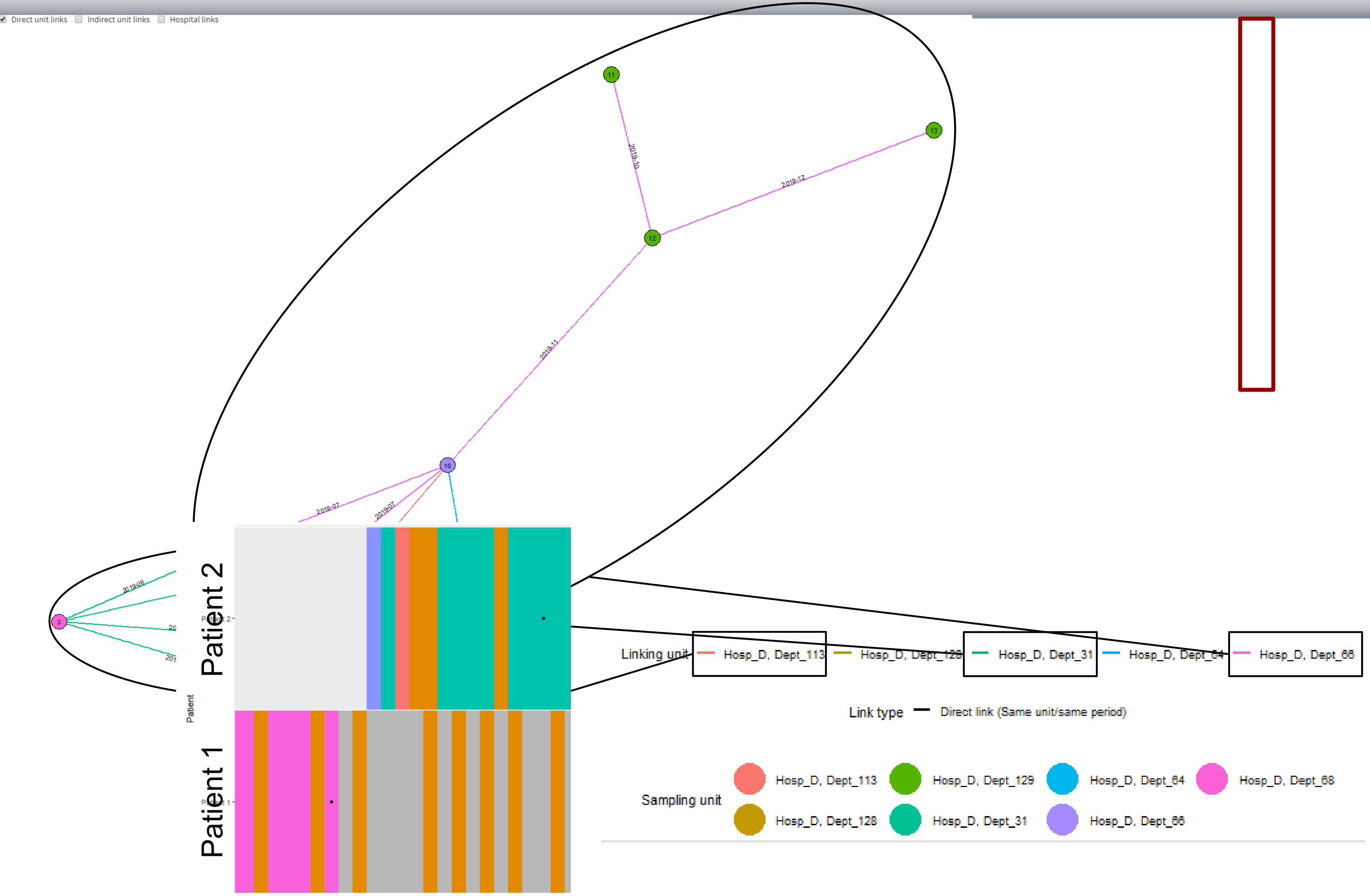
Dept_31

Patient 1
Patient 2
Patient 4

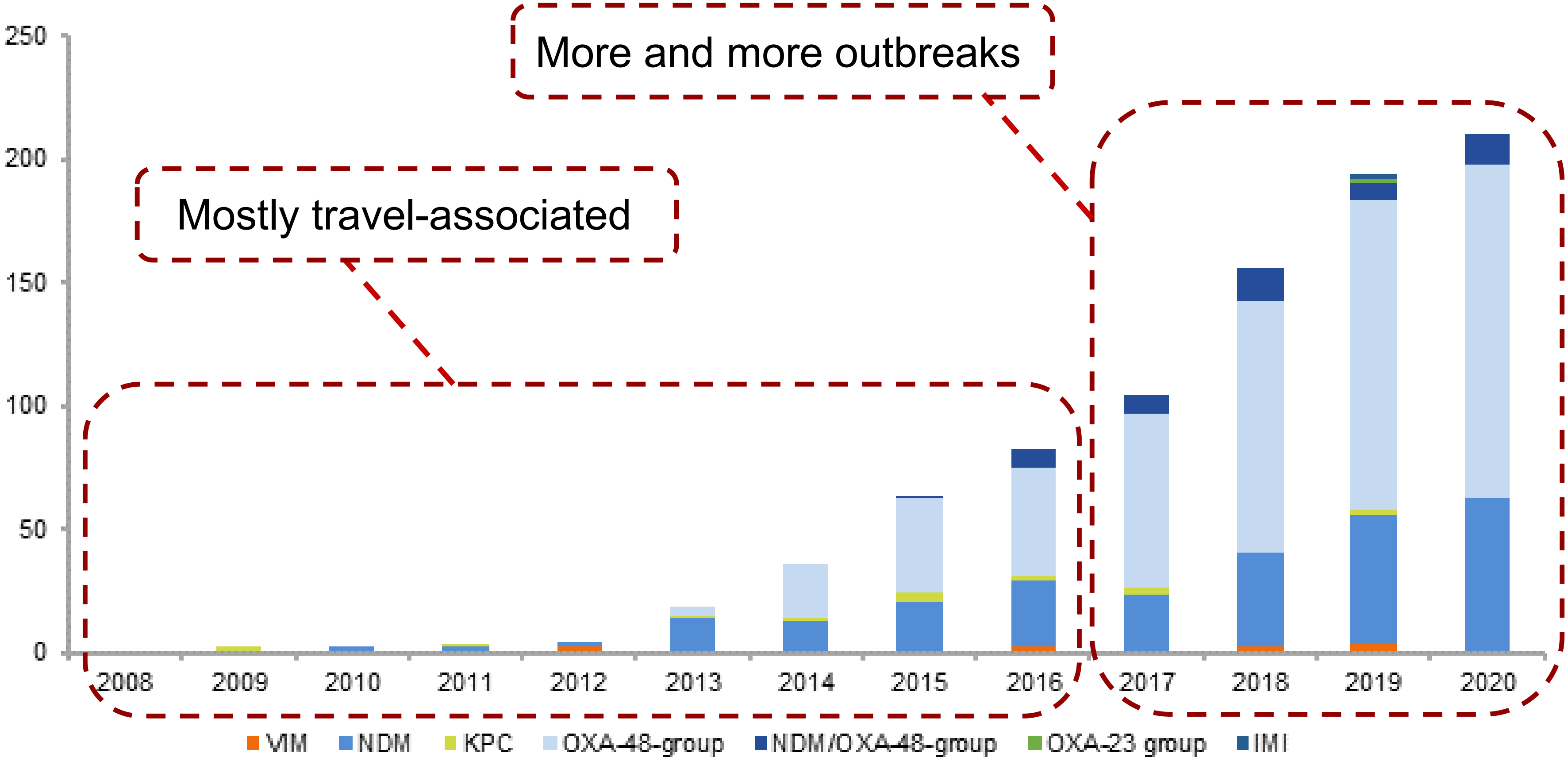
Dept_66

Patient 1

EPILINX – DIRECT NETWORK



CPO* NATIONAL SURVEILLANCE IN DENMARK



More and more outbreaks

Mostly travel-associated

All isolates are submitted to WGS (Illumina) and analyzed (Ridom SeqSphere+) to detect genomic (clonal) clusters across departments, hospitals and regions.

All clonal clusters are analyzed in EpiLinx to confirm nosocomial outbreaks.

---

* Carbapenemase producing organisms

THE ”KURS” NATIONAL OUTBREAK DATABASE

DTU

STATENS SERUM INSTITUT

DANMAP 2020

Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

Statens Serum Institut
National Food Institute, Technical University of Denmark

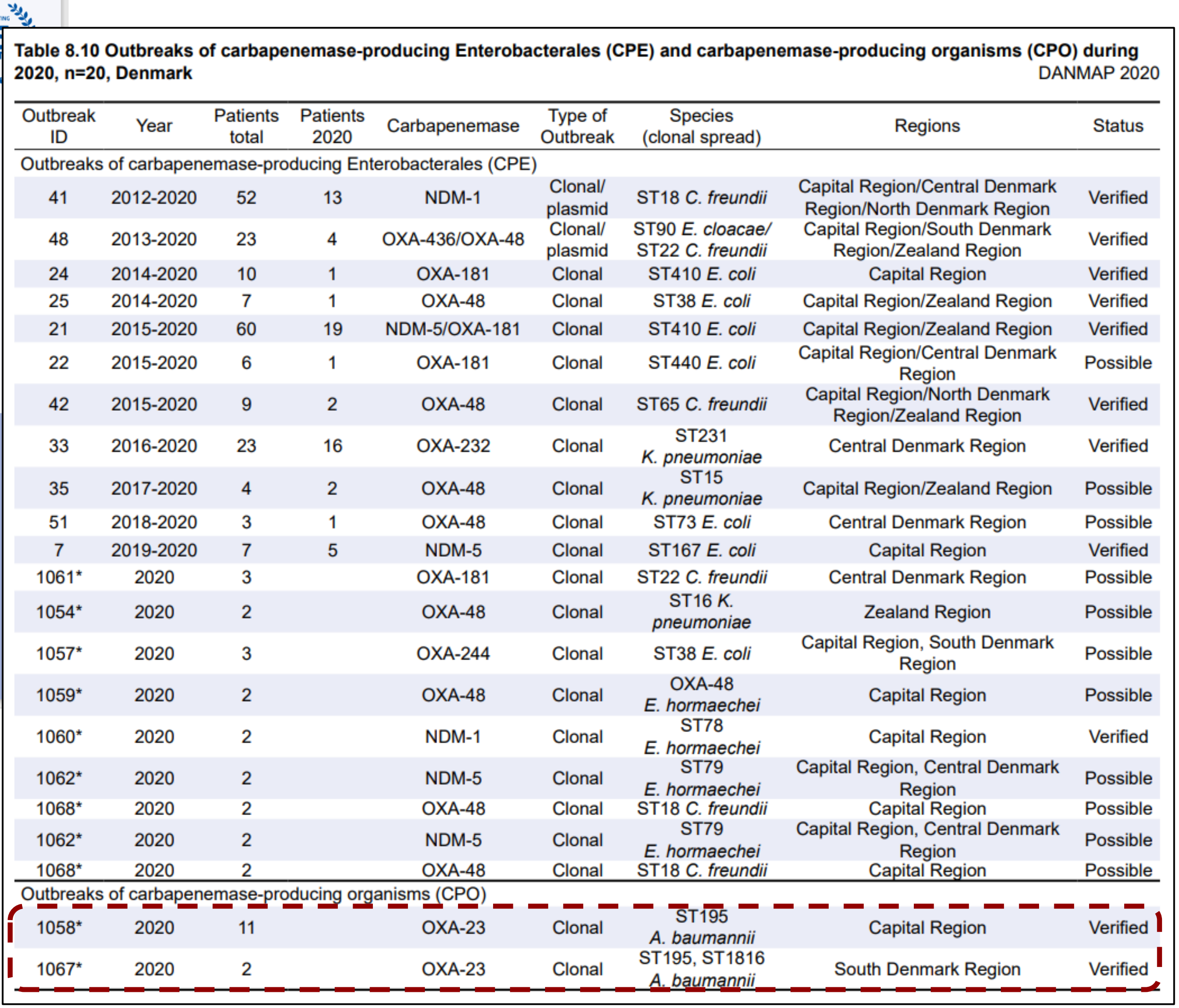


Table 8.10 Outbreaks of carbapenemase-producing Enterobacterales (CPE) and carbapenemase-producing organisms (CPO) during 2020, n=20, Denmark DANMAP 2020

Outbreak ID	Year	Patients total	Patients 2020	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions	Status
Outbreaks of carbapenemase-producing Enterobacterales (CPE)								
41	2012-2020	52	13	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	Capital Region/Central Denmark Region/North Denmark Region	Verified
48	2013-2020	23	4	OXA-436/OXA-48	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	Capital Region/South Denmark Region/Zealand Region	Verified
24	2014-2020	10	1	OXA-181	Clonal	ST410 *E. coli*	Capital Region	Verified
25	2014-2020	7	1	OXA-48	Clonal	ST38 *E. coli*	Capital Region/Zealand Region	Verified
21	2015-2020	60	19	NDM-5/OXA-181	Clonal	ST410 *E. coli*	Capital Region/Zealand Region	Verified
22	2015-2020	6	1	OXA-181	Clonal	ST440 *E. coli*	Capital Region/Central Denmark Region	Possible
42	2015-2020	9	2	OXA-48	Clonal	ST65 *C. freundii*	Capital Region/North Denmark Region/Zealand Region	Verified
33	2016-2020	23	16	OXA-232	Clonal	ST231 *K. pneumoniae*	Central Denmark Region	Verified
35	2017-2020	4	2	OXA-48	Clonal	ST15 *K. pneumoniae*	Capital Region/Zealand Region	Possible
51	2018-2020	3	1	OXA-48	Clonal	ST73 *E. coli*	Central Denmark Region	Possible
7	2019-2020	7	5	NDM-5	Clonal	ST167 *E. coli*	Capital Region	Verified
1061*	2020	3		OXA-181	Clonal	ST22 *C. freundii*	Central Denmark Region	Possible
1054*	2020	2		OXA-48	Clonal	ST16 *K. pneumoniae*	Zealand Region	Possible
1057*	2020	3		OXA-244	Clonal	ST38 *E. coli*	Capital Region, South Denmark Region	Possible
1059*	2020	2		OXA-48	Clonal	OXA-48 *E. hormaechei*	Capital Region	Possible
1060*	2020	2		NDM-1	Clonal	ST78 *E. hormaechei*	Capital Region	Verified
1062*	2020	2		NDM-5	Clonal	ST79 *E. hormaechei*	Capital Region, Central Denmark Region	Possible
1068*	2020	2		OXA-48	Clonal	ST18 *C. freundii*	Capital Region	Possible
1062*	2020	2		NDM-5	Clonal	ST79 *E. hormaechei*	Capital Region, Central Denmark Region	Possible
1068*	2020	2		OXA-48	Clonal	ST18 *C. freundii*	Capital Region	Possible
Outbreaks of carbapenemase-producing organisms (CPO)								
1058*	2020	11		OXA-23	Clonal	ST195 *A. baumannii*	Capital Region	Verified
1067*	2020	2		OXA-23	Clonal	ST195, ST1816 *A. baumannii*	South Denmark Region	Verified

THAT’S ALL, FOLKS!!

Questions, please?

Beta-lactam resistance in *Acinetobacter baumannii*

Valeria Bortolaia, DVM, PhD
Statens Serum Institut (SSI)

Funded by the European Union

Objectives

- Learn/Rehearse
  - mechanisms of beta-lactam resistance in *A. baumannii*
  - classification and nomenclature of beta-lactamases
- List the most common carbapenemases detected in *A. baumannii*
- Discuss opportunities and challenges in WGS-based detection of carbapenem resistance in *A. baumannii*

Outline

This session consists of the following elements:

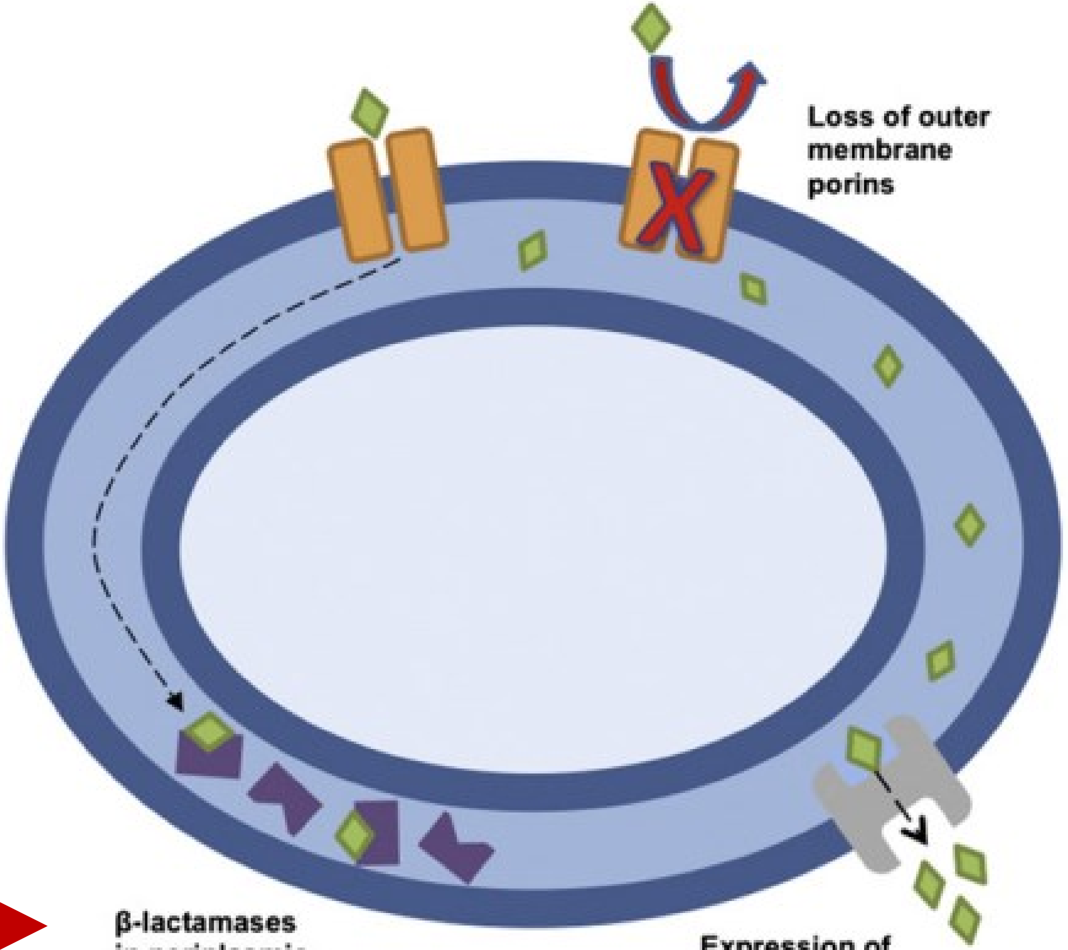
1. Brush-up

- mechanisms of beta-lactam resistance in *A. baumannii*
- beta-lactamases

2. Carbapenem resistance in *A. baumannii*

3. WGS-based detection of carbapenem resistance in *A. baumannii*

Beta-lactam resistance in *A. baumannii*



Loss of outer membrane porins

β-lactamases in periplasmic space

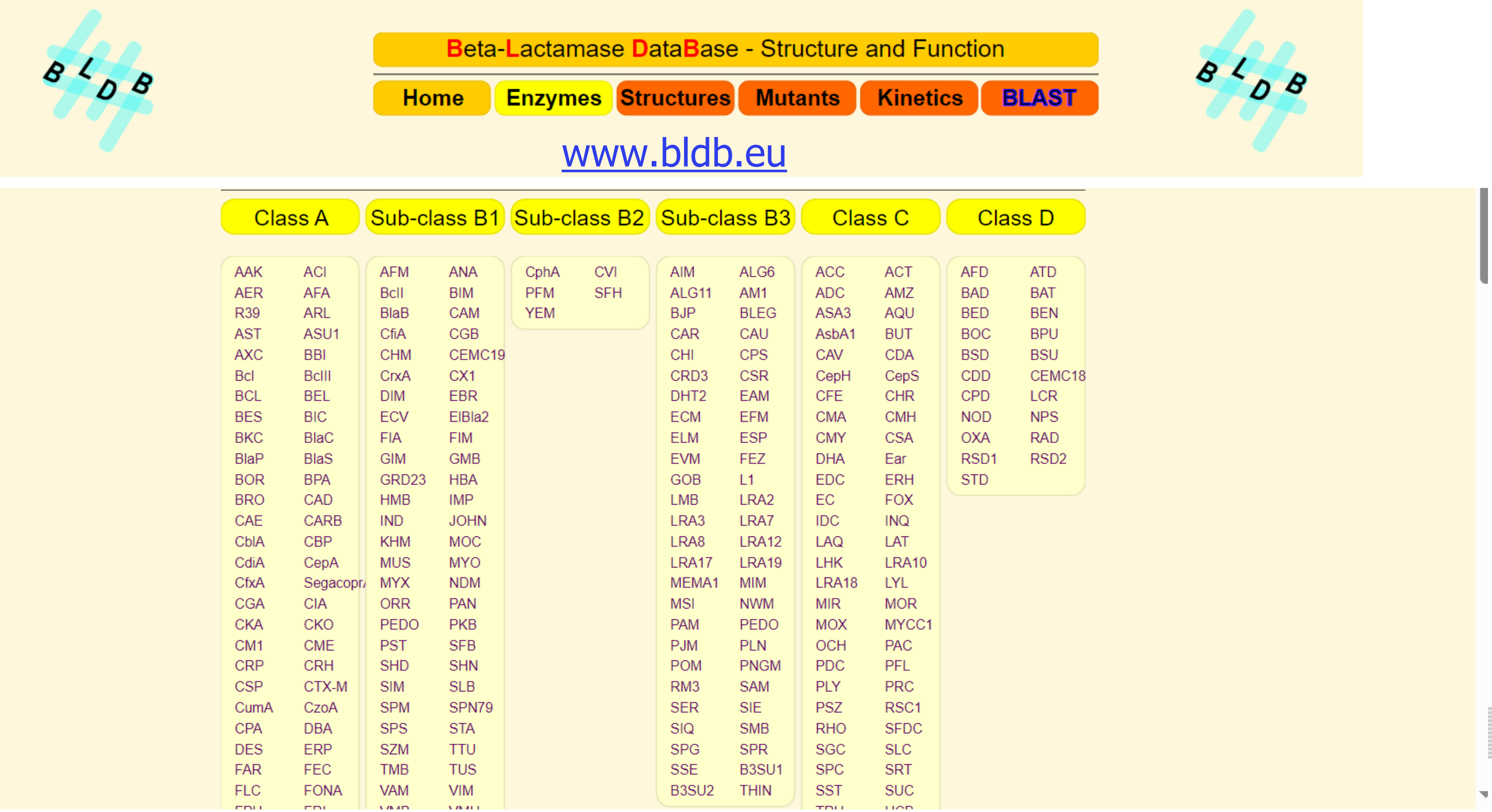
Expression of transmembrane efflux pump

Modified from Tang et al., 2014

Beta-lactamases – short brush-up

Type of classification	Rationale for classification
Molecular classification (Bush, 2013)	it is based on the amino acid sequence. It divides β-lactamases into **class A, C, and D** enzymes (which utilize serine for β-lactam hydrolysis) and **class B** metalloenzymes which require divalent zinc ions for substrate hydrolysis
Functional classification (Bush, 2013)	it takes into account **substrate and inhibitor profiles** (attempt to group the enzymes in ways that can be correlated with their phenotype in clinical isolates)
Clinical classification (Giske et al., 2009)	**operational definitions** to guide antimicrobial therapy

A useful resource for information on beta-lactamases



Beta-Lactamase DataBase - Structure and Function

www.bldb.eu

Class A		Sub-class B1		Sub-class B2		Sub-class B3		Class C		Class D	
AAK	ACI	AFM	ANA	CphA	CVI	AIM	ALG6	ACC	ACT	AFD	ATD
AER	AFA	BcII	BIM	PFM	SFH	ALG11	AM1	ADC	AMZ	BAD	BAT
R39	ARL	BlaB	CAM	YEM		BJP	BLEG	ASA3	AQU	BED	BEN
AST	ASU1	CfiA	CGB			CAR	CAU	AsbA1	BUT	BOC	BPU
AXC	BBI	CHM	CEMC19			CHI	CPS	CAV	CDA	BSD	BSU
BcI	BcIII	CrxA	CX1			CRD3	CSR	CepH	CepS	CDD	CEMC18
BCL	BEL	DIM	EBR			DHT2	EAM	CFE	CHR	CPD	LCR
BES	BIC	ECV	ElBla2			ECM	EFM	CMA	CMH	NOD	NPS
BKC	BlaC	FIA	FIM			ELM	ESP	CMY	CSA	OXA	RAD
BlaP	BlaS	GIM	GMB			EVM	FEZ	DHA	Ear	RSD1	RSD2
BOR	BPA	GRD23	HBA			GOB	L1	EDC	ERH	STD	
BRO	CAD	HMB	IMP			LMB	LRA2	EC	FOX		
CAE	CARB	IND	JOHN			LRA3	LRA7	IDC	INQ		
CblA	CBP	KHM	MOC			LRA8	LRA12	LAQ	LAT		
CdiA	CepA	MUS	MYO			LRA17	LRA19	LHK	LRA10		
CfxA	Segacopr/	MYX	NDM			MEMA1	MIM	LRA18	LYL		
CGA	CIA	ORR	PAN			MSI	NWM	MIR	MOR		
CKA	CKO	PEDO	PKB			PAM	PEDO	MOX	MYCC1		
CM1	CME	PST	SFB			PJM	PLN	OCH	PAC		
CRP	CRH	SHD	SHN			POM	PNGM	PDC	PFL		
CSP	CTX-M	SIM	SLB			RM3	SAM	PLY	PRC		
CumA	CzoA	SPM	SPN79			SER	SIE	PSZ	RSC1		
CPA	DBA	SPS	STA			SIQ	SMB	RHO	SFDC		
DES	ERP	SZM	TTU			SPG	SPR	SGC	SLC		
FAR	FEC	TMB	TUS			SSE	B3SU1	SPC	SRT		
FLC	FONA	VAM	VIM			B3SU2	THIN	SST	SUC		

Beta-lactamase types and variants definitions

Each beta-lactamase type (e.g. NDM-type, OXA-type, etc.) is further divided into variants: e.g. NDM-1, NDM-2, …, OXA-23, OXA-51, …

Annotation

Gene	Protein
$\textit{bla}_{\text{NDM-1}}$	NDM-1
$\textit{bla}_{\text{OXA-48}}$	OXA-48
$\textit{bla}_{\text{OXA-51}}$	OXA-51
etc.	etc.

IMPORTANT!

Variants are defined **based on amino acid sequences**

Example (hypothetical CARBA):

a. ”ATG TTC CC**G**” is ”MFP”

b. ”ATG TTC CC**A**” is ”MFP”

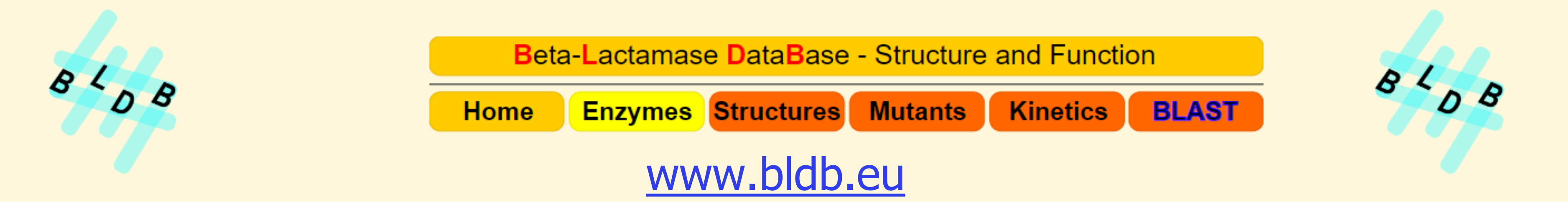
c. ”ATG TTC CTA” is ”MFL”

CARBA-1 They are the same CARBA!

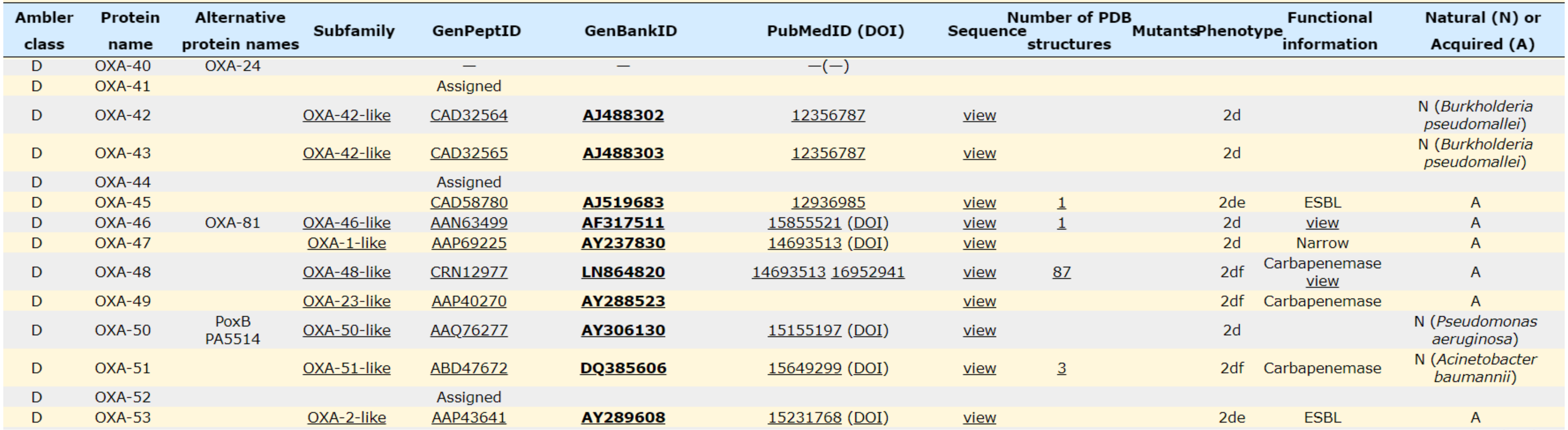
CARBA-2 It is a different CARBA!

Closely related variants can have different affinity for different beta-lactam substrates (i.e. different phenotypes)

A useful resource for information on beta-lactamases

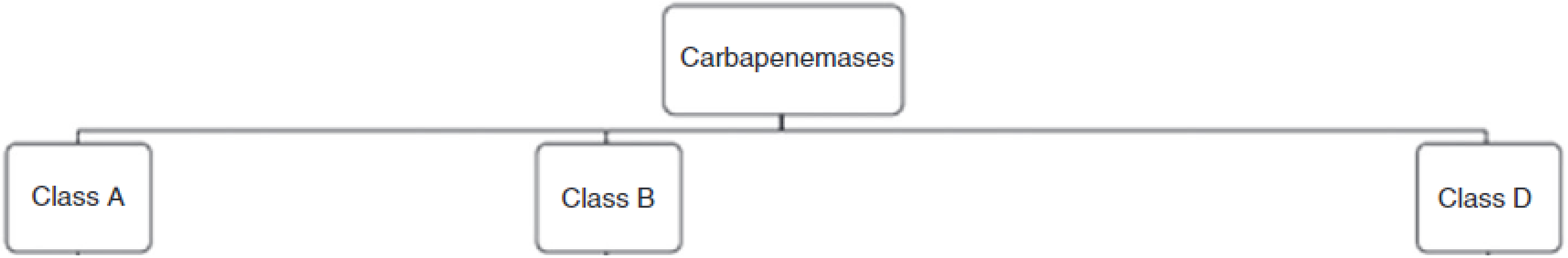

Beta-Lactamase DataBase - Structure and Function

www.bldb.eu



Ambler class	Protein name	Alternative protein names	Subfamily	GenPeptID	GenBankID	PubMedID (DOI)	Sequence	Number of PDB structures	Mutants	Phenotype	Functional information	Natural (N) or Acquired (A)
D	OXA-40	OXA-24		—	—	—(—)						
D	OXA-41			Assigned								
D	OXA-42		OXA-42-like	CAD32564	**AJ488302**	12356787	view			2d		N (*Burkholderia pseudomallei*)
D	OXA-43		OXA-42-like	CAD32565	**AJ488303**	12356787	view			2d		N (*Burkholderia pseudomallei*)
D	OXA-44			Assigned								
D	OXA-45			CAD58780	**AJ519683**	12936985	view	1		2de	ESBL	A
D	OXA-46	OXA-81	OXA-46-like	AAN63499	**AF317511**	15855521 (DOI)	view	1		2d	view	A
D	OXA-47		OXA-1-like	AAP69225	**AY237830**	14693513 (DOI)	view			2d	Narrow	A
D	OXA-48		OXA-48-like	CRN12977	**LN864820**	14693513 16952941	view	87		2df	Carbapenemase view	A
D	OXA-49		OXA-23-like	AAP40270	**AY288523**		view			2df	Carbapenemase	A
D	OXA-50	PoxB PA5514	OXA-50-like	AAQ76277	**AY306130**	15155197 (DOI)	view			2d		N (*Pseudomonas aeruginosa*)
D	OXA-51		OXA-51-like	ABD47672	**DQ385606**	15649299 (DOI)	view	3		2df	Carbapenemase	N (*Acinetobacter baumannii*)
D	OXA-52			Assigned								
D	OXA-53		OXA-2-like	AAP43641	**AY289608**	15231768 (DOI)	view			2de	ESBL	A

Carbapenemases in *A. baumannii*


Carbapenemases

Class A

Class B

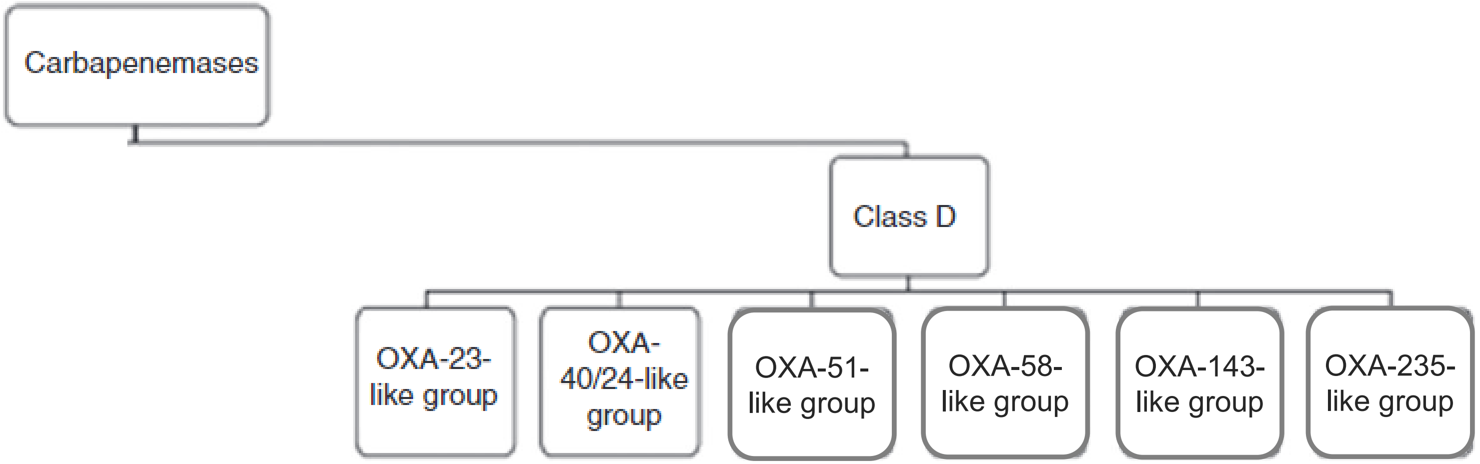
Class D

Modified from Nguyen and Joshi, 2021

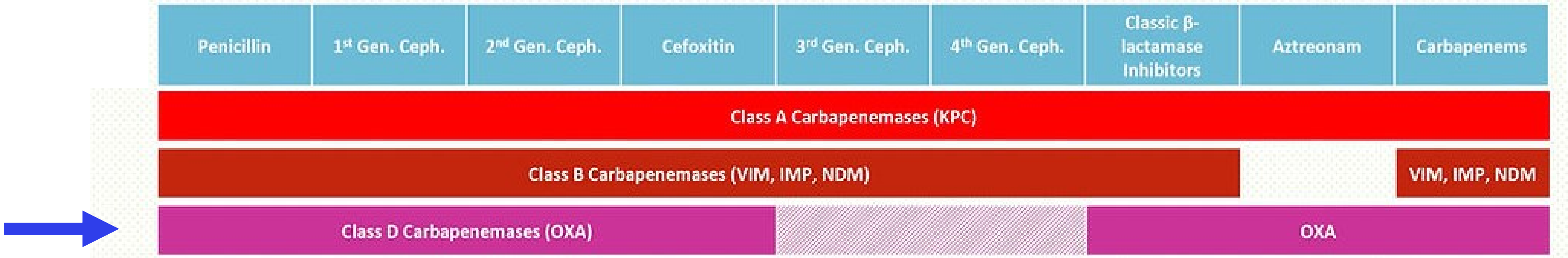
Penicillin	1st Gen. Ceph.	2nd Gen. Ceph.	Cefoxitin	3rd Gen. Ceph.	4th Gen. Ceph.	Classic β-lactamase Inhibitors	Aztreonam	Carbapenems
Class A Carbapenemases (KPC)								
Class B Carbapenemases (VIM, IMP, NDM)								VIM, IMP, NDM
Class D Carbapenemases (OXA)				▨	▨	OXA		

Modified from www.icureach.com

Carbapenemases in *A. baumannii*

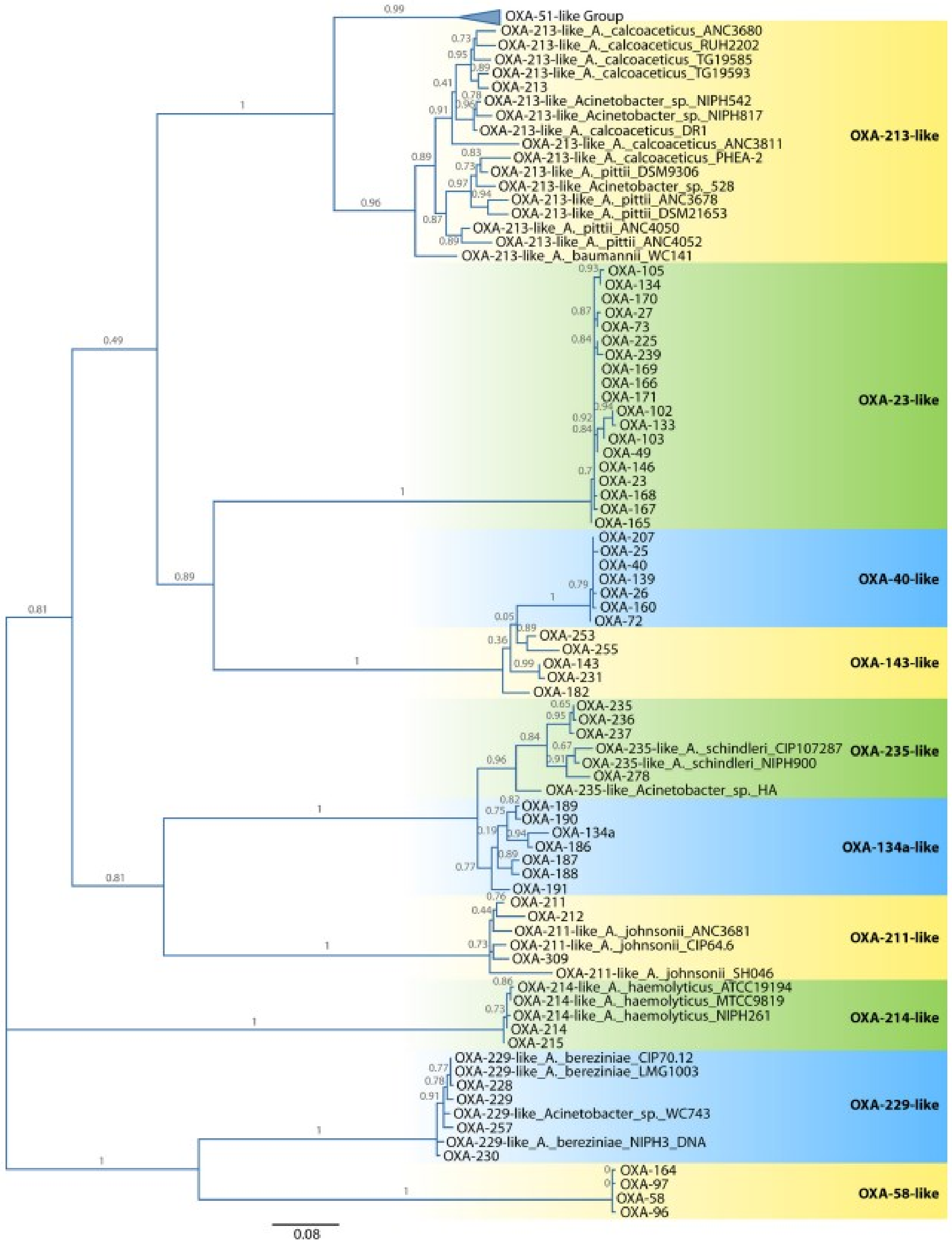


Modified from Nguyen and Joshi, 2021



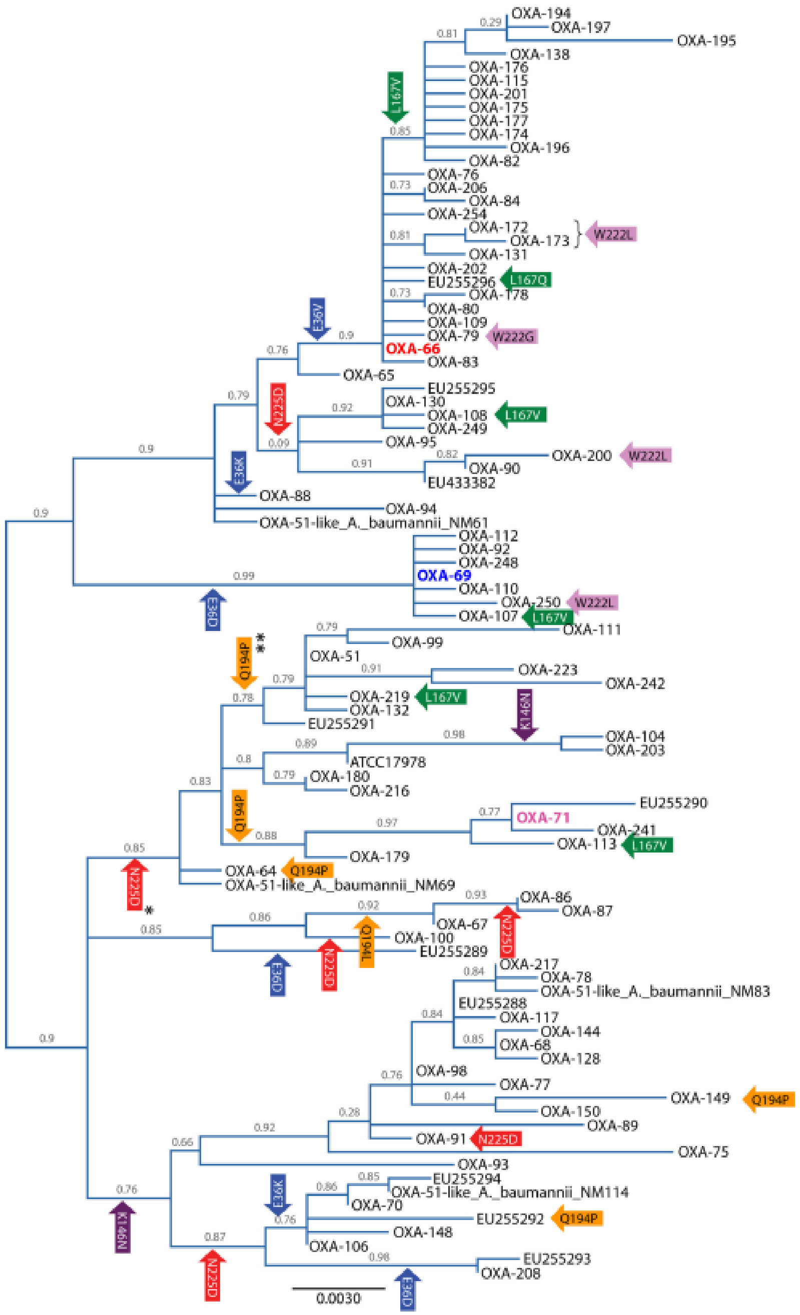
Modified from www.icureach.com

OXA-type beta lactamases



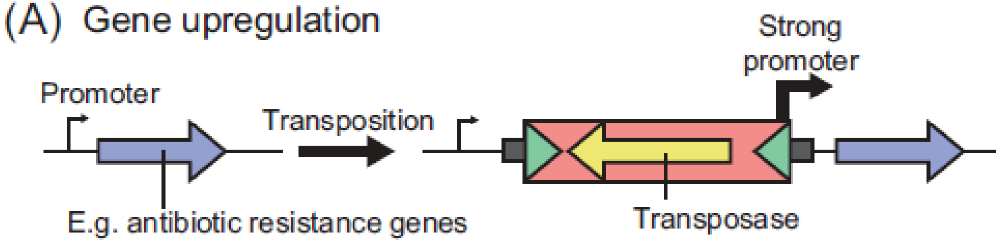
Evans et al., 2014

OXA-51-like beta lactamases

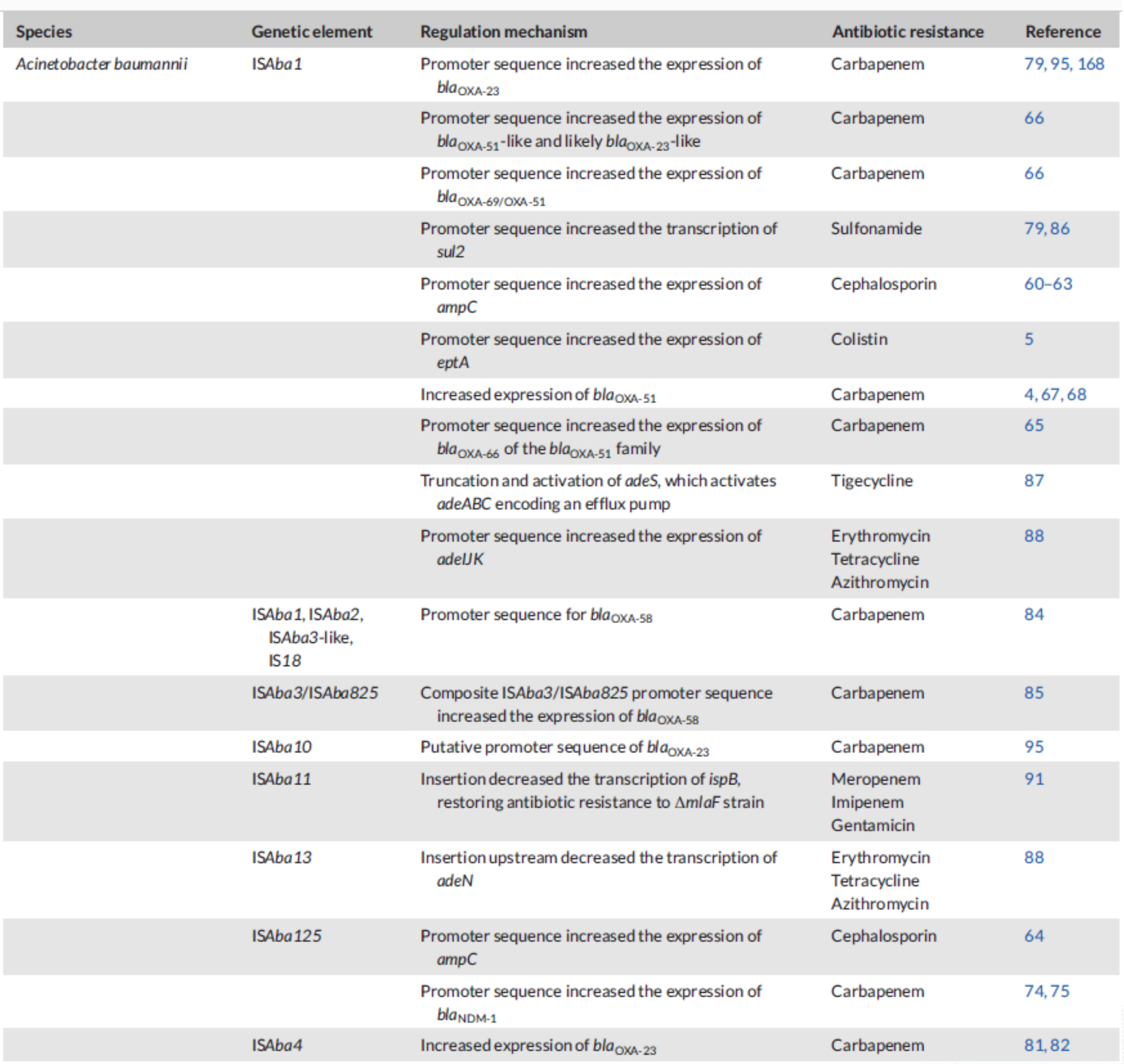


Evans et al., 2014

Insertion sequences play an important role in carbapenem resistance in *A. baumannii*

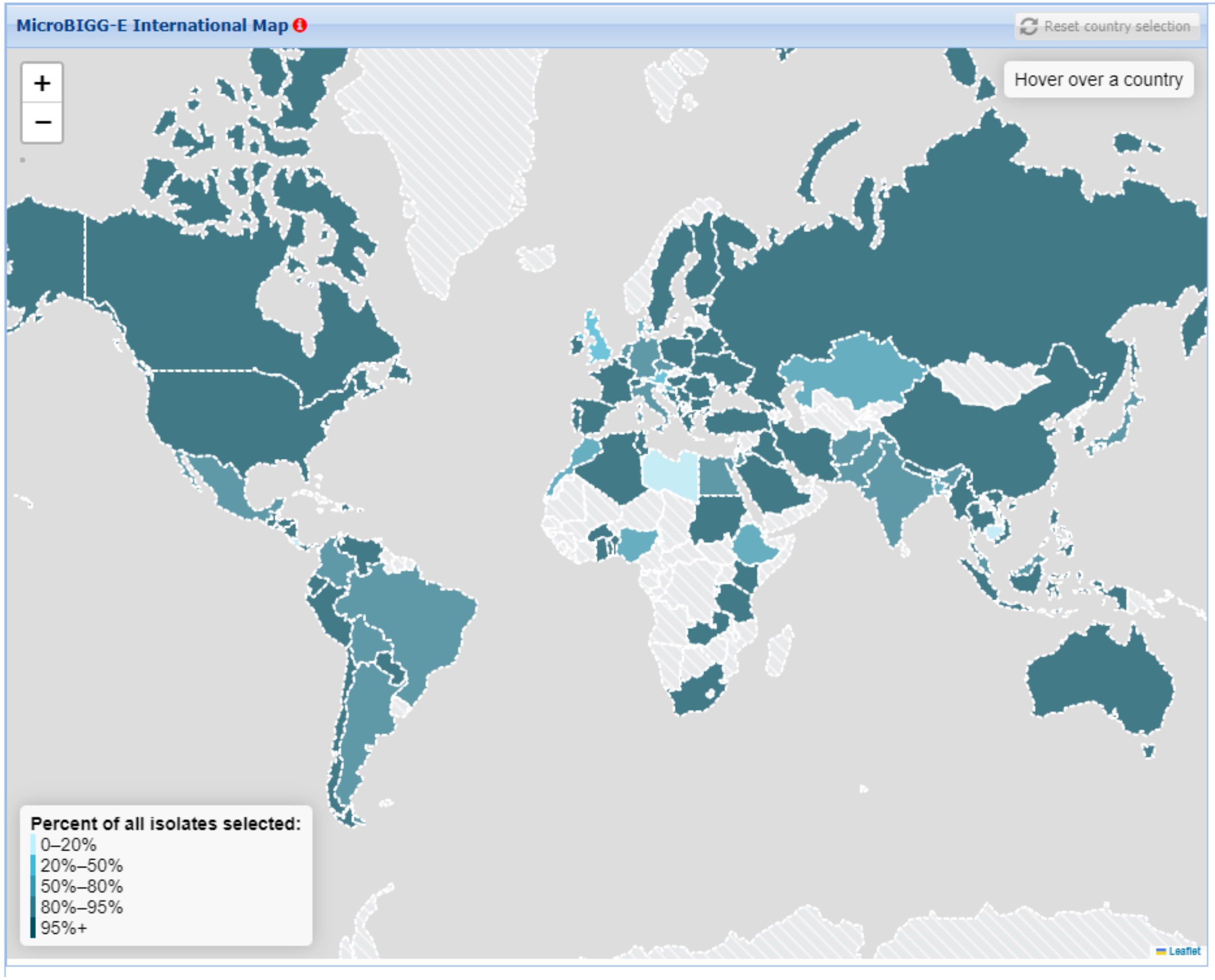


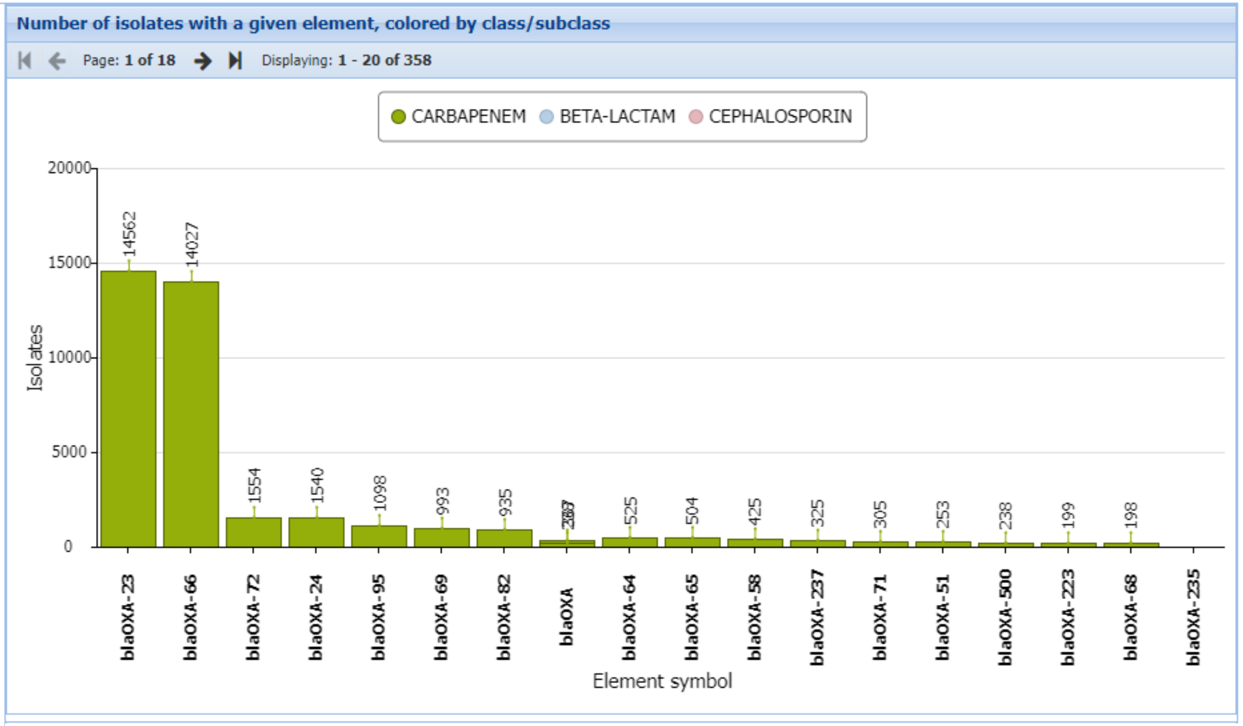
Modified from Noel et al., 2022



Species	Genetic element	Regulation mechanism	Antibiotic resistance	Reference
Acinetobacter baumannii	IS*Aba1*	Promoter sequence increased the expression of *bla*$_{\text{OXA-23}}$	Carbapenem	79, 95, 168
		Promoter sequence increased the expression of *bla*$_{\text{OXA-51}}$-like and likely *bla*$_{\text{OXA-23}}$-like	Carbapenem	66
		Promoter sequence increased the expression of *bla*$_{\text{OXA-69/OXA-51}}$	Carbapenem	66
		Promoter sequence increased the transcription of *sul2*	Sulfonamide	79, 86
		Promoter sequence increased the expression of *ampC*	Cephalosporin	60–63
		Promoter sequence increased the expression of *eptA*	Colistin	5
		Increased expression of *bla*$_{\text{OXA-51}}$	Carbapenem	4, 67, 68
		Promoter sequence increased the expression of *bla*$_{\text{OXA-66}}$ of the *bla*$_{\text{OXA-51}}$ family	Carbapenem	65
		Truncation and activation of *adeS*, which activates *adeABC* encoding an efflux pump	Tigecycline	87
		Promoter sequence increased the expression of *adeIJK*	Erythromycin Tetracycline Azithromycin	88
	IS*Aba1*, IS*Aba2*, IS*Aba3*-like, IS*18*	Promoter sequence for *bla*$_{\text{OXA-58}}$	Carbapenem	84
	IS*Aba3*/IS*Aba825*	Composite IS*Aba3*/IS*Aba825* promoter sequence increased the expression of *bla*$_{\text{OXA-58}}$	Carbapenem	85
	IS*Aba10*	Putative promoter sequence of *bla*$_{\text{OXA-23}}$	Carbapenem	95
	IS*Aba11*	Insertion decreased the transcription of *ispB*, restoring antibiotic resistance to Δ*mlaF* strain	Meropenem Imipenem Gentamicin	91
	IS*Aba13*	Insertion upstream decreased the transcription of *adeN*	Erythromycin Tetracycline Azithromycin	88
	IS*Aba125*	Promoter sequence increased the expression of *ampC*	Cephalosporin	64
		Promoter sequence increased the expression of *bla*$_{\text{NDM-1}}$	Carbapenem	74, 75
	IS*Aba4*	Increased expression of *bla*$_{\text{OXA-23}}$	Carbapenem	81, 82

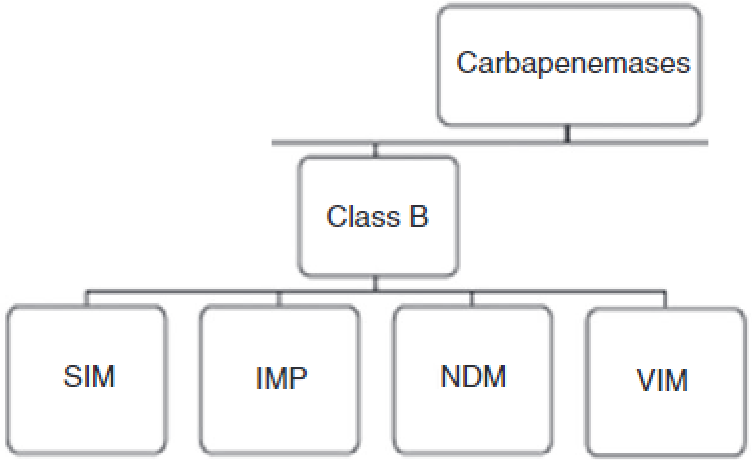
OXA - MicroBIGG-E Map





[MicroBIGG-E Map (nih.gov)](https://www.ncbi.nlm.nih.gov/pathogens/microbigge/)

Class B carbapenemases in *A. baumannii*

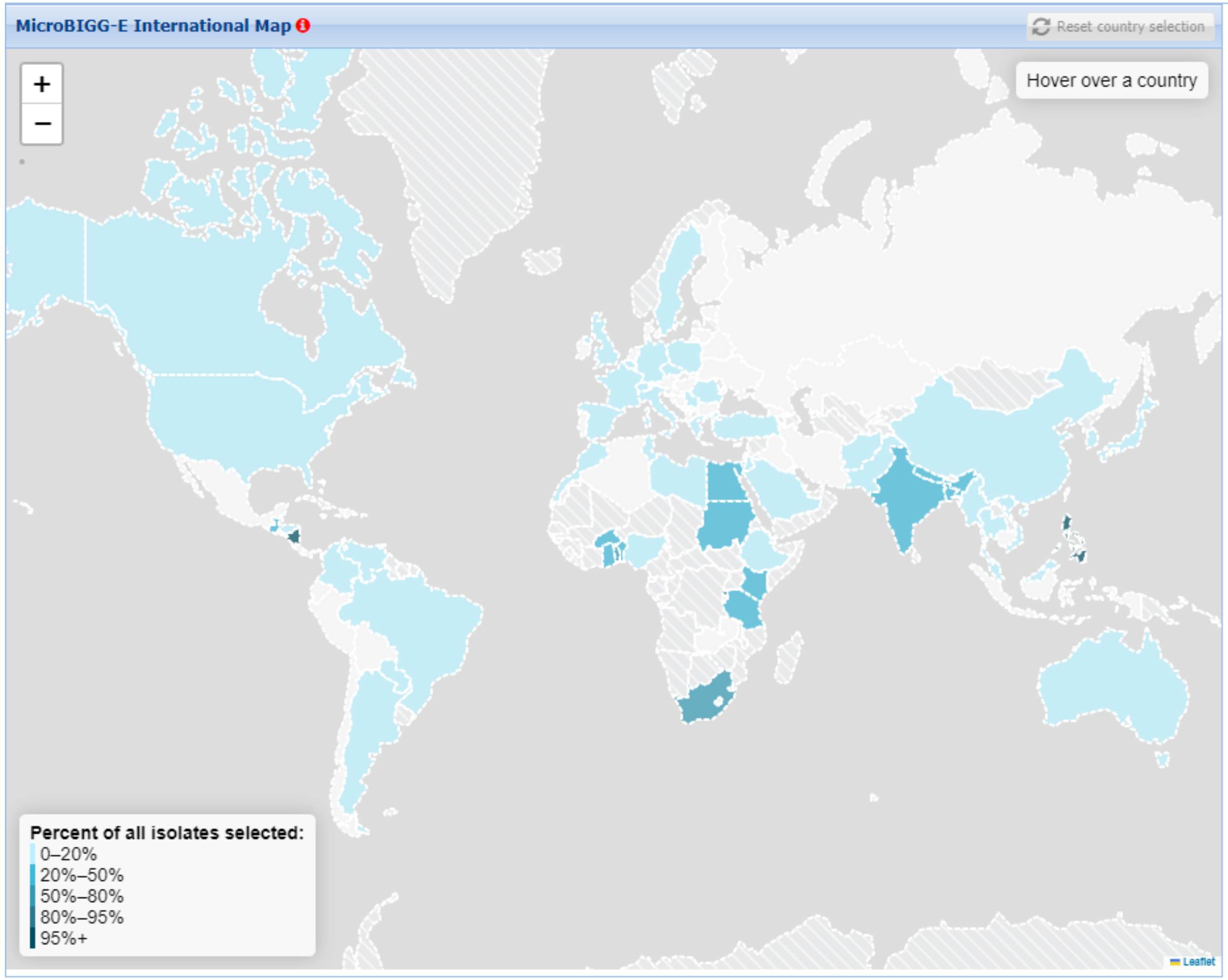


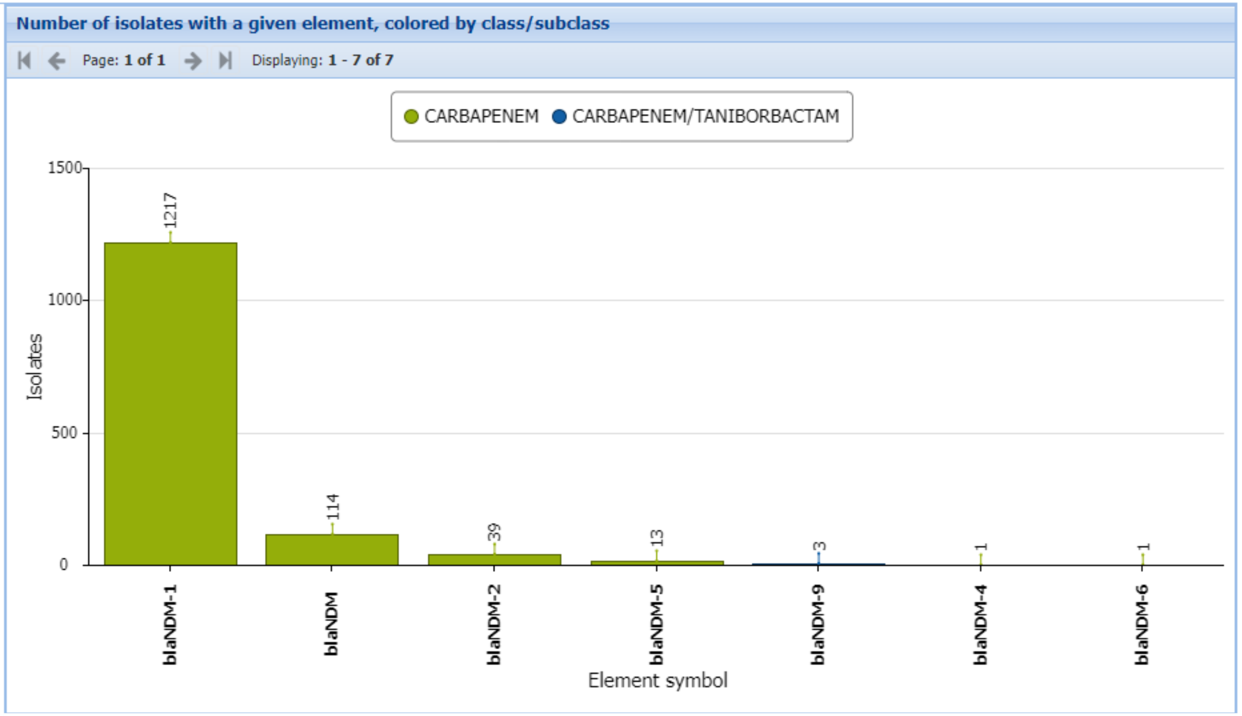
Modified from Nguyen and Joshi, 2021

Penicillin	1st Gen. Ceph.	2nd Gen. Ceph.	Cefoxitin	3rd Gen. Ceph.	4th Gen. Ceph.	Classic β-lactamase Inhibitors	Aztreonam	Carbapenems
Class A Carbapenemases (KPC)								
Class B Carbapenemases (VIM, IMP, NDM)								VIM, IMP, NDM
Class D Carbapenemases (OXA)				▨	▨	OXA		

Modified from www.icureach.com

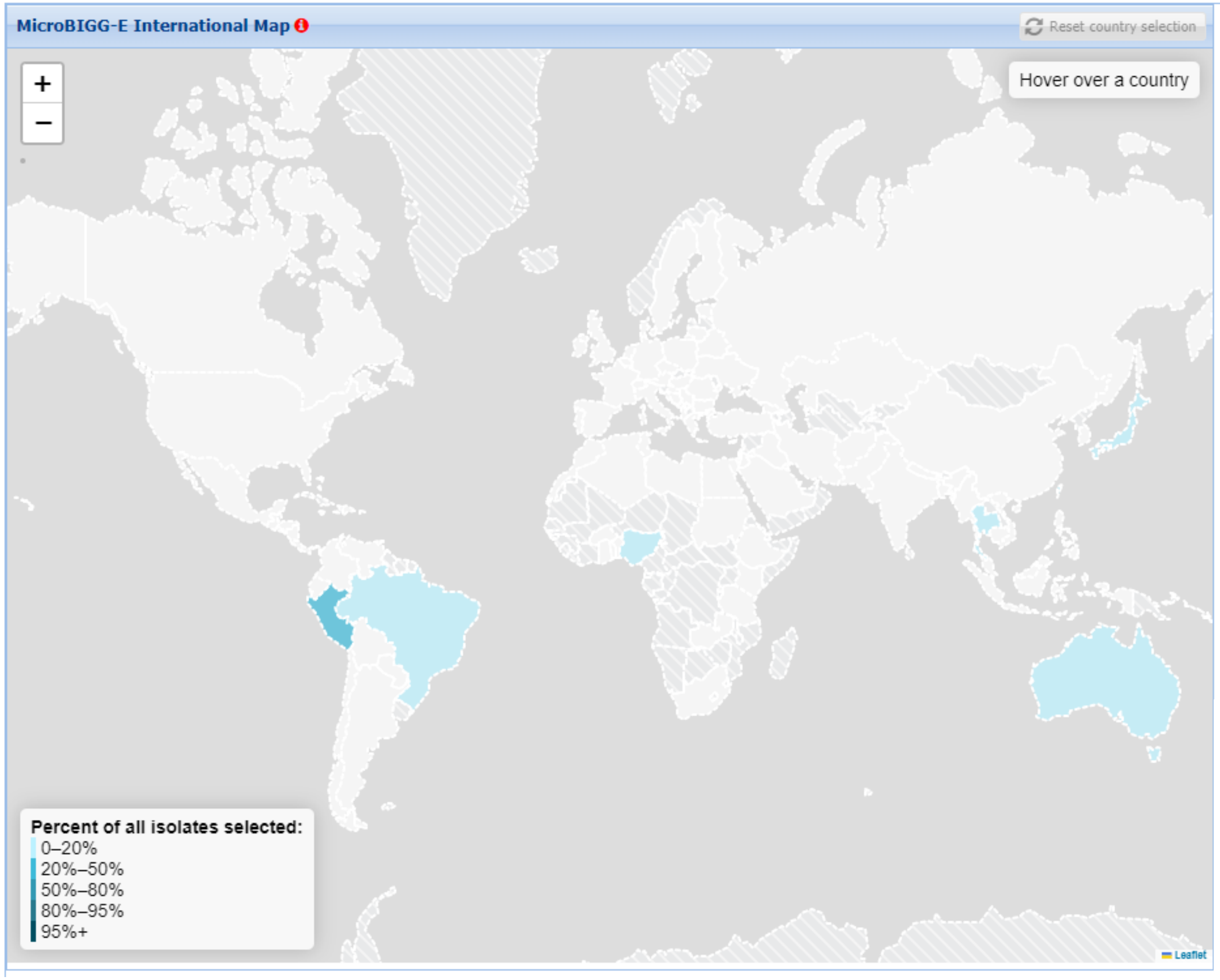
NDM - MicroBIGG-E Map

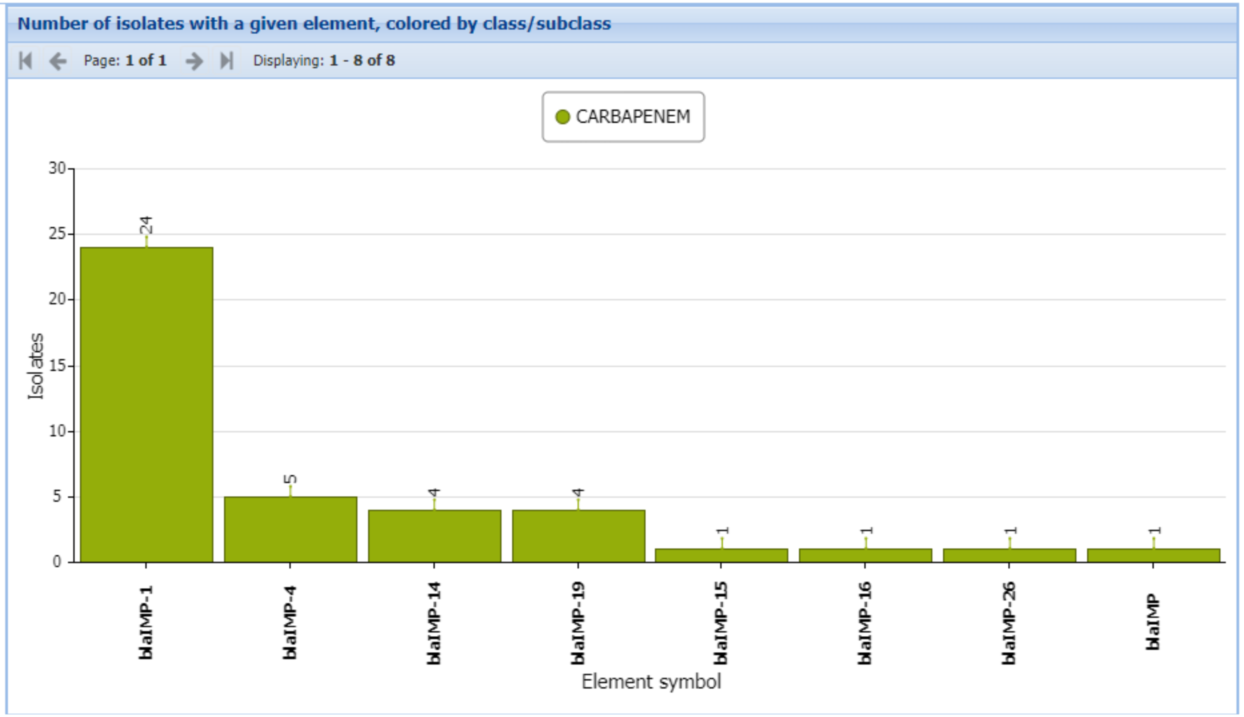




MicroBIGG-E Map (nih.gov)

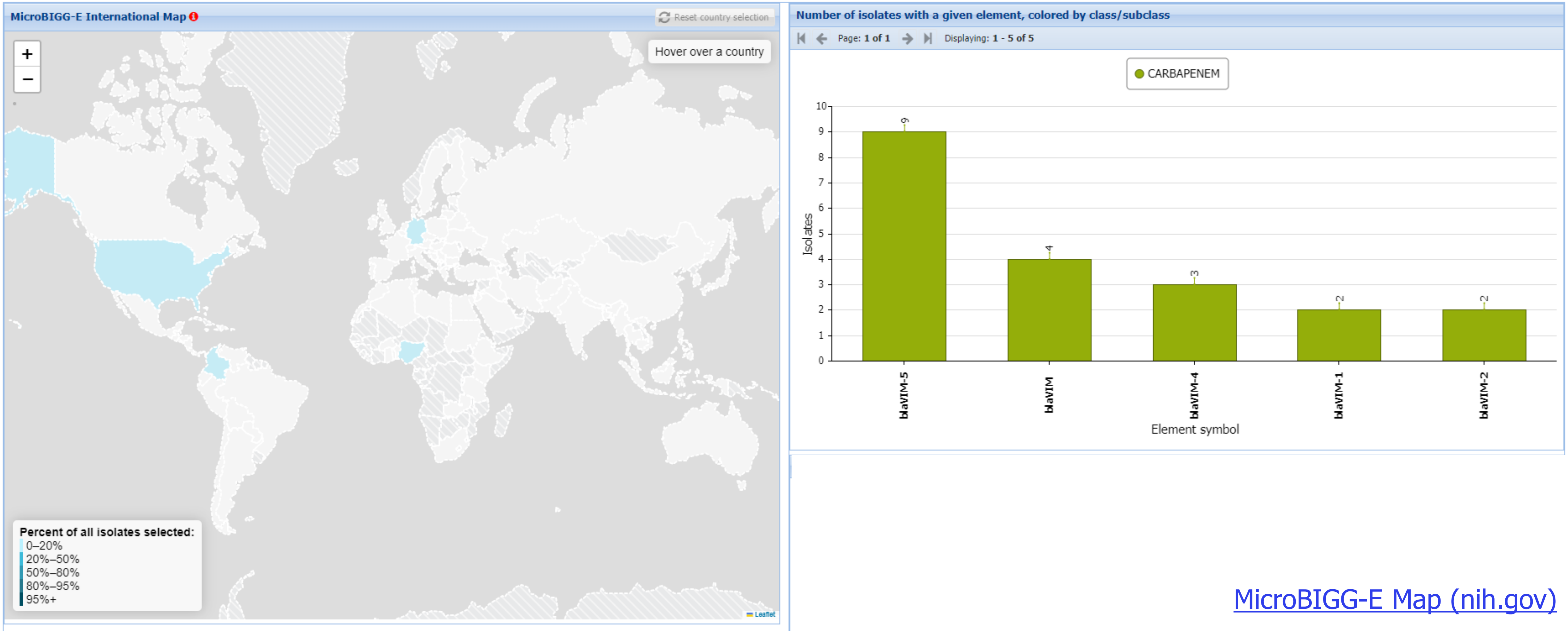
IMP - MicroBIGG-E Map





MicroBIGG-E Map (nih.gov)

VIM - MicroBIGG-E Map



MicroBIGG-E Map (nih.gov)

Class A carbapenemases in *A. baumannii*

Carbapenemases

Class A

GES

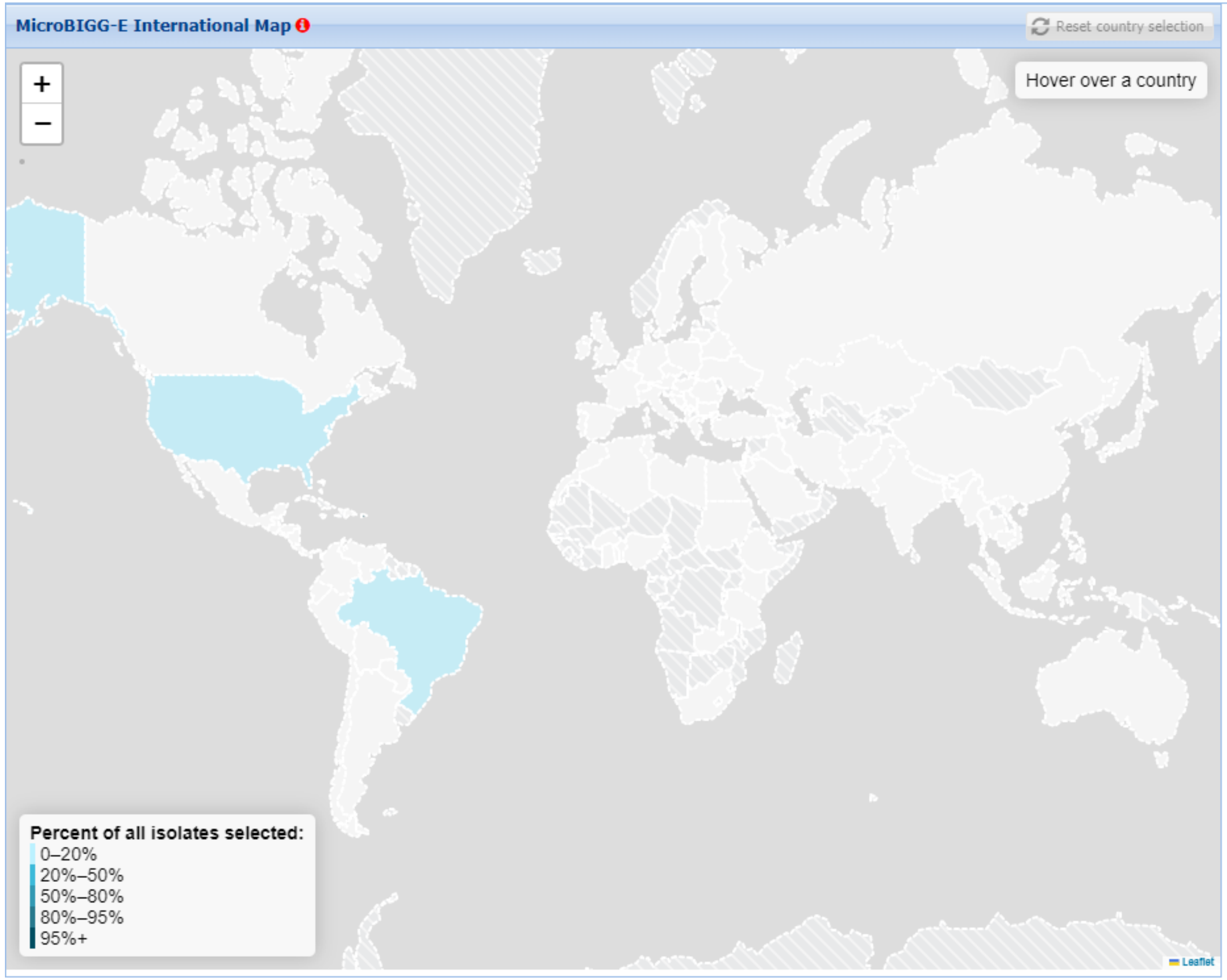
KPC

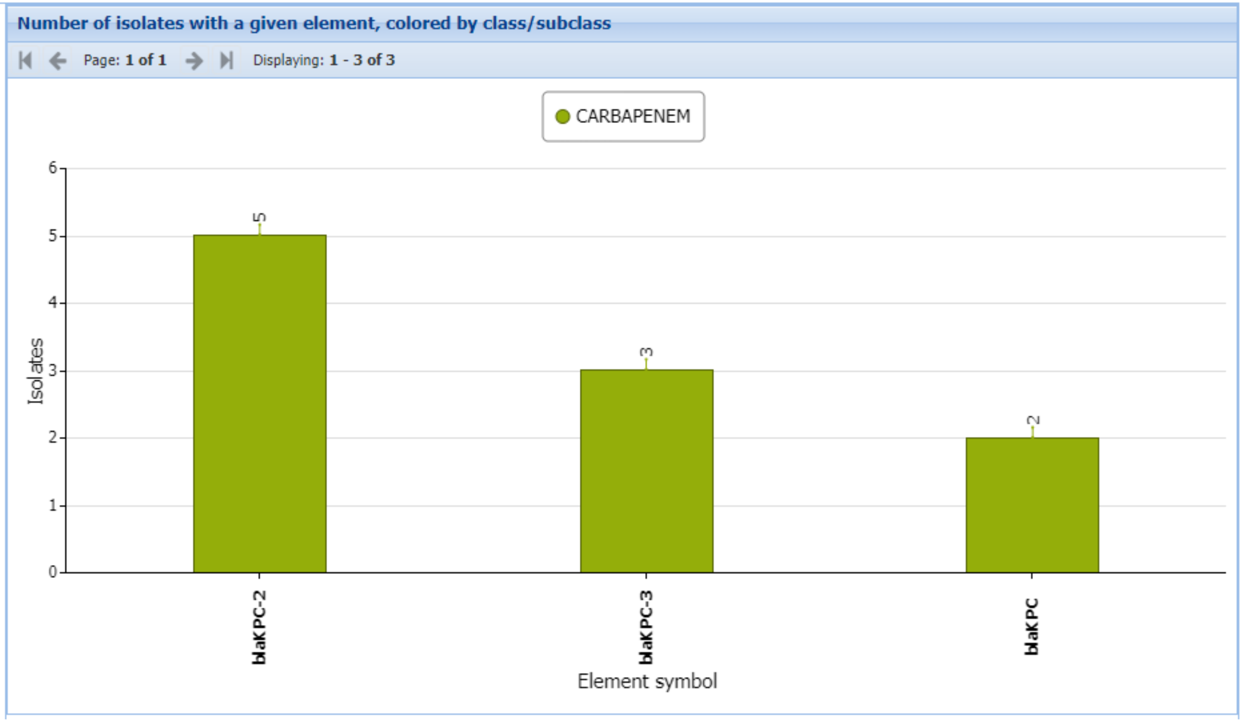
Modified from Nguyen and Joshi, 2021

	Penicillin	1st Gen. Ceph.	2nd Gen. Ceph.	Cefoxitin	3rd Gen. Ceph.	4th Gen. Ceph.	Classic β-lactamase Inhibitors	Aztreonam	Carbapenems
→	Class A Carbapenemases (KPC)								
	Class B Carbapenemases (VIM, IMP, NDM)								VIM, IMP, NDM
	Class D Carbapenemases (OXA)				▨	▨	OXA		

Modified from www.icureach.com

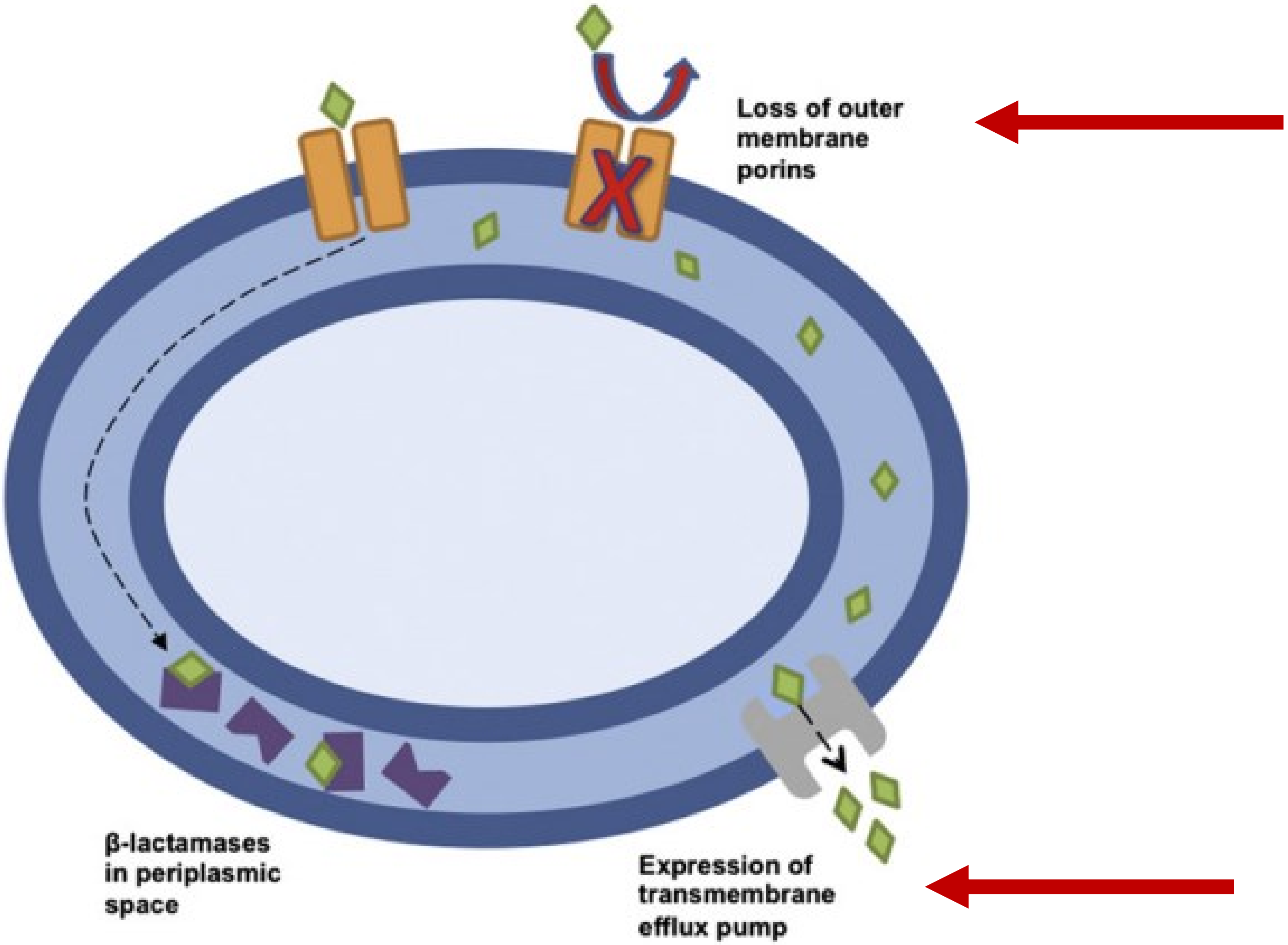
KPC - MicroBIGG-E Map





MicroBIGG-E Map (nih.gov)

Beta-lactam resistance in *A. baumannii*



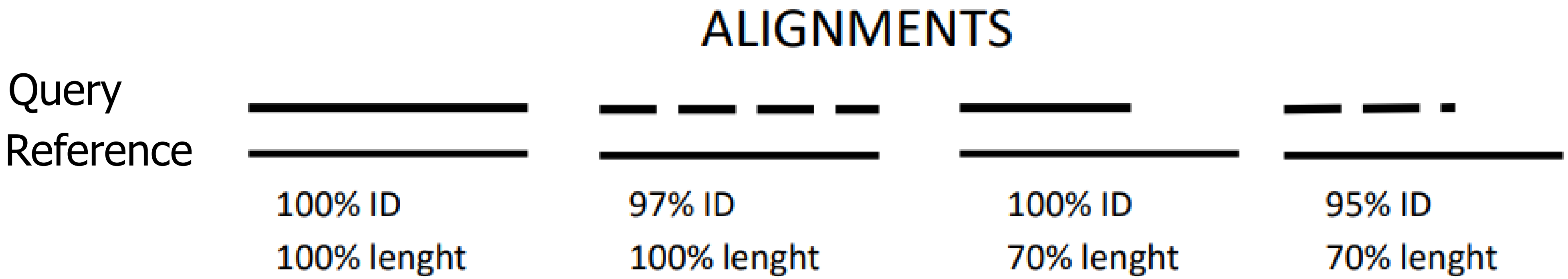
Modified from Tang et al., 2014

Carbapenem resistance by mechanisms other than carbapenemases in *A. baumannii*

- Loss of outer membrane protein CarO (Mussi et al., 2005)

- Increased expression of AdeABC efflux system (Roy et al., 2022)

Critical parameters for WGS-based detection of AMR:
Identity and coverage



Example (hypothetical CARBA):

a. ”ATG TTC CC**G**” is ”MFP”

b. ”ATG TTC CC**A**” is ”MFP”

c. ”ATG TTC CTA” is ”MFL”

CARBA-1

CARBA-2

Example 1

Database (i.e. the repository of reference sequences) contains sequence a only.

Query: isolate with sequence b

Output:

- $bla_{\text{CARBA-1}}$ w. 100% coverage, 89% ID (if nt. level)
- CARBA-1 w. 100% coverage, 100 % ID (if aa. level)

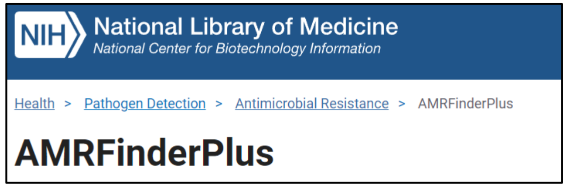
Example 2

Database contains sequence a only.

Query: isolate with sequence c

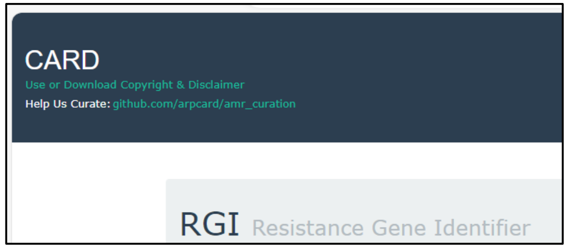
Output: 100% coverage, < 100% ID (both at nt. and at aa. level)

Tools/databases for WGS-based detection of AMR: output and gaps for CRAb

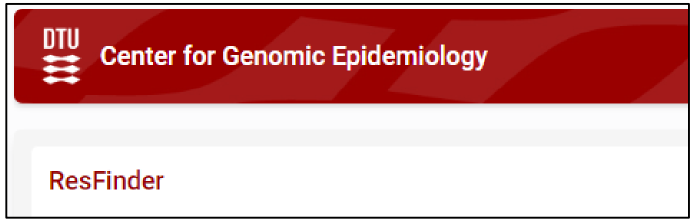

NIH National Library of Medicine
National Center for Biotechnology Information

Health > Pathogen Detection > Antimicrobial Resistance > AMRFinderPlus

AMRFinderPlus


CARD
Use or Download Copyright & Disclaimer
Help Us Curate: github.com/arpcard/amr_curation

RGI Resistance Gene Identifier


DTU Center for Genomic Epidemiology

ResFinder

Output	Gaps/Warnings
Carbapenemase-encoding genes	• Intrinsic/Acquired • Default identity and coverage may not lead to exact allelic variant
Selected mutations of chromosomal genes	• Databases differ greatly for genes/mutations included
Insertion sequences	• Not straightforward to correlate IS & AMR genes
Predicted phenotypes	• RGI and AMRFinderPlus report at class level • Validation of tools/databases is incomplete

In summary

- Beta-lactam resistance is mediated by various mechanisms, with the most studied being enzymatic inactivation by beta-lactamases (in Gram-neg.)
- Beta-lactamases are a large group of enzymes for which different classification systems have been developed
- OXA-type carbapenemases are the most widespread carbapenem resistance determinants in *A. baumannii*, followed by NDM, IMP, VIM and KPC
- Detection of carbapenemases in WGS data should be carefully evaluated: intrinsic or aquired carbapenemase?
  - If intrinsic, further analysis are needed to enable prediction of phenotype
  - If acquired, analysis of MGEs would be of great epidemiological importance
- Output of current bioinformatic tools and databases for WGS-based detection of AMR requires careful interpretation

Thank you for your attention!