Genomic characterization of *Klebsiella pneumoniae*

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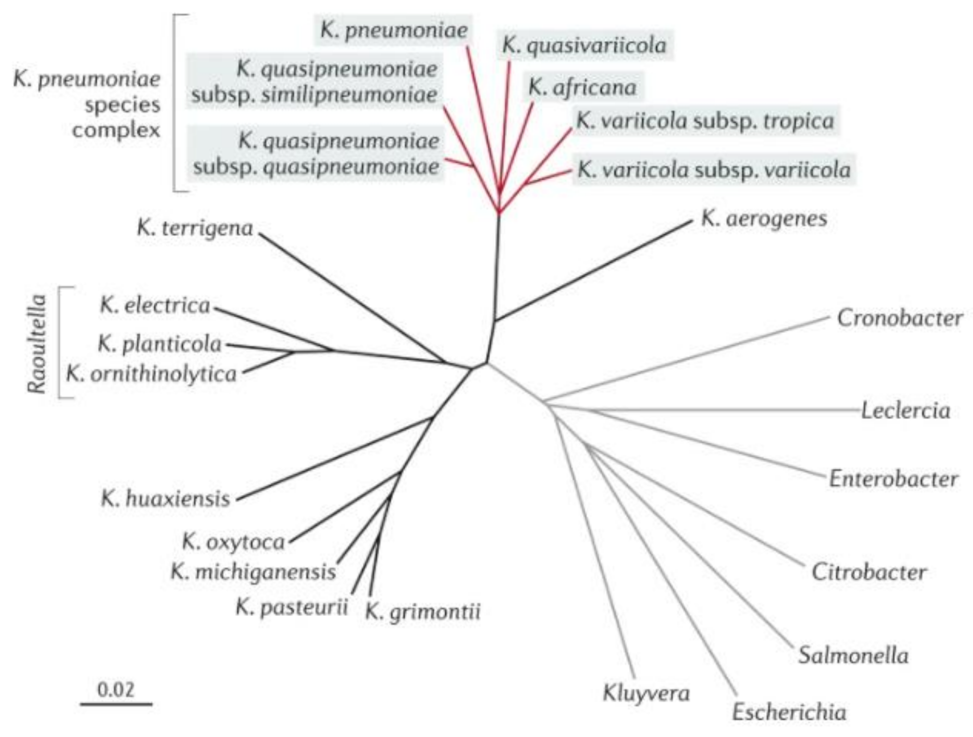
ERFAN Laboratory training course 17-21 October 2022

“Detection and characterization of *Listeria monocytogenes*, *Klebsiella pneumoniae* and *Salmonella* spp.”

Klebsiella pneumoniae: taxonomy



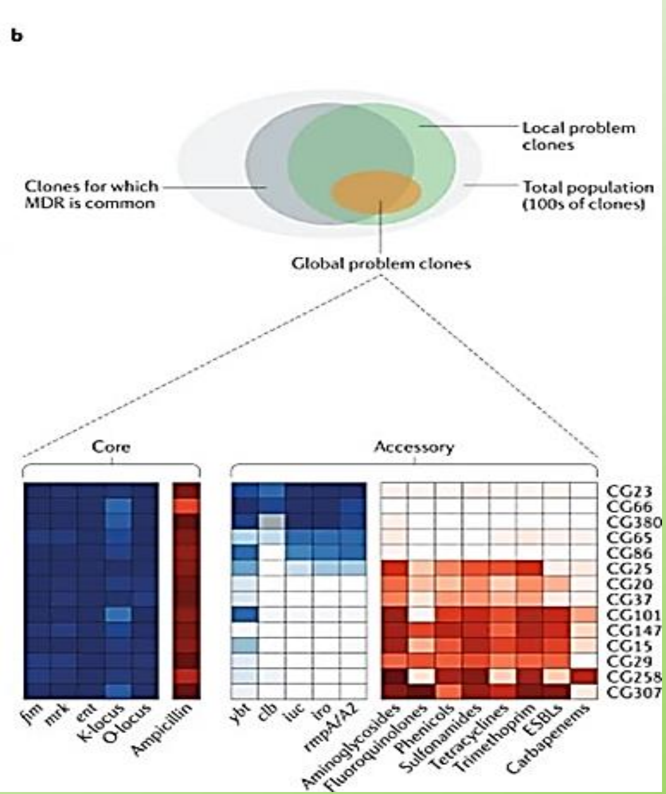
Wyres et al., 2020

K. pneumoniae and bacteria belonging to *K. pneumoniae* species complex **KpSC** (red branches) that includes seven phylogroups (Kp1 to Kp7):

- ***Klebsiella pneumoniae* (Kp1)**
- *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* (Kp2)
- *Klebsiella quasipneumoniae* subsp. *similpneumoniae (Kp4)*
- *Klebsiella variicola* subsp. *variicola* (Kp3)
- *Klebsiella variicola* subsp. *tropica* (Kp5)
- *Klebsiella quasivariicola* (Kp6)
- *Klebsiella africana* (Kp7)

Klebsiella pneumoniae: genome

Typical *K. pneumoniae* genomes are ~5–6 Mbp in size, encoding ~5,000–6,000 genes. Approximately 1,700 genes are conserved in all members of the species (**core genes**), whereas the remainder are variably present (**accessory genes**)



Klebsiella pneumoniae: DNA extraction

QIAGEN

QIAamp® DNA Mini Kit (250)

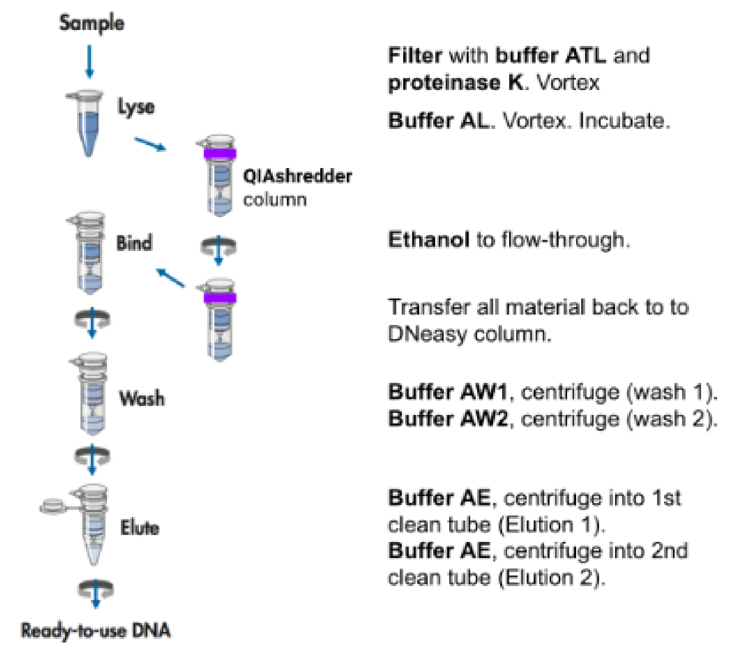
Cat. No. 51306

Store at room temperature (15–25°C)

For molecular biology applications
Not intended for diagnostic purposes

Lot No. 148038419

00513060000001480384 19



Filter with buffer ATL and **proteinase K**. Vortex

Buffer AL. Vortex. Incubate.

Ethanol to flow-through.

Transfer all material back to to DNeasy column.

Buffer AW1, centrifuge (wash 1).
Buffer AW2, centrifuge (wash 2).

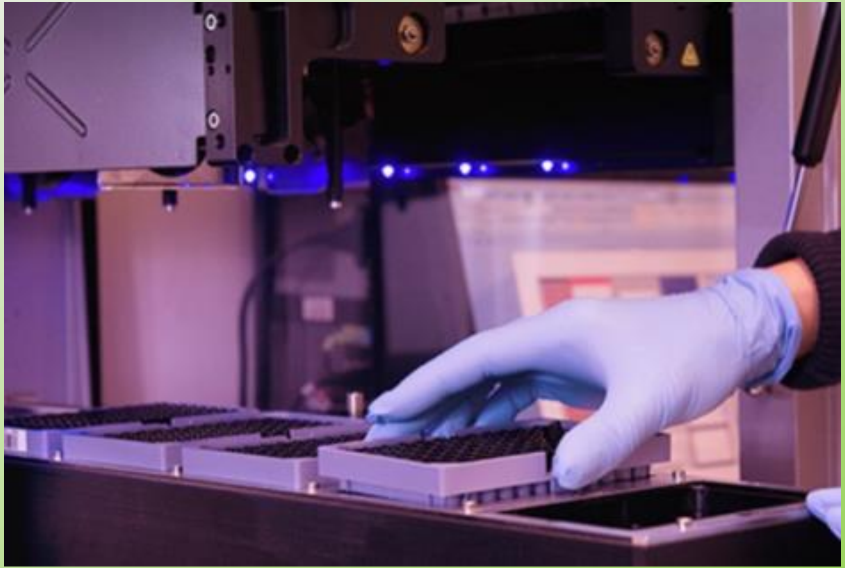
Buffer AE, centrifuge into 1st clean tube (Elution 1).
Buffer AE, centrifuge into 2nd clean tube (Elution 2).

Gram negative bacteria: starting from pure culture without incubation with lysozime at 37°C, but directly in ATL buffer + Proteinase K at 56° C.

Klebsiella pneumoniae:WGS

Genomics approaches have advanced our understanding of *K. pneumoniae* taxonomy, ecology and evolution as well as the diversity and distribution of clinically relevant determinants of pathogenicity and antimicrobial resistance.

IZSAM National Reference Center (NRC) for Genomic Sequences of Pathogenic Microorganisms: database and bioinformatics analysis



Klebsiella pneumoniae:WGS

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IZSAM NRC for Genomic Sequences:

- Creates a national platform for the collection and storage of genomic sequences of pathogenic microorganisms and for the performance of bioinformatics analyses, archiving and sharing of results;
- provides technical-scientific assistance to the Ministry of Health and the competent authorities;
- promotes research activities in the sector of competence;
- collaboration and coordination with other administrations and organizations.

Klebsiella pneumoniae: GENPAT platform

BioinfoDB - Laboratorio Klebsiella. Per visualizzare il **Motivo Attivo** clicca qui.

Schede dati Campione Genpat

Codice	Specie	Host	Matrice	Data P...	Punto Prelievo	Sequence Type	Richiedente
2019.TE.17588.1.10	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	1290	ISTITUTO ZOOPRO...
2019.TE.17588.1.11	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	636	ISTITUTO ZOOPRO...
2019.TE.17588.1.12	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	636	ISTITUTO ZOOPRO...
2019.TE.17588.1.13	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	636	ISTITUTO ZOOPRO...
2019.TE.17588.1.14	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	636	ISTITUTO ZOOPRO...
2019.TE.17588.1.15	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	704	ISTITUTO ZOOPRO...
2019.TE.17588.1.16	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	-	ISTITUTO ZOOPRO...
2019.TE.17588.1.17	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	104	ISTITUTO ZOOPRO...
2019.TE.17588.1.18	KLEBSIELLA PNEU...			27/08/...	IZSAM - Igiene e Te...	45	ISTITUTO ZOOPRO...

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Klebsiella pneumoniae: GENPAT platform

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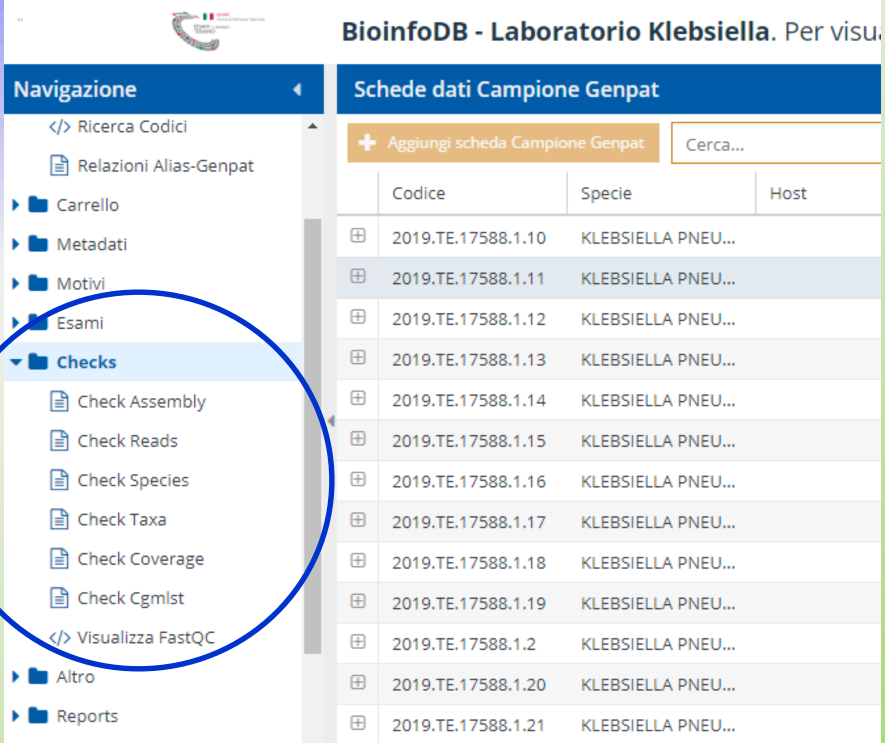

BioinfoDB - Laboratorio Klebsiella. Per visu

Navigazione

- Ricerca Codici
- Relazioni Alias-Genpat
- Carrello
- Metadati
- Motivi
- Esami
- **Checks**
  - Check Assembly
  - Check Reads
  - Check Species
  - Check Taxa
  - Check Coverage
  - Check Cgmlst
  - Visualizza FastQC
- Altro
- Reports

Schede dati Campione Genpat

	Codice	Specie	Host
⊞	2019.TE.17588.1.10	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.11	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.12	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.13	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.14	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.15	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.16	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.17	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.18	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.19	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.2	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.20	KLEBSIELLA PNEU...	
⊞	2019.TE.17588.1.21	KLEBSIELLA PNEU...	

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Klebsiella pneumoniae: GENPAT platform

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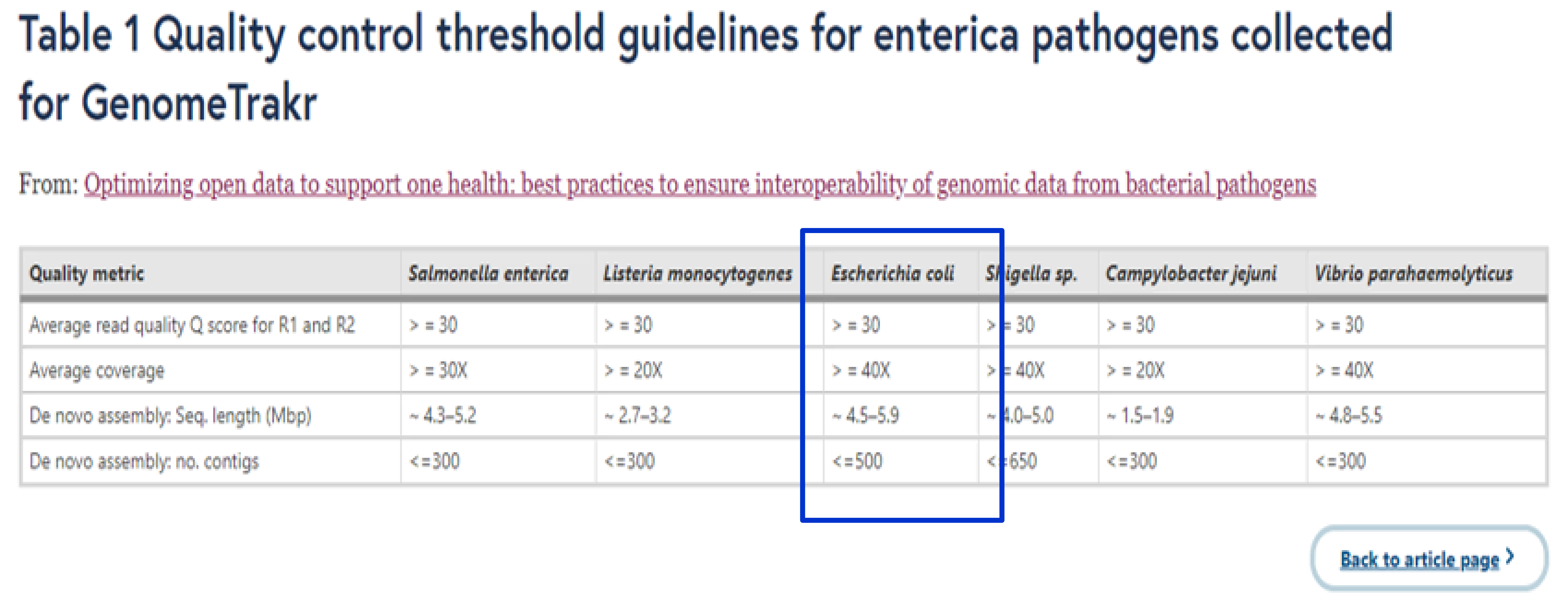


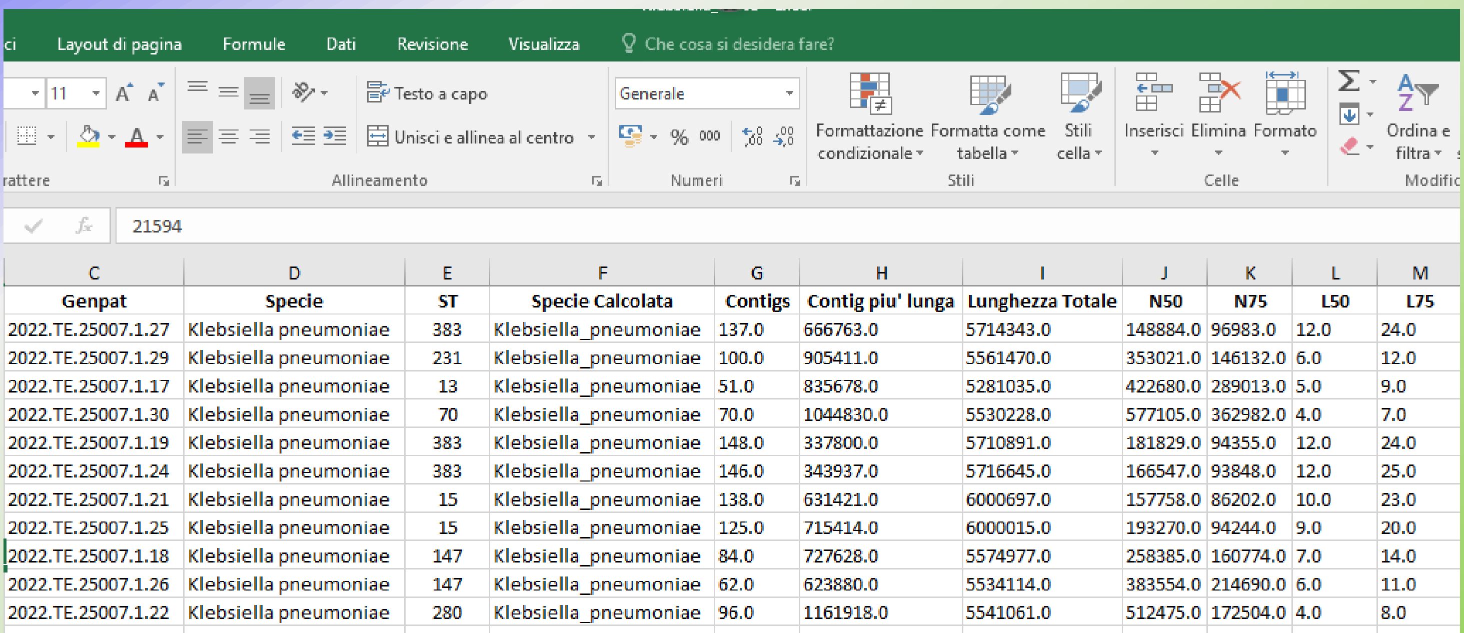
Table 1 Quality control threshold guidelines for enterica pathogens collected for GenomeTrakr

From: Optimizing open data to support one health: best practices to ensure interoperability of genomic data from bacterial pathogens

Quality metric	***Salmonella enterica***	***Listeria monocytogenes***	***Escherichia coli***	***Shigella sp.***	***Campylobacter jejuni***	***Vibrio parahaemolyticus***
Average read quality Q score for R1 and R2	> = 30	> = 30	> = 30	> = 30	> = 30	> = 30
Average coverage	> = 30X	> = 20X	> = 40X	> = 40X	> = 20X	> = 40X
De novo assembly: Seq. length (Mbp)	~ 4.3–5.2	~ 2.7–3.2	~ 4.5–5.9	~ 4.0–5.0	~ 1.5–1.9	~ 4.8–5.5
De novo assembly: no. contigs	< = 300	< = 300	< = 500	< = 650	< = 300	< = 300

Klebsiella pneumoniae: GENPAT platform

Check Assemply Report



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C	D	E	F	G	H	I	J	K	L	M
Genpat	**Specie**	**ST**	**Specie Calcolata**	**Contigs**	**Contig piu' lunga**	**Lunghezza Totale**	**N50**	**N75**	**L50**	**L75**
2022.TE.25007.1.27	Klebsiella pneumoniae	383	Klebsiella_pneumoniae	137.0	666763.0	5714343.0	148884.0	96983.0	12.0	24.0
2022.TE.25007.1.29	Klebsiella pneumoniae	231	Klebsiella_pneumoniae	100.0	905411.0	5561470.0	353021.0	146132.0	6.0	12.0
2022.TE.25007.1.17	Klebsiella pneumoniae	13	Klebsiella_pneumoniae	51.0	835678.0	5281035.0	422680.0	289013.0	5.0	9.0
2022.TE.25007.1.30	Klebsiella pneumoniae	70	Klebsiella_pneumoniae	70.0	1044830.0	5530228.0	577105.0	362982.0	4.0	7.0
2022.TE.25007.1.19	Klebsiella pneumoniae	383	Klebsiella_pneumoniae	148.0	337800.0	5710891.0	181829.0	94355.0	12.0	24.0
2022.TE.25007.1.24	Klebsiella pneumoniae	383	Klebsiella_pneumoniae	146.0	343937.0	5716645.0	166547.0	93848.0	12.0	25.0
2022.TE.25007.1.21	Klebsiella pneumoniae	15	Klebsiella_pneumoniae	138.0	631421.0	6000697.0	157758.0	86202.0	10.0	23.0
2022.TE.25007.1.25	Klebsiella pneumoniae	15	Klebsiella_pneumoniae	125.0	715414.0	6000015.0	193270.0	94244.0	9.0	20.0
2022.TE.25007.1.18	Klebsiella pneumoniae	147	Klebsiella_pneumoniae	84.0	727628.0	5574977.0	258385.0	160774.0	7.0	14.0
2022.TE.25007.1.26	Klebsiella pneumoniae	147	Klebsiella_pneumoniae	62.0	623880.0	5534114.0	383554.0	214690.0	6.0	11.0
2022.TE.25007.1.22	Klebsiella pneumoniae	280	Klebsiella_pneumoniae	96.0	1161918.0	5541061.0	512475.0	172504.0	4.0	8.0
2022.TE.25007.1.32	Klebsiella pneumoniae	231	Klebsiella_pneumoniae	123.0	452689.0	5465422.0	338180.0	144683.0	7.0	14.0
2022.TE.25007.1.23	Klebsiella pneumoniae	383	Klebsiella_pneumoniae	164.0	460126.0	5702807.0	125340.0	87203.0	14.0	27.0

Klebsiella pneumoniae:WGS

- KLEBORATE

  https://kleborate.erc.monash.edu/

- KAPTIVE

  https://kaptive-web.erc.monas

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Klebsiella pneumoniae:WGS

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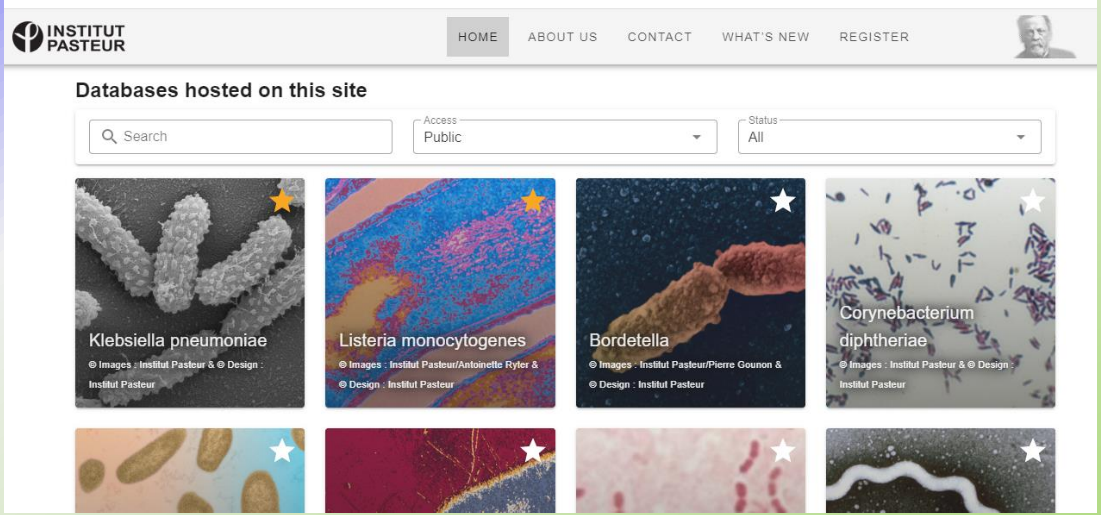
- BIGSdb Pasteur (sgcgMLST_629)

  https://bigsdb.pasteur.fr/



Databases hosted on this site

Access: Public

Status: All

Klebsiella pneumoniae
© Images : Institut Pasteur & © Design : Institut Pasteur

Listeria monocytogenes
© Images : Institut Pasteur/Antoinette Ryter & © Design : Institut Pasteur

Bordetella
© Images : Institut Pasteur/Pierre Gounon & © Design : Institut Pasteur

Corynebacterium diphtheriae
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This website provides access to genomic data and genotypic definitions for isolates of the ***K. pneumoniae*** species complex (also called ***K. pneumoniae*** sensu lato, i.e., including ***K. pneumoniae, K. quasipneumoniae, K. variicola*** and related taxa) based on Multilocus Sequence Typing (MLST), core genome MLST (cgMLST), ribosomal MLST (rMLST), capsular typing (wzc and wzi sequencing) and MLST of virulence gene clusters.

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Klebsiella PasteurMLST database

This database contains data for a collection of isolates that represent the total known diversity of Klebsiella pneumoniae and closely related species. For every allelic profile in the profiles/sequence definition database there is at least one corresponding isolate deposited here. Isolates submitted to this database are (in general) those that represent novel allelic profiles and consequently it should be noted that the database does not represent an unbiased population sample.

Record versions: **current**; Record creation: **all time**

Isolate count: 11,690 (▲ 112 [month])

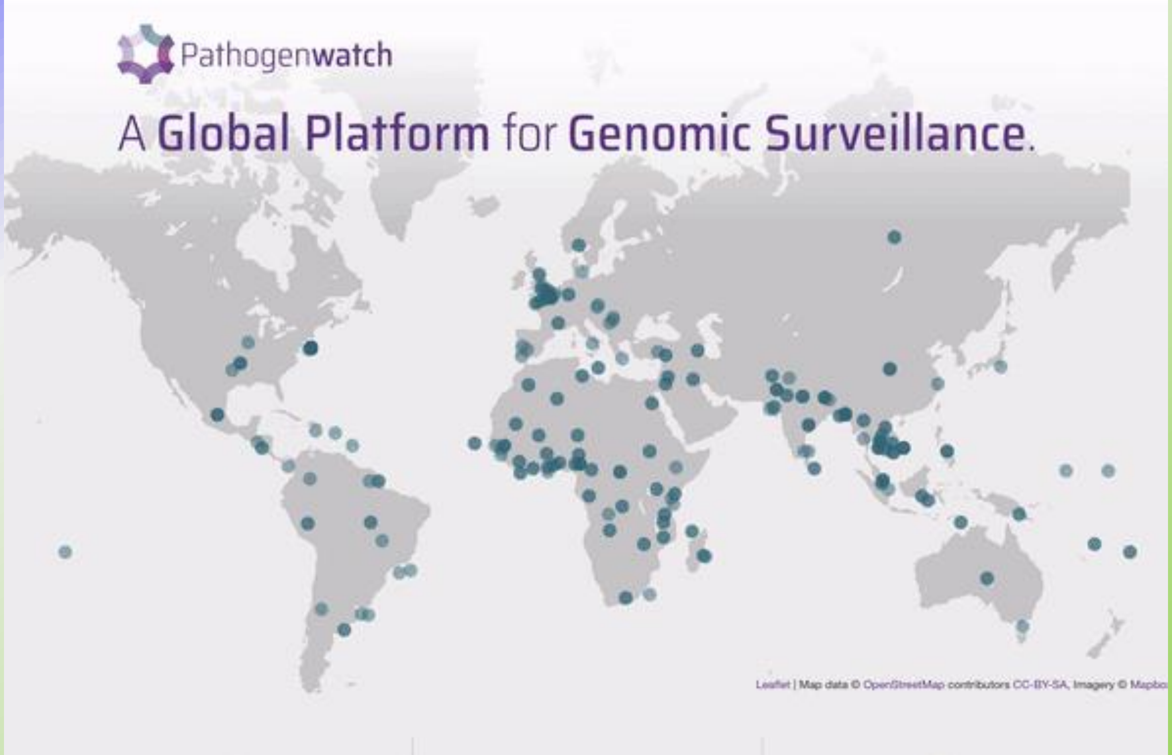
Genome count: 6,433 (▲ 64 [month])

Continent — Top 5 values

Value	Frequency
Europe	5,058
Asia	3,662
North America	934
Africa	923
South America	573

Klebsiella pneumoniae:WGS

- Pathogenwatch https://pathogen.watch/



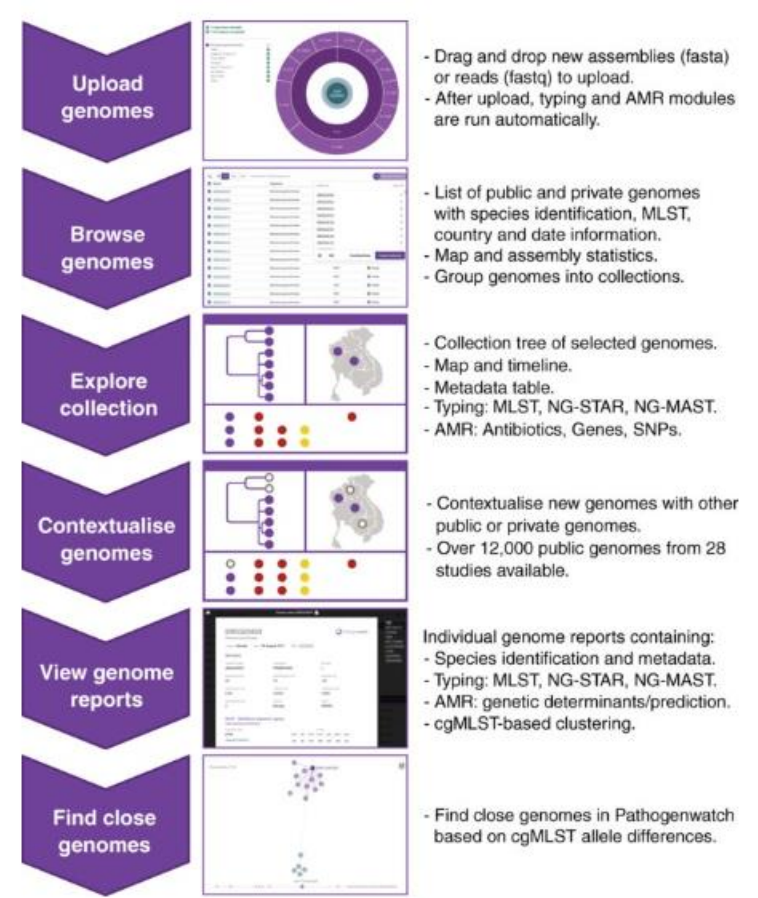
Pathogenwatch

A Global Platform for Genomic Surveillance.

Leaflet | Map data © OpenStreetMap contributors CC-BY-SA, Imagery © Mapbo

Pathogenwatch workfow

New genomes can be uploaded and combined with public data for contextualisation. The collection view allows data exploration through a combined phylogenetic tree, a map, a timeline and the metadata table, which can be switched to show typing information (multi-locus sequence typing, MLST) as well as known genetic AMR mechanisms . Genome reports summarise the metadata, typing and AMR marker results for individual isolates and allow finding other close genomes in Pathogenwatch based on core genome MLST (cgMLST).



Upload genomes

- Drag and drop new assemblies (fasta) or reads (fastq) to upload.
- After upload, typing and AMR modules are run automatically.

Browse genomes

- List of public and private genomes with species identification, MLST, country and date information.
- Map and assembly statistics.
- Group genomes into collections.

Explore collection

- Collection tree of selected genomes.
- Map and timeline.
- Metadata table.
- Typing: MLST, NG-STAR, NG-MAST.
- AMR: Antibiotics, Genes, SNPs.

Contextualise genomes

- Contextualise new genomes with other public or private genomes.
- Over 12,000 public genomes from 28 studies available.

View genome reports

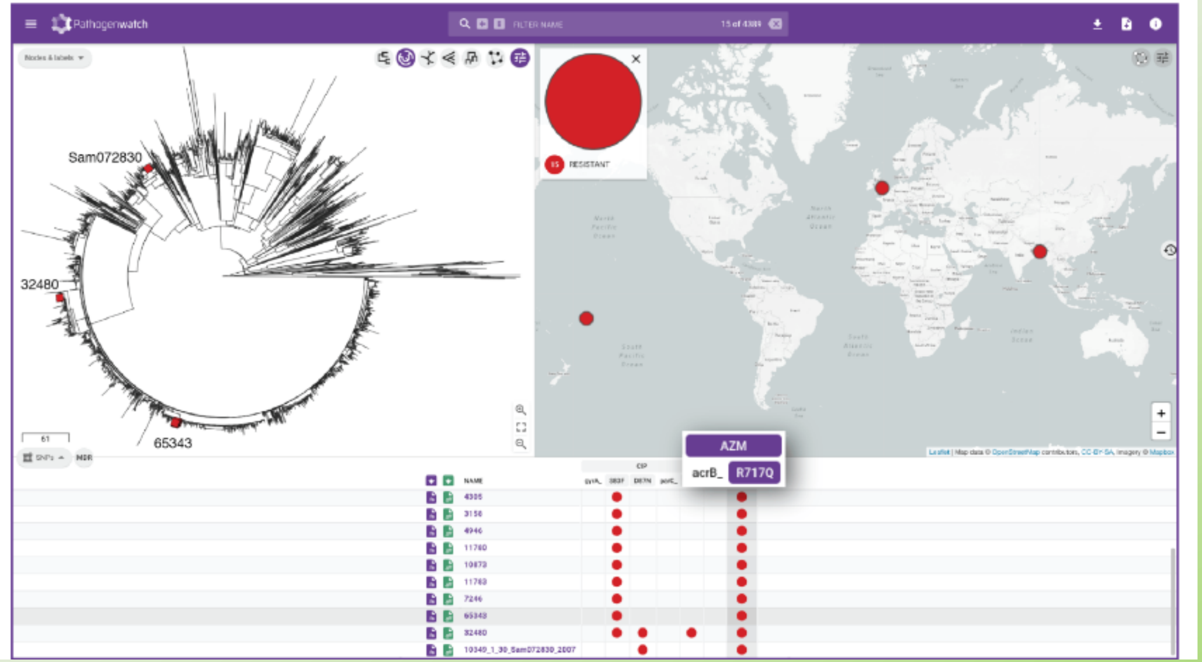
Individual genome reports containing:

- Species identification and metadata.
- Typing: MLST, NG-STAR, NG-MAST.
- AMR: genetic determinants/prediction.
- cgMLST-based clustering.

Find close genomes

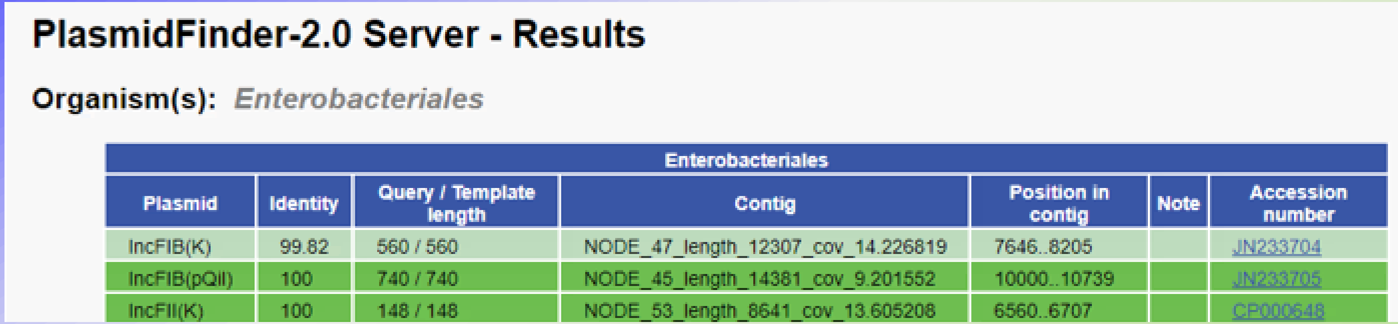
- Find close genomes in Pathogenwatch based on cgMLST allele differences.

Pathogenwatch workfow



Main display of a Pathogenwatch collection, showing a phylogenetic tree, a map and a table of SNPs (single-nucleotide polymorphisms) associated with AMR.

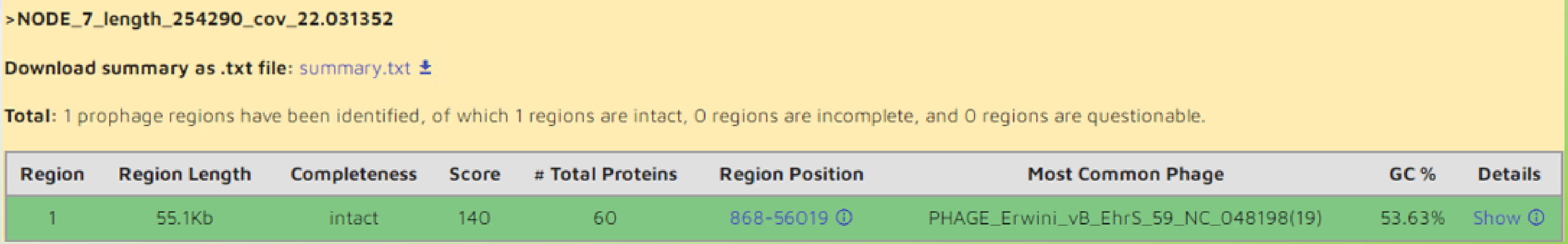
Plasmidfinder & Phaster



PlasmidFinder-2.0 Server - Results

Organism(s): *Enterobacteriales*

Enterobacteriales						
Plasmid	**Identity**	**Query / Template length**	**Contig**	**Position in contig**	**Note**	**Accession number**
IncFIB(K)	99.82	560 / 560	NODE_47_length_12307_cov_14.226819	7646..8205		JN233704
IncFIB(pQil)	100	740 / 740	NODE_45_length_14381_cov_9.201552	10000..10739		JN233705
IncFII(K)	100	148 / 148	NODE_53_length_8641_cov_13.605208	6560..6707		CP000648



>NODE_7_length_254290_cov_22.031352

Download summary as .txt file: summary.txt

Total: 1 prophage regions have been identified, of which 1 regions are intact, 0 regions are incomplete, and 0 regions are questionable.

Region	Region Length	Completeness	Score	# Total Proteins	Region Position	Most Common Phage	GC %	Details
1	55.1Kb	intact	140	60	868-56019 ⓘ	PHAGE_Erwini_vB_EhrS_59_NC_048198(19)	53.63%	Show ⓘ

WGS Applications

- Next Generation Sequencing Platforms
- Reduced costs, short response times, accuracy, higher level of discrimination
- Accredited method
- Quick response in emergency situations (e.g. Sars-Cov2)

Applications:

- **Genomic characterization of isolates**
- **Surveillance and outbreak investigations**
- **Analytical genomics**

Characterization

- Antimicrobial-resistance genes
- Virulence factors
- Genes related to the ability to form biofilms
- Genes related to tolerance to disinfectants and environmental stress

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Characterization

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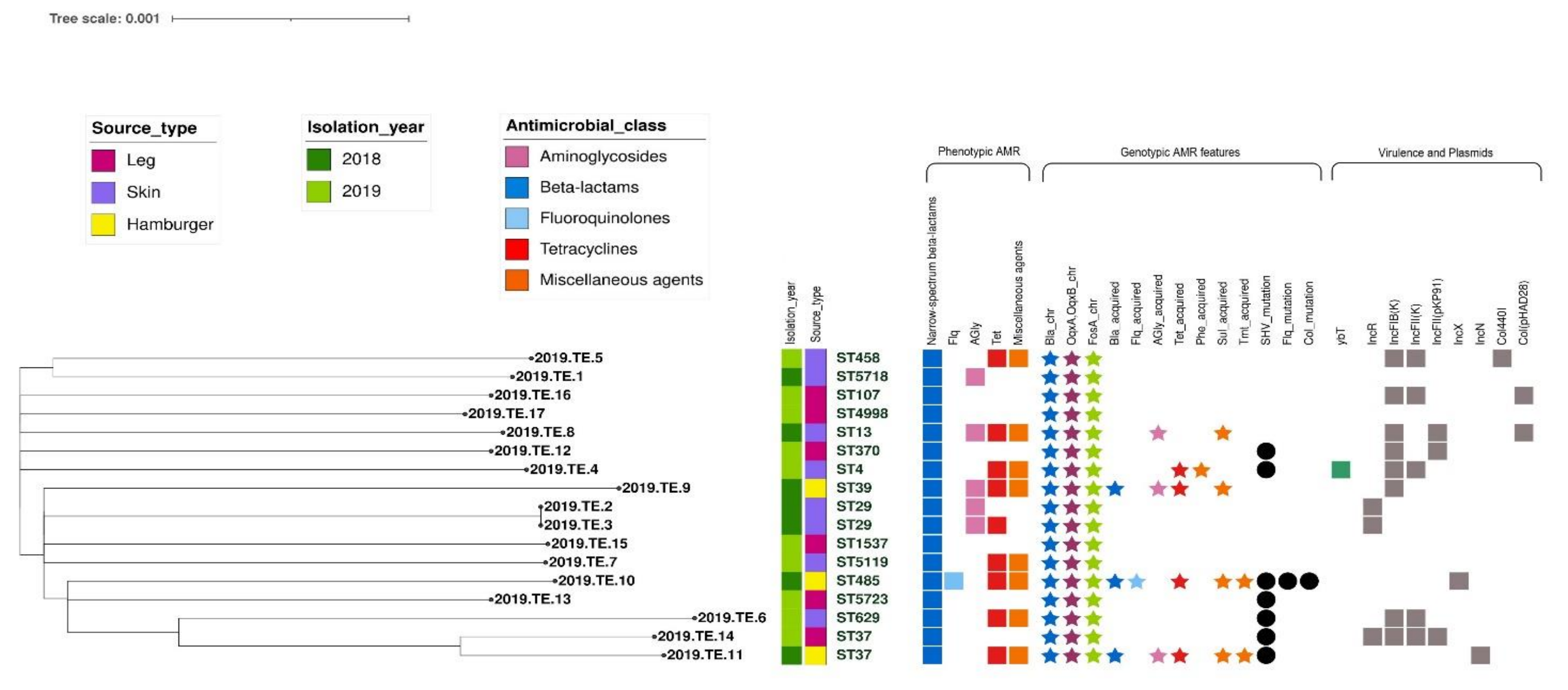
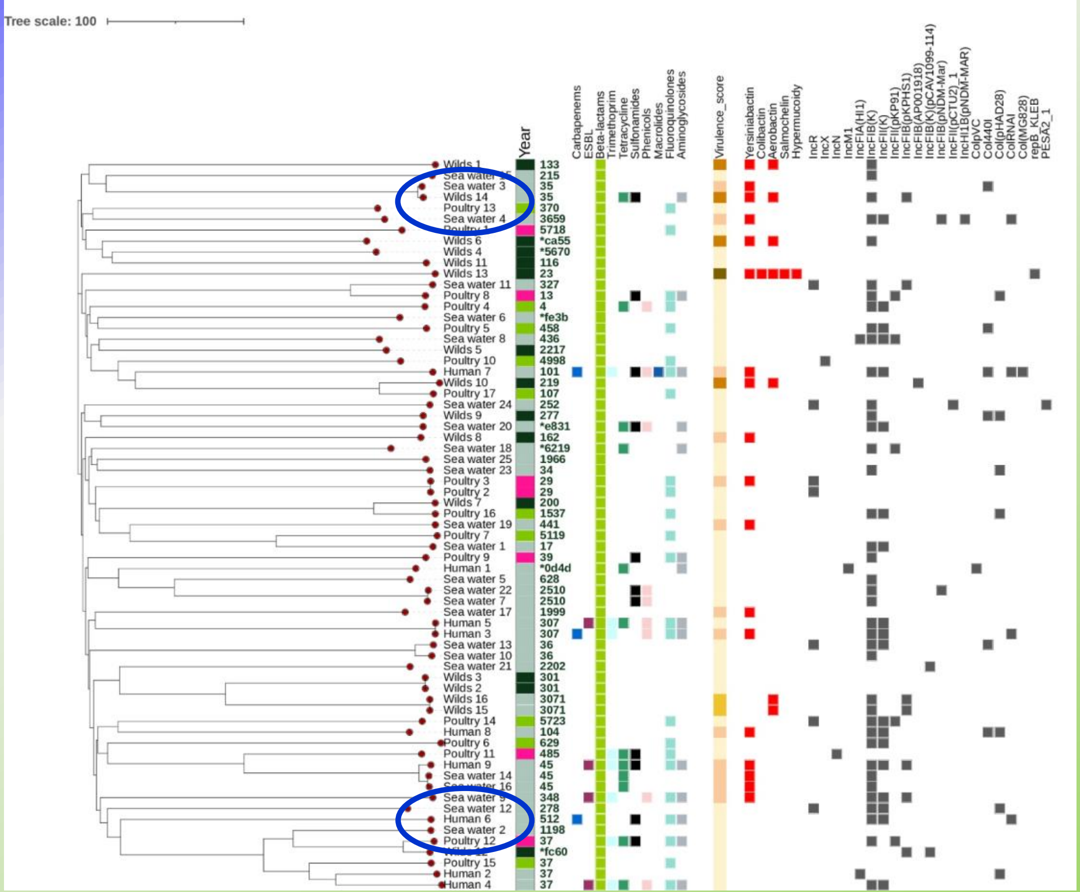
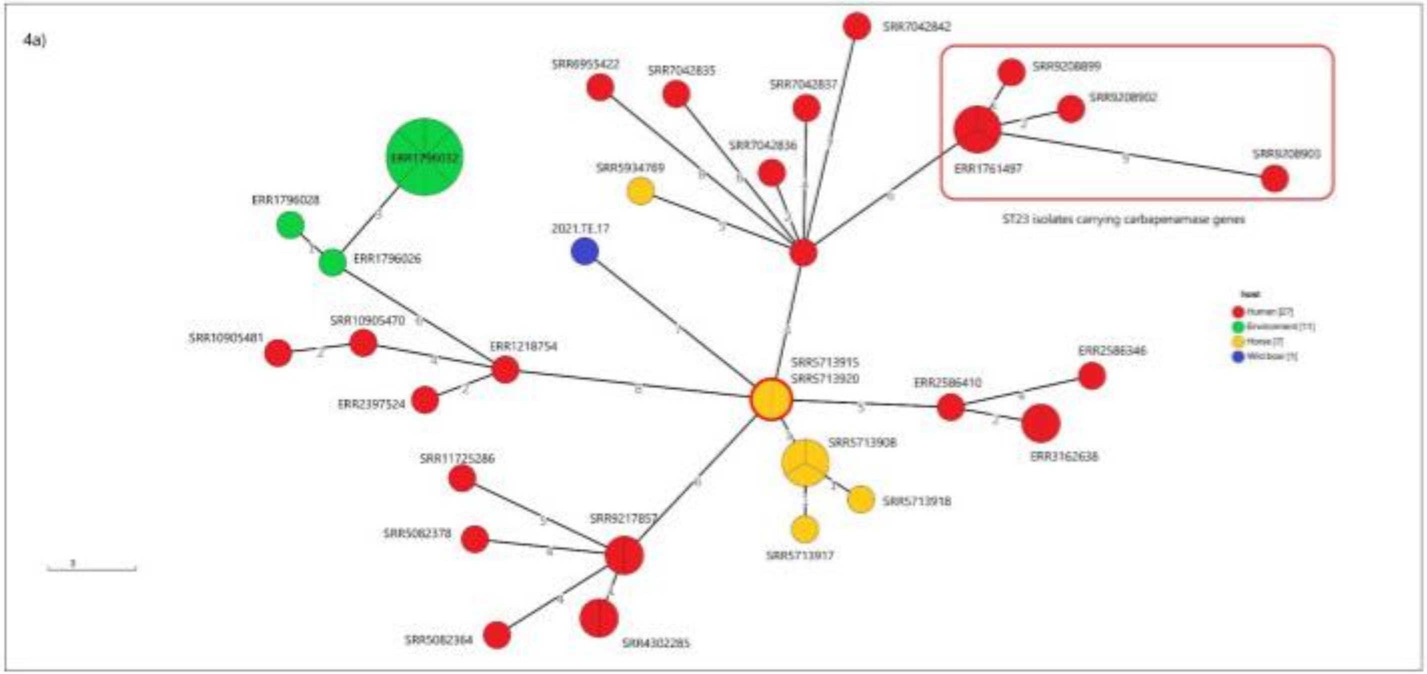


Characterization



Surveillance



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Surveillance

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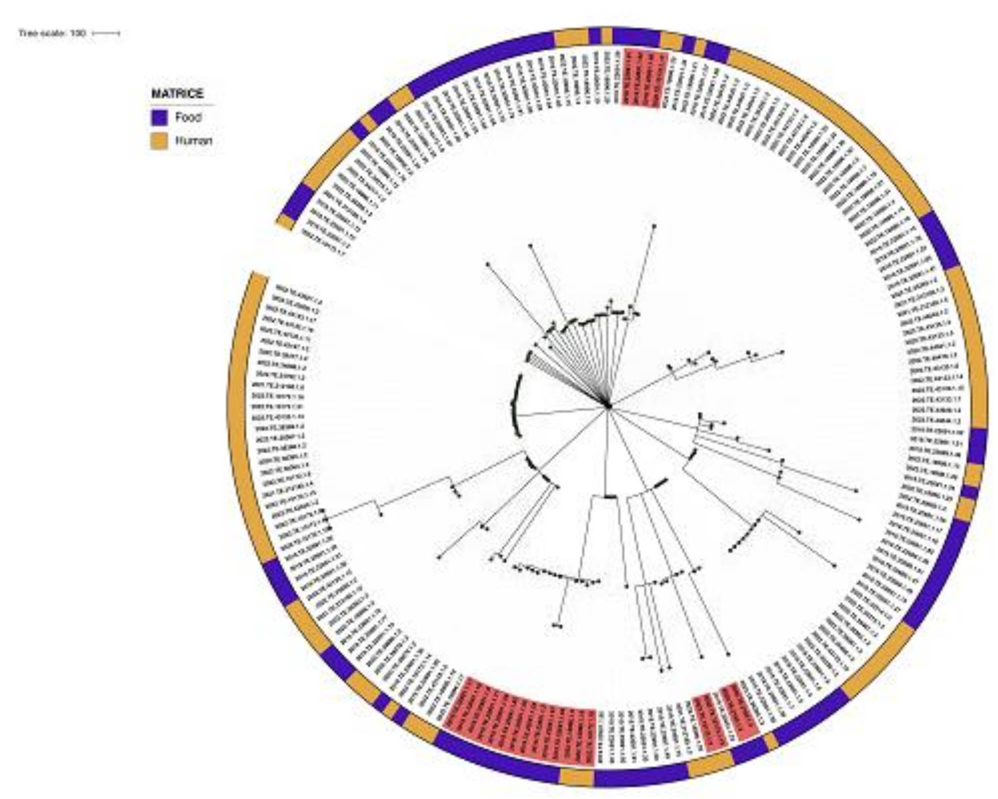

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Analytical genomics

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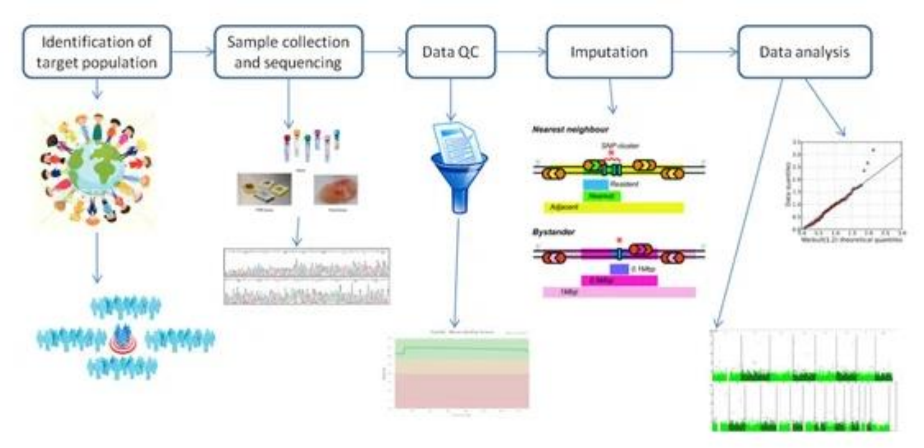
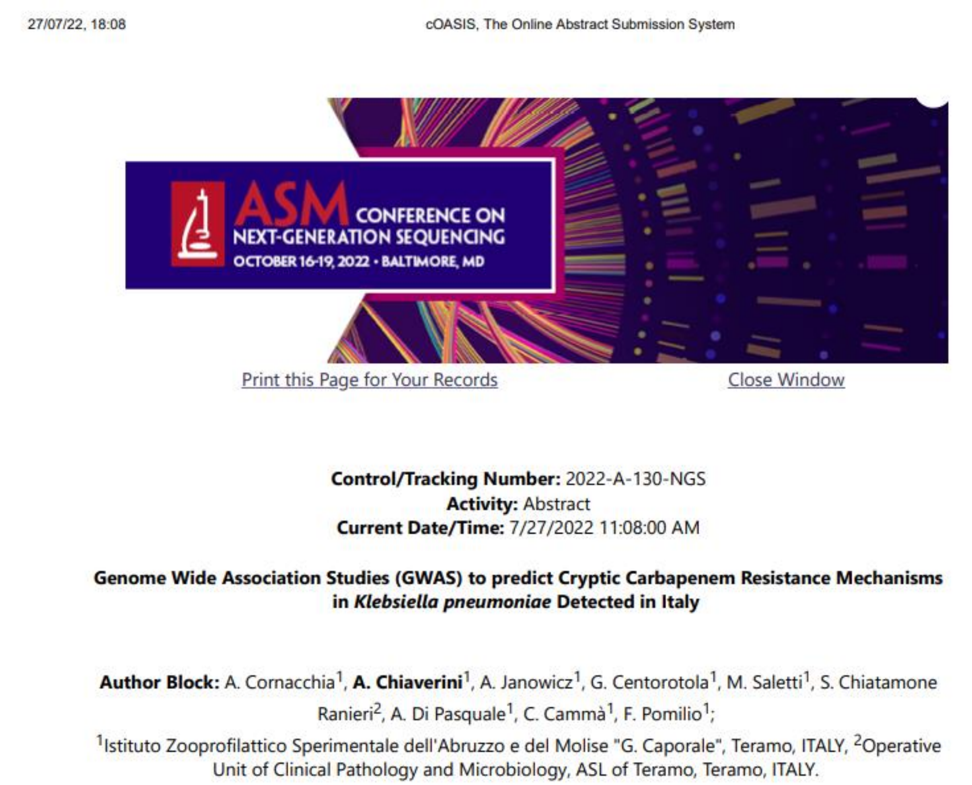
Genome Wide Association Study (GWAS)

A genome-wide association study (GWAS) is a research approach used to identify genomic variants that are statistically associated with a risk for a disease or a particular trait. The method involves surveying the genomes of many people or bacterial isolates, looking for genomic variants that occur more frequently in those with a specific disease or trait compared to those without the disease or trait. Once such genomic variants are identified, they are typically used to search for nearby variants that contribute directly to the disease or trait.



Analytical genomics

Genome Wide Association Study (GWAS)



27/07/22, 18:08

cOASIS, The Online Abstract Submission System

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Control/Tracking Number: 2022-A-130-NGS
Activity: Abstract
Current Date/Time: 7/27/2022 11:08:00 AM

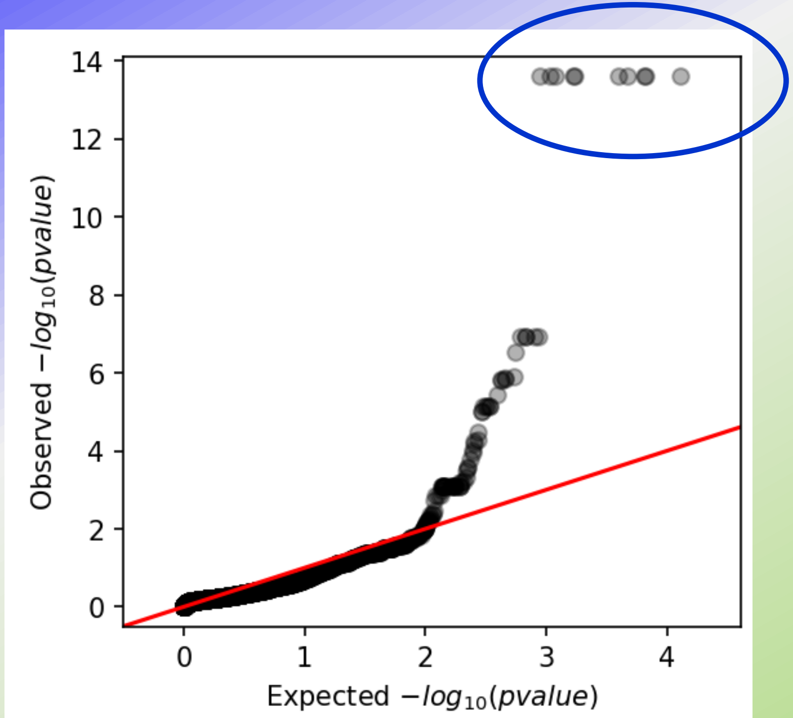
Genome Wide Association Studies (GWAS) to predict Cryptic Carbapenem Resistance Mechanisms in *Klebsiella pneumoniae* Detected in Italy

Author Block: A. Cornacchia[1], **A. Chiaverini**[1], A. Janowicz[1], G. Centorotola[1], M. Saletti[1], S. Chiatamone Ranieri[2], A. Di Pasquale[1], C. Cammà[1], F. Pomilio[1];
[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, ITALY, [2]Operative Unit of Clinical Pathology and Microbiology, ASL of Teramo, Teramo, ITALY.

Although the main carbapenem resistance determinants are well-studied, potential cryptic mechanisms are still unknown. A genome wide association study (GWAS) to reveal novel association between phenotype and genotype was performed , to understand the inconsistencies between phenotypic and genotypic profiles of resistance to carbapenems observed in *Kp* strains circulating in Italy.

Analytical genomics

Genome Wide Association Study (GWAS)



- We analyzed 320 *Kp* strains from foods, environmental samples, animals and clinical cases isolated between 2018-2020 in Italy.
- For a total of 40 *Kp* phenotypically resistant to carbapenems, we identified 14 strains which did not carry any known genetic determinants explaining their AMR phenotype.
- The GWAS revealed that the inconsistent carbapenem resistant strains were characterized by the presence of nine genes involved in peptidoglycan synthesis and multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) efflux pump.

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THANK YOU FOR YOUR ATTENTION!

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