Antimicrobial Resistance, Virulence and Plasmid Prediction using Whole Genome Sequences (WGS)

Introduction to Bioinformatics – Module 1

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Introduction

- Antimicrobial resistance (AMR) is a ”silent pandemic”
  - Resistance to last resort antibiotics
  - Depleting antibiotics reserves

- Virulence factors promote pathogenicity in bacteria

- Likely mobility of AMR/Virulence genes by plasmids

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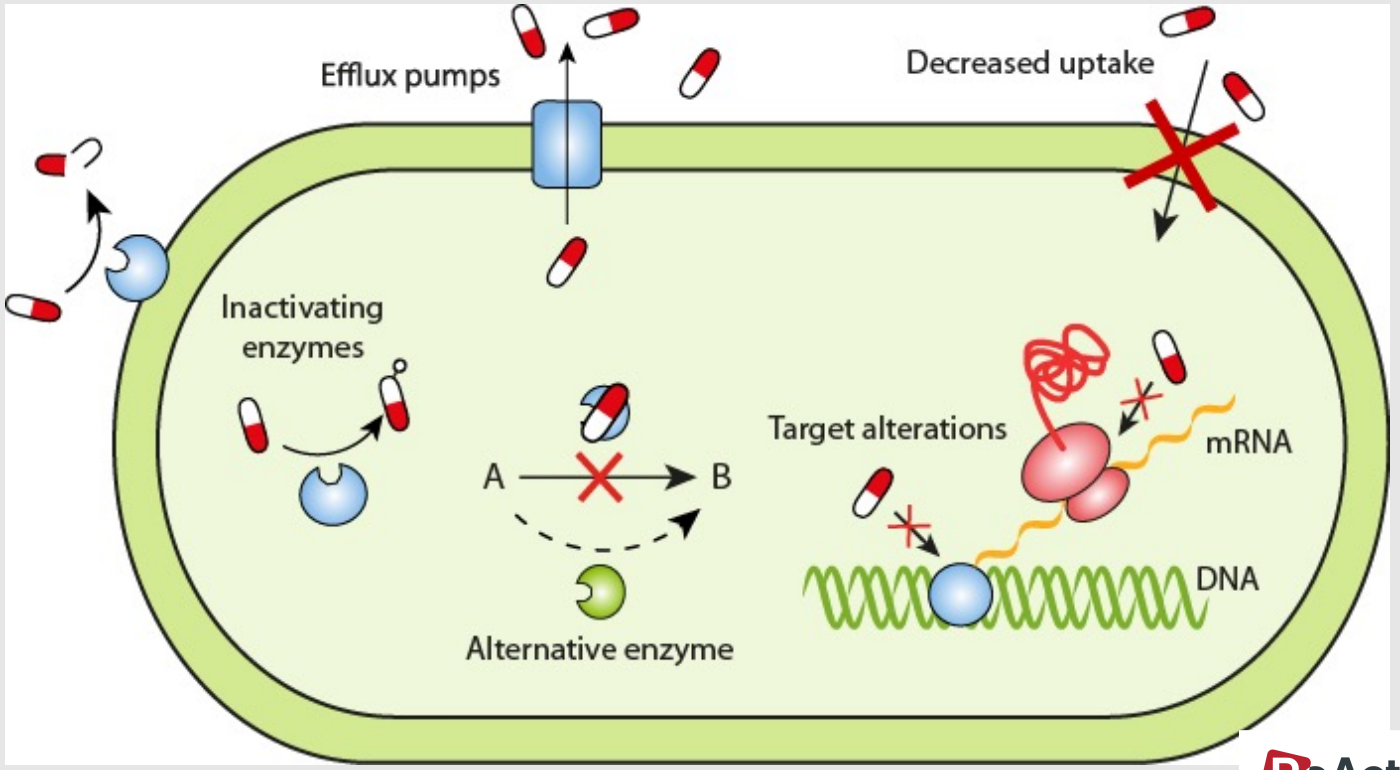
Mechanism of antibiotic resistance



ReAct

Aminoglycosides
Ansamycin
Beta-lactams
Fluoroquinolones
Polymixins
Fosfomycin
Glycopeptides
Lincosamides
Macrolides
Nitrofurans
Oxazolidinones
Phenicol
Quinolones
Sulphonamides
Trimethoprim
Tetracyclines
etc…

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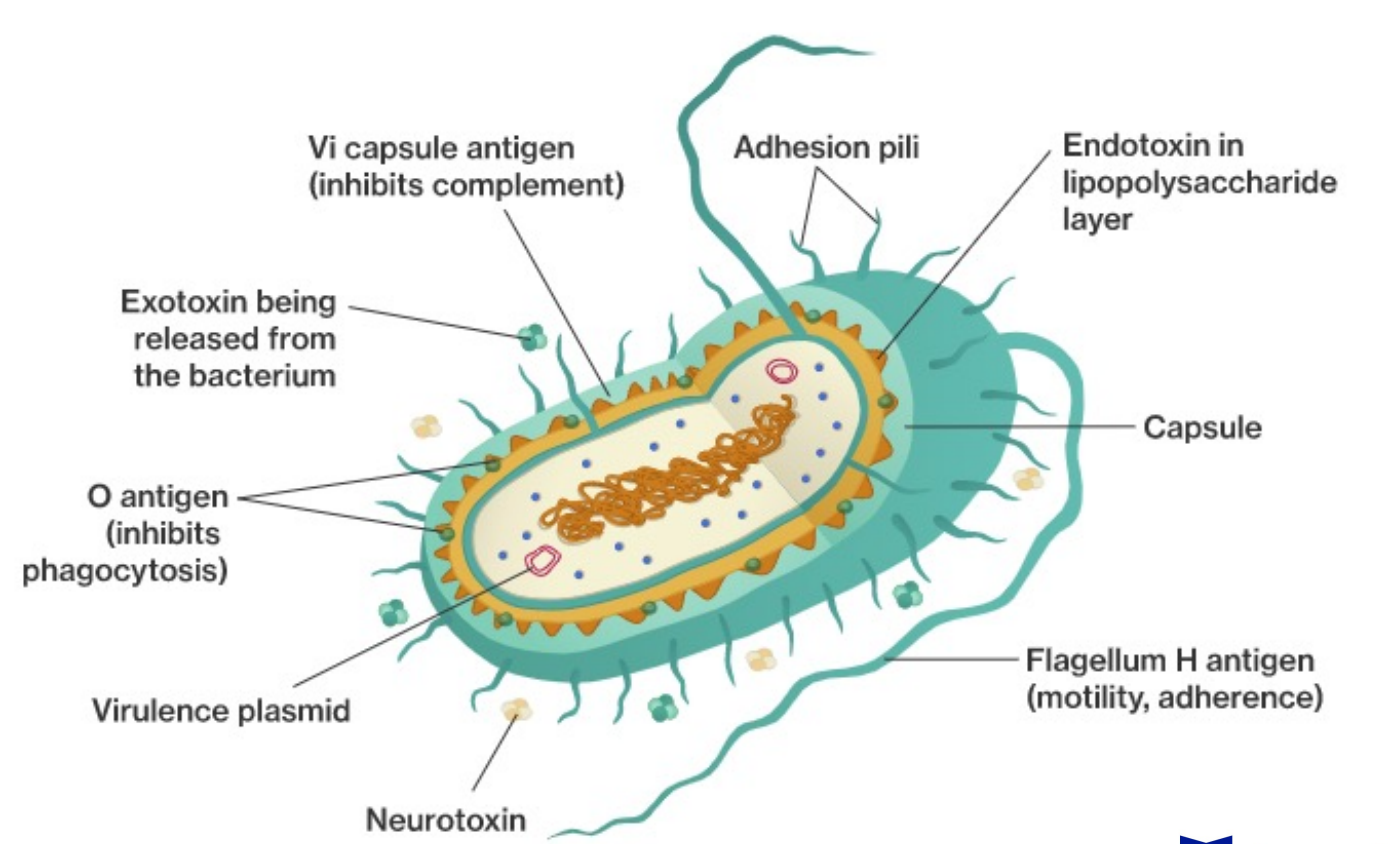
Virulence factors in bacteria



Course Hero

e.g., Capsules
Adhesins
Toxins

1. Invade host
2. Cause disease
3. Evade host defences

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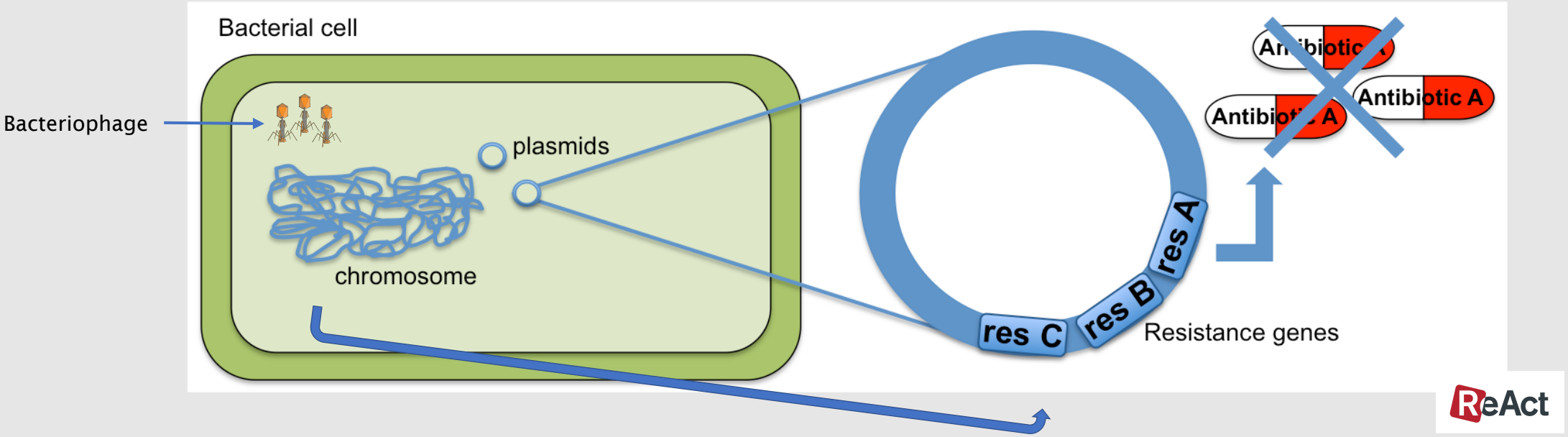
DNA structures in a bacterial cell



ReAct

A Bacterial cell may harbour genetic materials in chromosomal or plasmid DNA that promote antibiotic resistance and/or pathogenicity.

Bioinformatic analysis for detection of antimicrobial resistance genes, virulence determinants and plasmid DNA from Whole Genome Sequence Data.

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How is AMR predicted using WGS?

Alignment and mapping of WGS against a reference database

- Determine antimicrobial resistance genes
- Chromosomal/point mutations in the sequence data

Databases

- Contain a total of all existing AMR genes
- Contain all existing chromosomal mutations specifying AMR.
- Mutations that lead to porin modifications and efflux pumps that can mediate AMR.

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Sequence alignment

- Sequence alignment are core of bioinformatics
- Matching between query and reference sequence

Query seq	ACTGACTT	GCACTGC	TGACGAAT	CTA_CACGAG
Target seq	ACTGACTT	GCTCTGC	TGA_GAAT	CTATCACGAG
Biological Event.	Conservation	Substitution	Insertion	Deletion
Alignment interpretation	Match	Mismatch	Gap	Gap

CACTTACTAGACTGTTAGACTAGCAGATACAGGACTAGACGAGCATCAT

CTGTCACTTACTAGACTGTTAGACTAGC_GATACAGCACTAGACGAGCATCATGGACTA

Coverage (width): 83%

Identity: 96%

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Some tools used in the prediction of AMR

- DTU CGE (ResFinder)
- ARIBA
- ARG-ANNOT
- MEGARes
- NCBI AMRFinder
- ABRICATE
- CARD
- ARDB
- Galileo AMR etc.

These tools may differ in many ways e.g.,

- Accessibility
- Formats of input and output
- Level of complexity
- User friendliness
- Computational power
- Time to complete analysis

*PHA4GE hAMRonization of output data

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ResFinder 4.1 AMR Prediction

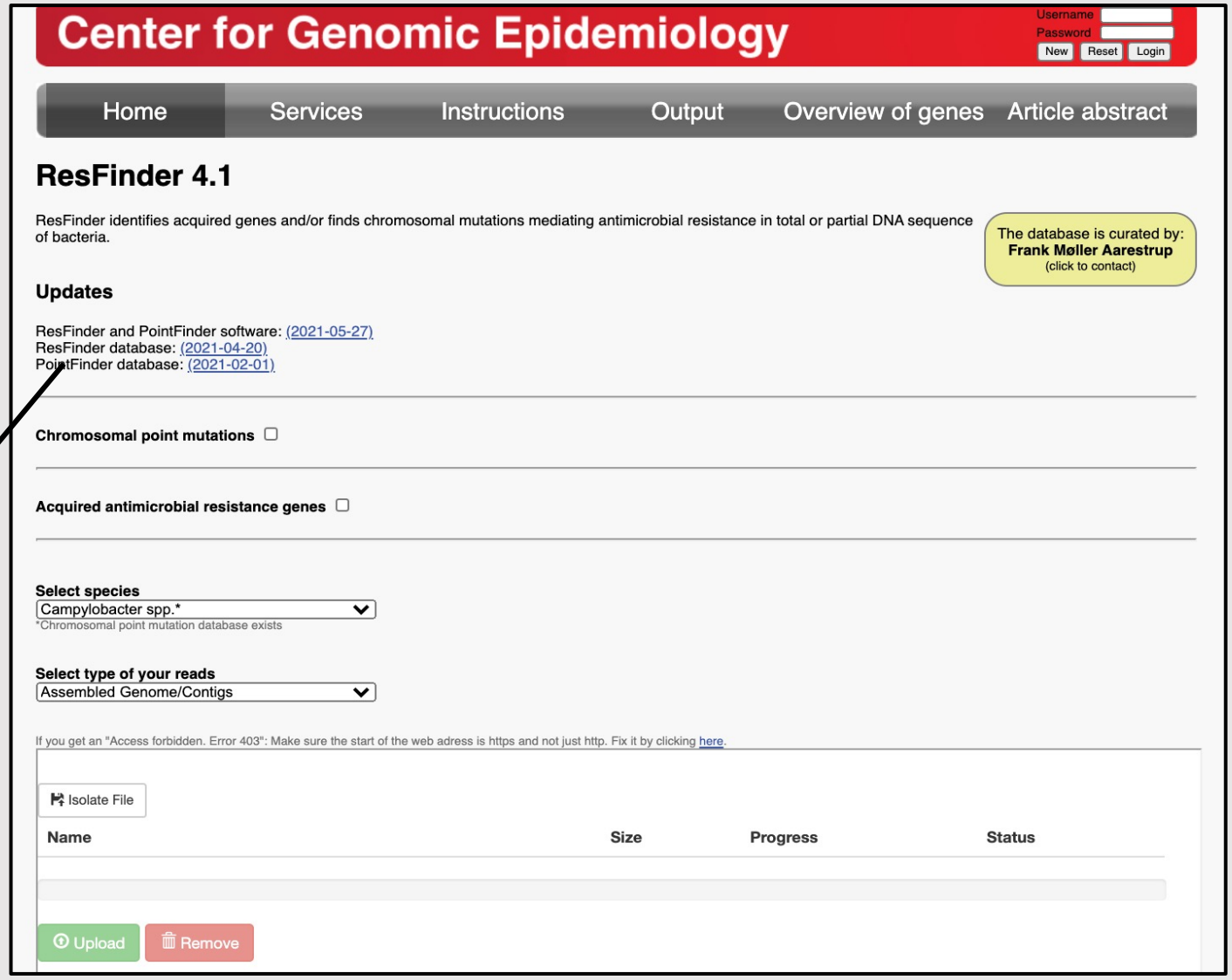
https://cge.cbs.dtu.dk/services/ResFinder/

Easy-to-use
Freely available
Good graphical-user interface

Updates

ResFinder and PointFinder software: (2021-05-27)
ResFinder database: (2021-04-20)
PointFinder database: (2021-02-01)

A database is only as good as its content, hence, need for regular updates.



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ResFinder 4.1 AMR Prediction

complete

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
vancomycin	glycopeptide	No resistance	
mupirocin	pseudomonic acid	No resistance	
tobramycin	aminoglycoside	No resistance	
virginiamycin m	streptogramin a	No resistance	
isepamicin	aminoglycoside	No resistance	
virginiamycin s	streptogramin b	No resistance	
hydrogen peroxide	peroxides	No resistance	
butirosin	aminoglycoside	No resistance	
ampicillin	beta-lactam	Resistant	blaSHV-70 (blaSHV-70_DQ013287), blaSHV-13 (blaSHV-13_AF164577), blaSHV-11 (blaSHV-11_X98101)
astromicin	aminoglycoside	No resistance	
lividomycin	aminoglycoside	No resistance	
sulfamethoxazole	folate pathway antagonist	Resistant	sul1 (sul1_X15024), sul2 (sul2_AY034138)
temocillin	beta-lactam	No resistance	
trimethoprim	folate pathway antagonist	Resistant	OqxA (OqxA_EU370913), OqxB (OqxB_EU370913), dfrA15 (dfrA15_AF221900)
oleandomycin	macrolide	No resistance	
unknown macrolide	macrolide	No resistance	
florfenicol	phenicol	No resistance	
fluoroquinolone	under_development	Resistant	acrR (p.R173G), acrR (p.F172S), acrR (p.K201M), acrR (p.F197I), acrR (p.P161R), acrR (p.G164_None491del), acrR (p.L195V)
quinupristin	streptogramin b	No resistance	
fosfomycin	fosfomycin	Resistant	fosA (fosA_ACZD01000244)
cephalothin	beta-lactam	Resistant	blaSHV-11 (blaSHV-11_X98101)

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Colour Schemes: ResFinder 4.1 AMR Prediction

Polymyxin									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
mcr-10	100.0	1620/1620	1..1620	NODE_17_length_49013_cov_8.249131	29724..31343	colistin	32116151	MN179494	

Dark green

- Indicates perfect match for the gene.
- Identity is 100% and the sequence length matches perfectly with reference gene in database

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Colour Schemes: ResFinder 4.1 AMR Prediction

Beta-lactam									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
blaSHV-70	99.8838559814	861/861	1..861	NODE_56_length_2052_cov_11.077829	173..1033	amoxicillin,ampicillin,aztreonam,cefepime,cefotaxime,ceftazidime,ceftriaxone,piperacillin,ticarcillin	16563703	DQ013287	Class A

Light green

Indicate imperfect identity due to variations in the bases of the query sequence compared to reference in database.

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Colour Schemes: ResFinder 4.1 AMR Prediction

Aminoglycoside									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
aph(3")-Ib	100.0	803/804	2..804	NODE_17_length_49013_cov_8.249131	38899..39701	streptomycin	9687410	AF024602	Alternative name strA, orfH

Grey

Indicate imperfect alignment of query and reference gene in database

Alignment is shorter than reference gene in database

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Genotypic prediction of antimicrobial resistance phenotype in *E. coli*

Research

Open Access

Use of whole genome sequencing of commensal *Escherichia coli* in pigs for antimicrobial resistance surveillance, United Kingdom, 2018

Check for updates

Like 0

Download

Emma Stubberfield[1] , Manal AbuOun[1] , Ellie Sayers[1,2] , Heather M O'Connor[3] , Roderick M Card[1] , Muna F Anjum[1]

Stubberfield et al., 2019

- Source: caecal content of pigs
- Sample size: 515 *E. coli* isolates
- Exposed to 9 antimicrobials across 7 drug classes

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Genotypic prediction of AMR phenotype in *E. coli*

Antibiotic	Ciprofloxacin	Cefotaxime	Ceftazidime	Gentamycin	Florfenicol	Ampicillin	Apramycin	SXT	Tetracycline	Overall
Specificity	96.1%	99.8%	99.8%	100.0%	100.0%	99.1%	100.0%	93.3%	95.0%	**98.9%**
Sensitivity	85.2%	90.0%	61.6%	95.7%	75.0%	95.9%	100.0%	88.7%	98.0%	**90.7%**
Kappa Correlation	0.814	0.9431	0.726	0.976	0.848	0.945	1.000	0.812	0.930	**0.914**

Results

- WGS/MIC specificity of 99%,
- Predictive value of a positive test was 98%
- kappa value of 0.914
- The majority of discrepancies were from false negative correlations.

isolates with reduced susceptibility lacking a relevant genetic resistance determinant, suggesting unknown resistance genes may be present in these bacteria.

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Prediction of virulence determinants and plasmid replicons types

WGS sequence data are examined for;

- Presence of absence of virulence determinants
- plasmid replicon types

Uses similar alignment and mapping of genes in a database

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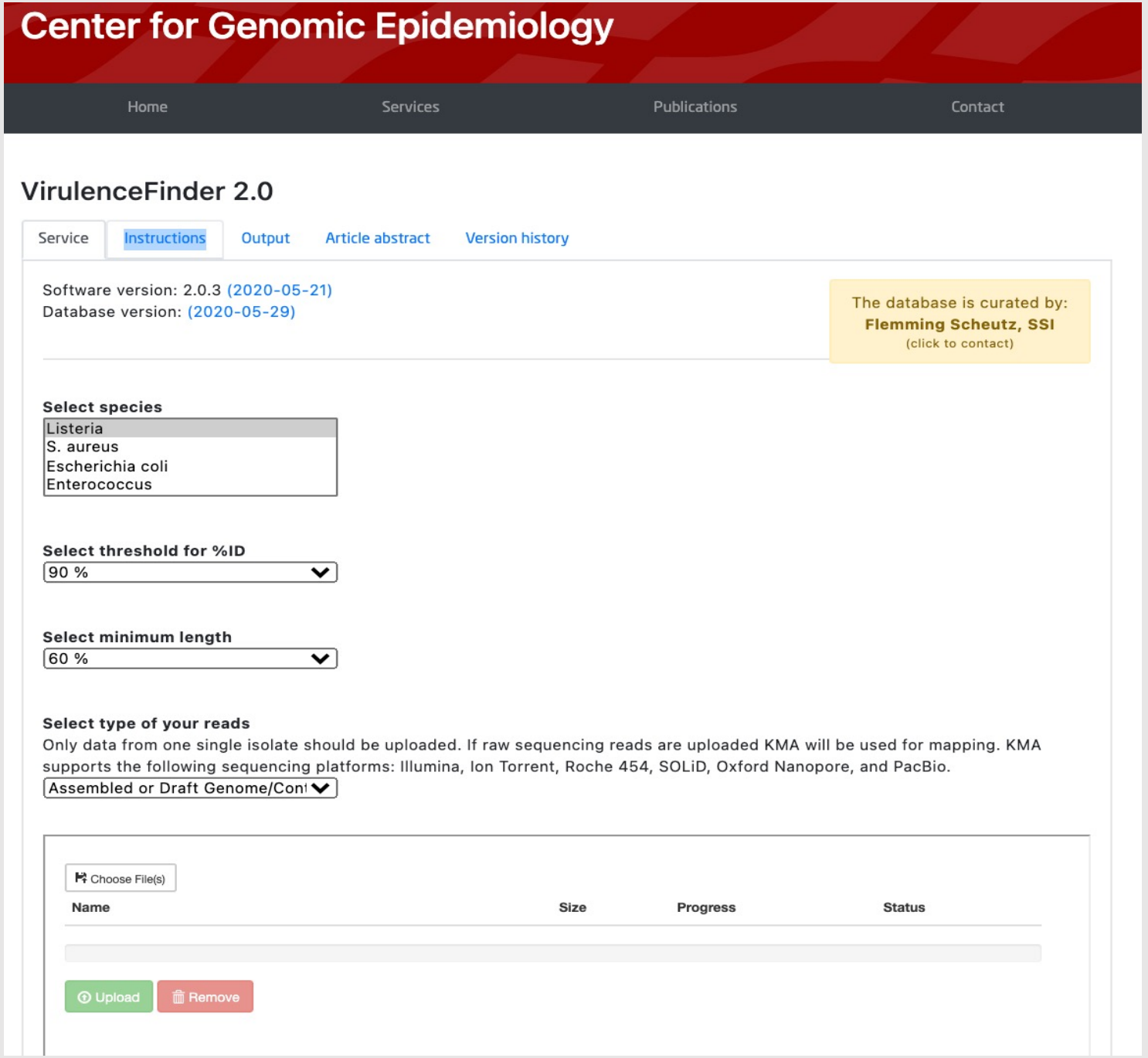
VirulenceFinder 2.0

Virulence Genes Prediction

https://cge.cbs.dtu.dk/services/VirulenceFinder/



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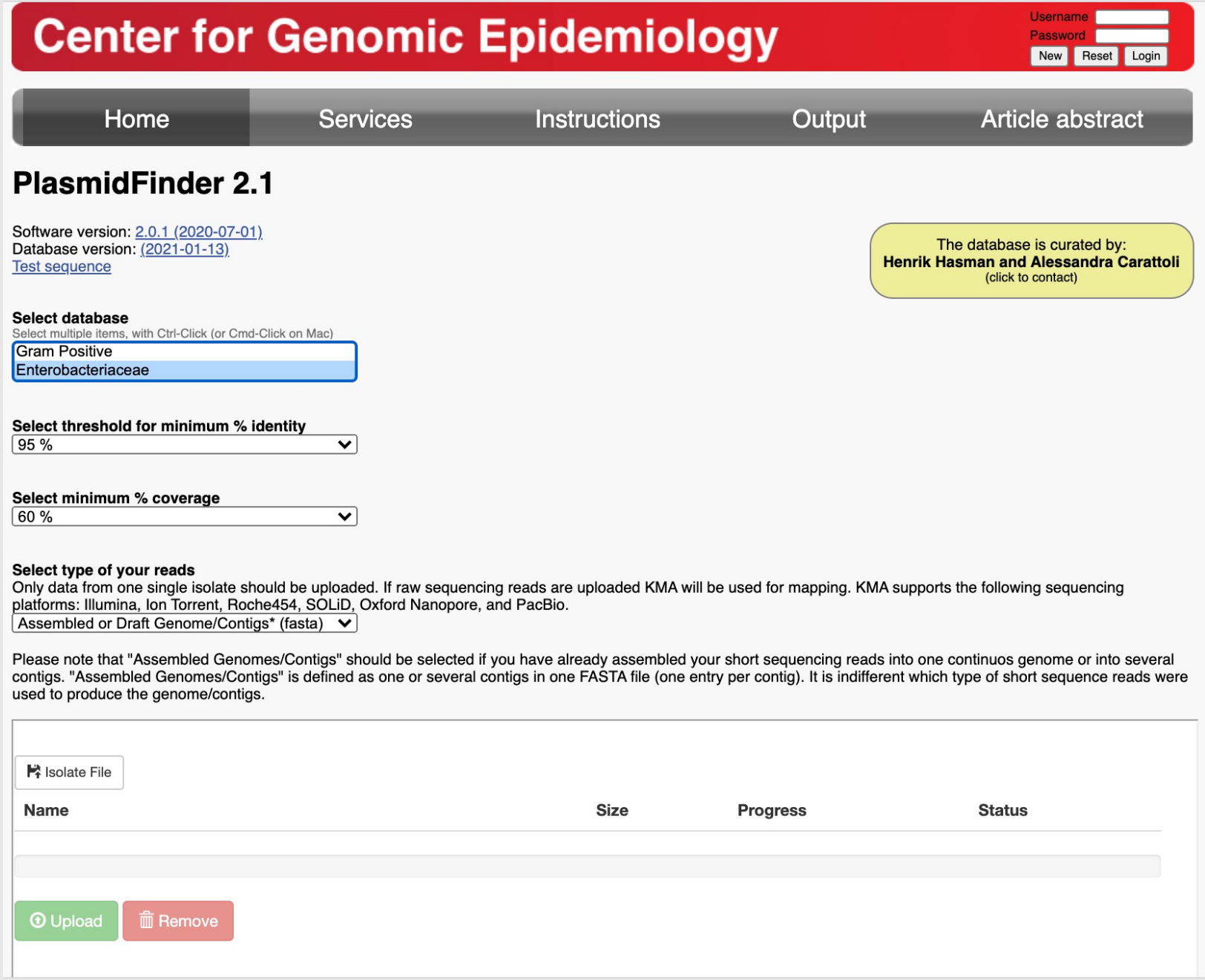
PlasmidFinder 2.1

Plasmid Replicon Prediction

https://cge.cbs.dtu.dk/services/PlasmidFinder/



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Benefits of WGS AMR, Virulence and Plasmid prediction

- They are vigorous and phenotypically confirmed methods that have been developed and improved over decades
- Provides more information (e.g., description) about detected genes in query sequence
- It can be used to detect new and emerging clones of antimicrobial resistance
- Phenotypic AMR aren’t flawless
- Important tool in AMR surveillance.

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Limitations to WGS prediction

Detect presence or absence

- no quantitative measurement of resistance/virulence
- genes might not be expressed in host

Detection of plasmid replicons may require further analysis to verify its carriage.

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Limitations to WGS prediction

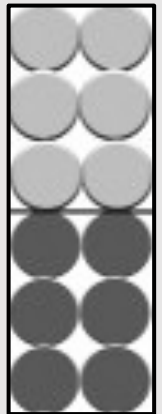
Your results are only as good as the content of database

No gene = no AMR /virulence
True or False?

Kieffer *et al.*, 2018
126 pig sample
8 positive for colistin resistant E. coli
1 possessed *mcr-3*

MIC = 4µg/ml

ECOFF= 2µg/ml



4µg/ml

8µg/ml

Limitations associated with laboratory determination of MIC

A novel resistance gene not yet in the database

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Thank you

Global Health Research Unit
Genomic Surveillance of AMR

RECTE SAPERE FONS

JUNO

The Fleming Fund

GPS
Global Pneumococcal Sequencing Project