

RESISTANCE DATABASES

NORDICAST WORKSHOP

GOTHENBURG

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Norwegian National Advisory Unit on
Detection of Antimicrobial Resistance

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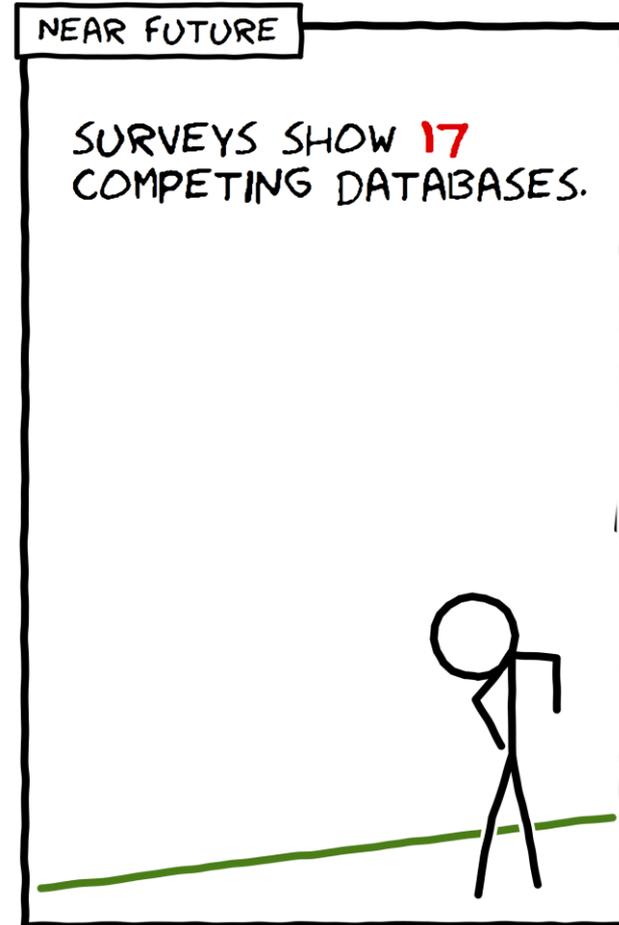
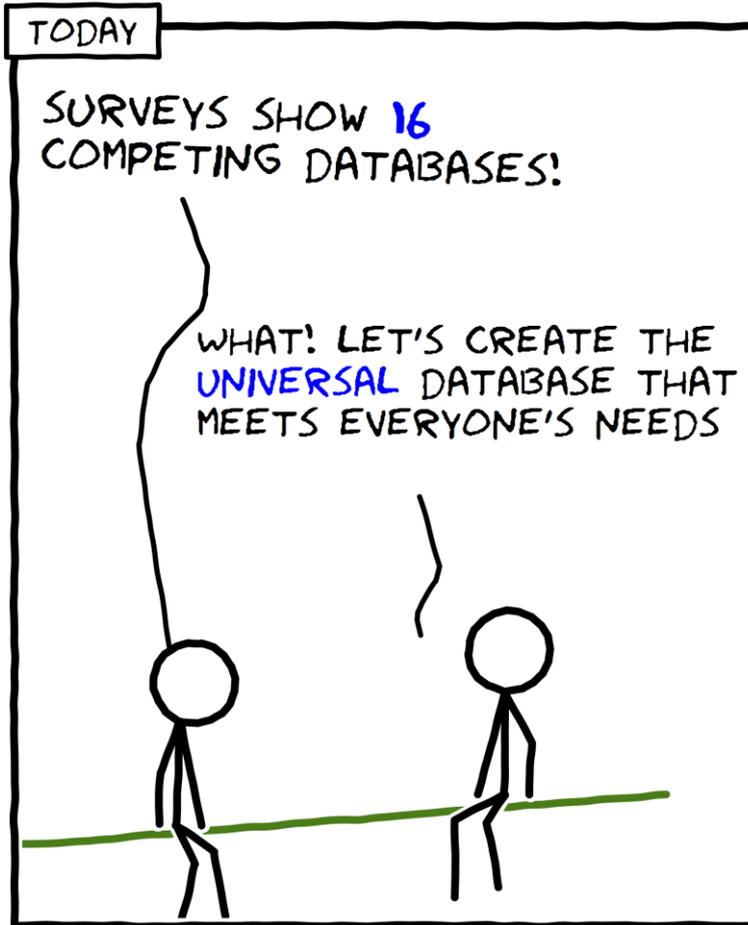
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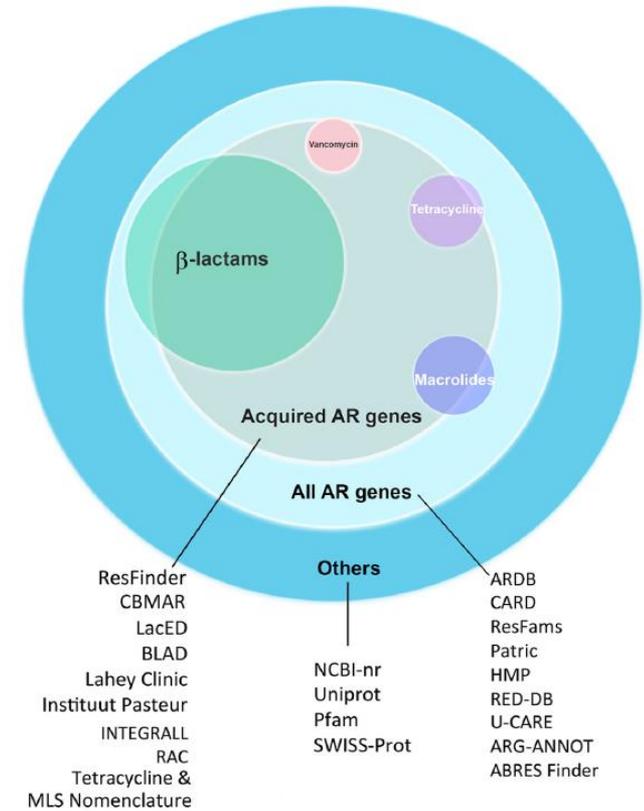
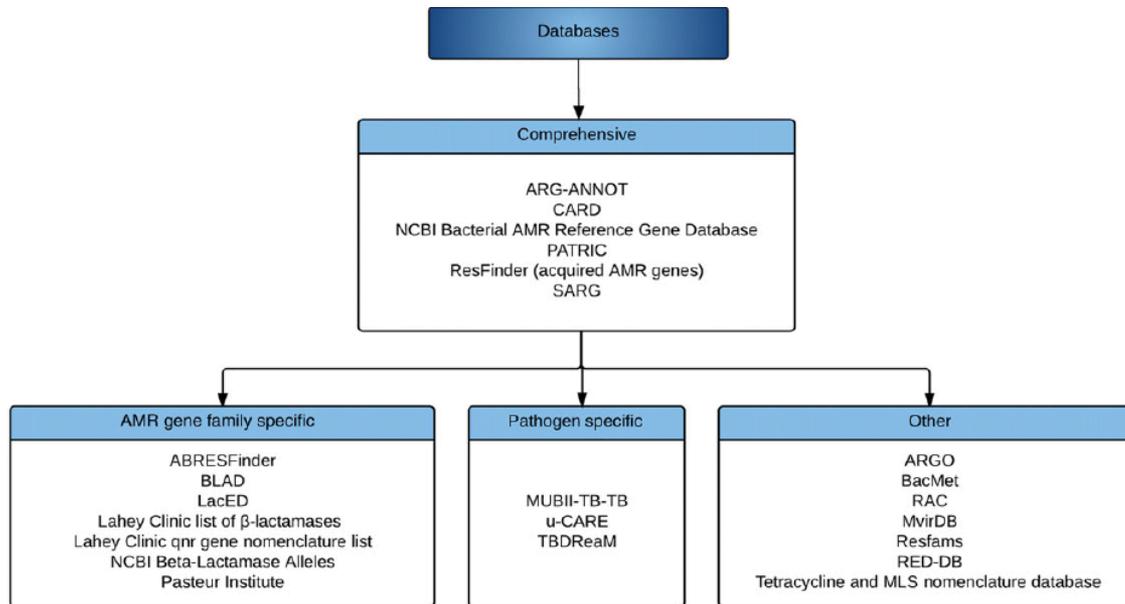


HOW BIOLOGICAL DATABASES PROLIFERATE





AMR DATABASES





CHALLENGES AMR DATABASES

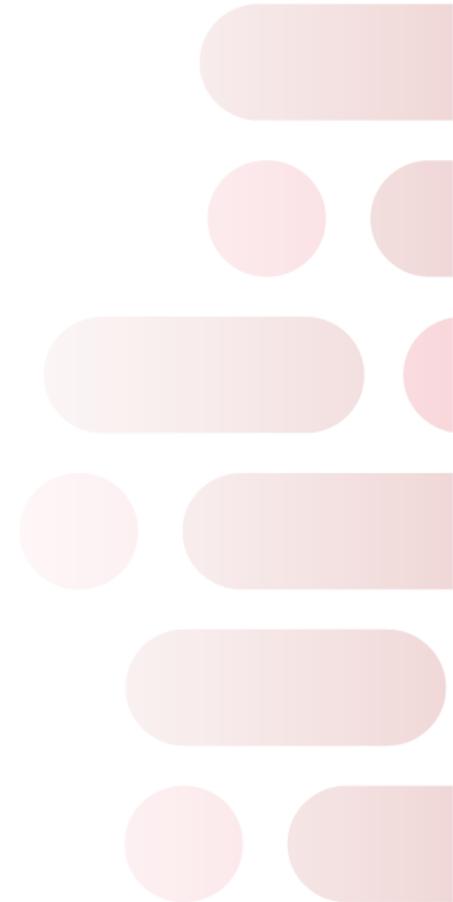
➤ No single database is complete:

- Resistance vs. non-resistance mutations in intrinsic genes
- Mutations in ribosomal RNA genes (e.g. linezolid resistance)
- Mutations in regulatory elements (e.g. efflux pump)
- Insertions (e.g. IS-element inactivation *mgrB* – colistin resistance)
- Resistance by absence (e.g. porin genes)
- Resistance operons (e.g. vancomycin resistance - VVE)



CHALLENGES AMR DATABASES

- Maintenance
 - Funding is often project driven
 - Update frequency varies
- Accessibility
 - Humans
 - Computers
- AMR gene nomenclature
 - Conflicting names
 - Generalized names/allele specific





CHALLENGES AMR DATABASES

➤ Genotype <-> phenotype:

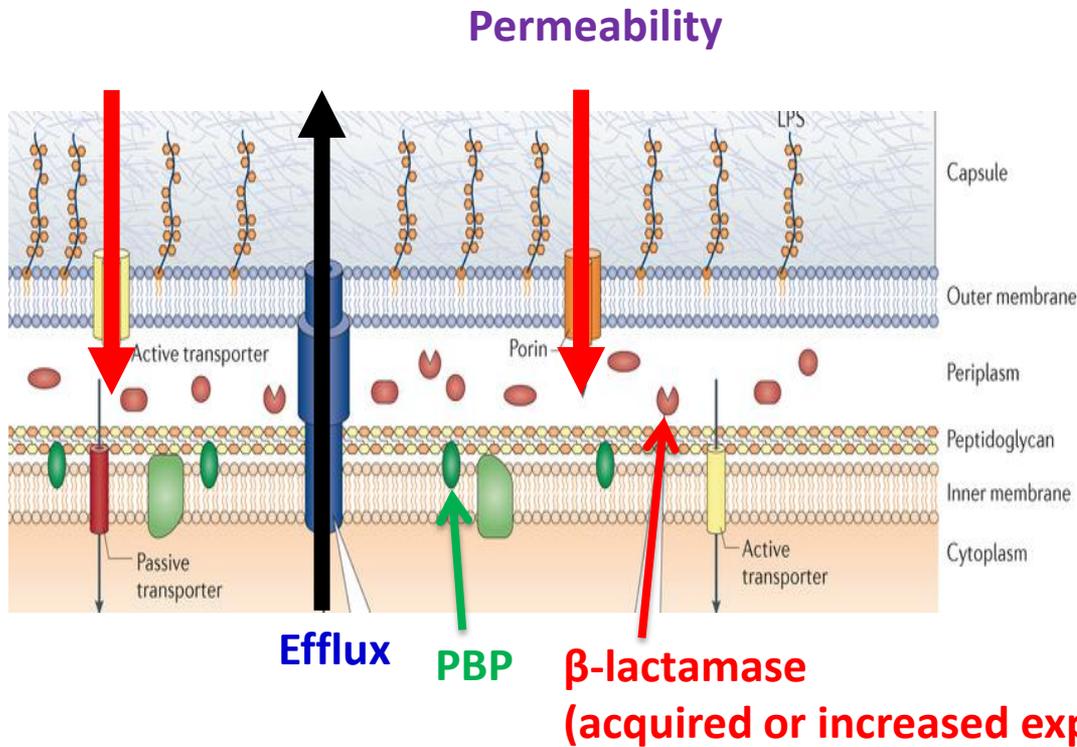
- Lack of linked metadata (e.g. MIC) to AMR databases
- No database predicts phenotypic resistance profile

E. coli: $bla_{\text{OXA-48}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$, *aadA-5*, *aac(3)-lid*, *gyrA* S83L, *parC* S80I, *dfrA-17*, *sul-1*

Antibiotic	Resistance genes	Genotypic Interpretation	MIC	Interpretation
Mecillinam	OXA-48, CTX-M-14	R	2	S
Piperacillin/tazobactam	OXA-48, CTX-M-14	R	128	R
Cefuroxime	CTX-M-14	R	>256	R
Cefotaxime	CTX-M-14	R	64	R
Ceftazidime	CTX-M-14	R	2	I
Ertapenem	OXA-48	R	1	I
Meropenem	OXA-48	R	0.25	S



MIC/ZONE DIAMETER



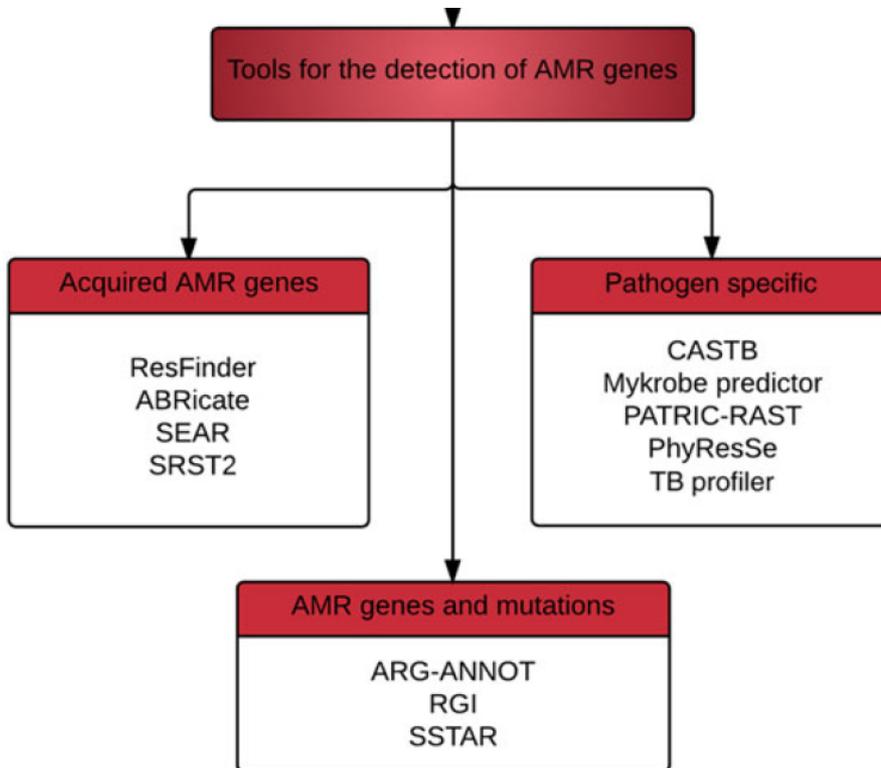
Reduced susceptibility/resistance: complex interplay of several factors
→ **MORE THAN PRESENCE**
ABSENCE OF A RESISTANCE
GENE/MUTATION

➤ Paradigm shift necessary:

- Genotypic detection -> phenotypic prediction based on genotype



IN-HOUSE OR «ONLINE» TOOLS?



➤ **CASTB, PhyResSe, TB profiler:**

- *M. tuberculosis*

➤ **Mykrobe predictor:**

- *M. tuberculosis* & *S. aureus*

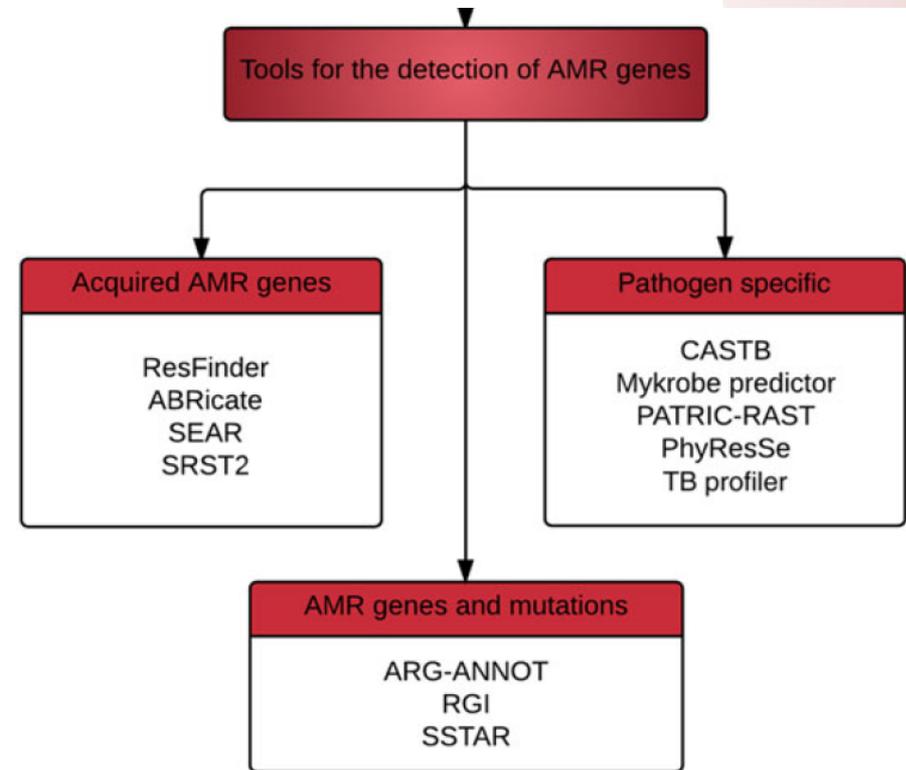
➤ **PATRIC-RAST:**

- Carbapenem-R *A. baumannii*
- MRSA
- β -lactam/co-trimoxazole-R *S. pneumoniae*



DIFFERENT TOOL – SAME DATABASE

- **3 main databases:**
 - ResFinder, ARG-ANNOT, CARD
- **ABRicate:**
 - ResFinder
- **SEAR, SSTAR:**
 - ARG-ANNOT
- **SRST2:**
 - ResFinder + ARG-ANNOT
- **RGI:**
 - CARD





BIOINFORMATIC METHODS/ALGORITHMS

- Continuous development of bioinformatics methods/ algorithms
 - ResFinder: Assembly/BLAST
 - SRST2/KmerResistance: Mapping raw reads

Table 2. Performance on the datasets

Dataset	Method	Accuracy		Performance		True pos. fraction	False pos. fraction
		SMC	MCC	TPP	FPP		
UK hospital full set	SRST2	95.92%	0.9026	0.9407	0.0331		
	ResFinder	96.04%	0.9052	0.9407	0.0314		
	KmerResistance	96.04%	0.9052	0.9407	0.0314		
UK hospital down-sampled	SRST2	73.43%	0.2679	0.0988	0.0		
	ResFinder	71.33%	0.1402	0.0277	0.0		
	KmerResistance	95.34%	0.8870	0.9051	0.0264		



CARD: Comprehensive Antibiotic Resistance Database

- Manually curated
- May 2017: 2353 reference sequences/944 SNPs
- Fasta files
- Ontology-driven database – ARO (Antibiotic Resistance Ontology)

Table 1. Major branches of the Antibiotic Resistance Ontology (ARO)

ARO branch	ARO accession	Description
Determinant of Antibiotic Resistance	ARO:3000000	Ontology terms describing genes, mutation, or genomic elements conferring antimicrobial resistance.
Mechanism of Antibiotic Resistance	ARO:1000002	Ontology terms describing mechanisms of antimicrobial resistance.
Antibiotic Target	ARO:3000708	Ontology terms describing targets of antimicrobial compounds.
Antibiotic Molecule	ARO:1000003	Ontology terms describing the chemical diversity of antimicrobial compounds.
Inhibitor of Antibiotic Resistance	ARO:0000076	Ontology terms describing molecules that inhibit antimicrobial resistance.



RGI – Resistance Gene Identifier (CARD)

➤ Protein Homolog

Reference Sequences & Detection Model Parameters
Model Type: protein homolog model
Model Definition: Models to detect proteins conferring antibiotic resistance, which include a reference protein sequence and a curated BLASTP cut-off.
E-Value Cut-off: 1e-100

Protein DNA

>gi|CA239946.1|-|NDM-1 [Klebsiella pneumoniae]
NELPNIHPVAKLSTALAAALMLSGCMPGERPTIGQQMETGQDFGDLVFRQLAPNVWQ
HTSYLDMPFGAVASNGLIIVKDGGRVIVDTAWTDDQTAQLLWIKQELNLPVALAVVTH
AHQDKMGMDALHAAGIATYANALSQQLAPQEGHVAQAHSLSLTAANGWVEPATAPNFGPL
KVFYFGPGRHSDNITVIGIDCTDIAFGGLIKDKAKSLGNLGDATERYASARAFGAAP
PKASIMVHSHAPDSRAAITBTARMADKLR

COPY

➤ Protein Variant:

- AMR mutations (SNPs)
- Nonsense mutations
- Insertions
- Deletions
- Frameshifts
- Differentiated by species

Reference Sequences & Detection Model Parameters
Model Type: protein variant model
Model Definition: A model to detect proteins that confer elevated resistance to antibiotic(s) relative to wild type. These models include reference sequences (which may or may not be a wild type sequence), a curated BLASTP cut-off, and mapped resistance variants.
E-Value Cut-off: 1e-150

PMID: 21300839

PMID: 17035499

PMID: 16377674

PMID: 17434825

PMID: 16584301

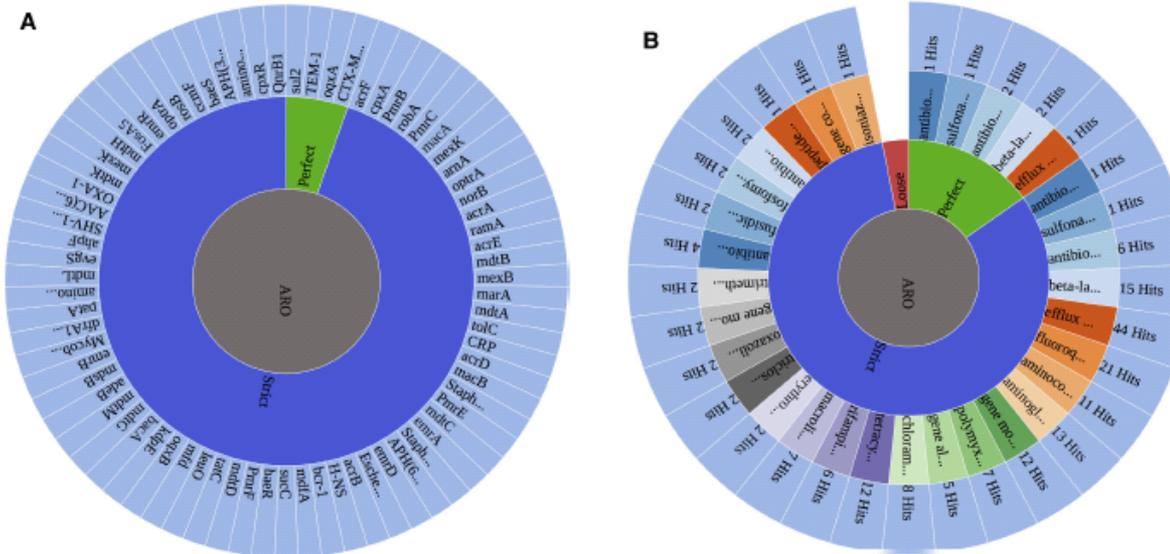
Protein DNA

>gi|CCP42728.1.1|[Mycobacterium tuberculosis gyrA conferring resistance to fluoroquinolones [Mycobacterium tuberculosis H37Rv]
NIDTTLPPDSDLRIEPEVDIEQEMQRSYIDYMSVIVGRALPEVRDGLKPVHRRVLYAMF
DSGFRPDRSHAKSARSVAETMGNYHHPGDASIIDSLVRMAQFWSLRYFLVDGQGNFGSPG
NDPFAAMRYTEARLTPLAEMLRIDEETVDFIPNYDGRVQEPVLPFRPFLLANGSGG
IAVGMATNIFPHNLRKLADAVFWALENHDADEETLAAVMGRVKGDFPTAGLIVGSGGT
ADAYKTRGSIIRMRGVVEEEDSRGRTSLIITELPYQVNHDFITSIAGQVRDGLAGIS
NIEDQSSDRVGLRIVIEIKRDAVAKVIVNINLKHITQLQTSFGANMLAIVDQVPRTLRLDQ
LIRYYVDHQLDVIIRRTTYLRKANERAMILRGLVKALDALDEVIALIRASPTVDIARAG
LIELLDIEIQAAILDMQLRRLAALERQRIIDDLAKIEAIEADLEDILAKPERQRGIYR
DELAEIVDRHGGDRRTRIIAADGVDSEDLIAREDVVVTITETGYAKRTKDLYRSQKRG
GKGVOGAGLKQDDIVAHFFVCSTHDLILFFTQGRVYRAKAYDLPEASRTARGQHVANLL
AFQPEERIAQVLIQIRGYTDAPYLVLATRNGLVKKSLTDFDNRSGGIVAVNLRDNDELV
GAVLCSAGDDELLVSANGQSIRFSATDEALRPMGRATSGVQGNRFNIDRLLSLNVVRES
TYLLVATSGGYAKRTAIEEYPVQGRGEGVITVMYDRRRGRVGLAIVDDSDSELYAVTSG
GGVIRTAARQVRRAGROTKVRLMNLGEGDTLLAIARNAEESGDDNAVNDANGADQGTGN

COPY



RGI – Output



RGI Results (Click on the RGI wheel to browse results)

Hit	Criteria	E-value	Percent Identity
oxqA	Perfect	0	100
APH(3)-VI	Perfect	0	100
TEM-1	Perfect	0	100
CTX-M-15	Perfect	0	100
armA	Perfect	0	100
CTX-M-15	Perfect	0	100
sul1	Perfect	0	100
NDM-1	Perfect	0	100
SHV-11	Perfect	0	100
mtrE	Perfect	0	100
OXA-9	Perfect	0	100
mphD	Perfect	0	100
QnrS1	Perfect	4.29719e-165	100
AAC(6)-Ib	Perfect	1.96996e-150	100
AAC(6)-Ib-cr	Perfect	1.16692e-148	100

Home | All Results | 92d70d0402505b23cee978be81622b-PS

[BACK](#)

NDM-1
(Perfect)

Name	Value
ARO Accession	3000589
ARO Category	determinant of beta-lactam resistance,antibiotic inactivation enzyme
bit score	555.829
E-value	0
Percentage Identity (of matching region)	100%
Percentage Identity (of matching region)	100%
Model type	protein homology model
Model Cut-off	1e-100
Source	NZ_CP020902.1_124
label	NZ_CP020902.1_124#128351#129163#1#ID=2_124:partial=00:start_type=ATQ:rbn_motif=A0GA:rbn_spacer=5-10bp:gc_cont=0.615
id	gnlBL_ORD_ID1913 hap_num=0
Start	128351
End	129163
Strand	+

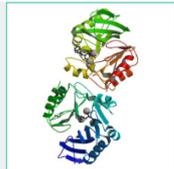
Predicted Sequence

```

HELPIEIHHPVAELSTALAAALHLGGCPHSEIRPTIGQQHETGQRFQDLVFRQLAPVAQ
HTSYLDRPFGAVASNGILVROGGKVLVDTAITDQTAQILINIKIQEINLPIVALAVTH
ANDKPHGGDLAAGAEIATYBAILSHQLANGPQHPAQHLLTFALAGASVPAITAFHIFPL
KVFYPSGHTSDHITVGGDITDITAFGGCLINDEKAKSLDRLGADQTEHYAASARAFGAAF
PKASIEVHSHAFQSRRAIHTARWAKLR
  
```

NDM-1

[Download Sequences](#)



PDB: 3Q6X | [Expand](#)

Accession	ARO:3000589
Synonym(s)	
Definition	NDM-1 is a metallo-beta-lactamase isolated from <i>Klebsiella pneumoniae</i> with nearly complete resistance to all beta-lactam antibiotics.
Classification	22 ontology terms Show
Parent Term(s)	+ ertapenem (confers resistance to drug) + meropenem (confers resistance to drug) + NDM beta-lactamase (is_a) + imipenem (confers resistance to drug) + amoxicillin-clavulanic acid (confers resistance to drug)
Sub-Term(s)	
Publications	Zhang H and Hao Q. 2011. FASEB J 25(8): 2574-2582. Crystal structure of NDM-1 reveals a common {beta}-lactam hydrolysis mechanism. (PMID 21507902)



RESFINDER

Center for Genomic Epidemiology Username Password

Home Services Instructions Output Overview of genes Article abstract

ResFinder 2.1

ResFinder identifies acquired antimicrobial resistance genes in total or partial sequenced isolates of bacteria.
Fasta file with test sequence: [Test sequence](#)
NOTE: Currently ResFinder focuses on acquired genes and does therefore not find chromosomal mutations (NAL, FUS, high-level CIP, RIF resistance, etc.)

The database is curated by: **Valeria Bortolaia** (click to contact)

View the [version history](#) of this server.

Select Antimicrobial configuration
Select multiple items, with Ctrl-Click (or Cmd-Click on Mac) - by default all databases are selected

Aminoglycoside
Beta-lactam
Colistin
Fluoroquinolone
Fosfomycin
Fusidic Acid

Select threshold for %ID
90 %

Select minimum length
60 %

Select type of your reads
Assembled Genome/Contigs*

If you get an "Access forbidden. Error 403". Make sure the start of the web address is https and not just http. Fix it by clicking [here](#).

Isolate File

Name	Size	Progress	Status
------	------	----------	--------

- **ONLY** acquired resistance genes
- **NOT** chromosomal mutations
- Batch upload of sequence data possible
- Assemblies/raw reads
- Updated frequently



RESFINDER - Output

Center for Genomic Epidemiology

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ResFinder-2.1 Server - Results

Aminoglycoside						
Resistance gene	%Identity	HSP length/Query	Contig	Position in contig	Predicted phenotype	Accession number
<i>aadA1</i>	100.00	789 / 789	LNGZ01000077.1	1178..1966	Aminoglycoside resistance	JQ480156
<i>aadA2</i>	100.00	792 / 792	LNGZ01000035.1	17182..17973	Aminoglycoside resistance	JQ364967
<i>aac(3)-IIa</i>	99.77	861 / 861	LNGZ01000055.1	1824..2684	Aminoglycoside resistance	X51534
<i>aacA4</i>	99.80	489 / 555	LNGZ01000128.1	1..489		KM278199
<i>armA</i>	100.00	774 / 774	LNGZ01000035.1	22662..23435	Aminoglycoside resistance	AY220558
<i>aph(3')-Ia</i>	100.00	816 / 816	LNGZ01000001.1	265..1080	Aminoglycoside resistance	V00359
<i>aph(3')-VIa</i>	98.46	780 / 780	LNGZ01000154.1	5729..6508	Aminoglycoside resistance	X07753

Beta-lactam						
Resistance gene	%Identity	HSP length/Query	Contig	Position in contig	Predicted phenotype	Accession number
<i>blaCTX-M-15</i>	100.00	876 / 876	LNGZ01000092.1	1076..1951	Beta-lactam resistance Alternate name; UOE-1	DQ302097
<i>blaNDM-1</i>	100.00	813 / 813	LNGZ01000154.1	2484..3296	Beta-lactam resistance	FN396876
<i>blaOXA-9</i>	100.00	840 / 840	LNGZ01000077.1	294..1133	Beta-lactam resistance	JF703130
<i>blaTEM-1A</i>	100.00	861 / 861	LNGZ01000089.1	423..1283	Beta-lactam resistance Alternate name; RblaTEM-1	HM749966
<i>blaOXA-1</i>	100.00	831 / 831	LNGZ01000107.1	675..1505	Beta-lactam resistance	J02967
<i>blaSHV-11</i>	100.00	861 / 861	LNGZ01000050.1	7233..8093	Beta-lactam resistance Alternate name; SHV-1-2a	HM751098

Colistin

No resistance genes found.

Fluoroquinolone

Display Settings: ▾ Send to: ▾
Bacterial Antimicrobial Resistance Reference Gene Database Accession: PRJNA313047 ID: 313047
 This Bioproject contains annotated sequence records for representative DNA sequences that encode proteins conferring or contributing to resistance to various antibiotics. [More...](#)

Accession	PRJNA313047
Data Type	Genome sequencing
Scope	Multispecies
Publications	1. Lahiri SD <i>et al.</i> , "Identification of Novel VEB β -Lactamase Enzymes and Their Impact on Avibactam Inhibition.", <i>Antimicrob Agents Chemother</i> , 2016 Apr 22;60(5):3183-6 More...
Submission	Registration date: 24-Feb-2016 NCBI
NCBI Links	<ul style="list-style-type: none"> NCBI Pathogen Detection National Database of Antibiotic Resistant Organisms More...
Related Resources	<ul style="list-style-type: none"> CARD Lahey Pasteur Institute Beta Lactamases ResFinder
Relevance	Agricultural, Medical, Industrial, Environmental, Evolution
Locus Tag Prefix	A7J11

Project Data:

Resource Name	Number of Links
SEQUENCE DATA	
Nucleotide (Genomic DNA)	4004
Protein Sequences	4004
PUBLICATIONS	
PubMed	1559
PMC	942

- Related information**
- Full text in PMC
 - Genomic DNA
 - Nucleotide
 - Protein
 - PubMed

- NCBI
- Nation Organi
- Submi
- NCBI I
- CARD
- Lahey
- Pasteur
- ResFit
- NCBI I

- Recei**
- B: R
 - A: bl
 - B:
 - B:
 - C:

[Health](#) > [Pathogen Detection](#) > [Beta Lactamase Data Resources](#)

Beta-Lactamase Data Resources

NOTE: THIS RESOURCE IS DEPRECATED AND REPRESENTS AN OLDER SNAPSHOT. FOR THE LATEST REFERENCE SET OF ANTIMICROBIAL RESISTANCE GENES/PROTEINS GO TO THIS PROJECT: [Bacterial Antimicrobial Resistance Reference Gene Database](#)

Along with assigning new allele numbers to [certain beta-lactamase families](#), NCBI has built the [Bacterial Antimicrobial Resistance Reference Gene Database](#) which contains the latest curated beta-lactamase protein and nucleotide sequences. Please use this resource for the most up to date information on antimicrobial resistance

We have deprecated two sets of beta-lactamase sequence files-these files are no longer actively maintained and may contain errors, inconsistencies, and outdated information:

- [Downloadable protein sequence files, nucleotide sequence files, and a table providing accessions and curated gene product names](#) for those beta-lactamase from families curated by NCBI for which there were nucleotide or protein accessions in Genbank on or before April 12, 2016.
- [Downloadable protein sequence files, nucleotide sequence files, and a table providing accessions and curated gene product names](#) for those beta-lactamases from the [Lahey Beta-Lactamase site](#) through to July 1, 2015 for which there were either nucleotide or protein accessions in Genbank.

Additional information about these resources is provided in the [Read Me file](#), and the parent directory of these files is found at <ftp://ftp.ncbi.nlm.nih.gov/pathogen/betalactamases/>.

To submit novel beta-lactamases, see this page: <https://www.ncbi.nlm.nih.gov/pathogens/submit-beta-lactamase/>

<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047>

<https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/>



Know your species!

- *oqxAB* – *K. pneumoniae*
- *bla*_{SHV-1/-11} – *K. pneumoniae*
- *bla*_{CTX-M} - *Kluyvera* spp.
- *bla*_{OXA-48-like} – *Shewanella* spp.
- *bla*_{cfiA} – *B. fragilis*
- *Etc, etc, etc,*

Fluoroquinolone						
Resistance gene	%Identity	HSP length/Query	Contig	Position in contig	Predicted phenotype	Accession number
<i>QnrS1</i>	100.00	657 / 657	LNGZ01000154.1	10386..11042	Quinolone resistance	AB187515
<i>aac(6)Ib-cr</i>	100.00	521 / 600	LNGZ01000128.1	1..521	Fluoroquinolone and aminoglycoside resistance	DQ303918
<i>oqxA</i>	99.23	1176 / 1176	LNGZ01000157.1	30757..31932	Quinolone resistance	EU370913
<i>oqxB</i>	98.86	3153 / 3153	LNGZ01000157.1	31956..35108	Quinolone resistance	EU370913



CONCLUSIONS

- No database is complete
- Antimicrobial resistance is not static
 - new mutations/resistance genes emerge
- Lack of genotypic -> phenotypic correlation
 - new models needed for prediction of phenotypes (clinical relevance)



NEED TO KNOW STRENGTHS AND WEAKNESSES OF EACH DATABASE AND BIOINFORMATIC APPROACH