

The Comprehensive Antibiotic Resistance Database

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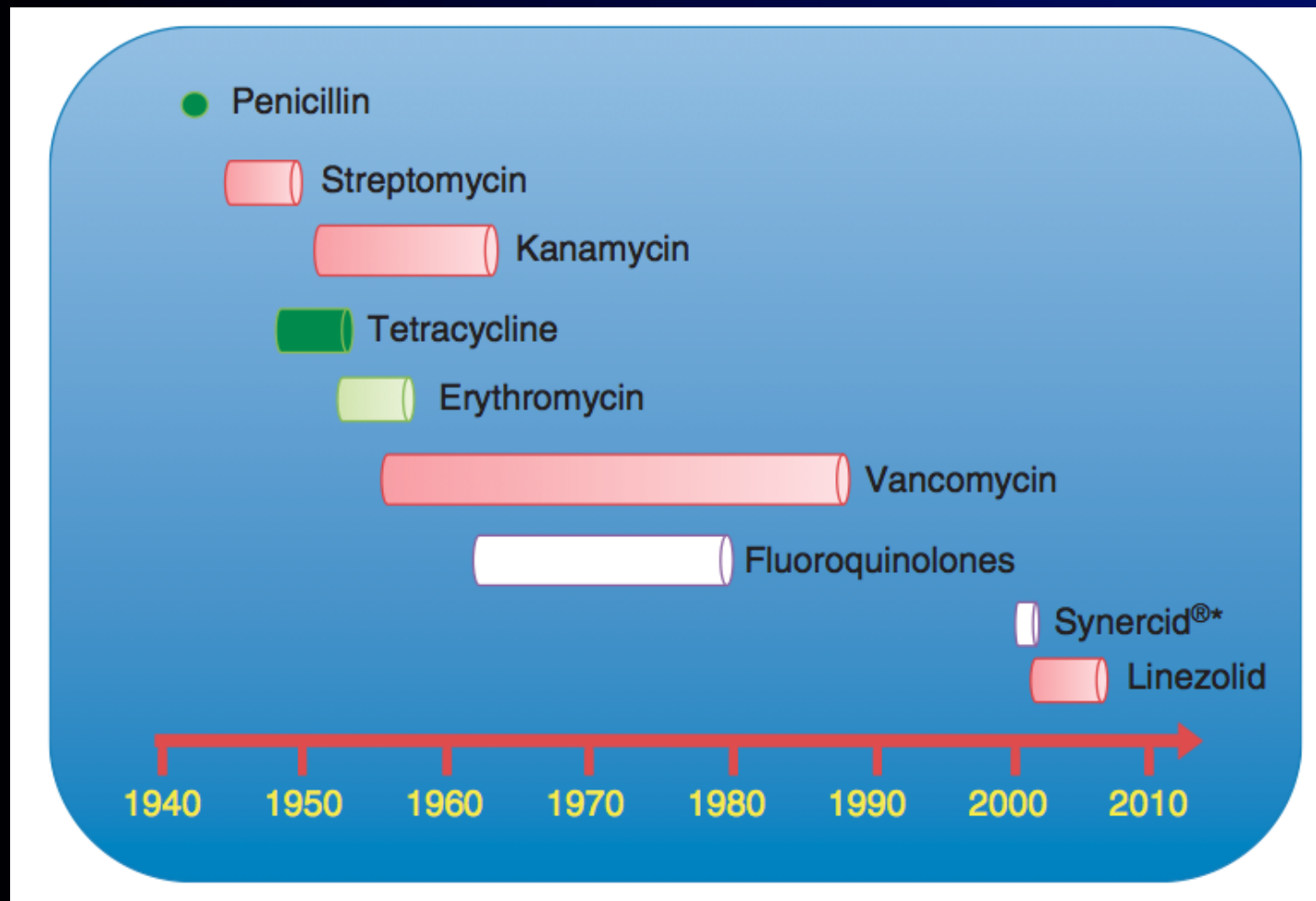


Motivation:

- Addressing antibiotic resistance requires research at molecular, clinical, population, agricultural, and environmental levels
- Common goals and objectives, but poorly integrated research findings
- Disparate sources of data, survey and experimental approaches, timelines, and research aims

Goal:

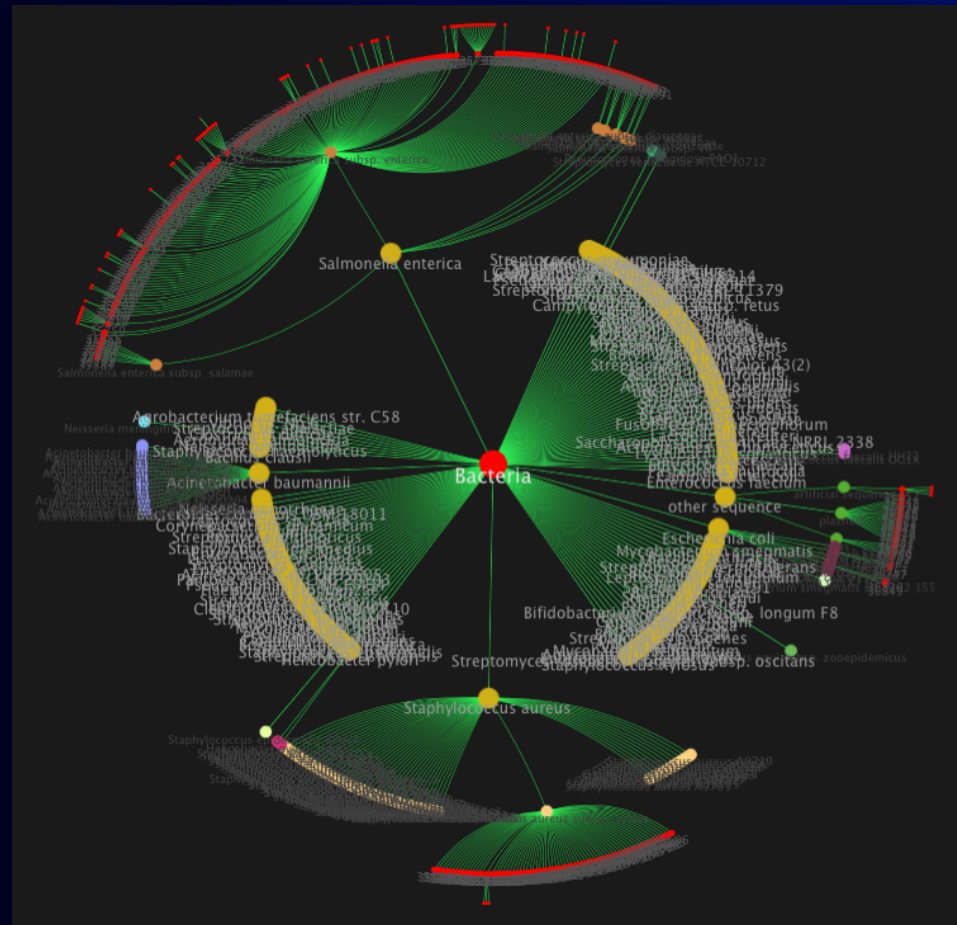
- Construction of a Comprehensive Antibiotic Resistance Database
- Development of predictive tools



Wright, G.D. 2010. The antibiotic resistome. *Expert Opin. Drug Discov.* 5: 1-10.

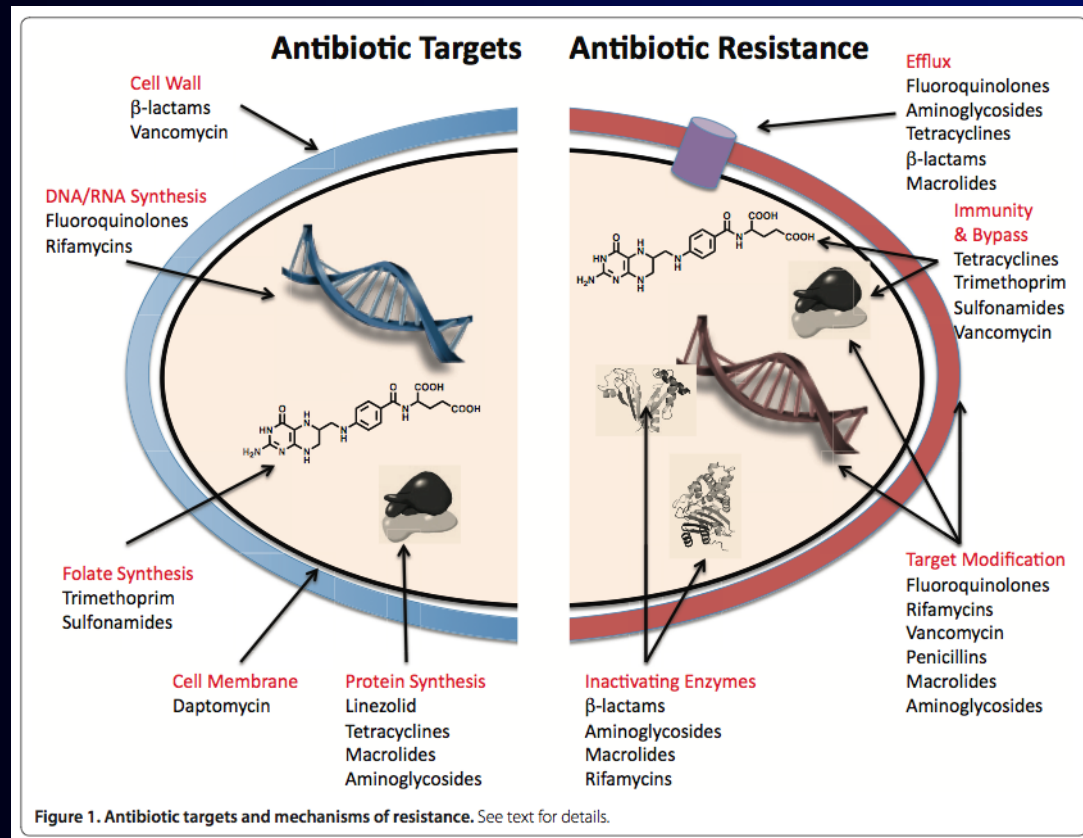
Challenges:

- Broad diversity of prokaryotic pathogens and strains



Challenges:

- Broad diversity of prokaryotic pathogens and strains
- Very diverse set of mechanisms and genes



Challenges:

- Broad diversity of prokaryotic pathogens and strains
- Very diverse set of mechanisms and genes
- Plasmid-mediated resistance, transposable elements, horizontal gene transfer

Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study



Karthikeyan K Kumarasamy, Mark A Toleman, Timothy R Walsh, Jay Bagaria, Fařhana Butt, Ravikumar Balakrishnan, Uma Chaudhary, Michel Doumith, Christian G Giske, Seema Irfan, Padma Krishnan, Anil V Kumar, Sunil Maharjan, Shazad Mushtaq, Tabassum Noorie, David L Paterson, Andrew Pearson, Claire Perry, Rachel Pike, Bhargavi Rao, Ujjwayini Roy, Jayanta B Sarma, Madhu Sharma, Elizabeth Sheridan, Mandayam A Thirunarayan, Jane Turton, Supriya Upadhyay, Marina Warner, William Welfare, David M Livermore, Neil Woodford

Summary

Background Gram-negative Enterobacteriaceae with resistance to carbapenem conferred by New Delhi metallo- β -lactamase 1 (NDM-1) are potentially a major global health problem. We investigated the prevalence of NDM-1, in multidrug-resistant Enterobacteriaceae in India, Pakistan, and the UK.

Methods Enterobacteriaceae isolates were studied from two major centres in India—Chennai (south India), Haryana (north India)—and those referred to the UK's national reference laboratory. Antibiotic susceptibilities were assessed, and the presence of the carbapenem resistance gene *bla*_{NDM-1} was established by PCR. Isolates were typed by pulsed-field gel electrophoresis of XbaI-restricted genomic DNA. Plasmids were analysed by S1 nuclease digestion and PCR typing. Case data for UK patients were reviewed for evidence of travel and recent admission to hospitals in India or Pakistan.

Findings We identified 44 isolates with NDM-1 in Chennai, 26 in Haryana, 37 in the UK, and 73 in other sites in India and Pakistan. NDM-1 was mostly found among *Escherichia coli* (36) and *Klebsiella pneumoniae* (111), which were highly resistant to all antibiotics except to tigecycline and colistin. *K pneumoniae* isolates from Haryana were clonal but NDM-1 producers from the UK and Chennai were clonally diverse. Most isolates carried the NDM-1 gene on plasmids: those from UK and Chennai were readily transferable whereas those from Haryana were not conjugative. Many of the UK NDM-1 positive patients had travelled to India or Pakistan within the past year, or had links with these countries.

Interpretation The potential of NDM-1 to be a worldwide public health problem is great, and co-ordinated international surveillance is needed.

Funding European Union, Wellcome Trust, and Wyeth.

Introduction

Bacteria from clinical and non-clinical settings are becoming increasingly resistant to conventional antibiotics. 10 years ago, concern centred on Gram-positive bacteria, particularly methicillin-resistant *Staphylococcus aureus* and

continents.^{1,2,3} Much of this dissemination is undetected, with resistant clones carried in the normal human flora and only becoming evident when they are the source of endogenous infections. The CTX-M-15 extended-spectrum β -lactamase (ESBL) encoded by *bla*_{CTX-M-15} was first reported

Lancet Infect Dis 2010;
10: 597–602

Published Online
August 11, 2010
DOI:10.1016/S1473-3099(10)70143-2

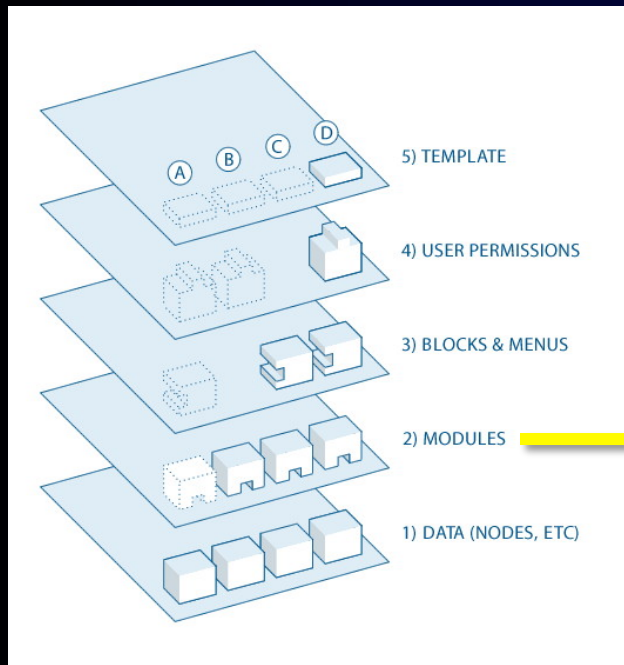
See Reflection and Reaction
page S78

Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, India (K K Kumarasamy MPhil, P Krishnan PhD); Department of Infection, Immunity and Biochemistry, School of Medicine, Cardiff University, Cardiff, UK (M A Toleman PhD, Prof T R Walsh PhD); Health Protection Agency Centre for Infections, London, UK (J Bagaria MD, R Balakrishnan MD, M Doumith PhD, S Maharjan MD, S Mushtaq MD, T Noorie MD, A Pearson PhD, C Perry PhD, R Pike PhD, B Rao MD, E Sheridan PhD, J Turton PhD, M Warner PhD, W Welfare PhD, D M Livermore PhD, N Woodford PhD); Department of Microbiology, Shaukat Khanum Cancer Hospital, Lahore, Pakistan (F Butt MD); Department of Microbiology,

Design Decisions:

- Development of a Antibiotic Resistance Ontology
- Initial focus upon molecular data
- Chado as the core organizing principle
- Use of a organismal ontology
- Ontology-centric interface
- Live curation of ontology and data
- Predictive tools

Drupal

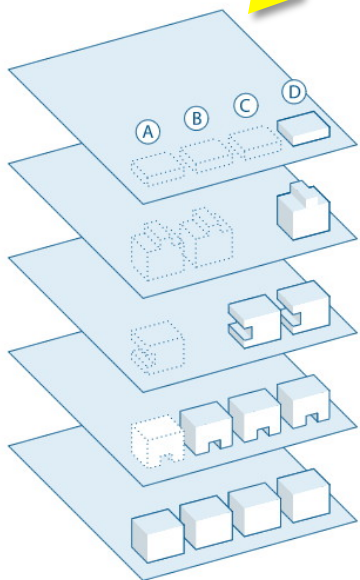


CARD modules

Chado



Drupal



- 5) TEMPLATE
- 4) USER PERMISSIONS
- 3) BLOCKS & MENUS
- 2) MODULES
- 1) DATA (NODES, ETC)

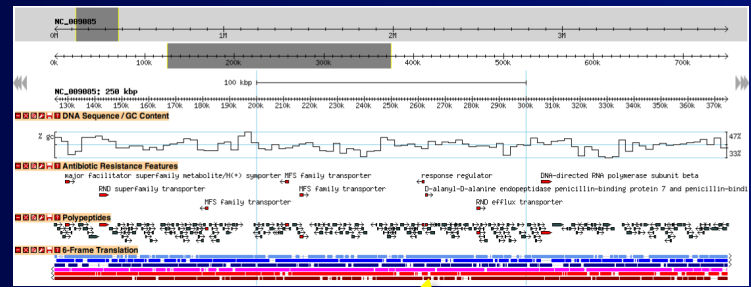
Live editing of Chado contents

Viroblast

```
>testpeptide on NC_011595.7059367.p01 hypothetical protein. Encoded by gene  
AB27A_000009. [Acinetobacter baumannii AB307-0294]  
Length = 335  
  
Score = 449 bits (1156), Expect = e-126, Method: Compositional matrix ad  
Identities = 201/330 (60%), Positives = 259/330 (78%)  
  
Query: 5 LRIVAVICCVWGTTHAQVVCVFDLLGKSGSEFYKQHEEALAAKQWADITLIFQDRA 64  
D + + + + + A VCVFELGK+G+SYKQHEEALAAK W +T L+P QDRA  
Sbjct: 6 LAMAAASVVGFSABAQAASVVCVFDLLGKAGDSFYKQHEEALAAKQWOTEINLVPQDRA 65  
  
Query: 65 LVDSRLREGKCDAAHTSMRARNYKFAISDAIGCVTSNIAQKAIYVLDQKNNHML 124  
+ D + GKCDIA MT-MRAR YKTFAGSID++GDV +N IAQ+A1+VFLD RN +M  
Sbjct: 66 VADNPFKAGKCAVMTANBARQVYKTFAGSIDGELGVVSNQIAQRA1YVLDAAHAAKMT 125  
  
Query: 125 TFMQNTREYVGVICGLLALVYRQDNASIEYVGTCTFALQYDAAQKIMVYDGAQD 184  
T G YEV GI +G A++FYRD+N+S+EK G RFA+L YD AQEIMV+ VQAQ +  
Sbjct: 126 TNLGKKKYEVAIGSPLGSAIFIVRDNINSVEKAAKGFVLYGDDAQKIMVQRVCAQV 185  
  
Query: 185 PSEITDFVVKFNSQVYEAIAAPVYFKPLEIKGIGTNGAMFFPVVYVFDLIARSD 244  
S+I++F KFN+GV++ +APY YKPLE+ KG+C NC +FV++VT DL+ R+DKP
```

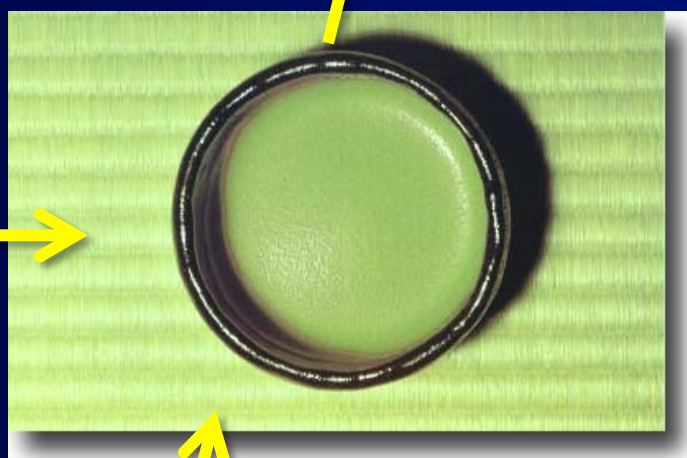
CARD modules

Gbrowse2



Bio:DB:Das:Chado

Chado



CARD_GFF_loader

molecular data

ORGANISM
organism_id
abbreviation
genus
species
common_name
comment

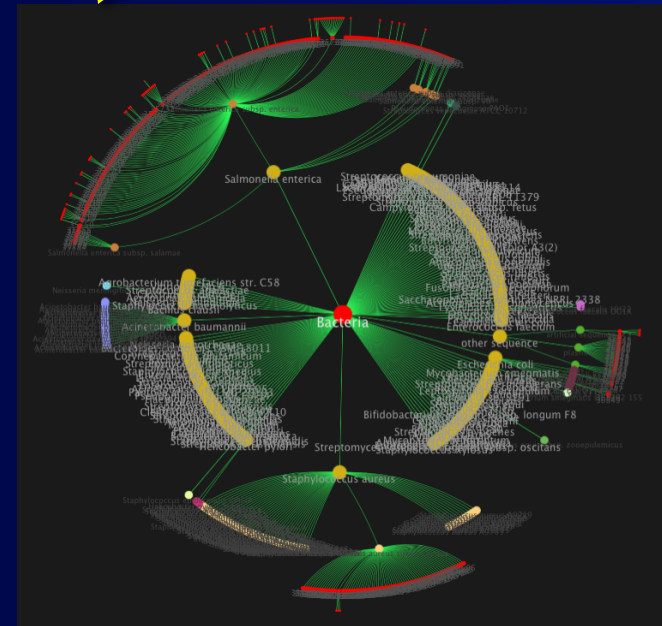
lacks complexity

FEATURE
feature_id
dbxref_id
organism_id
name
uniquename
residues
seqlen
md5checksum
type_id
is_analysis
is_obsolete
timeaccessioned
timelastmodified

1-to-many

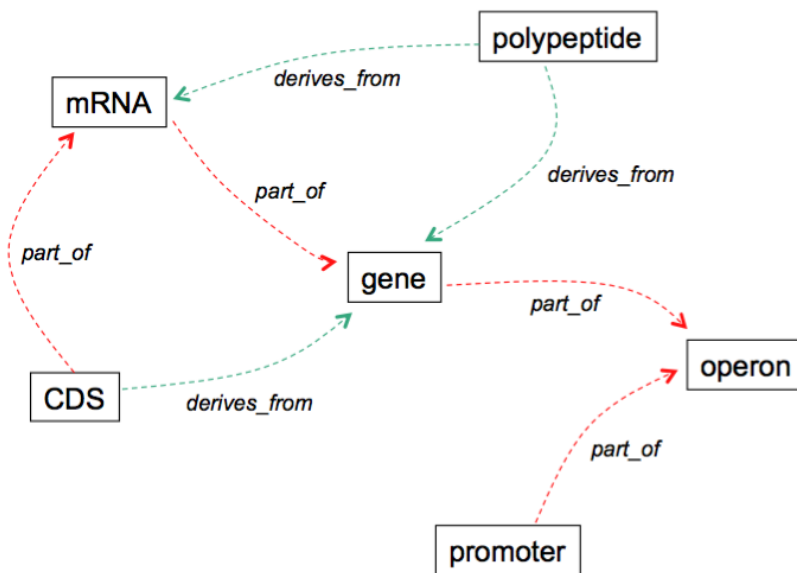
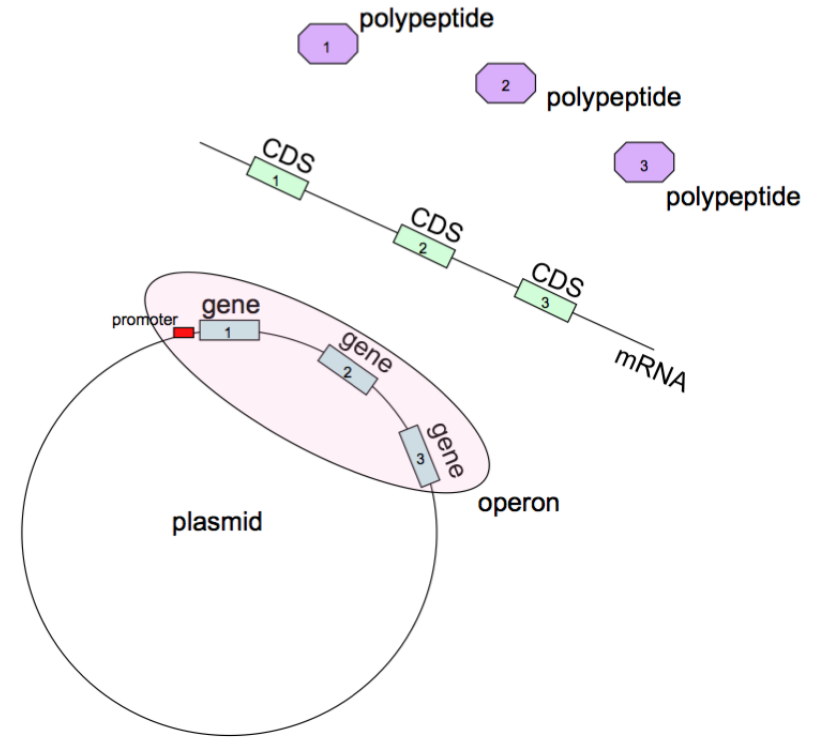
FEATURE_CVTERM
feature_cvterm_id
feature_id
cvterm_id
pub_id
is_not
rank

NCBI Taxonomy



Prokaryote Issues

- The prokaryotic gene model
- Chado and SOFA compliance
- Circular genomes and plasmids



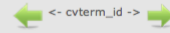
NDM-1

ARO:3000589 | Antibiotic Resistance Ontology



List all 4 sequences tagged for all forms of NDM-1.

Add Edit



Sources

- 1 → natural plasmid

Molecular Data

- 2 → gene
- 1 → PCR product
- 2 → polypeptide

Antibiotic Resistance

Clicking on numbers will list only those sequences precisely tagged for the term listed.

- 4 → NDM-1

Definition

NDM-1 is a metallo-beta-lactamase discovered from a Swedish patient carrying a *Klebsiella pneumoniae* infection after hospitalization in India.

External Data

Classification

is_a NDM beta-lactamase

* additional resistance information may be available at higher or lower levels of classification
[Visualize Ontology Subtree \[new window\]](#)

Taxonomic Distribution of Molecular Data:

- 2 *Escherichia coli*
- 2 *Klebsiella pneumoniae*

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
King D and Strynadka N. 2011. *Protein Sci* 20(9): 1484-1491. Crystal structure of New Delhi metallo-beta-lactamase reveals molecular basis for antibiotic resistance. PMID 21774017

[More from PubMed...](#)

Editor Notes

Ontology ClickSpan:

- NDM-1 (4 features)

Synonyms

Bioinformatics

[RegEx] NDM-1

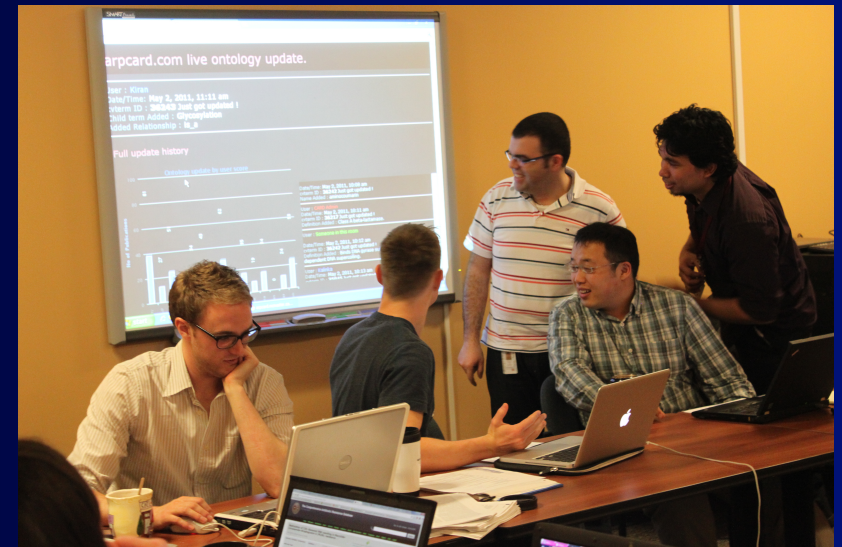
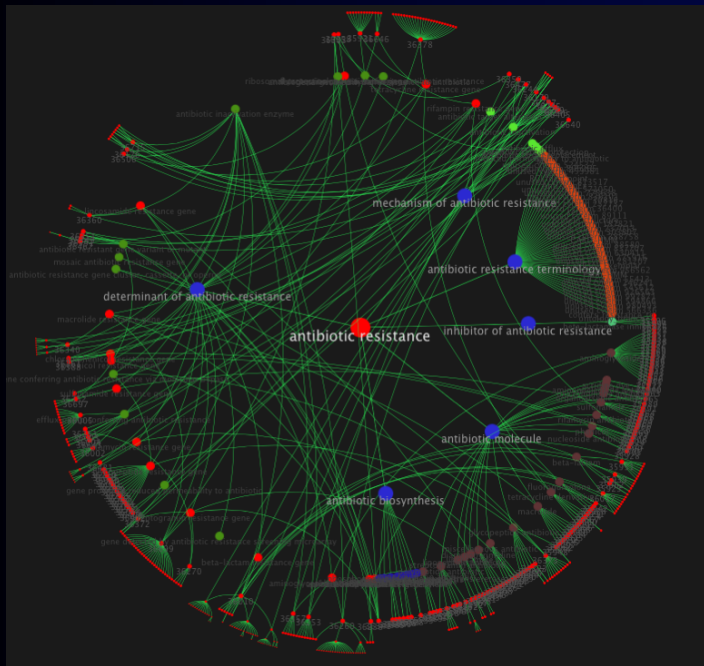
Experimental Data



Name: 3Q6X

Description: Crystal structure of NDM-1 in complex with hydrolyzed ampicillin

[View Structure](#) | [View Fragments](#)



Data so far:

- 22 prokaryotic genomes, 108 plasmids, 3 transposable elements
- 74.7 Mbp of genome/plasmid sequence
- 67,990 protein-coding genes
- 194 species, subspecies, or strains
- 543 antibiotic resistance ontology (ARO) terms
- 2,560 ARO tagged protein-coding genes

Ontology Centric Website

aminoglycoside resistance gene

ARO:3000104 | Antibiotic Resistance Ontology



Editing Not Allowed.

List all 212 sequences tagged for all forms of aminoglycoside resistance gene.

Sources

- 13 → circular double stranded DNA chromosome
- 11 → natural plasmid
- 2 → transposable element

Molecular Data

- 47 → cloned region
- 103 → gene
- 103 → polypeptide
- 5 → region
- 1 → repeat region

Antibiotic Resistance

Clicking on numbers will list only those sequences precisely tagged for the term listed.

- 43 → aminoglycoside acetyltransferase (AAC)
- 58 → aminoglycoside phosphotransferase (APH)
- 9 → ANT(2'')
- 28 → ANT(3'')
- 14 → ANT(4')
- 10 → ANT(6)
- 32 → ANT(9)
- 4 → APH(2'')
- 4 → APH(3'')
- 6 → APH(3')
- 4 → APH(4)
- 6 → APH(6)

Definition:

Genes conferring resistance to aminoglycoside antibiotics.

External Data:

Classification:

is_a determinant of antibiotic resistance
confers_resistance_to aminoglycoside

includes:

- aminoglycoside acetyltransferase (AAC)
- aminoglycoside nucleotidyltransferase (ANT)
- aminoglycoside phosphotransferase (APH)

* additional resistance information may be available at higher or lower levels of classification
[Visualize Ontology Subtree \[new window\]](#)

Taxonomic Distribution of Molecular Data:

8 *Acinetobacter baumannii* AB0057
4 *Acinetobacter baumannii* AB307-0294
4 *Acinetobacter baumannii* ACICU
14 *Acinetobacter baumannii* AYE
2 *Acinetobacter baumannii* SDF
2 *Bacillus circulans*
2 *Campylobacter fetus* subsp. *fetus*
2 *Campylobacter jejuni*
2 Cloning vector pBGS9-
2 Cloning vector pUCGM
2 *Enterobacter cloacae*
4 *Enterococcus casseliflavus*
6 *Enterococcus faecalis*
4 *Enterococcus faecium*
2 *Enterococcus gallinarum*
20 *Escherichia coli*
2 *Escherichia coli* O157:H7 str. Sakai
2 IncJ plasmid R391
11 *Klebsiella pneumoniae*
2 *Legionella pneumophila* 130b
2 *Mycobacterium fortuitum*
2 Plasmid NR79
2 Plasmid pWP14a
2 *Pseudomonas aeruginosa*
2 *Salmonella enterica*

Synonyms

Bioinformatics

[RGI focal point]

Experimental Data

Ontology Centric Website

Acinetobacter baumannii

NCBITaxon:470 | NCBI Taxonomy



Too many sequences,
please browse sub-categories.

Editing Not Allowed.

List all 86445 sequences tagged for all forms of *Acinetobacter baumannii*.

Sources

- 6 → circular double stranded DNA chromosome
- 17 → natural plasmid

Molecular Data

- 21133 → CDS
- 6 → cloned region
- 1 → gap
- 21700 → gene
- 1 → minus 10 signal
- 1 → minus 35 signal
- 21133 → mRNA
- 16 → ncRNA
- 3 → origin of replication
- 1 → PCR product
- 21133 → polypeptide
- 7 → protein binding site
- 705 → pseudogene
- 7 → recombination feature
- 4 → region
- 51 → repeat region
- 1 → ribozyme
- 2 → RNA 6S
- 102 → rRNA
- 415 → tRNA

Definition:

External Data:

Classification:

is_a Bacteria

includes:

- [Acinetobacter baumannii 1656-2](#)
- [Acinetobacter baumannii AB0057](#)
- [Acinetobacter baumannii AB056](#)
- [Acinetobacter baumannii AB058](#)
- [Acinetobacter baumannii AB059](#)
- [Acinetobacter baumannii AB307-0294](#)
- [Acinetobacter baumannii AB900](#)
- [Acinetobacter baumannii ACICU](#)
- [Acinetobacter baumannii ATCC 17978](#)
- [Acinetobacter baumannii ATCC 19606](#)
- [Acinetobacter baumannii AYE](#)
- [Acinetobacter baumannii MDR-ZJ06](#)
- [Acinetobacter baumannii SDF](#)
- [Acinetobacter baumannii WBA9388](#)
- [Acinetobacter baumannii WBL7215](#)
- [Acinetobacter baumannii WBL7355](#)

* additional resistance information may be available at higher or lower levels of classification

[Visualize Ontology Subtree \[new window\]](#)

Taxonomic Distribution of Molecular Data:

279 [Acinetobacter baumannii](#)
15419 [Acinetobacter baumannii AB0057](#)
13988 [Acinetobacter baumannii AB307-0294](#)
15240 [Acinetobacter baumannii ACICU](#)
13658 [Acinetobacter baumannii ATCC 17978](#)
48 [Acinetobacter baumannii ATCC 19606](#)
15132 [Acinetobacter baumannii AYE](#)
12681 [Acinetobacter baumannii SDF](#)

Synonyms

[Acinetobacter baumannii](#)
[Acinetobacter baumannii](#)
[Bacterium anitratum](#)

Bioinformatics

Experimental Data

Ontology Centric Website

natural_plasmid



Editing Not Allowed.

SO:0001476 | Sequence Ontology | Visualize SO Graph

List all 108 sequences tagged for all forms of natural_plasmid.

Sources

108 → natural plasmid

Molecular Data

- 1342 → CDS
- 2 → cloned region
- 2 → fragmentary
- 1352 → gene
- 17 → minus 10 signal
- 16 → minus 35 signal
- 17 → mobile genetic element
- 1342 → mRNA
- 1 → ncRNA
- 8 → origin of replication
- 2 → oriT
- 1342 → polypeptide
- 5 → promoter
- 12 → protein binding site
- 65 → pseudogene
- 16 → recombination feature
- 86 → region
- 171 → repeat region
- 2 → ribosome entry site

Antibiotic Resistance

Clicking on numbers will list only those sequences precisely tagged for the term listed.

- 7 → aminoglycoside
- 14 → aminoglycoside acetyltransferase (AAC)

Definition:

A plasmid that occurs naturally.

External Data:

Classification:

is_a plasmid
is_a extrachromosomal_mobile_genetic_element

includes:

- natural_transposable_element

* additional resistance information may be available at higher or lower levels of classification

[Visualize Ontology Subtree \[new window\]](#)

Taxonomic Distribution of Molecular Data:

- 4 Acinetobacter baumannii
- 1 Acinetobacter baumannii AB0057
- 2 Acinetobacter baumannii ACICU
- 2 Acinetobacter baumannii ATCC 17978
- 1 Acinetobacter baumannii ATCC 19606
- 4 Acinetobacter baumannii AYE
- 3 Acinetobacter baumannii SDF
- 1 Enterococcus faecalis
- 3 Escherichia coli
- 1 Escherichia coli O157:H7 str. Sakai
- 2 Klebsiella pneumoniae
- 1 Salmonella enterica subsp. enterica serovar Typhi
- 68 Staphylococcus aureus
- 1 Staphylococcus aureus subsp. aureus COL
- 2 Staphylococcus aureus subsp. aureus ED98
- 1 Staphylococcus aureus subsp. aureus JH1
- 1 Staphylococcus aureus subsp. aureus JH9
- 1 Staphylococcus aureus subsp. aureus MSSA476
- 1 Staphylococcus aureus subsp. aureus Mu50
- 1 Staphylococcus aureus subsp. aureus N315
- 1 Staphylococcus aureus subsp. aureus ST398
- 3 Staphylococcus aureus subsp. aureus USA300_FPR3757
- 2 Staphylococcus aureus subsp. aureus USA300_TCH1516
- 1 Staphylococcus aureus subsp. aureus USA300_TCH959

Publications:

More from PubMed...

Synonyms

natural plasmid

Bioinformatics

Experimental Data

Ontology Centric Website

natural_plasmid

SO:0001476 | Sequence Ontology | Visualize SO Graph



Editing Not Allowed.

List all 108 sequences tagged for all forms of natural_plasmid.

Sources

108 → natural plasmid

Molecular Data

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- 1352 → gene
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Antibiotic Resistance

Clicking on numbers will list only those sequences precisely tagged for the term listed.

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- 14 → aminoglycoside acetyltransferase (AAC)

Definition:

A plasmid that occurs naturally.

External Data:

Classification:

is_a plasmid
is_a extrachromosomal_mobile_genetic_element

Includes:

- natural_transposable_element

* additional resistance information may be available at higher or lower levels of classification
[Visualize Ontology Subtree \[new window\]](#)

Taxonomic Distribution of Molecular Data:

- 4 Acinetobacter baumannii
- 1 Acinetobacter baumannii AB0057
- 7 Acinetobacter baumannii ACICU
- 2 Acinetobacter baumannii ATCC 17978
- 1 Acinetobacter baumannii ATCC 19606
- 4 Acinetobacter baumannii AYE
- 3 Acinetobacter baumannii SDF
- 1 Enterococcus faecalis
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- 2 Klebsiella pneumoniae
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- 68 Staphylococcus aureus
- 1 Staphylococcus aureus subsp. aureus COL
- 2 Staphylococcus aureus subsp. aureus ED98
- 1 Staphylococcus aureus subsp. aureus JH1
- 1 Staphylococcus aureus subsp. aureus JH9
- 1 Staphylococcus aureus subsp. aureus MSA476
- 1 Staphylococcus aureus subsp. aureus Mu50
- 1 Staphylococcus aureus subsp. aureus N315
- 1 Staphylococcus aureus subsp. aureus ST398
- 3 Staphylococcus aureus subsp. aureus USA100_FPR3757
- 2 Staphylococcus aureus subsp. aureus USA300_TCH1516
- 1 Staphylococcus aureus subsp. aureus USA300_TCH959

Publications

More from PubMed...

Synonyms

natural plasmid

Bioinformatics

Experimental Data

cvterm_crossref
materialization tables

Feature Page

blaCTXM-1 protein.

Encoded by gene blaCTXM-1.

BLASTP → [CARD Polypeptides](#)
TBLASTN → [CARD Genes](#)
[CARD Genomes and Plasmids](#)
HMMER → [Pfam Protein Domains](#)

Editing Not Allowed.

Related Features

[X92506.gene](#) (gene)
[X92506.gene.t01](#) (mRNA)

Antibiotic Resistance

(based on curation)

CTX-M beta-lactamase
[gene detected by antibiotic resistance screening microarray](#)

Sequence Ontology

[polypeptide](#)

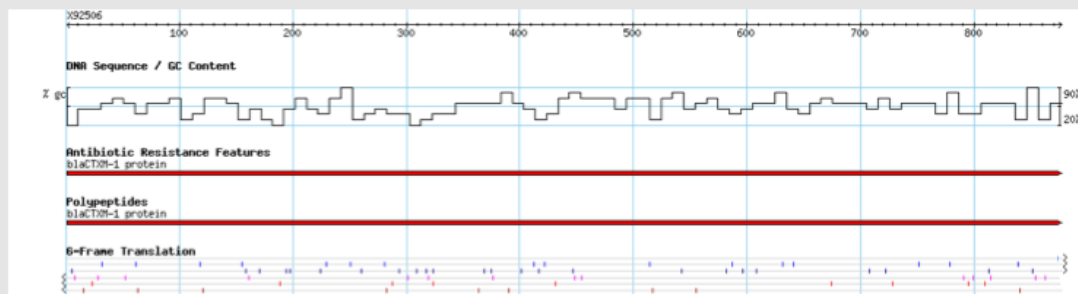
Organism

[Escherichia coli](#)

Gene Ontology

(based on uncurated, automated annotation)

E.coli bla(CTX-M-1) gene (CARD: [X92506](#))



Coordinates: 1 - 876 (+ strand). Length: 876 bp (291 aa). Click on the image to view sequence in GBrowse, a tool for working with molecular sequences. [\[new window\]](#)

>X92506.gene.p01

```
MVKKSLRQFTLMATATVTLGSGVPLYAQTADVQQKLAELERQSGGRLGVALINTADNSQILYRADERFAMCSTSKVMAVAAVLKKSESEPNLLNQRVEI  
KKSDLVNYNPIAEKHVDGTMSLAELSAALQYSDNVAMNKLIHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPDRTTSPRAMAQLRNLTLGK  
ALGDSQRAQLVTHMKGNTTGAASIQAGLPASWVVGDKTGSVDYGTNDIAVIWPKDRAPLILVTYFTQPQPKAESRRDVLASAARKIVTNGL
```

Publications:

Bauernfeind A, et al. 1996. Antimicrob Agents Chemother 40(2): 509-513. Sequences of beta-lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other beta-lactamases. PMID 8834913

Bioinformatics

CARD: [X92506.gene.p01](#)
PMID:8834913

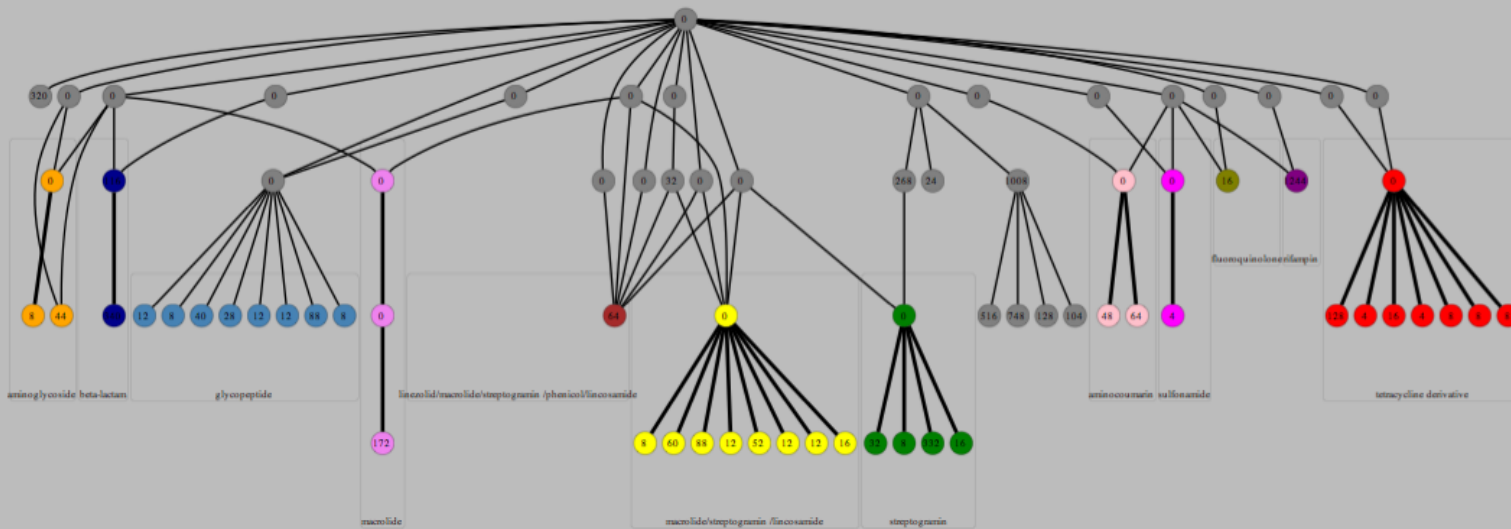
Experimental Data

Resistance Gene Identifier

Predicted Antibiotic Resistance Genes

Get ! Big ! Small !

Download Image



The figure to the left gives an ontological classification of the Antibiotic Resistance Gene results, with specific terms (e.g. NDM-1 beta-lactamase) being at the bottom and general terms (e.g. macrolide resistance gene) being at the top. Numbers within the circles represent the number of BLASTP hits (e-value $1e-30$) to genes in CARD generated from your predicted ORFs. Where possible, colour is used to indicate range of resistance, but circles in grey may also be informative. Hover or click on the circle to view the predicted Antibiotic Resistance genes and BLASTP details.

Saved ORFs

Choose File test-1900kb.fasta

Annotate

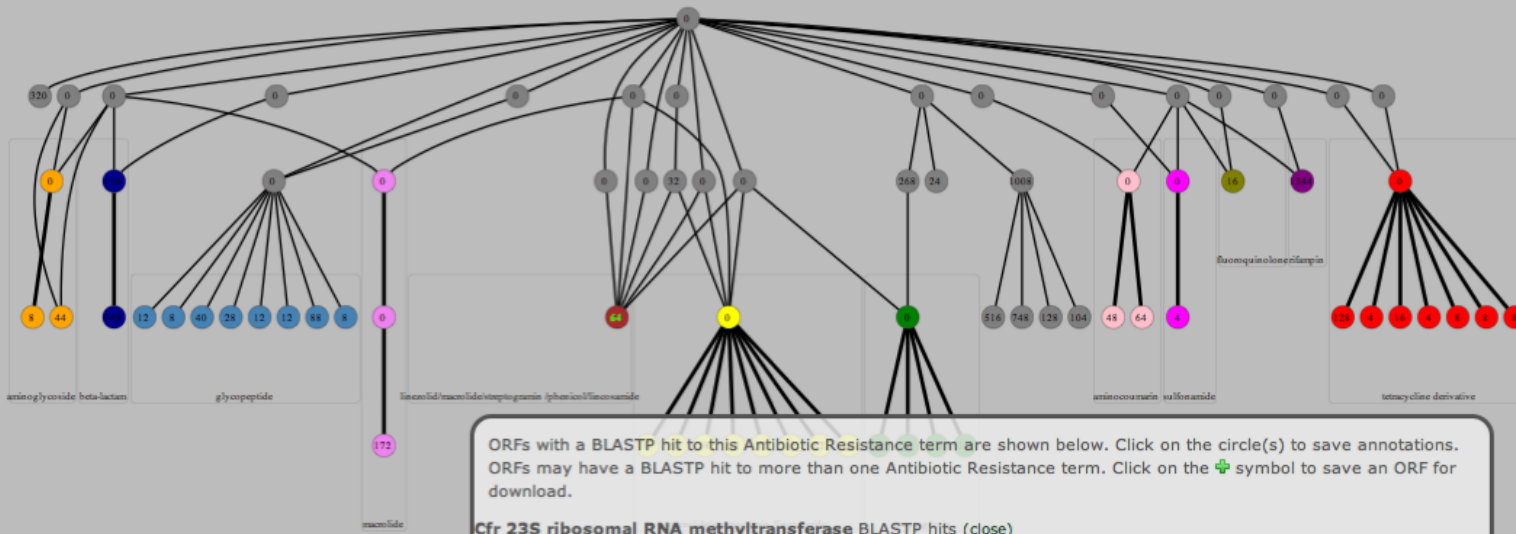
1985 of 1985 ORFs summarized (100.00%)

Resistance Gene Identifier

Predicted Antibiotic Resistance Genes

Get! Big! Small!

Download Image



The figure to the left gives an ontological classification of the Antibiotic Resistance Gene results, with specific terms (e.g. NDM-1 beta-lactamase) being at the bottom and general terms (e.g. macrolide resistance gene) being at the top. Numbers within the circles represent the number of BLASTP hits (e-value < 1e-30) to genes in CARD generated from your predicted ORFs. Where possible, colour is used to indicate range of resistance, but circles in grey may also be informative. Hover or click on the circle to view the predicted Antibiotic Resistance genes and BLASTP details.

Saved ORFs

Choose File test-1900kb.fasta

1985 of 1985 ORFs summarized (100.00%)

Annotate

ORFs with a BLASTP hit to this Antibiotic Resistance term are shown below. Click on the circle(s) to save annotations. ORFs may have a BLASTP hit to more than one Antibiotic Resistance term. Click on the + symbol to save an ORF for download.

Cfr 23S ribosomal RNA methyltransferase BLASTP hits (close)

>contig0-orf1373

Perform *de novo* BLASTP against (new window): GenBank | CARD

CARD BLASTP Hit	Hit Length	Mismatches	Bit Score	E-Value
radical SAM protein [Cfr 23S ribosomal RNA methyltransferase]	345	142	397	7e-112
radical SAM protein [Cfr 23S ribosomal RNA methyltransferase]	345	142	397	7e-112
radical SAM protein [Cfr 23S ribosomal RNA methyltransferase]	345	142	397	7e-112
radical SAM enzyme, Cfr family [Cfr 23S ribosomal RNA methyltransferase]	367	211	228	5e-61
ribosomal RNA large subunit methyltransferase N [antibiotic]	363	221	218	3e-58

Future work:

- Ontology development
- Custom HMMs
- SNP models
- Data curation
- Minimum inhibitory concentration (MIC)
- Prevalence
- Metagenomics

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