Anti-Microbial Resistance DB (AMR-DB) User Manual

The AMR-DB is a Centers for Disease Control and Prevention (CDC)-funded database resource. It started as a comprehensive non-redundant database of anti-microbial resistance gene and protein sequences derived from 5 major AMR databases (CARD, Resfinder, ARG-ANNOT, Lahey/NCBI and MEGARes), which was used to query GenBank to enable global analysis of AMR from sequenced bacterial genomes. The layout of the website consists of 5 main sections: Home, Analysis, Primer, Support, and Contact.

1. Home

The AMR-DB home page dashboard provides a high-level summary of AMR-DB contents. Menu and submenus provide functionality to browse and search data, AMR information on world map, AMR-Finder that allows users to find AMR genes in AMR-DB reference database. Primer menu allow users to browse and search catalog of AMR primers loaded in AMR-DB, and it allow users to primer finder on existing AMR-DB reference database. Secure admin menu is only available to registered users with admin role, and it allow users to edit data in AMR-DB.

1.1. Header Menu

The right corner of menu allows the user to sign-up or sign-in into AMR-DB (see below). Only registered users can sign-in (1). The horizontal menu across the top of the page has four sections in addition to the (A) home button: B) Analysis, C) Primer D) "support", E) "contact" page, and F) Admin (only appears when signed in as a registered user).

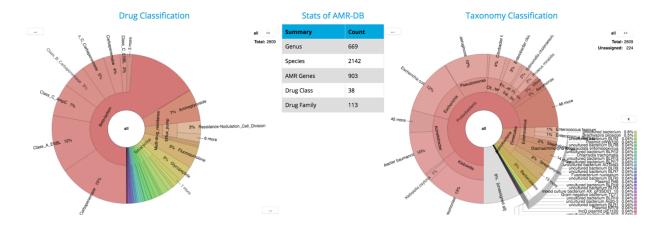


- 1. **Sign In/Sign Up:** The user must register and sign in to edit AMR-DB.
- A. **Home**: goes to the home page
- B. **Analysis**: The location of all the tools to view, browse and search the AMR-DB. Tools available from this page include:

- AMR-Map: View an interactive world map showing the location of antimicrobial resistance genes in AMR-DB.
- **Sequence Similarity Search Module:** Search user-supplied sequences for similar sequences in AMR-DB.
- Browse and Search: Explore and word-search contents of AMR-DB.
- C. **Primer**: Allows the user to validate user-supplied oligonucleotide primers used in diagnostic PCR.
 - Primer Catalog: List of 24 primers used to detect common AMR genes
 - **Primer Finder**: Search AMR-DB for the location of primer-binding sites
- D. **Support**: Provides help and information for the user to use the site or examine how the data was gathered and processed
- E. Contact: Form to get support
- F. **Admin**: Enables a registered user secure access to submit new data, edit existing data, add new primer sequences to the Primer Catalog, and rebuild AMR-Finder reference database. This tab only appears once the registered user is signed in.

1.2. **Body**

The body of the main page shows the stats of the AMR-DB along with two dynamic sun-burst charts (Krona graphs) displaying the drug classification and taxonomy classification data present in database (see below). A separate table also display the total count of AMR genes, drug class/family/sub-class and number of different genus with their species present in AMR-DB.

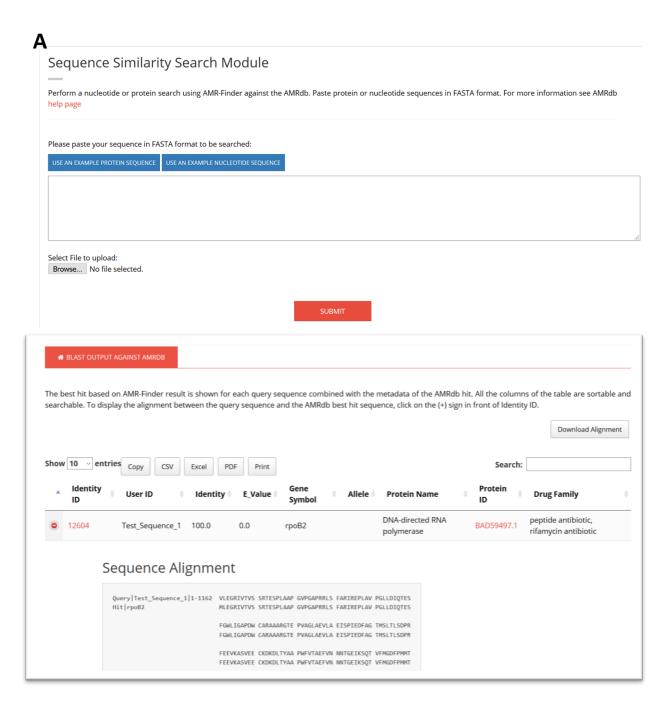


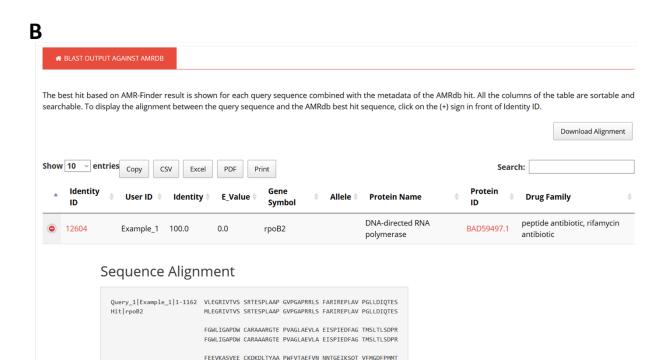
2. Analysis

2.1. **AMR-Map**

2.2. Sequence Similarity Search Module

The AMR-Finder tool allows users to find homologous AMR genes based on user-provided input multi-FASTA sequences (**A**) and allows users to access the gene information pages (see Section B.3) and sequence alignments for the best hits and download results and AMR sequences (**B**).





2.3. Browse and Search

The Browse and Search tool enables users to view, search, and export the data contained within the AMR-DB based on different classifications such as gene name, drug targets, threat level and organism names. Users can perform a global search (**A**) across all data fields using the search function or can tailor their search to specific data fields using the advanced search (**B**) option. The results of both a global (**A**) and advanced (**B**) search are displayed in tabular format with the most commonly searched data types preferentially displayed. Users can display detailed information about each gene by clicking on the Identity ID link to open a detailed gene information page (**C**)

FEEVKASVEE CKDKDLTYAA PWFVTAEFVN NNTGEIKSQT VFMGDFPMMT

Α

AMRdb Browse/Search Module

Browse and search the data contained within the AMRdb. Commonly searched data types are preferentially displayed. Each data type is also sortable.

Example search function: To display all Escherichia coli genes of the beta-lactam drug family, enter "Escherichia coli beta-lactam" in the main search bar. AMRdb data will be filtered and only those AMR genes found in Escherichia coli AND that belong to the beta-lactam drug family are displayed. For more information see help page.

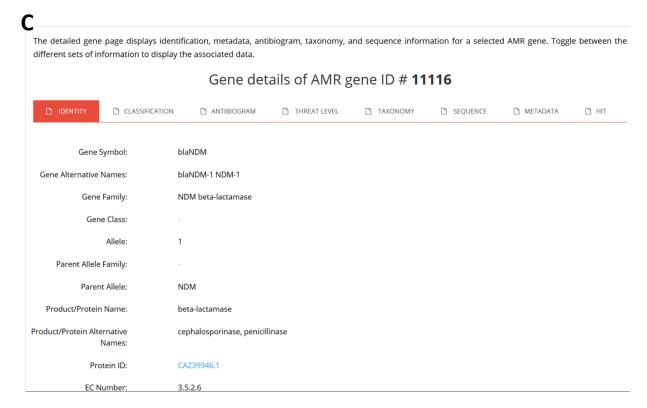
							Ex	port
Show 10 × entr	ries					Search:	Advanced S	earch
Identity ID 🏝	Gene Symbol 💠	Allele 🛊	Protein/Product Name	Protein ID 🍦		\$	Organism	\$
11116	blaNDM	1	beta-lactamase	CAZ39946.1	penam, cephamycin, carbapenem, cephalosporin		Klebsiella pneumoniae	
11117	blaNDM	10	beta-lactamase	AGT37351.1	penam, carbapenem cephalosporin, cephamycin		Klebsiella pneumoniae	
11118	blaNDM	11	beta-lactamase	AJE61443.1			Escherichia coli	
11119	blaNDM	12	beta-lactamase	BAO79439.1	penam, carbapenem cephalosporin, cephamycin		Escherichia coli	
11120	blaNDM	13	beta-lactamase	BAQ02518.1	penam, carbapenem cephalosporin, cephamycin		Escherichia coli	

В

Advance Search

Field Name	Operator	Values	And / Or	+ Add
Gene Symbol	=	aac(6')	AND ~	Delete Row
Taxon Genus ~	=	Escherichia	AND ~	Delete Row
Taxon Species V	= ~	coli	AND ~	Delete Row
Mol Type ~	=	Plasmid	AND ~	Delete Row
Specimen Collection Date	>=	2005	AND ~	Delete Row

Submit



3. Primer

3.1. Primer Catalog

The primer catalog tool provides a listing of all primers stored in the AMR-DB. Users can perform a global search of the available primers using the search function to identify specific primers of interest.

Primer Catalog

Primer Catalog provides listing and advance search of all primers available in the system. For more information see help page.

Primer	* Target	→ FWD	
СС	Screening of AmpC	CCTCCGTCAGCTCAGATACA	TTTACTAGGTGCAAGCCAGACA
ACT	Screening of AmpC	CGTTACGCCGCTGATGAAAG	CCAGGGTAAGGCCTTTCCTG
AIM	Screening of MBL	ATGAAACGTCGCTTCACCCTGCTG	TCAAGGCCGCGCGCCGCTGGA
CMY	Screening of AmpC	GGGGCATATGATGAAAAAATCGTTA	CCGGATCCTCAACCGGCCAACTG
CMY	Screening of AmpC	AACACACTGATTGCGTCTGA	TCCTGGGCCTCATCGTCAGTTAT
CTX	Screening of ESBL	CGCTTTGCGATGTGCAG	ACCGCGATATCGTTGGT
OHA	Screening of AmpC	GCAAAGCCAGTATGCGTACG	CAGTTGTTGCGCCCGTTTTA
=OX	Screening of AmpC	TAGTCTGGGCCAGCCATTTG	GTAACCGGATTGGCCTGGAA
GES	Screening of Carbapenemases	CTTCATTCACGCACTATTAC	TAACTTGACCGACAGAGG
GIM	Screening of MBL	AACTTCCAACTTTGCCATGC	TCGACACCTTGGTCTGAA

3.2. Primer Finder

The Primer Finder is a web-based tool that enables in silico testing of the performance of PCR and RT-PCR primers used for ARG diagnostics. It will support multiple use cases (ARG-specific primers and flanking gene-specific primers). Unless the user specifies an input sequence or GenBank accession to test (see screenshot below), Primer Finder will first check primer-binding to ARGs internal to the AMR-DB (default) from a user-provided multi-FASTA sequence of primers or pasted sequence of forward and reverse primers. Primer Finder will produce an output report of the predicted binding sites, target(s) hit, amplicon sequence, length, position, number of degenerate bases and mismatches per primer, strand, gene annotations overlapping the amplicon and amplicon FASTA file. If the user specifies an input sequence or GenBank accession to query primer-binding sites, possibly to check primers external to ARGs (a.k.a., flanking primers), Primer Finder will first find the ARGs on the input sequence using our newly-developed AMR-Finder, and annotate ARGs within the input sequence, search for primer-binding sites and generate a report. The produced report displays multiple binding sites for primers on the specified genome(s). When using user-supplied sequences to query, it's critical to have as much of the target genome (in the case of single isolate screening by PCR) provided so that primer-binding specificity and amplicon length can be verified.

Primary Finder

PCR primer finder assesses the specificity of primers on AMR genes or a target genome. PCR primer finder uses open source Simulate_PCR tools for predicting both desired and off-target amplification products. For more information see AMRdb help page	
Primer Forward Sequence:	
USE AN EXAMPLE PRIMER FORWARD SEQUENCE	
	/
Select File to upload: Browse No file selected.	<u>///.</u>
Primer Reverse Sequence:	
USE AN EXAMPLE PRIMER REVERSE SEQUENCE	
	//.
Select File to upload: Browse No file selected.	
Reference:	
AMR-DB(default)	
O Browse No file selected.	
GenBank Accession	
SUBMIT	

4. Support

Access the user and admin manuals through this tab.

5. Contact

Contact page allows user to submit questions, comments or issues to the AMR-DB Team.

Contac	ons about or would like assistance using the ARMdb, please
contact us using th	
Name*	
Email*	
	1
Subject*	
	:
Message*	
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6. Admin

After the user signs into the AMR-DB, a new menu will be shown (F) "ADMIN", which has three sub-menus: F.1.) Submit New Data, and F.2.) Edit Data, and F.3.) Submit New Primer.

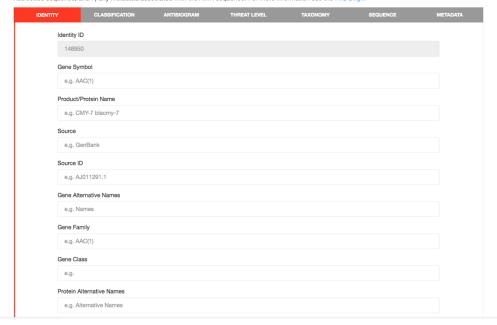
6.1. Submit Data.

When the user submits a new entry in the database, the system will automatically assign a unique Identity ID for the new submission. The user needs to provide the identity details in the IDENTITY tab, taxonomy information in the CLASSIFICATION tab, any antibiogram data under the ANTIBIOGRAM tab, CDC antibiotic threat level under the THREAT LEVEL tab, sequence information in their respective fields of the SEQUENCE tab, and other metadata information under the METADATA tab.

Some of the data (e.g., "gene symbol") is required, while some are optional (e.g., "antibiogram data"). Once the user completes data entry, clicking on the "next" button at the bottom of the tab will shift to the next tab. The data submission button is in the last tab.

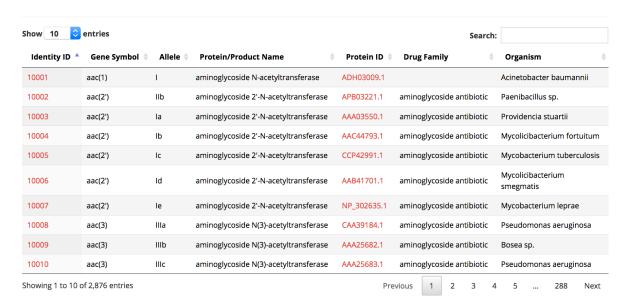
Submit new data to the AMRdb

This page allows the user to submit new AMR data into the AMRdb. The page is split into six different sections or tabs: 1) Identification details of the AMR entry, 2) drug classification and SNP variant information, 3) antibiogram data if any, 4) threat level of the organism, 5) taxonomy of the organism, 6) protein and nucleotide sequence, and 7) any metadata associated with the AMR sequence. For more information see the help page.



6.2. Edit Data.

The second sub-menu option from the Admin menu, "Edit Data", with similar appearance to the Browse and Search Page, allows the user to edit any existing ID in the AMR-DB. The page layout is like the ID description page except all the fields are editable and there is a submit button after the last tab.



6.3. Submit New Primer.

This page enables the registered user to add new primer sequences to the Primer Catalog. The four data fields are: Primer (e.g., IMP), Target (e.g., Screening of MBL), FWD (i.e., forward primer sequence), and REV (i.e., reverse primer sequence). Sequence of primers is the conventional 5'->3' orientation. Hit "SUBMIT" when completed.

6.4. Rebuild Reference Database

This page enables the registered user to rebuild reference database by pulling data from MySql database. During build, all new reference sequences added in the MySql database will be added to AMR-Finder reference database. Hit "REBULD" to initiate the reference database build process