Biotechnology Reports 29 (2021) e00604

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Antibiotic resistance: Global health crisis and metagenomics

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ARTICLE INFO

Article history:
Received 17 January 2020
Received in revised form 11 January 2021
Accepted 18 February 2021

Keywords:
Metagenomics
MDR (multidrug-resistant bacteria)
Resistome

ABSTRACT

Antibiotic resistance is a global problem which affects human health. The imprudent use of antibiotics (medicine, agriculture, aquaculture, and food industry) has resulted in the broader dissemination of resistance. Urban wastewater & sewage treatment plants act as the hotspot for the widespread of antimicrobial resistance. Natural environment also plays an important role in the dissemination of resistance. Mapping of antibiotic resistance genes (ARGS) in environment is essential for mitigating antimicrobial resistance (AMR) widespread. Therefore, the review article emphasizes on the application of metagenomics for the surveillance of antimicrobial resistance. Metagenomics is the next generation tool which is being used for cataloging the resistome of diverse environments. We summarize the different metagenomic tools that can be used for mining of ARGs and acquired AMR present in the metagenomic data. Also, we recommend application of targeted sequencing/ capture platform for mapping of resistome with higher specificity and selectivity.

1. Introduction: Antimicrobial Resistance

Microbes constitute 70 % of the total biomass of the earth's biosphere and play a vital role in the sustainability of the environment. For several billions of years, the planet belonged to them and them alone. Microbial cells have learned to rapidly adapt themselves against toxic compounds since they colonized planet Earth [1]. Their short generation time ensures the transfer of acquired characters to the next generation leading to the evolution of stable genetic determinants. Thus, the microbial gene transfer acts as the primary driving force for microbial diversity [2]. Antibiotic resistance originates when bacteria adapt and grow in the presence of antibiotics [3]. While their adaptability ensures their survival, this trait is emerging as a threat to human and animal health worldwide [4,5].

Antimicrobial resistance occurs naturally through genetic changes [6]. However, overexploitation of antibiotics in medicine, agriculture, food industries (meat production) is accelerating this process. Antimicrobial-resistant microbes are present in people, animals, food, and the environment (in water, soil, and air) [7–9]. It can spread among people, and from animals to person. Antimicrobial resistance currently accounts for over 7 million deaths annually, and it will reach around 10 million deaths by the year 2050, accounting for about 100 trillion USD worldwide [5,10]. Bacteria resistant to the first line of antimicrobials infect around 2 million people in the USA each year, accounting for about 20 billion US dollar. Similarly, antimicrobial resistance accounts for more than 30,000 deaths in the European Union and nearly 900,000 disability-adjusted lives per year [3].

The situation of antibiotic resistance has also become very acute in BRIC countries, i.e., Brazil, Russia, India, and China [11,12]. During the period 2010–2015, the consumption of antibiotics (DDD/1000 individuals/ day) increased in China (89 %), Tunisia (69 %) and India (13 %) respectively [13].

India is among the world's largest consumers of antibiotics [14]. Poor infection control, inadequate sanitary conditions, higher burden of diseases, lack of stewardship and the unregulated use of antibiotics are critical factors which encourage the dissemination of antimicrobial resistance in developing countries [9,15,16]. The local temperature also affects the abundance and distribution of ARGs in the environment. A study conducted in the USA found that rising population density and the local temperature was consistent with the increasing number of antibiotic-resistant pathogens such as *Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus* [17,18]. World Health Organization (WHO) has already expressed its concern over antimicrobial resistance, stating, "no action today, no cure tomorrow" and "post-antibiotic era" may soon become reality of the 21st century [11]. WHO has also proposed a global action plan to combat antimicrobial resistance and urged its members to implement national action plan by 2017 [19,20]. Most antibiotics used today, whether penicillin to carbapenems, microbes have evolved resistance to such compounds [21]. Due to the continuous expansion in antibiotic resistance mechanisms, our ability to treat

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https://doi.org/10.1016/j.btre.2021.e00604

common infectious diseases is diminishing [22]. It is compromising the treatment of infectious diseases and undermining many other advances in healthcare and medicine [23].

Discovery of antibiotic was mainly based on the inhibition of the growth of target microbes. The use of inhibitory approach has failed to yield novel antibiotics [24,25]. Understanding the origins, progression, and mechanisms of transfer of resistance is essential for adequately addressing this public health issue [26]. Mitigating antibiotic resistance requires a coordinated approach involving physicians, pharmacists, veterinarians, patients, and farmers. Antibiotics are removed from wastewater using two principal mechanisms i.e., sorption and biodegradation. Conventional wastewater treatment plants are unable to remove pharmaceuticals, antibiotic residues, and ARGs. Advanced engineered biological wastewater treatment plants have the potential to remove approximately 48%–77% of antibiotic residues from wastewater [13]. Therefore, novel tools and techniques are required for the removal of antibiotic residues and ARGs. Application of these techniques will reduce the abundance of antibiotic residues and ARGs in the environment and their subsequent dissemination [27]. There is a need for a powerful agency to monitor the antibiotic consumption patterns and implement regulations to prevent the loss of active drugs to resistance. It should also encourage the development of novel therapeutics having deleterious activities against microbial pathogens causing infectious diseases [28–30]. Therefore, the present review provides an update of the latest research emphasizing on the network involved in the evolution & dissemination of antibiotic resistance and its consequences on human and environment health. It also highlights the advantage of metagenomics as a platform for monitoring the abundance of ARGs and the discovery of novel antimicrobials.

2. Critical knowledge gaps associated with environmental antimicrobial resistance

As we know that antibiotic resistance has emerged as a global threat, its mitigation will require "one health perspective" approach involving human, animal, and environmental health. Environment plays an essential role in the evolution and dissemination of antimicrobial resistance. Still, there are certain knowledge gaps associated with progression and transmission of antibiotic resistance that are yet to be answered. The understanding of these knowledge gaps is essential for the mitigation of antimicrobial resistance (Larsson et al. 2018). In the year 2017, WHO held a meeting in Brazil to address the current gaps associated with antimicrobial resistance [19]. These critical issues about antimicrobial resistance have been discussed below:

2.1. Anthropogenic practices driving the evolution and transmission of antimicrobial resistance

Antibiotics and ARGs are ubiquitous in diverse niches. Anthropogenic activities play a critical role in the environmental dissemination of antimicrobial resistance. Aquatic ecosystem serves as a hotspot for the dissemination of antimicrobial resistance [16,31–34]. The pharmaceutical industry, aquaculture, and wastewater treatment plants are among the significant anthropogenic sources and drivers of ARGs in the environment. However, to what extent these sources contribute to the selection of antimicrobial resistance (AMR) is still underexplored [35–37]. Recently few studies have shown that pharmaceutical industry effluent and wastewater discharge alters the microbial community and antibiotic richness in receiving water bodies [32,34,38]. Antibiotics are also being used for livestock production (therapeutic and prophylactic purpose) and at a subtherapeutic concentration to boost growth which is also an essential factor contributing in the environmental selection and dissemination of AMR [39]. It also leads to the selection of mobile genetic elements (MGEs) conferring multiple antibiotic and metal resistance [40]. The higher concentration of pharmaceuticals and personal care products, chemicals, drugs, and heavy metals in sewage and wastewater has increased opportunities for genetic exchange between environmental bacteria and human pathogens leading to the evolution of multidrug-resistant pathogens [40–42]. Horizontal gene transfer (HGT) acts as a principal mechanism for the dissemination of antibiotic resistance in the natural and clinical environment [43,44]. It accounts for over 75 percent resistance genes exchange between environmental microbes and clinical pathogens (Fig. 1) [45,46].

MGEs (plasmids, transposons, or integrons) also act as vectors for the dissemination of ARGs [47,48]. HGT occurs chiefly by three mechanisms: conjugation, transformation, and transduction [48]. In conjugation, DNA molecules move by cell contact between donor and recipient cells. During transformation, short fragments of naked DNA are readily taken up by naturally competent bacteria. In transduction transfer of DNA is mediated via bacteriophages [49]. It occurs most likely between phylogenetically diverse bacteria sharing the same ecological niche (e.g., human gut flora) [50–52].

2.2. Inadequate surveillance of antimicrobial resistance and lack of social awareness

The lack of stewardship towards antimicrobial resistance is amplifying its epidemiology at the regional and global scale [53]. Inadequate research funding, lack of surveillance are critical factors which contribute to the dissemination of resistance in developing countries. Southeast Asia is now being recognized as a hotspot for the evolution and spread of resistance [39]. India was the largest consumer of antibiotics in the year 2010 [14]. However, both the lack of uniform access to vaccines and overuse of antibiotics are among major factors contributing to the antimicrobial resistance [54]. It causes enormous socioeconomic losses and morbidity and mortality. For example, pneumonia and sepsis are the leading causes of childhood deaths in India. In the year 2005–2006, a large number of children died after suffering from pneumonia. Only one-third number of children availed the medical advice, out of which only 13 percent were treated by using antibiotics. Thus, infant mortality may have been reduced by treating them with antibiotics. About 215,000 children below five-year age die annually despite the availability of the vaccine against the causative organisms *Streptococcus pneumoniae* and *Haemophilus influenza* [3,54]. Indian Council of Medical Research (ICMR) is an apex body of government of India, which is now collecting data from tertiary referral hospitals for understanding the factors contributing to antimicrobial resistance. It was observed that about 80 % of cases of infections caused by *Klebsiella* and *E. coli* were resistant against the third generation cephalosporins.

More than 50 % of cases of nosocomial infections were attributed to carbapenems resistant *Actinobacter baumanii* and *Pseudomonas aeruginos*a. Rise in the number of fluoroquinolone- and cephalosporin-resistant *Salmonella typhi* were found since 2013 [55]. Overuse of colistin antibiotics to combat carbapenem resistant bacteria resulted in the evolution of gram-negative colistin-resistant bacteria [56]. Very little information is available regarding the application of antimicrobials in animal husbandry in India. Drugs which are prescribed for people's treatment are also used for treating animal illness and preventing their transmission. Recently, Food Safety and Standard Authority of India had given its directives to implement strict rules for reducing the use of antibiotics in meat production and for livestock [57,58]. Therefore,

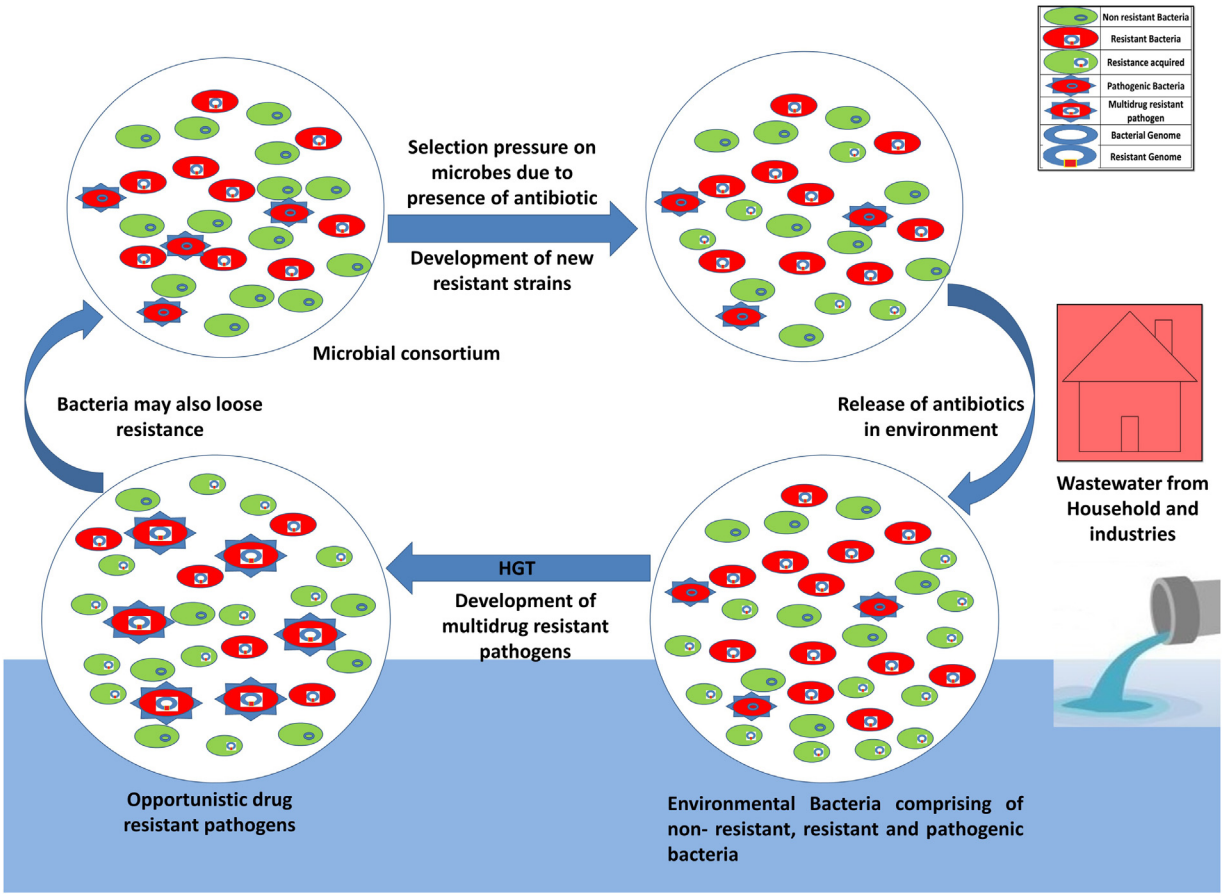

Fig. 1. Dissemination of antimicrobial resistance via Horizontal gene transfer.

these critical issues require the attention of the government and policymakers.

3. The evolution of antimicrobial resistance in bacteria

The total amount of resistance gene associated with an ecosystem is known as "resistome" [23,59]. Both terrestrial and aquatic ecosystem harbors complex resistome. This resistome acts as a reservoir of resistance genes for human pathogens [23,60]. The resistance is as old as bacterial metabolism (D'Costa et al. 2011). The presence of ARGs in Lechuguilla Cave, New Mexico, and 30,000-year-old Beringian permafrost sediments confirmed that antibiotic resistance is the natural and ancient phenomenon [61]. Mostly antimicrobial drug resistance originates in nonpathogenic environmental microbes. These microbes have evolved the mechanism to sense, interact, and metabolize small molecules that modulate the activities of antibiotics [22,62]. An understanding of the evolution of resistance can provide insight for the design of new antibiotics capable of evading resistance [63]. Antimicrobial resistance was first discovered in *Escherichia coli* and *Staphylococcus aureus* strains shortly after the discovery of penicillin [64]. No new antimicrobials/antibiotics were discovered since the last two decade, which represents an innovation gap during the genomic era [65,66]. Soil microorganisms naturally produce most antimicrobials in clinical use, and they act as the source of resistance genes found in clinically relevant bacteria [67,68].

Metagenomic mining has provided insight on the evolutionary mechanism of resistance, suggesting that microbes have acquired resistance genes long before the "antibiotic era" (Finley et al. 2013; [4]). It is now evident from previous studies that ARGs are ancient and natural part of the genome of environmental bacteria [22]. In nature, microbes often express antimicrobial-resistance genes as their defense mechanism to combat antimicrobial compounds or toxin produced by competitors in the same ecological niche [69]. The rapid increase in the abundance of antibiotic-resistant pathogens has exposed our knowledge limitation about the evolutionary and environmental processes undergoing in microbial ecosystems [70]. Multidrug resistant bacteria (MDR) has acquired intelligentsia to metabolize antimicrobials, and may also transfer these properties to genetically and taxonomically different bacterial species [71,72]. Recently published reports have shed light on the mechanism and type of resistance microbes have acquired even against last resort antibiotics.

3.1. Microbial diversity as a bioresource for bioactive molecules (secondary metabolites) with antimicrobial potential

Bioactive molecules represent a significant source of approved drugs and still may play a vital role [73,74]. Secondary metabolites find various applications in the sphere of medicine and are commercially used for combating disease as antimicrobials, antitumor, antiviral and anti-aging agents. In nature, microbes are often exposed to extreme conditions and adaptation under these conditions leads to the production of secondary metabolites [75–77]. Bioactive molecules or secondary metabolites are the product of secondary metabolism. They are not required for normal growth, development, or proliferation of host organism. They empower microbial cells to survive the interspecies competition and also helps in cellular communication [78,79]. Screening for microbial metabolites has provided many life-saving drugs and

antibiotics. These biomolecules provide new scope for treating previously "untreatable diseases," and help in reducing mortality rates. The finding accelerated the search for new potential antibiotics using microbial diversity as bioresource. Because of their enormous potential Davies, in 2013 regarded secondary metabolites as real “evolutionary molecules” [80]. Despite their biological novelty, the commercial applications of these biomolecules are mostly underexplored. Because, a vast majority of microbial species (upto 99 %) are ‘uncultured’ and do not grow under laboratory conditions and this phenomenon is popularly known as the “Great Plate Count Anomaly” [81,82]. Understanding why uncultivated microorganisms are uncultivated is an essential first step to access and use both their taxonomic and catabolic potential. There is an increased need for the new drug to treat infectious diseases and to encounter antimicrobial resistance. Novel approaches are required for discovering such new bioactive molecules from nature [83]. Therefore, techniques like ichip and diffusion chambers were developed for culturing uncultured microbes. These techniques mimic their natural habitat, making microorganisms to think that they are growing in their natural environment.

In ichip, soil samples are diluted to such an extent that only one bacterial cell enters a single channel. After covering the device with semipermeable membranes, it is placed back in the soil. The diffusion of nutrients and growth factors enables the growth of uncultured bacteria. It offers an advantage over the traditional culture-based approach that it can cover up to 50 % diversity, whereas “the latter” can cover only1% of the actual diversity associated with soil samples [84]. “Ling et al. [85]” applied the multichannel ichip technique for the discovery of a novel antimicrobial compound. After the screening of over 10,000 extracts, Teixobactin was discovered. It acts by blocking protein synthesis and does not affect DNA metabolism. It shows antimicrobial activity against drug-resistant pathogens like *S. aureus* and *M. tuberculosis* strains. Therefore, the above finding may provide the new avenue for the novel discovery of compounds having antimicrobial potential but no resistance [85].

Similarly, the diffusion chamber technology is used for growing uncultured actinomycetes as a source of new antimicrobials. These new cultivation techniques allow access to the previously inaccessible microorganisms. The advancement of the mass spectrometry technique enables the rapid detection and identification of trace amounts of environmental substrates. Thus, it can be used for the discovery of multiple antibiotics directly from the environment, prediction of their structure, and biosynthetic pathway [86]. Recent technical advancement in DNA sequencing

Table 1
Metagenomic database and tools used for the mining of antibiotic resistant genes and the discovery of secondary metabolites.

Name	Distinct Feature	Reference Data	Input Data	Reference
MGRAST	Open-submission platform it provides in-depth analysis of metagenomic data	200,000	Raw & assembly	[99]
MEGAN	Open source software used to explore taxonomic diversification of the dataset	SILVA, eggnog, KEGG, SEED	assembly	[100]
antiSMASH	Open source software used for the discovery of antibiotics and secondary metabolites	6200 complete bacterial genomes &18,576 draft genomes	Raw	[101]
IMG-ABC	Largest publicly available database of experimentally verified and predicted biosynthetic gene clusters	730,000 BCs in 40,034 microbial genomes and >310 000 BCs in 2416 metagenomes	assembly	[102]

Name	Distinct Feature	Reference Data	Input Data (assembly based)	Reference
ResFinder	Open source software identifies acquired resistance genes present in metagenomic data	1400 resistance gene	assembly	[103]
ARG-ANNOT	Identifies existing and new antibiotic resistance genes in bacterial genomes	>1800 resistance gene	assembly	[104]
ARGs-OAP	Improved automated classification and enumeration of ARG-like sequences	4246 resistance gene	assembly	(Yin et al., 2018)
PointFinder	Open source software for detection of chromosomal point mutations associated with antimicrobial resistance	NA	NA	[105]
AMR Finder	AMRFinder uses reference dataset to identify AMR genes	4579 resistance gene	assembly	[106]
BusyBee	Open source software used to explore taxonomy and functional profile of the metagenomic data	reference-independent binning tools	assembly	[107]

Name	Distinct Feature	Reference Data	Input Data (raw sequences)	Reference
SRST2	allows rapid and accurate annotation of genes, alleles, and multi-locus sequence types	>900 WGS	Raw data	[108]
SEAR	Open source software for the detection of horizontally acquired antimicrobial resistance genes	ARG-ANNOT database based	Raw data	[109]
PATRIC	Curation, integration, and visualization of virulence factors in PATRIC database	22,000 WGS; 4891 virulent gene sequences	Raw data	[110]
KmerResistance	tool for identification of acquired antimicrobial resistance genes	NA	Raw data	[103]
DeepARG	Open source software for profiling of ARGs in metagenomic data	CARD, ARDB & UniProt	Raw data/ assembled	[111]
SSTAR	it can be customized as per requirement (particular pathogen groups and resistance mechanisms)	ARG-ANNOT	Raw data	[112]

and bioinformatics tools (i.e. Metagenomics) has also made the discovery of novel biosynthetic gene clusters encoding for secondary metabolites easier [87–89].

3.2. Metagenomic approach for monitoring ARGs abundance and discovery of new antimicrobials

Biological novelty usually dictates chemical novelty, and the possibility of microbial diversity to yield new antibiotic is tremendous [65,67,83]. Recently, genome mining approaches have provided insight that the microbial dark matter has enormous potential to synthesize variety bioactive small molecules [90,91]. The properties of these molecules are yet to be deciphered [92,93]. Therefore, the paradigm has shifted towards metagenome sequencing for metabolite discovery. The rapid advancement of genome sequencing technology has revolutionized almost every aspect of biological research, including the hunt for antimicrobials or other bioactive compounds [88,89]. Metagenomics is the next-generation toolbox for microbial ecologists and is the most direct, unbiased means to interrogate the functional potential of microbial communities [60,94,95]. Metagenomics is being used for the discovery of a wide range of enzymes and bioactive metabolites from the soil and marine samples [96]. Thus, the application of the metagenomic approach will increase the chances of discovering novel antimicrobials [97,98]. Metagenomic tools and database used for screening of ARGs were listed in Table 1 and are discussed in the preceding section.

3.2.1. Metagenomic tools for mining secondary metabolite gene cluster

1 **MG-RAST:** MG-RAST stands for "Metagenomic Rapid Annotation using Subsystem Technology." It is an open-source database which acts both as the repository of metagenomic data and allows taxonomic and functional annotation of the metagenome. It uses MD5- based non-redundant protein database (M5nr) pipeline for the annotation of sequences using multiple databases such as Kyoto Encyclopedia of Genes and Genomes, National Center for Biotechnology Information, Joint Genome Institute (KEGG, SEED, NCBI, JGI, etc.). It can be used for the analysis of both 16SrRNA amplicon sequencing and Whole-genome sequencing data [99].
2 **MEGAN Community Edition:** MeTaGEnomeANalyzer community provides an interactive exploration of the taxonomic and functional diversity of large metagenomic datasets. A new updated version of MEGAN has been released recently and renamed as MEGAN Community Edition (CE). Taxonomic profiling is done through NCBI based taxonomy, while a functional annotation is assigned by different approaches such as SEED, KEGG, etc. The present version of MEGANCE comes with additional tools such as "DIAMOND" which allows high-throughput DNA-to-protein alignment. Further, now it offers a functional classifier called InterPro2GO, gene-centric read assembly, principal coordinate analysis of taxonomy and function for the metagenomic data [100].
3 **antiSMASH.4:** antiSMASH stands for "antibiotics and secondary metabolite analysis shell'. It is also an open-source database which allows the mining of biosynthetic gene clusters encoding for secondary metabolites and antimicrobials present in bacterial genome or metagenome. A recently updated version, i.e., AntiSMASH.4 has been released. It has the potential to predict gene cluster boundaries by using Cluster-Finder method or may predict better substrate specificity for NRP adenylation domain using SANDPUMA algorithm. Now it can also perform a comparative analysis of trans-AT polyketides synthase peptide cluster using modified domain-level alignment tool [101].
4 **IMG-ABC:** Secondary metabolites are diverse compounds produced by microbes having antibacterial, antiviral, and anticancerous activity. They are encoded by clusters of co-located genes called biosynthetic gene clusters (BCs). A database called the Atlas of Biosynthetic gene Clusters (https://img.jgi.doe.gov/abc/) within the Integrated Microbial Genomes system (IMG-ABC) was created for the analysis of biosynthetic gene clusters and secondary metabolites in bacterial genomes and metagenomes. BCs were predicted and annotated across all microbial genomes and a set of metagenomes using a combination of Clusterfinder and antiSMASH. After the creation of the database, it is continuously updating itself by importing genomes of new isolates and annotating it by using antiSMASH. It also obtains data from NCBI. At present, the IMGABC database contains more than 730,000 BCs in 40,034 isolate microbial genomes and >310 000 BCs in 2416 metagenomes [102].

3.2.2. Metagenomic tools for monitoring the abundance of antibiotic-resistant genes in the environment

Metagenomics has now emerged as a viable tool for monitoring antimicrobial resistance. Various publicly available metagenomic tools have been developed for monitoring the abundance of ARGs in the natural and bioengineered systems [3,113]. Theses bioinformatics tools can be broadly classified into two categories depending upon the data requirement, i.e., raw sequences and assembled sequences. Some of which are highlighted here:

3.2.2.1. Assembly based methods. Short reads obtained from sequencing techniques such as Illumina are assembled as contigs and are then annotated by using reference databases. Assembly based approaches are time-consuming and expensive. It requires higher coverage for annotation of both known and novel antibiotic resistance genes.

1 **Resfinder**: Resfinder is a web tool which utilizes BLAST for the identification of acquired antimicrobial resistance genes. It may detect horizontally acquired resistance genes in metagenome data. As input data, it takes raw reads, assembled sequences or partial or complete genome sequences from four sequencing platforms. It aligns query sequence data to the 1411 sequences present in 1862 GenBank files with 100 % similarity. The default parameter for annotation of acquired resistance genes is 98 % for identity and 60 % for coverage [103]. Also, Resfinder offers several advantages like it can predict the site of resistance genes present in the genome. It can even predict how newly discovered acquired resistance genes vary from the reference genes concerning insertions, deletions, and SNPs. ResFinder can be accessed at www.genomicepidemiology.org. ResFinder will continuously be updated as new resistance genes are identified [103,114].
2 **ARG-ANNOT**: Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) was designed for identifying existing and putative new antibiotic resistance (AR) genes in bacterial genomes. It utilizes Bio-Edit software for analyzing antibiotic resistance sequences using local BLAST program. About 1689 all antibiotic-resistant genetic determinants were collected from diverse sources such as published works and publicly available database such as NCBI and GenBank to create a database. ARG-ANNOT efficacy was checked using 100 random sequences. The results indicated that it has the potential to annotate even short sequences (17–40 bp) with significant E values. Further, comparative analysis of ARG-ANNOT and ResFinder gene analyzer using *Acinetobacter baumanii* and *Staphylococcus aureus* genomes showed that the latter is more robust in the annotation of AR genetic determinants [104].
3 **ARGs-OAP:** ARGs-OAP is an online analysis pipeline for the detection and quantification of ARGs present in the

metagenome of environmental samples. It utilizes SARG as reference database, which is a combination of Antibiotic Resistance Database (ARDB) and Comprehensive Antibiotic Resistance Database (CARD). It uses UBLAST and BLASTX approach for annotation of ARGs in the metagenome. It may provide a platform for the comparative analysis of global metagenomic data [115]. In the year 2018, an upgraded version of ARGs-OAP version 2.0 was launched. It contains SARG 2.0 reference database which has three times more antibiotic resistance sequences than previous SARG database. Sequences were obtained from CARD, ARDB database, and recently published studies of environmental metagenomes. ARGS-OAP now utilizes the Hidden Markov Model (HMM) model for the identification of antibiotic-resistant genes. It allows rapid annotation and classification of ARGs sequences present in the metagenomic data. It also allows quantification of the cell using a single-copy marker gene. It can be accessed through http://smile.hku.hk/SARGs [116].

4 **PointFinder**: Genetic mutation is an important mechanism responsible for the acquisition of antimicrobial resistance. Most of the tools present to date were unable to predict whether resistance was acquired by horizontal gene transfer or by a chromosomal genetic mutation. Therefore, PointFinder tool was designed for the detection of antimicrobial resistance acquired by chromosomal genetic variation using metadata. PointFinder has two databases created by obtaining acquired ARGs from recently published studies. Chromosomal gene database contains ARGs in FASTA format, and chromosomal mutation database provides information regarding codon positions and substitutions conferring antimicrobial resistance. It utilizes the BLASTn algorithm for annotation of resistance genes using chromosomal gene database. Sequences showing similarity more significant than 80 % are further analyzed using chromosomal mutation database for mismatches. PointFinder allows options to select the user to see all mismatches between the query and reference sequences or known mismatches found from the chromosomal database. Thus, PointFinder tool has the potential to identify chromosomal point mutation leading to antimicrobial resistance [105].

5 **NCBI-AMRFinder:** AMR Finder tool was designed by the National Centre for Biotechnology Information (NCBI) to identify antimicrobial resistance genes in whole-genome sequencing data using Bacterial Antimicrobial Resistance Reference Gene Database. AMRFinder accuracy was compared against ResFinder (2017). AMRFinder missed 16 loci that Resfinder found, while Resfinder missed 1147 loci AMRFinder identified. It was found that AMRFinder has a higher potential for antibiotic resistance gene detection than ResFinder [106]. It can be accessed through https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder

6 **BusyBee**: BusyBee is a web-based server for the metagenomic analysis [107]. Both taxonomic and ARGs annotation can be performed using BusyBee. It may use assembled contigs (Illumina) or long reads (Oxford Nanopore technologies) as input sequences in fasta format. Input sequences are categorized into population-level genomic bins using reversible compression step combined with bootstrapping based binning. It utilizes Prokka as a tool for the rapid annotation of microbial genomes [117]. The translated coding sequences are then searched against the ResFams (collection of antibiotic resistance genes) using hmmsearch from HMMER (v3.1b2; http://hmmer.janelia.org/) [118]. In BusyBee input sequences are represented as individual points in the 2D scatter plot. ARGs are represented as points of larger size and in dark color. The BusyBee Web server is available for use at https://ccb-microbe.cs.uni-saarland.de/busybee.

3.2.2.2. Raw sequences based. In this approach reads are directly annotated as antibiotic resistance gene determinants by comparing reads to available sequences in the reference databases. Read based approaches are fast and allow resistome profiling of large datasets. The annotation of resistance genes is dependent on the available information in the reference database.

1 **SRST2:** SRST2 is a read mapping-based tool which allows rapid and accurate detection of genes, alleles, and multi-locus sequence types (MLST) from WGS data. Analysis performed using >900 genomes from common pathogens confirmed that SRST2 has higher accuracy as compared to assembly-based methods for both the gene detection and allele annotation [108]. Further, it was also applied in the hospital environment for surveying microbial genomes. With rising incidences of antimicrobial resistance in pathogenic bacteria, SRST2 offers a reliable option for rapidly identifying clinically relevant information available in whole genome sequencing data. Source code is available from http://katholt.github.io/srst2/.

2 **SEAR**: SEAR stands for Search Engine for Antimicrobial Resistance. It is a cloud compatible pipeline and web interface that allows rapid detection of antimicrobial resistance genes present in both environmental metagenomes and sequencing data from clinical isolates. It consists of Perl, Shell, and R scripts that call on several pieces of open-source software and utilize a customizable reference database to annotate ARGs direct from short-read sequencing data. It provides gene information, abundance estimation, and the reconstructed sequence of antimicrobial resistance genes; it also includes web links for additional information on each gene [109]. The pipeline utilizes clustering and read mapping to annotate full-length genes with the user-defined database. It also uses the local alignment of annotated genes to a range of online databases to provide additional information. SEAR can be downloaded from: http://computing.bio.cam.ac.uk/sear/SEAR_WEB_PAGE/SEAR.html.

3 **PATRIC**: PATRIC stands for Pathosystems Resource Integration Center (http://www.patricbrc.org). PATRIC provides researchers an online resource that stores and integrates a variety of data types such as genomics, transcriptomics, three-dimensional protein structures, and associated metadata. Datatypes are summarized for individual genomes and across taxonomic levels. Genomes available in PATRIC are consistently annotated using the Rapid Annotations using Subsystems software (RAST). PATRIC provides multiple options for researchers to find data of interest. It also allows a private workspace where user can store their private data and provides a suite of tools to perform comparative genomic or transcriptomic analysis [110].

4 **KmerResistance:** k-mers (fragments of the DNA sequence of length k) is used to map the raw WGS data against reference databases, and not only identify the resistance genes but also determine the species. Mapping against the species reference is then used to normalize the antimicrobial resistance prediction. KmerResistance was built upon KmerFinder, which was created for bacterial typing from raw WGS data. KmerFinder and KmerResistance examine the number of co-occurring k-mers between the query genome and a database of resistance genes. To minimize false hits, each k-mer is annotated to the gene with the highest number of unique k-mer matches. After this, the k-mers mapping to the best hit are removed, and the procedure is repeated. Since Kmer Resistance detects genes directly from the raw WGS data, some noise due to contaminating sequences is expected. To avoid this, the Kmer Resistance biases the threshold according to the quality of the data, measured by the coverage and depth of the predicted species genome using a scheme similar to KmerFinder. By doing this, an estimate of both depth and coverage are determined. The coverage is the fraction of the

genome covered by at least one k-mer, and the depth is the average number of times the k-mers in the hit are present in the input data. KmerResistance can also be used for species prediction [119].

5 **DeepARG**: DeepARG was designed for the direct annotation of ARGs present in the metagenomic data without human intervention. It is available in both command line and a web-based version. In the command line version, it accepts input data either as FASTA or BLAST tabular file format. If the input sequence is in FASTA format, the software first performs the sequence search and then annotate the antibiotic-resistant genes. If the input is in BLAST tabular file format, the software annotates resistant genes directly. In the web version, raw metagenomic sequence file (FASTQ format) can be uploaded directly for the identification of resistance gene (http://bench.cs.vt.edu/deeparg) [111].

6 **SSTAR:** Metagenome sequencing is now being used as a routine method for identifying genes associated with antimicrobial resistance (AR). Metagenomic analysis poses challenges to many microbiologists. SSTAR software with a graphical user interface has the potential to identify known ARGs present in the whole genome sequencing data. SSTAR stands for Sequence Search Tool for Antimicrobial Resistance [112]. It utilizes a locally executed BLASTN search against a customizable database combines with the graphical user interface for the identification of antimicrobial resistance (AR) genes. It can be customized as per requirements (for particular pathogen groups and resistance mechanisms). It has the potential to detect new variants of known antimicrobial resistance genes. SSTAR is platform-independent and is compatible with both Windows and UNIX operating systems. SSTAR and it's manual, are freely available fromhttps://github.com/tomdeman-bio/Sequence-Search-Tool-for-Antimicrobial-Resistance-SSTAR.

4. Concluding remarks

The present review article deals with the role of anthropogenic practices and environmental factors responsible for the dissemination of antimicrobial resistance. It also describes various tools and databases available for the monitoring of abundance of ARGs and discovery of BCs in the environmental metagenome. Further, the discovery of new antibiotics has become a necessity to mitigate antimicrobial resistance against existing antimicrobials. The development of novel tools and techniques for the removal of pharma/antibiotic residues, pathogens from environmental/engineered settings is also necessary. It also focuses on the necessity of implementation of strict laws by government agencies/policymakers and adoption of safety measures (increasing social awareness) to mitigate the antimicrobial resistance.

Declaration of Competing Interest

The authors declares that there is no competing interest.

Acknowledgment

The authors wish to acknowledge Director, CSIR-NEERI for providing the necessary facility for this work. Dr. Shailendra Yadav is grateful to DST: SERB for the national post-doctoral fellowship: FILE NO. PDF/2016/001389. The manuscript has been checked for the plagiarism using iThenticate software at the NEERI Knowledge resource center: KRC no.: CSIR-NEERI/KRC/2019/SEP/DRC/1.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2021.e00604.

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