REVIEW

Clinical Perspective of Antimicrobial Resistance in Bacteria

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Abstract: Antimicrobial resistance (AMR) has become a global clinical problem in recent years. With the discovery of antibiotics, infections were not a deadly problem for clinicians as they used to be. However, worldwide AMR comes with the overuse/misuse of antibiotics and the spread of resistance is deteriorated by a multitude of mobile genetic elements and relevant resistant genes. This review provides an overview of the current situation, mechanism, epidemiology, detection methods and clinical treatment for antimicrobial resistant genes in clinical important bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), extended-spectrum β-lactamase-producing *Enterobacteriaceae*, acquired AmpC β-lactamase-producing *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae* (CPE), multidrug-resistant (MDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa.*

Keywords: antimicrobial resistant, genes, antibiotic resistance mechanisms, epidemiology, detection methods

Introduction

The global spread of antimicrobial resistance (AMR) is a serious threat to global public health. AMR not only causes soaring economic burden on health care but also increases morbidity and mortality. When microorganisms (such as bacteria, fungi, viruses, and parasites) are exposed to antimicrobial drugs (such as antibiotics, antifungals and antivirals), they respond and develop AMR. As a result, the anti-microbial drugs become less effective gradually. Infections persistent in human body promotes the risk of spread. As O'neill et al described in 2016,[2] death rates due to resistant infections were 700,000 per year and the infectious population will reach 50M people globally in 2050. On one hand, the supply of new antibiotics is insufficient to keep pace with the increase of AMR pathogens. On the other hand, unnecessary use of antibiotics globally further selectively enriches AMR pathogens, increasing health risks. In addition, AMR will compromise the effective therapies against other diseases such as cancer chemotherapy, treatment of HIV and malaria. It is important to understand the AMR mechanisms and develop rapid point-of-care diagnostic test.

The abuse of antimicrobial drugs increases the background concentration of antibiotics, and creates niches favoring AMR bacteria. It is reported that horizontal gene transfer (HGT) mechanism could play an important role to the rapid dissemination of genes for AMR via natural ecosystem and human microbiome.[3–10] There are three mechanisms of HGT: gene transformation, conjugation and transduction. Gene transformation involves bacterial uptake of naked DNA from environment, conjugation is usually plasmid-mediated process between a donor and a recipient bacterium, and transduction is associated with bacterial phage infection. All three HGT mechanisms can rapidly spread genes for AMR among microbial community in natural environment and human microbiome, and be selected in the presence of antimicrobial drugs. Specific terminologies describing acquired resistance are explained in Box 1. Gene database involved in AMR have been well documented.[11] To date, 263 pathogens, 4937 reference sequences have been collected by Comprehensive Antibiotic Resistance Database (CARD, https://card.mcmaster.ca/).

In 2017, the World Health Organization (WHO) published a list of bacteria for which researches and drugs were urgently needed.[12] In the list, carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Enterobacteriaceae* and ESBL-producing *Enterobacteriaceae* were listed as critical priority pathogens, while vancomycin-resistant *Enterococcus* (VRE) *faecium*, methicillin-resistant *Staphylococcus aureus* (MRSA) were listed as high priority pathogens. What's more, penicillin-non-susceptible *Streptococcus pneumoniae* was listed as medium priority pathogens. Now many reviews are reported about certain AMR rather than a broader conclusion of AMR. Our study summarizes the prevalent AMR genes, AMR mechanisms, epidemiology, detection methods and medication. Hence, taking morbidity and mortality into consideration, in this review, we focus on 3 gram-positive bacteria including MRSA, VRE, penicillin-resistant *Streptococcus Pneumoniae* (PRSP); and 5 gram-negative bacteria including extended-spectrum β-lactamase-producing *Enterobacteriaceae*, acquired *AmpC* β-lactamase-producing *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae* (CPE), multidrug-resistant (MDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The antimicrobial resistant bacteria discussed are all clinically important with wide prevalence or/and high risk of death.

AMR in Gram-Positive Bacteria

Methicillin-Resistant *Staphylococcus aureus*

From 1950s to 1980s, MRSA developed from a newcomer to a worldwide superbug and is still spread around not only nosocomial but also outside hospital. MRSA are generally coupled with high mortality and morbidity which arouses great concerns due to its clinical importance. *mecA* is the most popular relevant gene mediating methicillin-resistant in *Staphylococcus aureus*. It encodes PBP2a, a transpeptidase with lower affinity for the β-lactam antibiotics, which mediates the methicillin resistance in MRSA. Except *mecA*, other homologues (*mecB*, *mecC*, *mecD*) are discovered recently. These methicillin-resistant genetic components are carried on staphylococcal cassette chromosome mec (SCCmec), a mobile genetic element (MGE).[13,14] *mecB* is often described in a transposon *mec* complex (Tn6045) in *Macrococcus caseolyticus*. Although *mecC* was reported in livestock-associated MRSA, *mecC* in MRSA can be transmitted between species.[15] *mecD* has been recently spotted in bovine and canine *M. caseolyticus* isolates.[16]

Vancomycin-Resistant Enterococcus

From 1970s, vancomycin was approved to treat *Enterococcus* which came with the appearance of VRE. Until early 1990s, VRE became a serious problem in America, and great efforts have been made to prevent VRE.[17] Vancomycin resistance in *Enterococcus* is related to various *van* genotypes, including *vanA*, *vanB*, *vanM* and another 7 types (*vanC/D/E/F/G/L/N*).[18] It is reported that *Enterococcus faecium* (*E. faecium*) features with high recombination rates due to the lack of CRISPR-cas loci, which protect the conservation of genomic DNA in other bacteria.[18] The *van* genes confer modifications to the d-Ala-d-Ala dipeptide at the C-terminus end of the translocated pentapeptide which is the key to formidable antimicrobial effect of glycopeptides against enterococci reduces the affinity of vancomycin binding by up to 1000 times, thus losing its efficacy.[18] Genes of *vanA*, *vanB* and *vanM* deserve more clinical attention because they tend to cause intermediate to high levels of resistance to vancomycin in *Enterococcus*. The *vanM*, *vanA*, *vanB*, and *vanD* is genetically and phenotypically similar, whereas *vanL* and *vanN* are similar to *vanC*.[19]

Penicillin-Resistant *Streptococcus Pneumoniae*

S. pneumoniae has six types of PBPs, of which three PBPs mutation are related to penicillin-resistance: PBP1A, PBP2X and PBP2B.[20] PBPs mutation is the main mechanism for PRSP to acquire penicillin-resistance. These resistant isolates of pneumococci survived after the selection of abundant treatment of bacterial infections with β-lactams and evolved gradually through accumulation of spontaneous mutations coupled with recombination of alleles from other β-lactam resistant group streptococci.[21]

Box 1 MDR, XDR and PDR

The standardized international terminology to describe acquired resistance was established in the 21st century. Multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) are created for public health use and epidemiological purposes to cover the acquired resistance spectrum of AMR bacteria, including *Staphylococcus aureus, Enterococcus spp.*, *Enterobacteriaceae* (other than *Salmonella* and *Shigella), Pseudomonas aeruginosa* and *Acinetobacter spp*. MDR was defined as "acquired non-susceptibility to at least one agent in three or more antimicrobial categories", XDR was defined as "non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (ie bacterial isolates remain susceptible to only one or two categories) " and PDR as "non-susceptibility to all agents in all antimicrobial categories".

To interpret an in-vitro antimicrobial susceptibility test results is to identify a MDR strain. When the test shows "resistance to multiple antimicrobial agents, classes or subclasses of antimicrobial agents", it proves to be a MDR strain which means it is "resistant to one key antimicrobial agent".

For XDR, the first understanding is based on the number of antimicrobials or classes or subclasses to which a bacterium is resistant, and the second one is whether they are "resistant to one or more key antimicrobial agents".

As for PDR, it demonstrates a complete drug-resistance to all routine antibiotics clinically.[1]

AMR in Gram-Negative Bacteria

AMR in Enterobacteriaceae

The most popular resistant mechanism in gram-negative bacteria is hydrolytic enzymes, especially β-lactamase, a group of enzymes, which can hydrolyze the β-lactam ring to break the amide bond and inactivate the antibacterial activity of drugs. Various mechanisms of antibacterial resistance to β-lactams reported include ESBLs, AmpC, and carbapenemases.[22]

Extended-Spectrum β-Lactamase-Producing (ESBL-Producing) *Enterobacteriaceae*

ESBLs are frequently detected in *Escherichia coli (E. coli)* and *Klebsiella pneumoniae*, which are representative MDR gram-negative bacteria.[23] Most of the ESBLs belong to Ambler class A and they are generally inhibited by clavulanic acid or tazobactam.[24] β-lactamases related to the AMR of ESBLs include CTX-M (bla_{CTX-M} genes), TEM (bla_{TEM} genes) and SHV (bla_{SHV} genes).[25] Recently, rapid worldwide spread of ST131 *E. coli* strains, which contain plasmids harboring CTX-M ESBL genes especially $bla_{CTX-M-15}$, has attracted great attention.[23] Some special OXA-type β-Lactamase, OXA-10 and OXA-13 to OXA-19, also hydrolyze extended spectrum cephalosporins and they are also regarded as ESBLs.[26] Currently, *E.coli* with CTX-Ms are the most widespread ESBLs-producing bacteria and CTX-M-15 is the most frequently detected resistant gene, followed by CTX-M-14, which is often found in South-East Asia.[27] Furthermore, recent epidemiology studies have discovered CTX-M-27 in Japan and Europe.[25] The population structure of ESBL-producing *E. coli* is dominated globally by a high-risk strain named ST131.[28]

Acquired AmpC β-Lactamase-Producing *Enterobacteriaceae*

AmpC β-lactamases, which are clinically important cephalosporinases, are mostly class C β-lactamases that also hydrolyze 3rd generation cephalosporins, but are not inhibited by clavulanic acid or tazobactam.[24] However, the name of AmpC is not accurate since several so-called enzymes in the literature actually belong to class A.[29] AmpC enzymes are encoded on chromosome and plasmids. The AmpC β-lactamases are at a low expression level in *E. coli* and the AmpC-encoded gene is absent in the chromosome of *Klebsiella* and *Salmonella* strains.[30] However, plasmid-expressed AmpC β-lactamases can cause relevant resistance in these bacteria. Plasmid-mediated AmpC enzymes have been named according to the resistant drugs (CMY, FOX, MOX, LAT), according to the type of enzyme (ACC, ACT) or based on the site of discovery, such as MIR-1 or DHA.[26]

Carbapenemase-Producing *Enterobacteriaceae*

Clonal spread from chromosomes and plasmid-mediated transmission contribute to continued rise in CPE incidence.[31] The bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{IMP} and bla_{VIM} are the dominant carbapenemase gene families.[32] These carbapenemase genes can be defined by Ambler classification system: Ambler class A (KPC enzymes); molecular class

Table 1 Antimicrobial Resistant Mechanisms of *Acinetobacter Baumannii* and *Pseudomonas Aeruginosa*

Resistant Mechanism	Type	Relevant Genes	References
Enzymatic drug inactivation	ESBLs[37]	bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA} and other genes	
	MBLs[63]	bla_{IMP} bla_{VIM}, bla_{SIM}, bla_{NDM}, bla_{SPM}, bla_{GIM}, bla_{DIM}, intI 1, intI 2	
	OXAs	$bla_{OXA-23-like}$, $bla_{OXA-40/24-like}$, $bla_{OXA-51-like}$, $bla_{OXA-58-like}$, $bla_{OXA-143-like}$	[64]
	AMEs	aadA1, aph(3')-IIb, aph(3')-I, aph(6)-Id, aac(3)-Id	[37,65]
	16S-RNA methylase	armA, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF, rmtG, rmtH, npmA	[66]
	tetracycline destructases	tetX	[67,68]
Drug target alteration	fluoroquinolone resistance	gyrA/B, parC/E	[69]
	polymyxin resistance	pmrA/pmrB/ mcr-1/lpxA/lpxC/lpxD	[70–72]
Permeability defects	tigecycline resistance	abrp	[73]
Efflux pumps	MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM		[74,75]
	Ade	adeA, adeB, adeC, adeRS, adeIJK, abeM	[68]
	QepA, OqxA		[41]

Abbreviations: ESBLs, extended-spectrum β-lactamases; MBLs, metallo-β- lactamases; OXAs, oxacillinases; AMEs, aminoglycoside-modifying enzymes; Ade, *Acinetobacter* drug efflux.

B (NDM, VIM, and IMP enzymes); and class D (OXA enzymes).[33] As the most important antibacterial agents for multidrug-resistant infections of gram-negative *Bacilli* spp, carbapenem and colistin are the last resource to treat such bacterial infections. Unfortunately, bacteria have evolved carbapenemase, one of their arsenals to resist drugs.

AMR in Non-Fermentative Bacteria

Multidrug-Resistant *Acinetobacter Baumannii* and *Pseudomonas Aeruginosa*

Acinetobacter baumannii and *Pseudomonas aeruginosa* are critical bacteria among global priority list of antibiotic-resistant bacteria that are identified by WHO[12] and these troublesome MDR bacteria have potentials to cause a wide prevalence of infections and outbreaks. *Acinetobacter baumannii* is naturally transformable to take naked DNA and *Pseudomonas aeruginosa* tends to exchange AMR genes by conjugation.[34] Both bacteria have various antimicrobial mechanisms, including enzymes, efflux pumps, modification of aminoglycosides, permeability defects, and alteration of target sites.[35] ESBLs, metallo beta lactamases (MBLs), oxacillinases (OXAs), aminoglycoside-modifying enzymes (AMEs), 16S-rRNA methylase are all relevant to producing of enzymes in these non-fermentative bacteria.[35–37] Efflux pumps are special mechanisms which can confer resistance to β-lactams, polymyxin, tigecycline and fluoroquinolones. Quinolone Resistance Determining region (QRDR) is quite important for *aeruginosa* and *baumannii*, involved in protein mutation and efflux pumps. The relevant drug resistance genes of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are listed in Table 1.

Antibiotic Resistance Mechanisms

AMR is naturally occurring as a reaction of microbial organisms to environment. However, the acquired resistance in clinical settings is the result of mutations in chromosomal genes or the transmission of external genomic determinants of resistance.[24] The AMR mechanisms mainly include enzymatic drug inactivation, drug target alteration, changes in outer membrane permeability and active efflux of antimicrobial compounds.

Enzymatic Drug Inactivation

Bacteria produce enzymes that can destroy antibiotics or cause them to lose their antibacterial action, causing the drug to be destroyed or fail before it acts on the bacteria cell.[22] There are three main kinds of drug inactivating enzyme: hydrolase (mainly β-lactamase), passivation enzyme (aminoglycoside inactivating enzyme, chloramphenicol acetyltrans-ferase, erythromycin esterase, etc.) and modified enzyme (aminoglycoside modifying enzyme). The enzymes encoded by both chromosomal and plasmid genes can target and cleave the vulnerable hydrolytically susceptible chemical bonds in drugs.[38] The enzymatic genes of the most clinical concerns harbor amidases that cut down the β-lactam ring of the penicillin and cephalosporin classes of drugs, including bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{CMY}, bla_{FOX}, bla_{MOX}, bla_{DHA}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA} and so on.[38]

Drug Target Alteration

There are many antibiotic-binding targets in the bacteria. The target site alteration can make the antibiotics difficult to bind to the bacteria, which is an important mechanism for drug resistance. This mechanism mainly reflects in the drug-resistance of gram-positive bacteria and polymyxin-resistance. For example, the PBP of *Staphylococcus aureus* is converted to PBP2a (encoded by the *mecA* gene), and the latter is a low-affinity binding protein, resulting in resistance to all β-lactam antibiotics.[24,29] The polymyxin resistance is mainly caused by the modification of the lipid A moiety of lipopolysaccharide (LPS), which is the primary target of polymyxin due to genes in PmrA/PmrB, PhoP/PhoQ, ParR/ParS, ColR/ColS or CprR/CprS two-component systems or plasmid-mediated *mcr* genes.[39]

Changes in Outer Membrane Permeability

In gram-negative bacteria, β-lactam antibiotics mainly pass through the outer membrane by hydrophilic channel proteins, and mutations leading to channel protein alteration or decreased expression will make the bacteria less sensitive to various β-lactams. For example, the loss of Opal D2 channel protein in patina causes resistance to imipenem.[24,29] The low outer membrane permeability of *Pseudomonas aeruginosa* gives it high intrinsic resistance to antiseptics and antibiotics.[40] The mutation in genes encoding outer membrane porins is expected to affect drug susceptibility.

Active Efflux of Antimicrobial Compounds

It is also known as the efflux pump system or the drug pumping system. The drug concentration in the bacteria is insufficient to exert an antibacterial effect, resulting in drug resistance. This process requires energy and acts on a variety of antibiotics. For example: *Staphylococcus aureus* resistance to quinolones; OprK protein of *Pseudomonas aeruginosa* outer membrane can transport various antibiotics to the outside of bacteria.[24,29,41] The most important efflux transporters are resistance-nodulation-division (RND), major facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE), small multidrug-resistance (SMR), and ATP-binding cassette (ABC) superfamilies or families. Each efflux families possess several important genes, such as *acrB, mdtF* in RND, *bcr, cmr* in MFS, *mdtK, yeeO* in MATE, *emrE, ydgE* in SMR and *macB* in ABC.[42]

Epidemiology

With severe clinical situation of antimicrobial resistant bacteria infections, some specialists and organizations have begun to monitor the prevalence of different drug-resistant organisms. Multilocus sequence typing (MLST), with high resolution, and whole genome sequencing (WGS), with comprehensive information, are the common methods to type antimicrobial resistant bacteria in the molecular level.

For Gram-positive bacteria, the main antimicrobial resistant bacteria include MRSA, VRE and PRSP. MRSAs are widely distributed all over the world, especially in South America, North America and Japan with high prevalence rate of over 40%, while PRSP is a challenge for Europe.[43–45] As for VRE, *Enterococcus faecium* is more ubiquitous than *Enterococcus faecalis*.[43–50]

As for gram-negative bacteria, the main antimicrobial resistant bacteria include cephalosporin- and/or carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae*, and carbapenem-resistant *Acinetobacter baumannii* and

Table 2 The Percentage of Prevalent Antimicrobial Resistant Bacteria from 9 Countries on 6 Continents

Continent	Country	Year	MRSA	VRE (Faecalis)	VRE (Faecium)	PRSP	CRkp	MDR-PA	MDR-AB	reference
Oceania	Australia	2017	19.0%	1.0%	50.0%	NF	1.0%	NF	NF	[48]
South America	Argentina	2017	45.0%	4.0%	69.0%	15.0%	20.0%	NF	NF	[43]
North America	USA	2016	NF	5.0%	68.0%	17.0%	3.0%	21.0%	NF	[23,51,52]
	Canada	2017	16.0%	NF	NF	16.5%	2.0%	21.0%	NF	[44]
Europe	UK	2019	6.0%	1.6%	22.2%	1.4%	0.7%	2.9%	1.2%	[23,45]
Asia	China	2019	32.2%	0.0%	1.4%	2.7%	6.4%	20.7%	56.1%	[23,50]
	India	2018	39.0%	7.0%	27.0%	3.0%	59.0%	NF	NF	[49]
	Japan	2017	41.0%	NF	NF	1.0%	0.0%	NF	NF	[43]
Africa	South Africa	2016	27.0%	1.0%	5.0%	NF	7.0%	NF	NF	[69]

Notes: NF, not found; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant Enterococcus; PRSP, penicillin-resistant *Streptococcus pneumoniae*; CRkp, carbapenemase-resistant *Enterobacteriaceae*; MDR-PA, multidrug-resistant *Pseudomonas aeruginosa*; MDR-AB, multidrug-resistant *Acinetobacter baumannii*.

Pseudomonas aeruginosa. The global spread of ESBL-producing bacteria in the community since the 2000s has threatened the public health. In recent years, the clinical medication and intensity of carbapenem antibiotics have increased year by year, and the carbapenem resistance rate of clinical strains (including *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) is of an increasing trend. MDR *Acinetobacter baumannii* and *Pseudomonas aeruginosa* may gradually turn into a major clinical challenge.[23,50]

To be more specific, information about global AMR is plotted in Table 2 to present the difference situation in 9 countries.[23,43–52] This table indicates the percentage of prevalent antimicrobial resistant bacteria in different countries of six continents relatively. The data were collected from references and several surveillance systems and monitoring reports. Australia is on behalf of Oceania, Argentina on behalf of South America, USA and Canada on behalf of South America, UK on behalf of Europe, China, India, and Japan on behalf of Asia and South Africa on behalf of Africa. The data of Australia were collected from Australian Group on Antimicrobial Resistance (AGAR),[46–48] Argentina, South Africa and Japan from The Center for Disease Dynamics, Economics & Policies,[43] USA from reports or articles published by Chong et al.[23] Monaco et al[51] and Sader et al.[52] Canada from Canadian Antimicrobial Resistance Alliance (CARA),[44] UK from article published by Chong Yong et al[23] and European Centre for Disease Prevention and Control (ECDC),[45] India from India Council of Medical Research[49] and China from article published by Chong et al[23] and China Antimicrobial Resistance Surveillance System (CARSS) monitoring report.[50] MRSA, VRE (*faecalis*), VRE (*faecium*), PRSP, ESBL, CRkp, MDR-PA and MDR-AB are included in the study.

According to obtained data, VRE (*faecium*), accounting for 50%, 68% and 69% ranks the first in Australia, USA and Argentina; MDR-PA (21%) in Canada; VRE (22.2%) in UK; MDR-AB (56.1%) in China; CRkp (59%) in India; MRSA (41% and 27%, respectively) in Japan and South Africa.[23,43–52]

Detection Methods
Antimicrobial Susceptibility Testing
Antimicrobial susceptibility tests (ASTs) include K-B test, dilution test, Epsilometer test (E-test) and commercialized automatic drug susceptibility detection and analysis systems. K-B test uses antibiotic-containing wafers or disks, sometimes added with enzyme inhibitors, to test whether specific bacteria strains are sensitive to specific antibiotics. Broth microdilution test, the gold standard of AST, is used to quantitatively determine the minimal inhibitory concentration (MIC) of antimicrobial agent to inhibit or kill the bacteria.[53] MIC is standardized by globally recognized institutions such as CLSI and EUCAST. To be more convenient, E-test comes into the market as a plastic strip with a gradient concentration of antimicrobial agents impregnated in it. With advanced technology, commercial systems with consistent and rapid results, including bioMérieux VITEK® 2 Automated instrument for ID/AST testing, Sensititre™ Complete Automated AST System and BD Phoenix™ automated identification and susceptibility testing system, are recommended for clinical microbiology laboratories.

Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)
MALDI-TOF MS is widely used in the rapid identification of bacteria based on the detection of proteins. Furthermore, MALDI-TOF MS can be utilized in AST with the combination of other techniques, such as surface-enhanced Raman scattering (SERS) and isotope incorporation.[54]

Molecular Biology Tests
With the rapid development of molecular biology, it's widely used in microbiology, especially in the tests of AMR genes of MRSA, tuberculosis and superbugs. Methods that are commonly used in laboratory include polymerase chain reaction (PCR), quantitative PCR (qPCR), Real-Time Fluorescent PCR Assay (RT-PCR), DNA Microarrays/Genechip and WGS, which are all in need of large volumes of blood and specific for antibiotic resistance gene identification.[54]

Table 3 Major Antibiotic Resistance Mechanisms, Detection Methods and Medical Recommendations for Popular Antimicrobial Resistance Genes of Antimicrobial Resistant Bacteria

	Popular Antimicrobial Resistance Genes	Major Antibiotic Resistance Mechanisms	Detection Methods	Medication Recommendation
Methicillin-resistant *Staphylococcus aureus*[77]	*mecA* *mecB* *mecC* *mecD*	Drug target alteration	MIC determination, disk diffusion tests or latex agglutination to detect PBP2a, genotypically using PCR	Vancomycin, linezolid, teicoplanin, daptomycin, sulfamethoxazole/ trimethoprim (SMZ/TMP)
Vancomycin-resistant *Enterococcus*[18]	*vanA* *vanB* *vanC* *vanD/E/F/G/L/M/N*	Drug target alteration	MIC determination, disk diffusion, the breakpoint agar method, PCR-based methods, DNA microarray-based	Linezolid, nitrofurantoin, fosfomycin (urinary tract infection)
Penicillin-resistant *Streptococcus pneumoniae*[20]	*pbp1a, pbp2x, pbp2b*	Drug target alteration	MIC or disk diffusion, PCR-based methods, DNA microarray-based	Levofloxacin, moxifloxacin, vancomycin
ESBL-producing *Enterobacteriaceae*[23,25–28]	bla_{CTX-M}, bla_{TEM}, bla_{SHV} bla_{OXA-10}, $bla_{OXA-13\ to\ 19}$	Enzymatic drug inactivation	ESBL gradient test, Combination disk diffusion test (CDT), Broth microdilution, Double disk synergy test (DDST), PCR-based methods, DNA microarray-based	Piperacillin/tazobactam, cefoperazone/sulbactam, oxycephalosporin, cefmetazole, cefminox, cefoxitin, ertapenem
Acquired AmpC β-lactamase-producing *Enterobacteriaceae*[24,26,29,30]	bla_{CMY}, bla_{FOX}, bla_{MOX}, bla_{LAT}, bla_{ACC}, bla_{ACT}, bla_{MIR-1}, bla_{DHA}	Enzymatic drug inactivation	inhibition of AmpC (cloxacillin or boronic acid derivatives), the Mast 'AmpC Detection Disc Set', the AmpC gradient test, PCR-based methods, DNA microarray-based	Ertapenem, imipenem/ cilastatin, meropenem, panipenem/betamipron, cefepime
Carbapenemase-producing *Enterobacteriaceae*[32,33,78]	bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{IMP}, bla_{VIM}	Enzymatic drug inactivation	Diffusion methods, combination disk, Carba NP test, CIM, Hodge test, DDS, Etest, PCR, DNA microarray	A combination of drugs is recommended. Available drugs include: polymyxin E (colistin), polymyxin B, ceftazidime-avibactam, fosfomycin, tigecycline, minocycline
Multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*[41,68–76]	bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}, bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{NDM}, bla_{SPM}, bla_{GIM}, bla_{DIM}, *intI 1, intI 2, adeA, adeB, adeC, adeRS, adeIJK, abeM, abrp, gyrA/B, pmrA/ pmrB/ mcr-1*	Enzymatic drug inactivation, efflux pumps, modification of aminoglycosides, permeability defects, and alteration of target sites	MIC determination, disk diffusion, Etest, PCR-based methods	A combination of drugs is recommended. Available drugs are as follow. *Acinetobacter baumannii*: polymyxin B *Pseudomonas aeruginosa*: Polymyxin E (colistin), cefoperazone/sulbactam + minocycline

Abbreviations: MIC, minimum inhibitory concentration; ESBL-producing, Extended-spectrum β-lactamase-producin.

Raman Micro-Spectroscopy

Raman micro-spectroscopy is a non-invasive and label-free technology which provides an intrinsic biochemical "finger-print" of a single bacterial cell.[55] Raman micro-spectroscopy coupled with the stable-isotope technique using heavy water

(D_2O rather than H_2O) has been proven as a universal method to evaluate in vivo metabolic activity of a cell.[56–60] In the presence of heavy water (D_2O), metabolically active cells will form a C-D band by incorporating D (deuterium) from D_2O via NADPH electron transport chain. It has been confirmed that C-D Raman biomarker in Single-Cell Raman Spectra quantitatively indicates general metabolic activity of a cell. The higher the metabolic activity, the higher the C-D intensity. When cells are exposed to D_2O and antibiotics in AST test, the C-D band in cells becomes detectable in as short a time as 20 minutes and reaches the highest value after 1–3 hours.[56–60] Raman micro-spectroscopy has also been applied to bacterial identification.[55,61]

Microfluidic Chip

Microfluidic Chip has been tested in identification and AST of multiple uropathogens for research, which combines the spatial resolution of the cell culture arrays and the color resolution from the chromogenic reaction. The AST is determined by MIC which is reflected from the degree of chromogenic reaction.[62,63]

Clinical Treatment for AMR Bacteria

Due to various AMR bacteria, clinical medication is in a dilemma where clinicians worried that no effective drugs to fight against superbugs in the future. Table 3 listed recommendations of medications for AMR bacteria according to update guidelines and references.

Conclusion

The study of drug-resistant bacterial genotypes, epidemiology, detection methods and clinical treatment is meaningful for monitoring, diagnosing and treating of drug-resistant colonies in nosocomial or community infections outbreaks. Quick and accurate detection is the primary assurance to handle worsened situation. Traditional AST still needs more research to maximize the revealed information and shorten the sample turn-around time. Under the guidance of the concept of "One Health", focus on the AMR genes should not only be restricted among people but also in food animals and even plants.

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All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

We have no conflict of interest.

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