ANTIBIOTIC RESISTANCE GENES AND RELATED *q*PCR DETCTION METHODS

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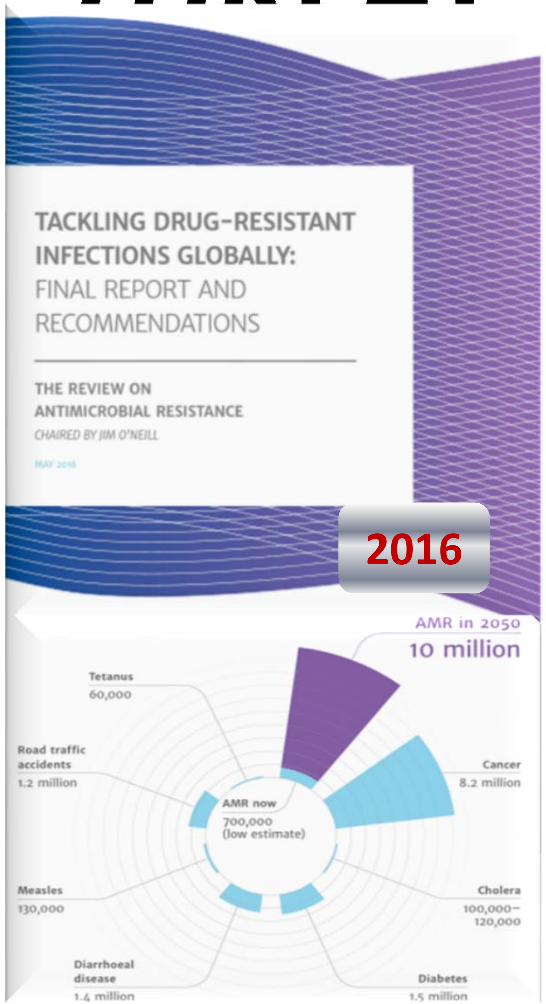
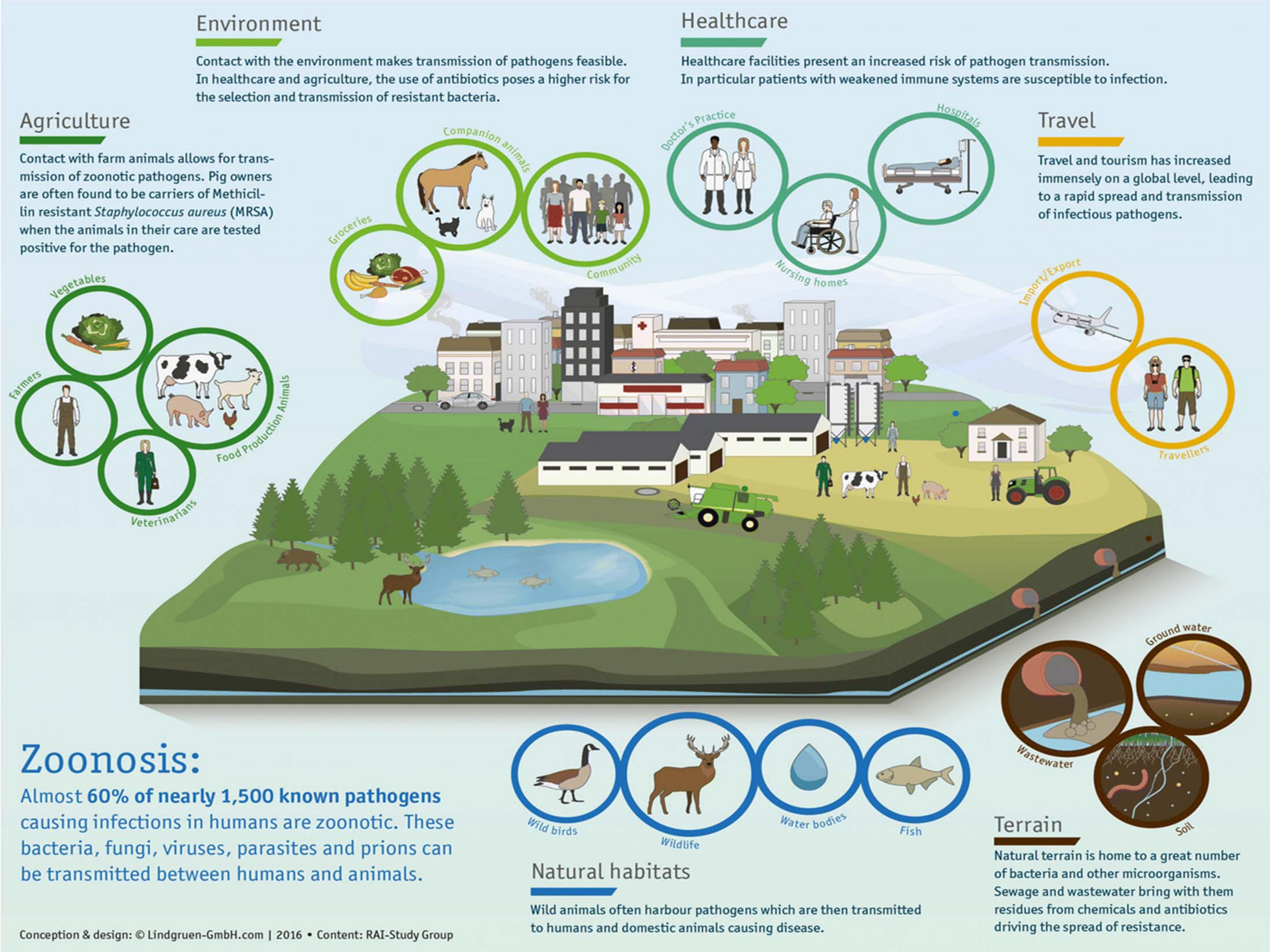
AMR, AN IMPORTANT PUBLIC THREAT



ONE HEALTH, ONE RESISTANCE

One Health is the collaborative effort of multiple health science professions to attain optimal health for people, domestic animals, wildlife, plants, and our environment. The drivers of antimicrobial resistance include antimicrobial use and abuse in human, animal, and environmental sectors and the spread of resistant bacteria and resistance determinants within and between these sectors and around the globe.

McEwen SA, et al. Microbiol


Environment
Contact with the environment makes transmission of pathogens feasible. In healthcare and agriculture, the use of antibiotics poses a higher risk for the selection and transmission of resistant bacteria.
Healthcare
Healthcare facilities present an increased risk of pathogen transmission. In particular patients with weakened immune systems are susceptible to infection.
Agriculture
Contact with farm animals allows for transmission of zoonotic pathogens. Pig owners are often found to be carriers of Methicillin resistant *Staphylococcus aureus* (MRSA) when the animals in their care are tested positive for the pathogen.
Travel
Travel and tourism has increased immensely on a global level, leading to a rapid spread and transmission of infectious pathogens.
Companion animals
Groceries
Community
Doctor's Practice
Hospitals
Nursing homes
Import/Export
Travellers
Vegetables
Farmers
Food Production Animals
Veterinarians
Ground water
Wastewater
Soil
Wild birds
Wildlife
Water bodies
Fish
Zoonosis:
Almost **60% of nearly 1,500 known pathogens** causing infections in humans are zoonotic. These bacteria, fungi, viruses, parasites and prions can be transmitted between humans and animals.
Natural habitats
Wild animals often harbour pathogens which are then transmitted to humans and domestic animals causing disease.
Terrain
Natural terrain is home to a great number of bacteria and other microorganisms. Sewage and wastewater bring with them residues from chemicals and antibiotics driving the spread of resistance.
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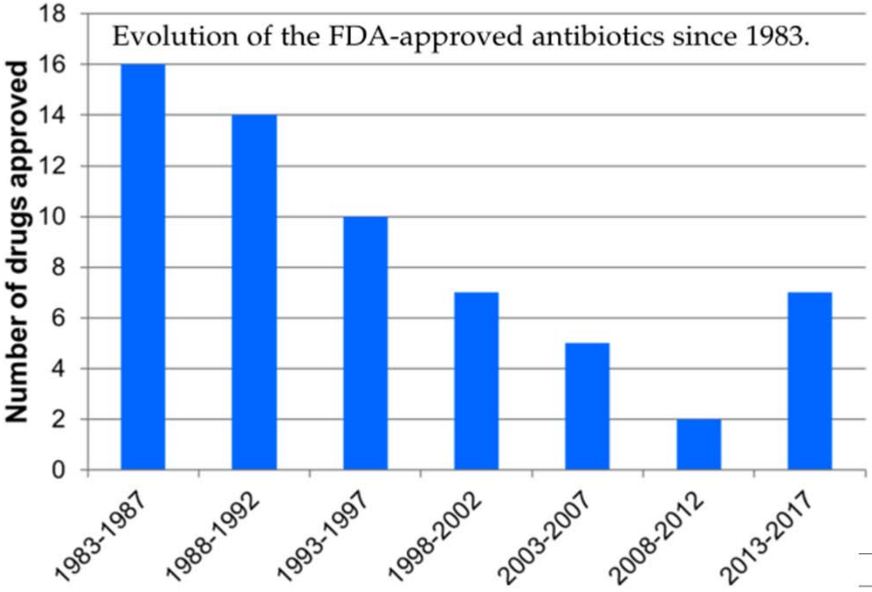
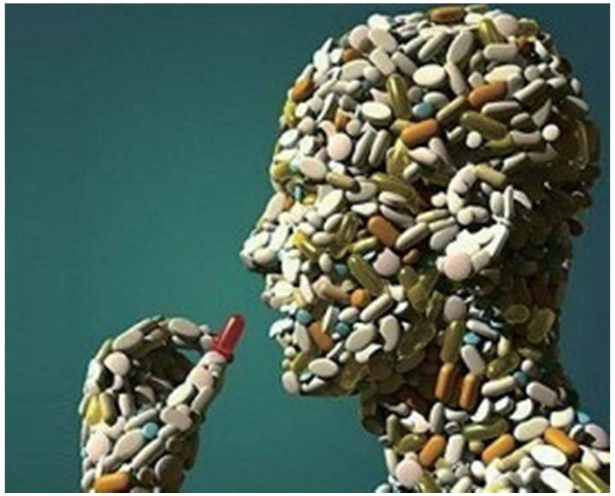
ANTIBIOTICS EVOLUTION





Less and less antibiotics were discovered and approved

List of systemic antibiotics approved by the FDA and EMA since 1999.

Antibacterial	Year Approved		Novel Mechanism?	Spectra
	FDA	EMA		
Quinupristin/dalfopristin	1999	2000	No	GPB
Moxifloxacin	1999	2001	No	GPB-GNB
Gatifloxacin *	1999	/	No	GPB-GNB
Linezolid	2000	2001	Yes	GPB
Cefditoren pivoxil	2001	/	No	GPB-GNB
Ertapenem	2001	2002	No	GNB-GPB
Gemifloxacin *	2003	/	No	GPB-GNB
Daptomycin	2003	2006	Yes	GPB
Telithromycin *	2004	2001	No	GPB
Tigecycline	2005	2006	Yes	GPB-GNB
Doripenem *	2007	2008	No	GNB-GPB
Telavancin	2009	2011	Yes	GPB
Ceftarolin fosamil	2010	2012	No	GPB-GNB
Ceftolozane-tazobactam	2014	2015	No	GNB-GPB
Tedizolid	2014	2015	No	GPB
Oritavancin	2014	2015	No	GPB
Dalbavancin	2014	2015	No	GPB
Ceftazidime-avibactam	2015	2016	No	GNB
Meropenem-vaborbactam	2017	2018	No	GPB-GNB
Delafloxacin	2017	/	No	GPB-GNB
Omadacycline	2018	/	No	GPB-GNB

Raphaël E. Duval et al. Mc

ANTIBIOTICS AND AMR

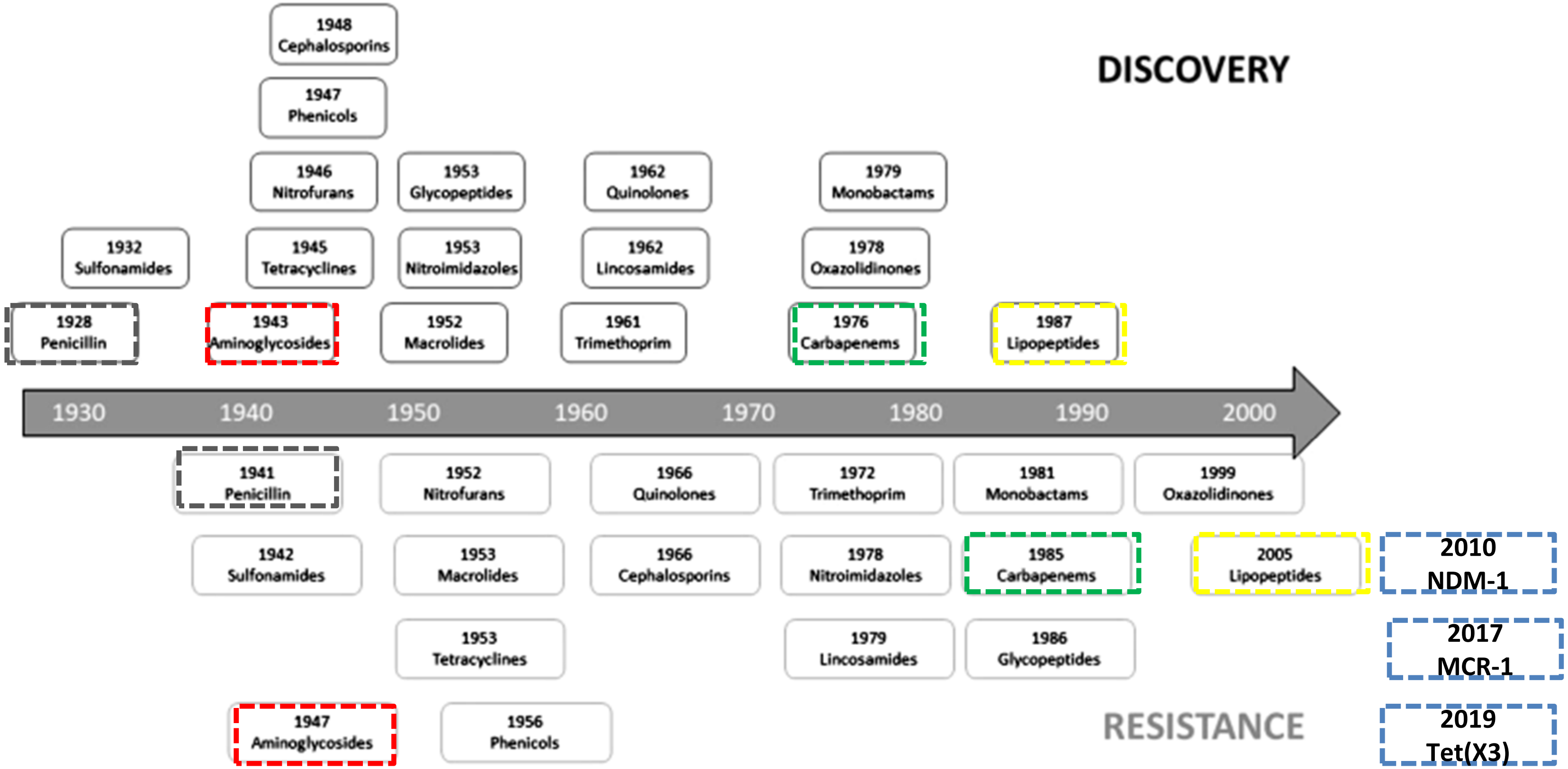


Figure 3. Antibiotics timeline from the end of the 1920s until today, indicating when the main antibiotic classes were discovered, and when the mechanisms of resistance to these antibiotics were first described.

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What is AMR and ARG

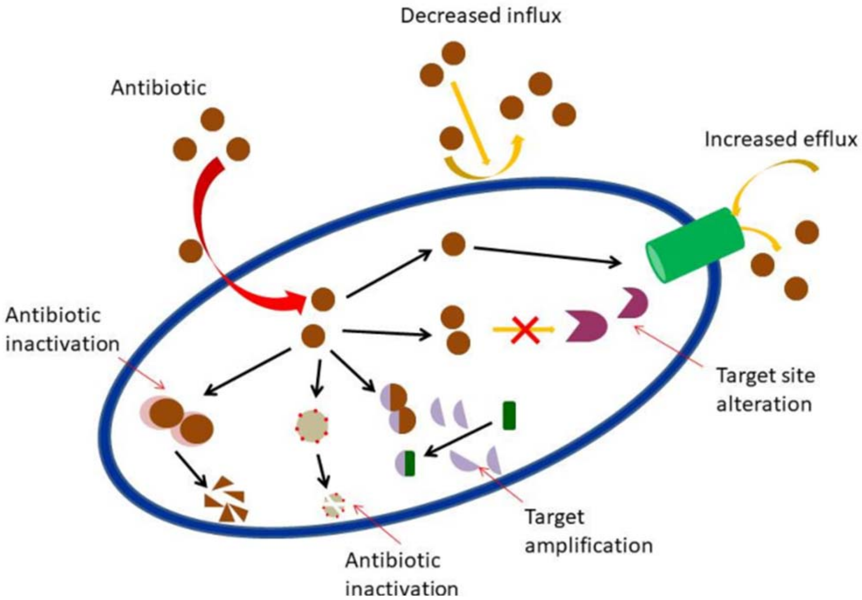
Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, viruses, fungi and parasites change in ways that render the medications used to cure the infections they cause ineffective.

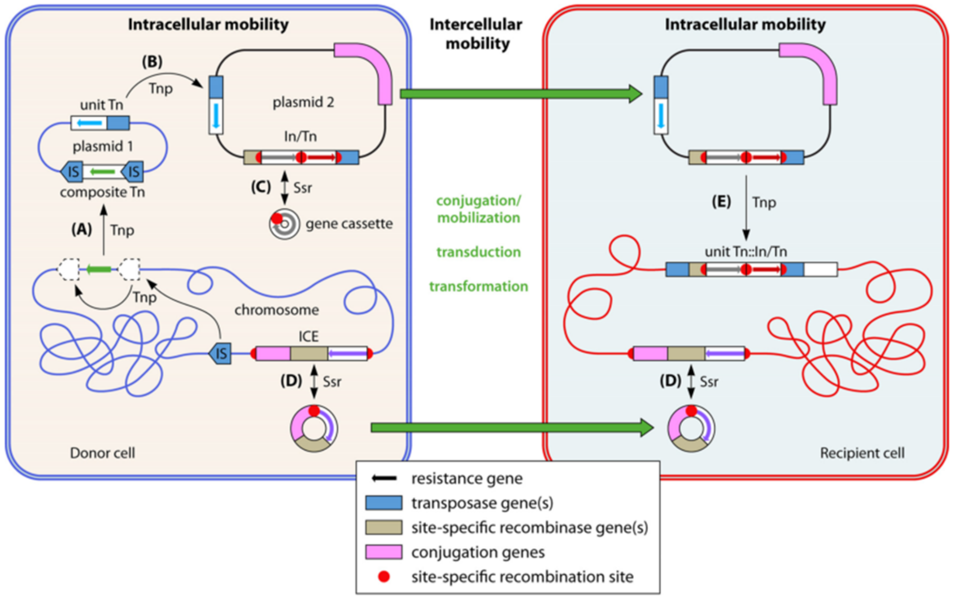
Antibiotic resistance (AR) occurs when bacteria change in response to the use of antibiotics used to treat bacterial infections (such as urinary tract infections, pneumonia, bloodstream infections) making them ineffective.

Antibiotic resistance gene (ARG) give contribution to bacterial antibiotic resistance or non-sensitive.

WHO. 2017.

What is AR and ARG





Mahizan NA, et al. 2019. Mol
Partridge SR, et al. 2019. Clir

Types of AR Mechanisms

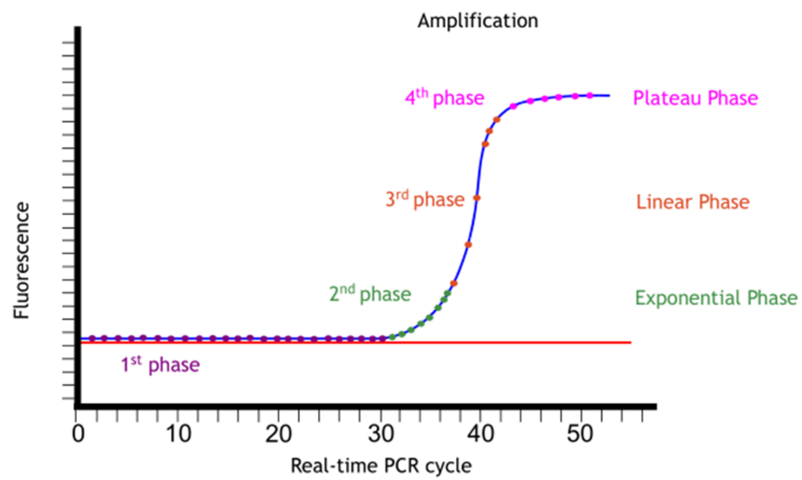
- Acquired resistance
  - Target modification
  - Decreased intracellular drug accumulation
  - Antibiotic inactivation
  - Coexisted and complex
- Intrinsic resistance

Types of AR Mechanisms

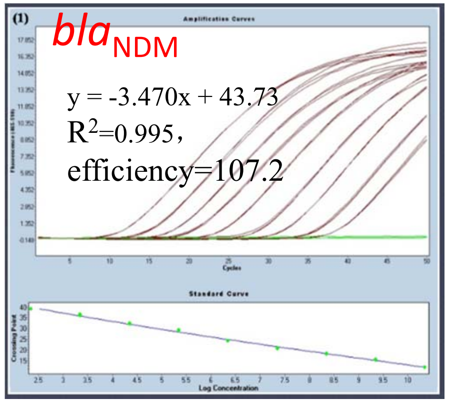
Antimicrobial agents	Target modification	Decreased intracellular drug accumulation	Antibiotic inactivation
Aminoglycosides	*rmt, nmp*		*aph, aac, ant*
β-lactams	*mec*		*bla*
Carbapenems			*bla*
Colistin	*mcr*		
Fosfomycin			*fos, fom*
Glycopeptides	*van*		
Lincosamides			*lnu*
Macrolides	*erm, car(B), mdm, tlr, lrm, srm, lmr, myr*	*mef(A), car(A), msr(A), msr(B), ole(B), ole(C), srm(B), tlr(C), vga, vga(B)*	*ere, vgb, mph, vat*
Oxazolidinones	*cfr, optrA*		
Quinolones	*qnr*	*qepAB*	*aac(6′)-Ib-cr*
Phenicols	*cfr*	*floR, cmlA, fex*	*cat*
Pleuromulitins		*msr(A), msr(SA), msr(SA)′, msr(B)*	*ere,vgb,vat*

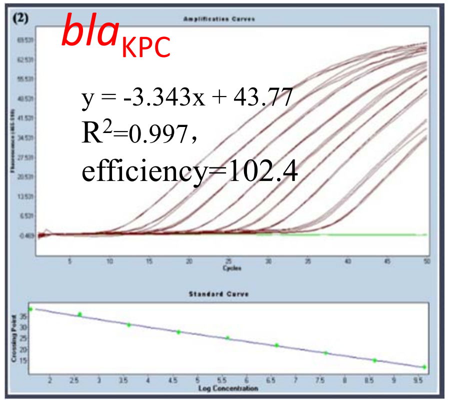
ARGs detection

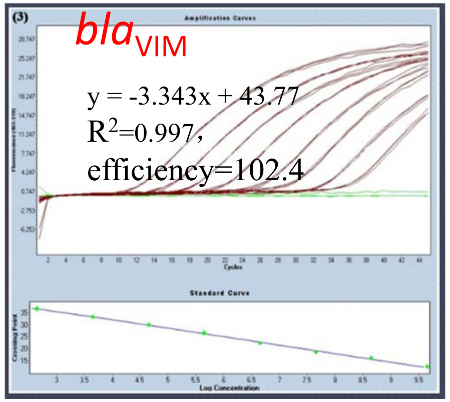
- **The use of PCR and related methods to detect the presence of ARGs in a bacterial isolate or even in samples from different environments is commonplace**
- **SybrGreen qPCR**
- **High-throughput qPCR assay by probe mothod**
- **Taqman-MGB**
- ***int*1, IS*CR*1, *bla* genes, *r***
- ***aac*(6′)-Ie-*aph*(2′)-Ia ,*a***
- ***optr*A, *aac*(6′)-Ib-cr …**

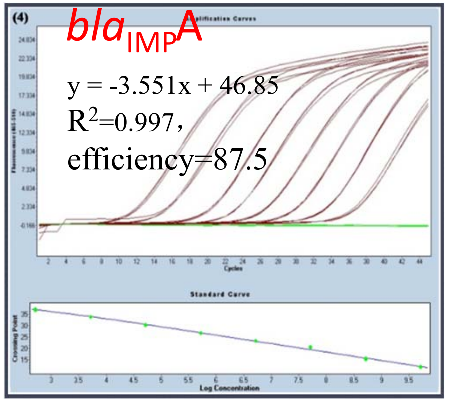


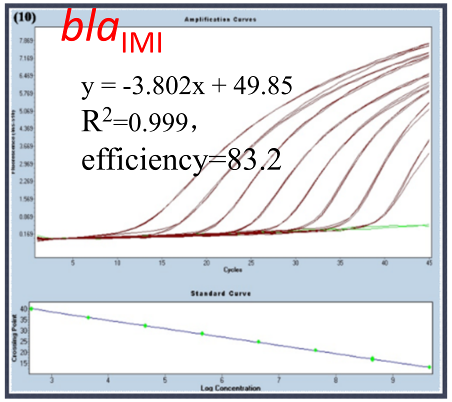
AGR harboring recombinant plasmids co

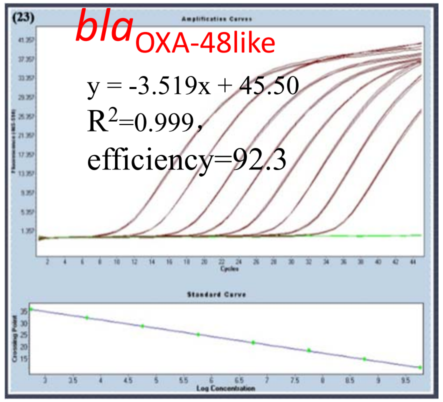






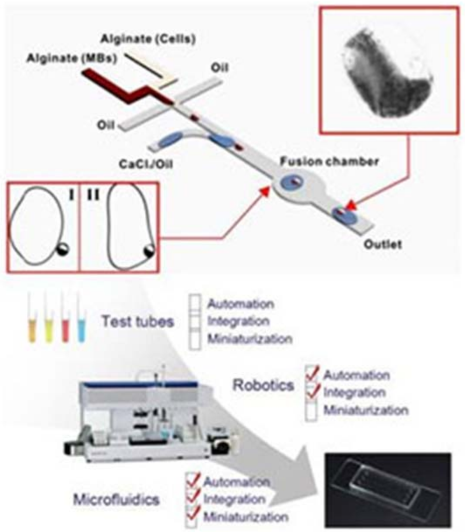




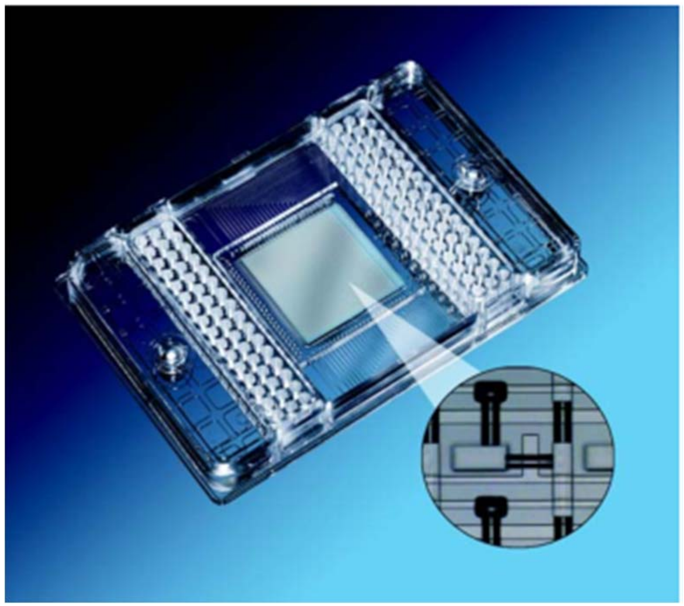


Microfluidics methdos

High-throughput *q*PCR assay by using int



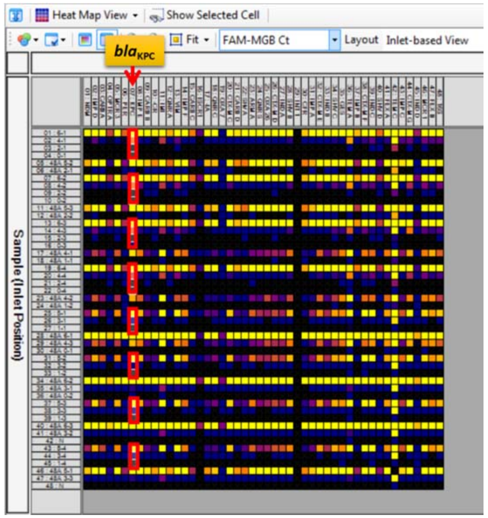
Lab on a chip

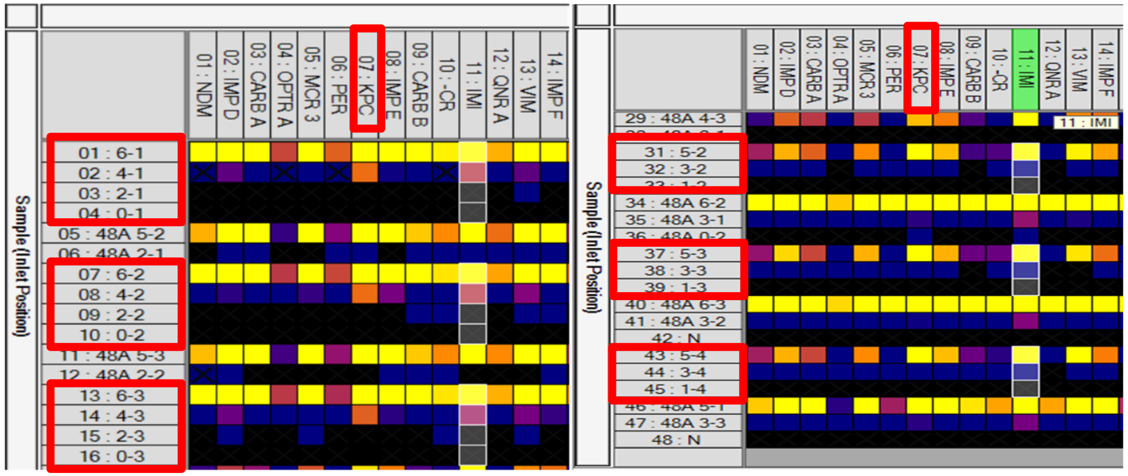


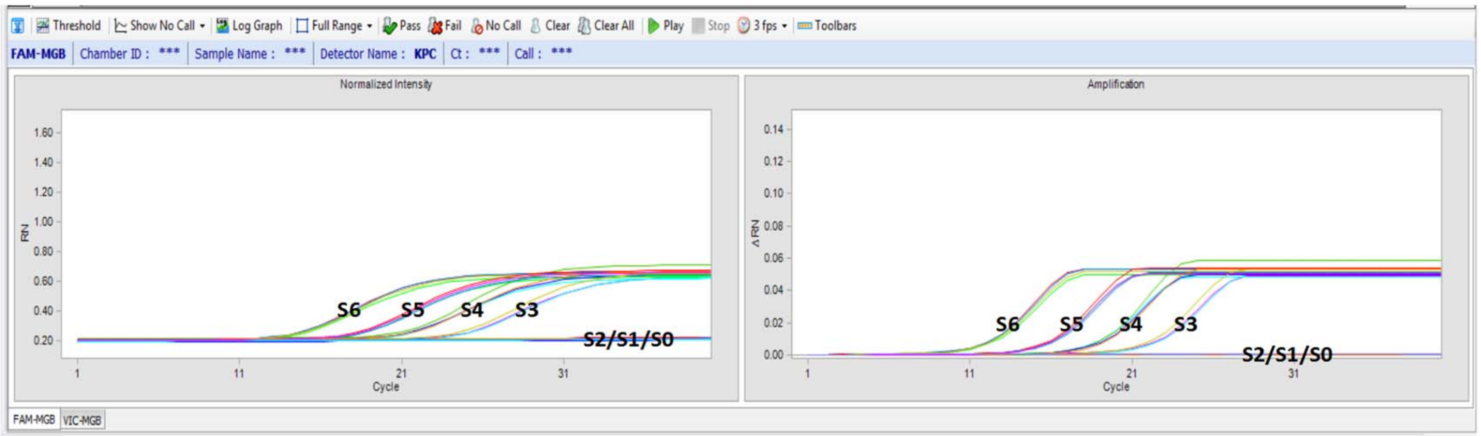
Target ARGs developed by high-throuput *q*PCR assay

Antimicrobial agent	No.[a]	Gene group	Number	Accession no. of reference sequences	Antimicrobial agent	No.	Gene group	Number	Accession no. of reference sequences
Carbapenems	1	*bla*$_{OXA-48}$-like	14	AY236073	other β-lactams	25	*bla*$_{SHV}$ D	2	JQ029959
	2	*bla*$_{IMP}$ A	13	S71932		26	*bla*$_{TEM}$ A	157	AF093512
	3	*bla*$_{IMP}$ B	5	AF290912		27	*bla*$_{TEM}$ B	4	J01749
	4	*bla*$_{IMP}$ C	4	AB074436		28	*bla*$_{OXA}$	7	JN596991
	5	*bla*$_{IMP}$ D	5	AY553332		29	*bla*$_{PER}$	6	AY740681
	6	*bla*$_{IMP}$ E	9	EF118171		30	*bla*$_{CARB}$ A	22	KJ934265
	7	*bla*$_{IMP}$ F	2	KF148593		31	*bla*$_{CARB}$ B	4	AF313471
	8	*bla*$_{NDM}$	15	FN396876		32	*bla*$_{CARB}$ C	3	AF030945
	9	*bla*$_{KPC}$	21	AF297554		33	*bla*$_{CARB}$ D	5	AF135373
	10	*bla*$_{VIM}$	38	JN982330	Fluoroquinolones	34	*qnr*A	8	GU295952
	11	*bla*$_{IMI}$	5	DQ173429		35	*qnr*C	1	EU917444
	12	*bla*$_{IND}$ A	6	AF219131		36	*qnr*S	4	FJ167861
	13	*bla*$_{IND}$ B	2	AF099139	Colistin	37	*mcr*-1	1	KP347127
	14	*bla*$_{IND}$ C	2	AF219127		38	*mcr*-3	1	KY924928
	15	*bla*$_{IND}$ D	2	GU186044	Aminoglycosides	39	*arm*A	1	[16]
Other β-lactams	16	*bla*$_{GES}$	22	AF156486		40	*aac*(6')-*Ie*-*aph*(2")-*Ia*	1	HQ015159
	17	*bla*$_{CTX-M}$ A	58	AF255298	Phenicols	41	*fex*A	1	KC222021
	18	*bla*$_{CTX-M}$ B	22	AJ416344		42	*fex*B	1	JN201336
	19	*bla*$_{CTX-M}$ C	11	FR682582	Multidrug	43	*cfr*	1	JF969273
	20	*bla*$_{CTX-M}$ D	48	HQ833652		44	*optr*A	1	KP396637
	21	*bla*$_{CTX-M}$ E	3	AY238472		45	*aac*(6')-*Ib*-cr	1	GU189577
	22	*bla*$_{SHV}$ A	132	AF148850	Gene spread related	46	*intI*1	1	[17]
	23	*bla*$_{SHV}$ B	4	LN515533		47	ISCR1	1	[17]
	24	*bla*$_{SHV}$ C	2	JQ341060	Internal processing control	48	*16S*rDNA	1	[9]

Plasmid standard curves of high-through







on of high-throughput *q*PCR assay for ARG

Table 3. Validation of the novel high-throughput real-time PCR array for the detection of AMR target genes contained within a plasmid mixture.

High-throughput assay	AMR target genes contained in plasmid mixture		Total
	Positive	Negative	
Positive reactions	9670[a]	476[b]	10146
Negative reactions	68[c]	24346[d]	24414
Total	9738	24822	34560

Sensitivity rate

SEN=99.30%

specificity rate

SPE=98.08%

positive predictive value

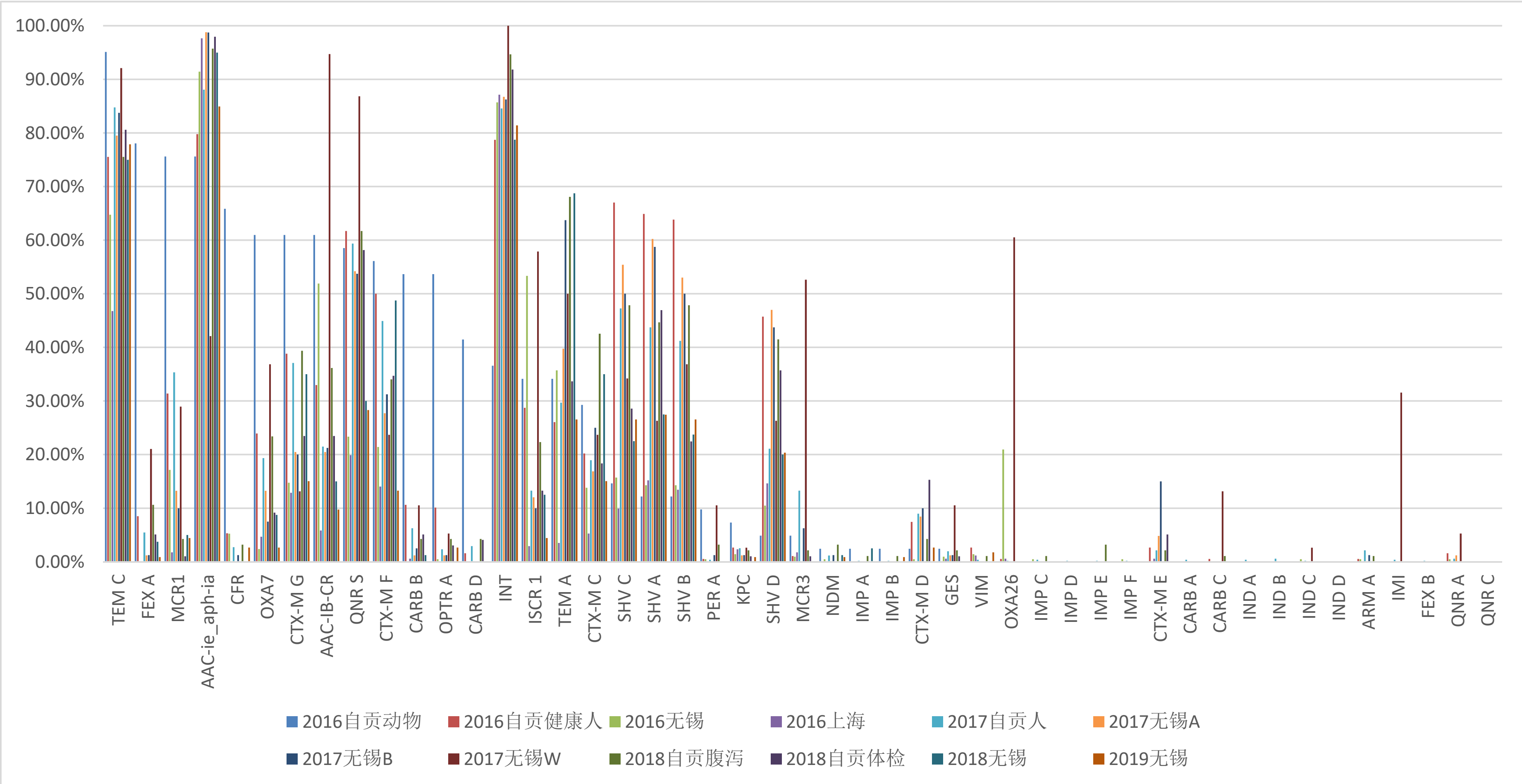
PPV=95.31%

negative predictive value

NPV=99.79%

ARGs detection by high-throughput *q*PCR in China

Data unpublished



Summary

- AMR, an important public threat
- Antibiotics, AMR and ARG
- Types of AR Mechanisms (AGRs)
- *q*PCR methods for ARGs detection
- High-throughput *q*PCR assay for ARGs detection

THANK YOU!

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