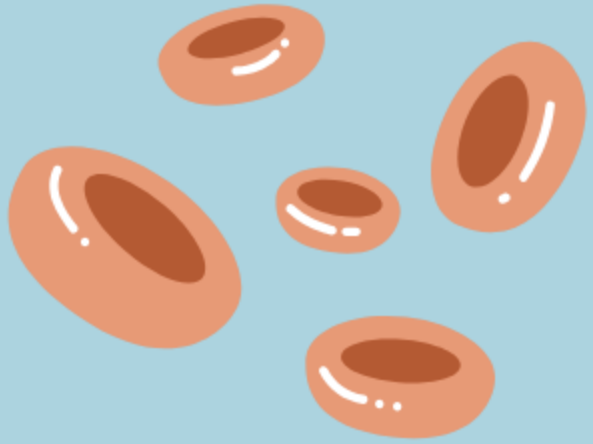
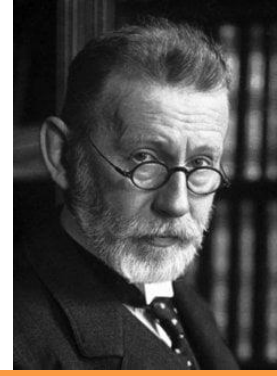
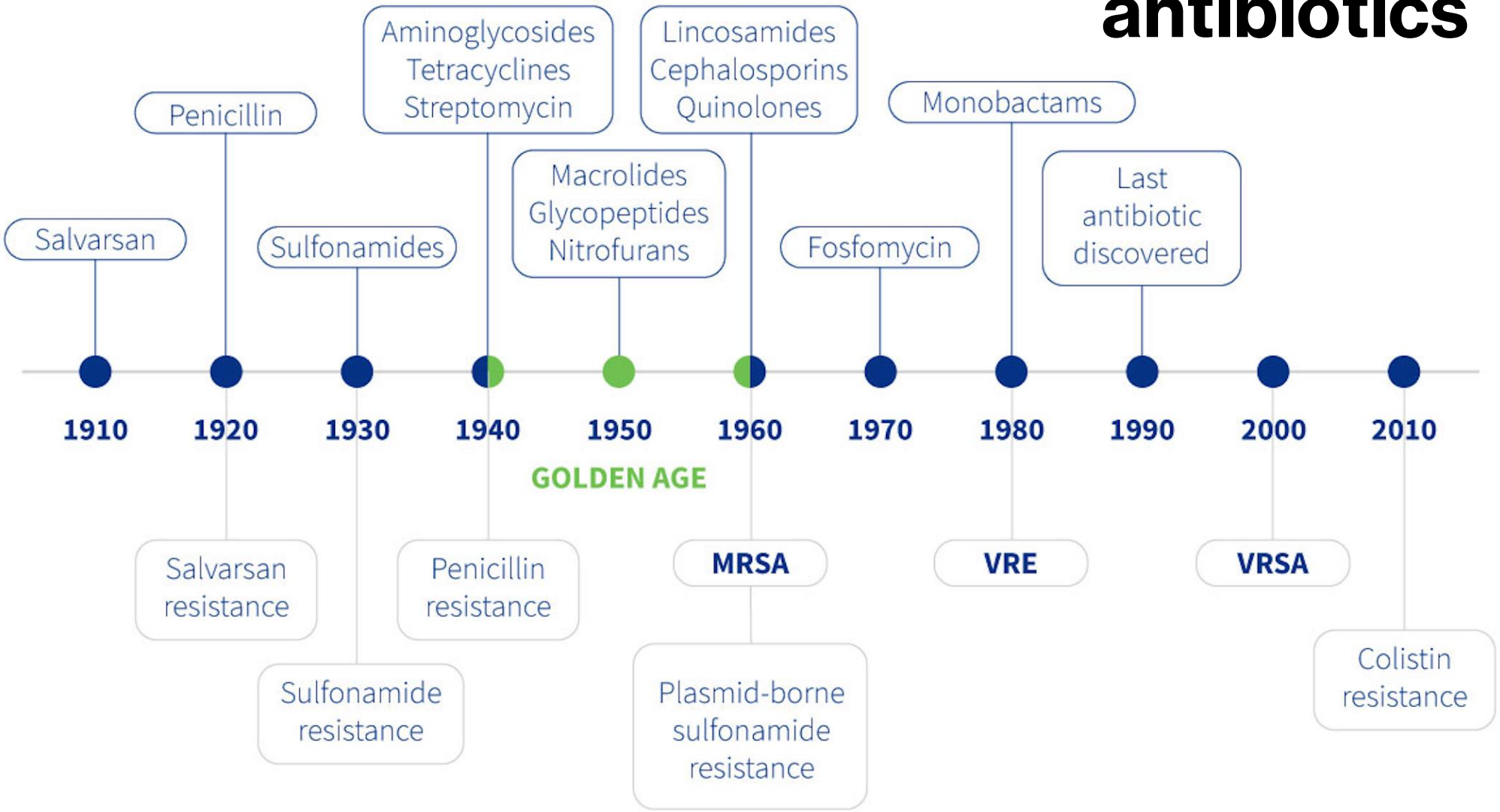
Antibiotic sensitivity screening



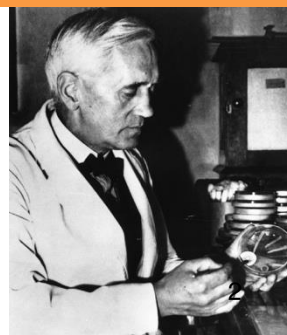
Sophie Holper & Camille Masanet

The history of antibiotics





- **1909:** Paul Ehrlich discovering the drug salvarsan to treat syphilis [1]
- **1928:** Alexander Fleming accidentally discovers Penicillin [2]



What is an antibiotic?

Main goal: Destroying or inhibiting the bacterial cell

Types of antibiotics:
- Bactericidal antibiotics
- Bacteriostatic antibiotics

Two possible work mechanisms:
- Preventing cell reproduction
  → bacteria can no longer multiply
- Disrupting essential cellular functions or processes
  → cell wall synthesis, protein synthesis

Do **NOT** work against viruses [4]

Different types of antibiotics

MIC = Lowest concentration of an antibiotic that **inhibits visible bacterial growth in 24h** [5]

MBC = Lowest concentration that **kills 99.9%** of the initial bacterial population. [5]

Different modes of action: [6]

Bactericidal	Bacteriostatic
MBC/MIC ratio is ≤4	MBC/MIC ratio is >4
e.g. Lipopeptides	e.g. Fusidic acid

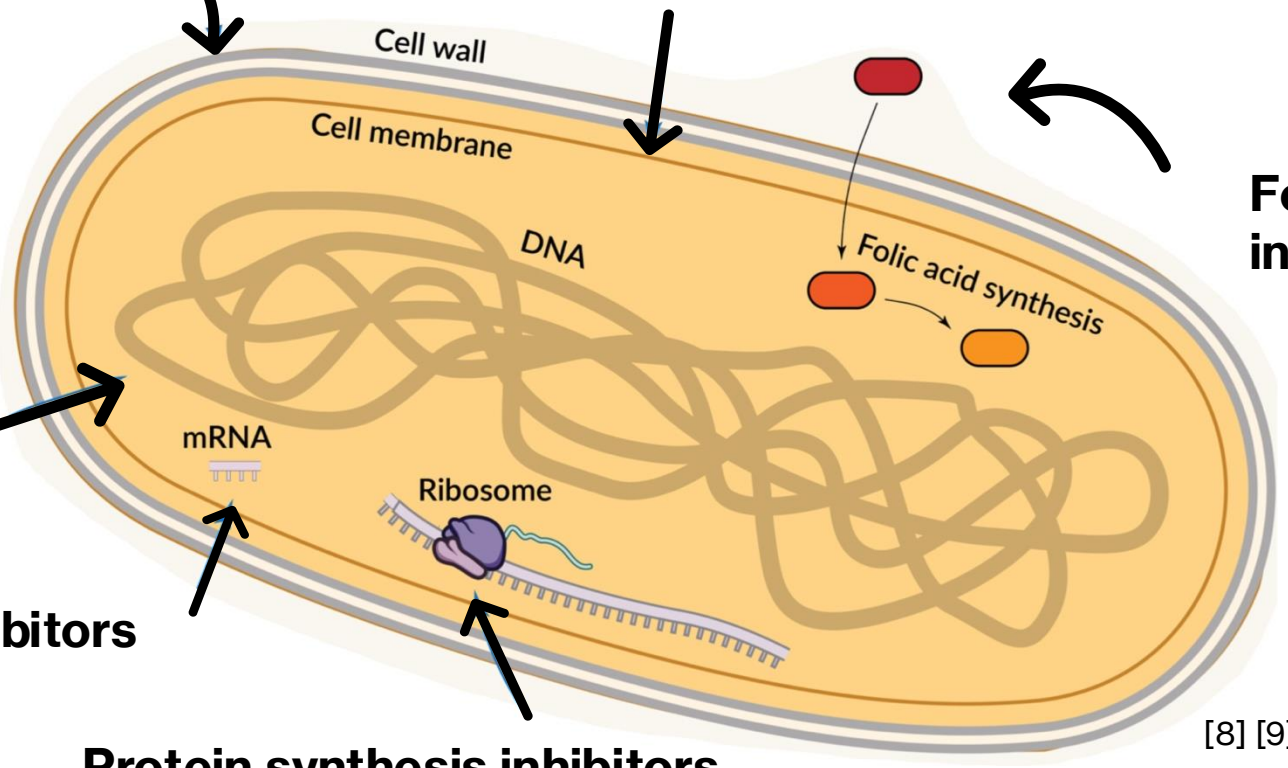
Different spectrums of action: [7]

Broad spectrum	Narrow spectrum
✓ act on a large number of bacteria ✓ useful when the infecting pathogen is unknown X Disruption of normal microbiome X Can induce microbial resistance	✓ Effective against a certain group of bacterial types ✓ Less detrimental effects on the host microbiome X Not sufficiently developed for all pathogens
e.g. ampicillin	e.g. penicillin

How do different antibiotics work?

Cell wall synthesis inhibitors
Block bacteria from building their protective outer wall, which makes them burst and die

Cell membrane disruptors
Damage the bacterial membrane, causing essential contents to leak out and killing the bacteria



Folate synthesis inhibitors

DNA gyrase inhibitors

RNA synthesis inhibitors

[8] [9]

Protein synthesis inhibitors
Disrupt the machinery bacteria use to build proteins, which they need for growth

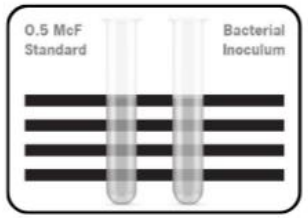
- Tetracyclines

Main methods for antibiotic sensitivity screening

1. Preparation of a bacterial suspension

- select an isolated colony

- create an dilution/inoculum using a 0.5 McFarland standard

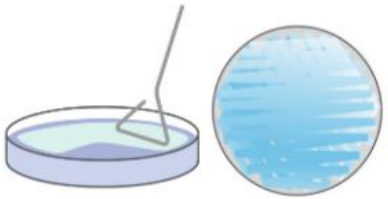


[10]

ideal to perform a sub-culture of the isolated colony

DISK DIFFUSION METHOD

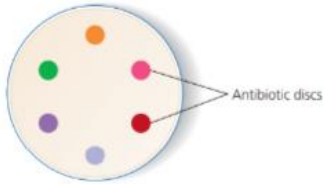
2. Inoculation on MHA (Mueller Hinton Agar) growth medium



spread plating (glass rod or swab)

10 min

3. Filter paper disk containing antibiotics are placed on the plate



incubate 16-20h
35 °C

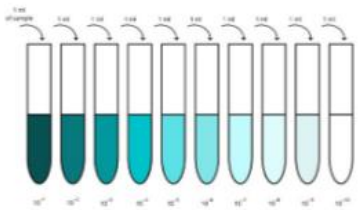
Result analysis

qualitative results

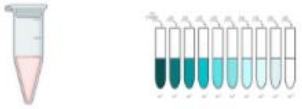
[11]

MIC METHOD

2. Serial dilution of the studied antibiotic



3. Preparation of the MIC plates

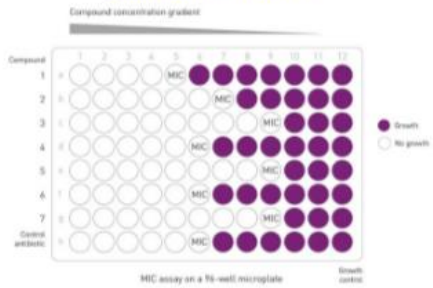


bacteria inoculum + antibiotic solutions

incubate 18-24h
35 °C

Result analysis

quantitative results



[5]

- Dilution in PBS for disk diffusion
- Inoculation in a growth medium (broth or agar) for MIC

Methods:

- J.E Rubin Visual Guide for disc diffusion
- Kowalska-Krochmal et al., 2021

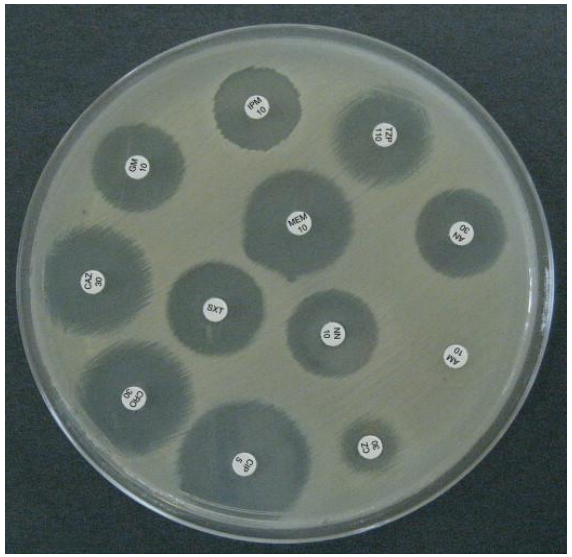
Interpreting results

Disk Diffusion method

[11]
[12]

Qualitative results

- susceptible
- intermediate
- resistant



Following CLSI guidelines

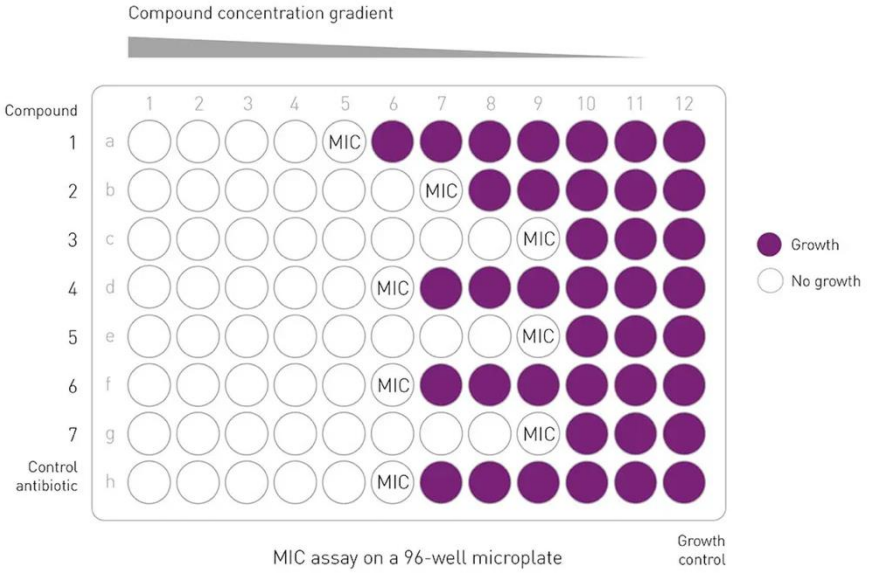
Simple, low cost, easy to interpret

Cannot distinguish between bactericidal and bacteriostatic effects

MIC method

[5]
[13]

Quantitative results (µg/mL or mg/L)



Quantifiable, tool for monitoring the spread of antibiotic resistance, proper dosing of medication

Tedious, risk of errors in the preparation of antimicrobial solutions, large amounts of reagents and space required

The rise of antibiotic resistance

It is estimated that by 2050, antibiotic resistant infections will account for over 10 million deaths annually. [14]

What are the bacterial resistance mechanisms?

1. Stop the antibiotic from reaching its target

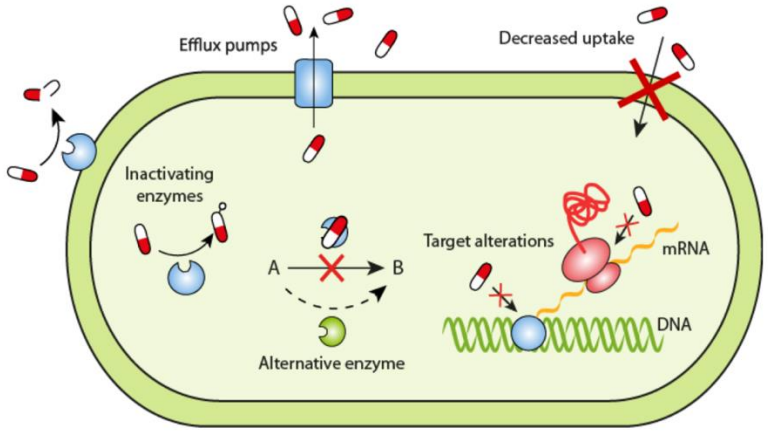


Figure 1. Antibiotic resistance strategies in bacteria. Courtesy of E. Wistrand-Yuen.

[15]

2. Modify or bypass the target of the antibiotic

- **Camouflage the target:** Changes in the structure of the target in the bacterium
- **Express alternative proteins:** Bacteria produce alternative proteins that can be used instead of the ones that are inhibited by the antibiotic.

[16]

Paper using the method

Applied and Environmental Microbiology, June 2010, p. 3444–3451 Vol. 76, No. 11
0099-2240/10/$12.00 doi:10.1128/AEM.02964-09
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Antibiotic Resistance Characteristics of Environmental Bacteria from an Oxytetracycline Production Wastewater Treatment Plant and the Receiving River▽†

Dong Li, Tao Yu, Yu Zhang, Min Yang,* Zhen Li, Miaomiao Liu, and Rong Qi

State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Received 8 December 2009/Accepted 3 April 2010

[17]

Motivation for the study

- Oxytetracycline (OTC) = broad-spectrum antibiotic
- High levels found in the effluent of **an OTC production plant** (North China Pharmaceutical Group Corporation in Hebei Province) which flows **into a river (used for irrigation)**
- Tetracycline resistance increased in enterococcal isolates taken from a nearby hospital
- Rising concern for microbial resistance → **Testing for multidrug resistance (MDR) in pathogens downstream from the industrial facility**

Research question

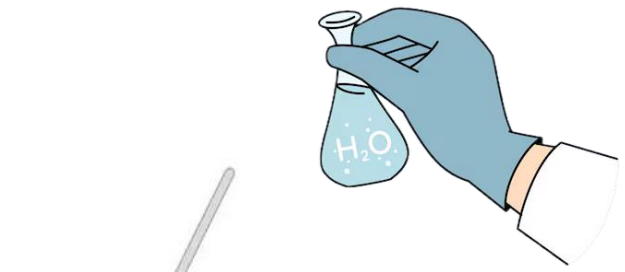
What are the **resistance characteristics** of bacterial isolates for 10 antibiotics found in the WW of the OTC production plant?

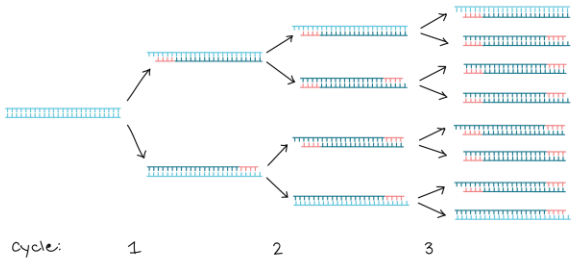
Paper using the method

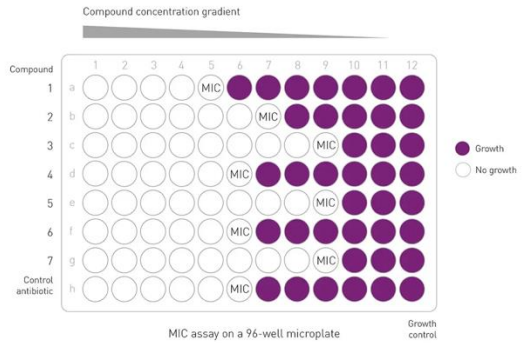
Methods used

1. Water samples from **WW effluent** + river water samples from **both downstream (20km) and upstream (5km)** sections were collected
2. Samples diluted + plated on R2A (non-selective agar) → **colonies formed and were isolated**
3. PCR performed on bacterial DNA → amplified products **were sequenced to identify bacterial strands and *tet* genes**
4. **Antibiotic susceptibility testing (MIC) for 10 antibiotics** with a concentration range of 0.25 to 1,024 mg liter:

resistance prevalence = number of strains resistant to the antibiotic / total number of strains in the population







Paper using the method

Main conclusions

- Bacteria from WW and downstream river showed **high resistance to most antibiotics**
- Despite treating only OTC wastewater, bacteria in the WWTP and downstream river did not show higher tetracycline resistance compared to other antibiotics (resistant to all)
- Most wastewater bacteria (86.8%) had **OTC MICs higher than the residual OTC** in the effluent (need more OTC to be killed than what is found in water)
- **More than 96 %** of the WW and RWD isolates exhibited **MDR**.

Research question: What are the resistance mechanisms used by bacteria against OTCs?

TABLE 3. Prevalence of bacterial isolates carrying *tet* genes in bacterial populations in wastewater and river water downstream

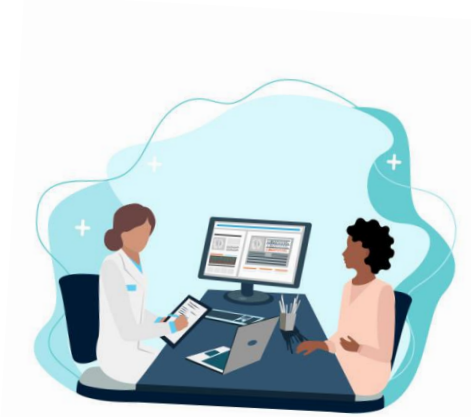
Mechanism of drug resistance[b]	Resistance gene	Prevalence of isolates (%) in[a]: WW	RWD
Efflux	*tet*(A)	69.3	62.1
	tet(B)	6.3	6.9
	tet(C)	42.9	21.8
	tet(D)	26.5	33.3
	tet(E)	2.1	0
	tet(G)	0	0
	tet(H)	0.5	1.1
	tet(J)	32.3	44.8
	tet(Z)	23.3	9.2
	tet(30)	2.6	9.2
	tet(K)	23.3	16.1
	tet(L)	37.6	31.0
	tet(AP)	11.6	12.6
	tet(Y)	22.2	27.6
Ribosomal protection	*tet*(M)	12.2	18.4
	tet(O)	9.0	16.1
	tet(S)	6.3	33.3
	tet(W)	28.0	67.8
	tet(Q)	0	0
	tet(T)	4.2	5.7
	otr(A)	16.9	8.0
	tet(BP)	0.5	0
Enzymatic modification	*tet*(X)	0	0

[a] WW, wastewater effluent; RWD, downstream river water.
[b] Efflux is the mechanism of utilizing energy-dependent membrane-associated efflux proteins which export tetracycline out of the cell; ribosomal protection is the mechanism of utilizing ribosomal protection proteins which interact with the ribosome and disrupt the tetracycline binding site; and enzymatic modification is the mechanism of utilizing tetracycline inactivation enzymes.

Possible solutions to mitigate antibiotic resistance

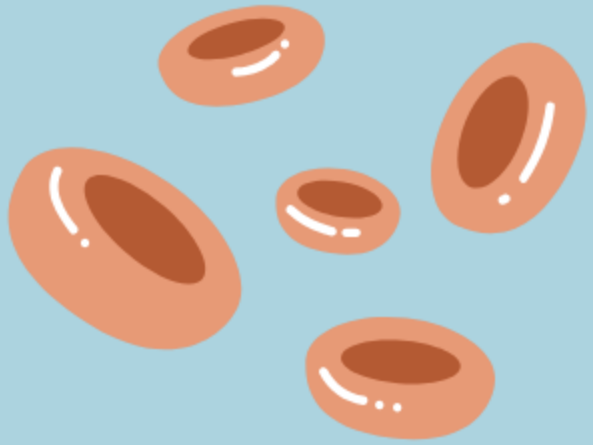
Main solutions being developed [18] [19]

- Improvements in **antibiotic stewardship** (making sure antibiotics are only prescribed when necessary)
- Development of **narrow-spectrum antibacterial agents**
- Development of **anti-virulence drugs:** preventing bacterial pathogenesis
- Development of **adjuvants that suppress bacterial resistance mechanisms**



Thank you for your attention !

Questions?



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