Antibiotics Sensitivity Screening

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ENV - 412

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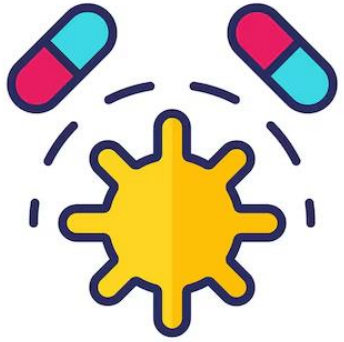
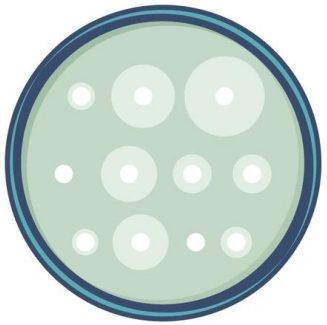


Antibiotic resistance



Antibiotic sensitivity screening

Scientific research and paper review

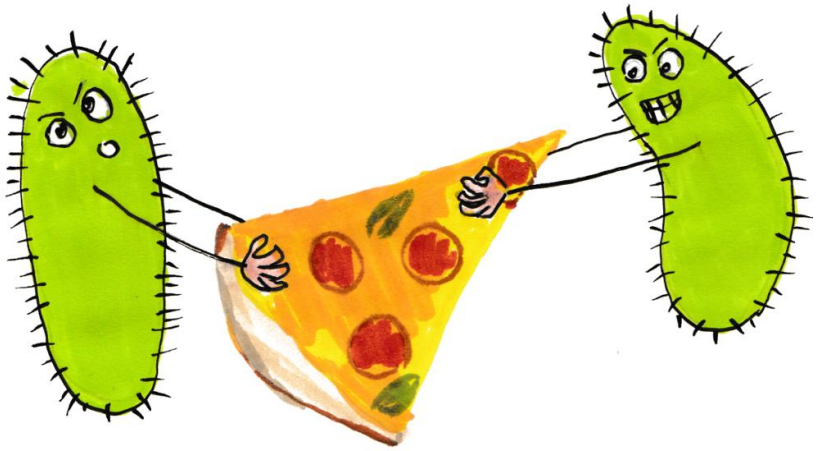
Introduction to antimicrobials

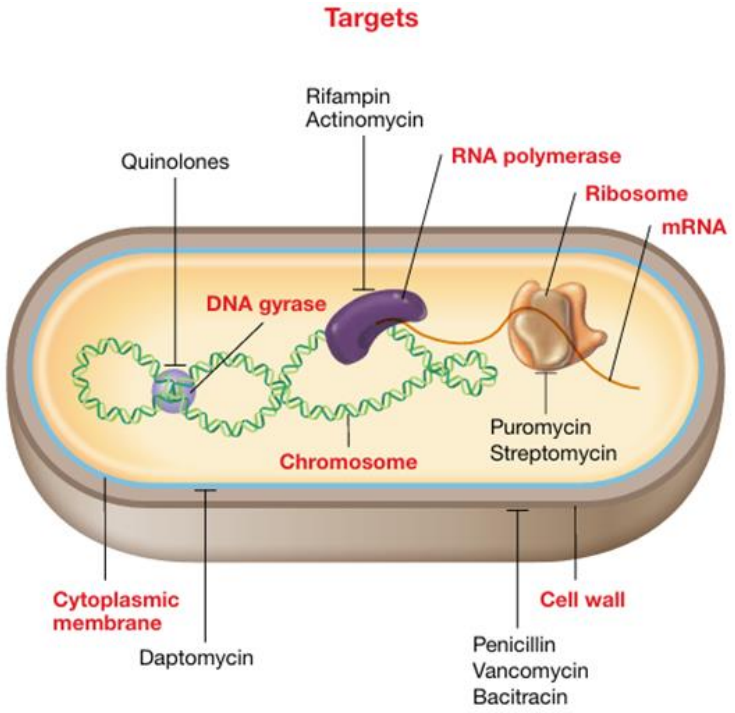
"Umbrella" term to indicate everything that inhibit or kills microbes. Includes **antibiotics** (which target bacteria), antifungals (which target fungi) and antivirals (which target viruses).

Antibiotics are antimicrobial agents naturally produced by microorganisms, characterized by their ability to kill (bactericidal) or inhibit (bacteriostatic) a bacteria's growth.

Classification according to their target:

- Antibiotics that target major molecular processes
- Antibiotics that target cell membrane and wall

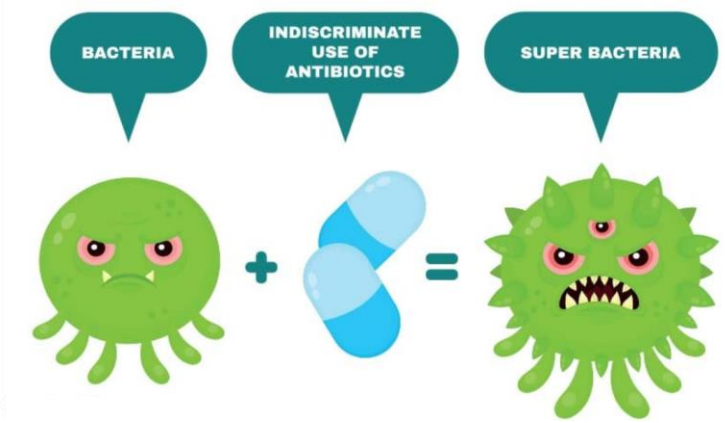




- Some are highly specialized, other can attack a wide range of bacteria.
- Important role in microbial interactions

Antibiotic resistance – background

Antibiotic resistance is a phenomenon where bacteria develop mechanisms to evade the effects of antibiotics
→ Bacteria no longer respond to antibiotics that were previously effective against them.



The **World Health Organization (WHO)** considers antimicrobial resistance to be among the top 10 global public health threats facing humanity and claims that the world is "running out of antibiotics".

The Antibiotic Resistance Crisis

- Over 70% of pathogenic bacteria are resistant to at least one commercially available antibiotic,
- By 2050, antibiotic-resistant infections are projected to cause up to 10 million deaths annually, with an economic burden of approximately $100 trillion globally,
- Antibiotic resistance complicates treatments like organ transplants, chemotherapy, and surgeries such as cesarean sections, increasing the risks.

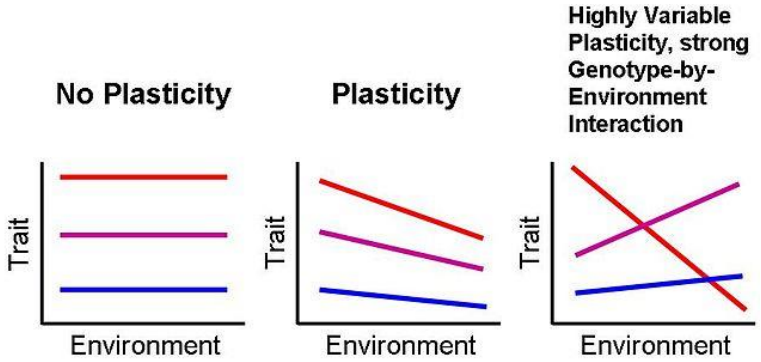
Main causes:

- Spread of resistance genes across bacterial species (health care centers, trade, lack of sanitation, …)
- Overuse of broad-spectrum antibiotics in *human medicine* and in *agriculture*,
- Lack of rapid diagnostic tools → inappropriate prescribing,
- Availability of few new antibiotic (due to pharmaceutical companies' reluctance)

Antibiotic resistance – Mechanisms

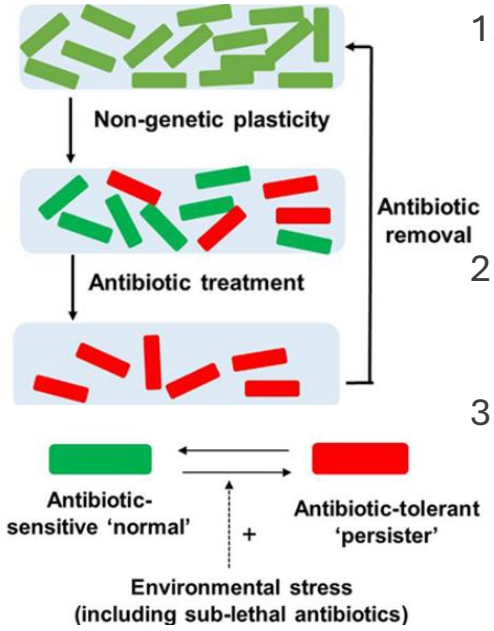
The bacterial response to antibiotics is the prime example of **bacterial adaptation and peak of evolution**.

→ Consequence of immense **genetic plasticity** of bacteria that allows bacteria to respond to a wide array of environmental threats



Genetic strategies of adaptation:

➢ Mutations in gene(s) associated with the mechanism of action of the antibiotic



1. A subset of cells from a susceptible population develops mutations in genes that affect the activity of the antibiotic,
2. The antibiotic eliminate the susceptible population, and the resistant mutant predominate,
3. Often the mutational changes are costly to cell homeostasis and are only maintained if the antibiotic is present.

➢ Acquisition of foreign DNA coding for resistance through horizontal gene transfers (HGT)

Environmental resistome: the collective set of all antibiotic resistance genes present in environmental microbial communities.

→ Resistance already present

→ Prolific source of antibiotic resistance genes

Transformation, i.e. incorporation of naked DNA

Integrons, i.e. integration of small mobile genetic elements called gene cassettes

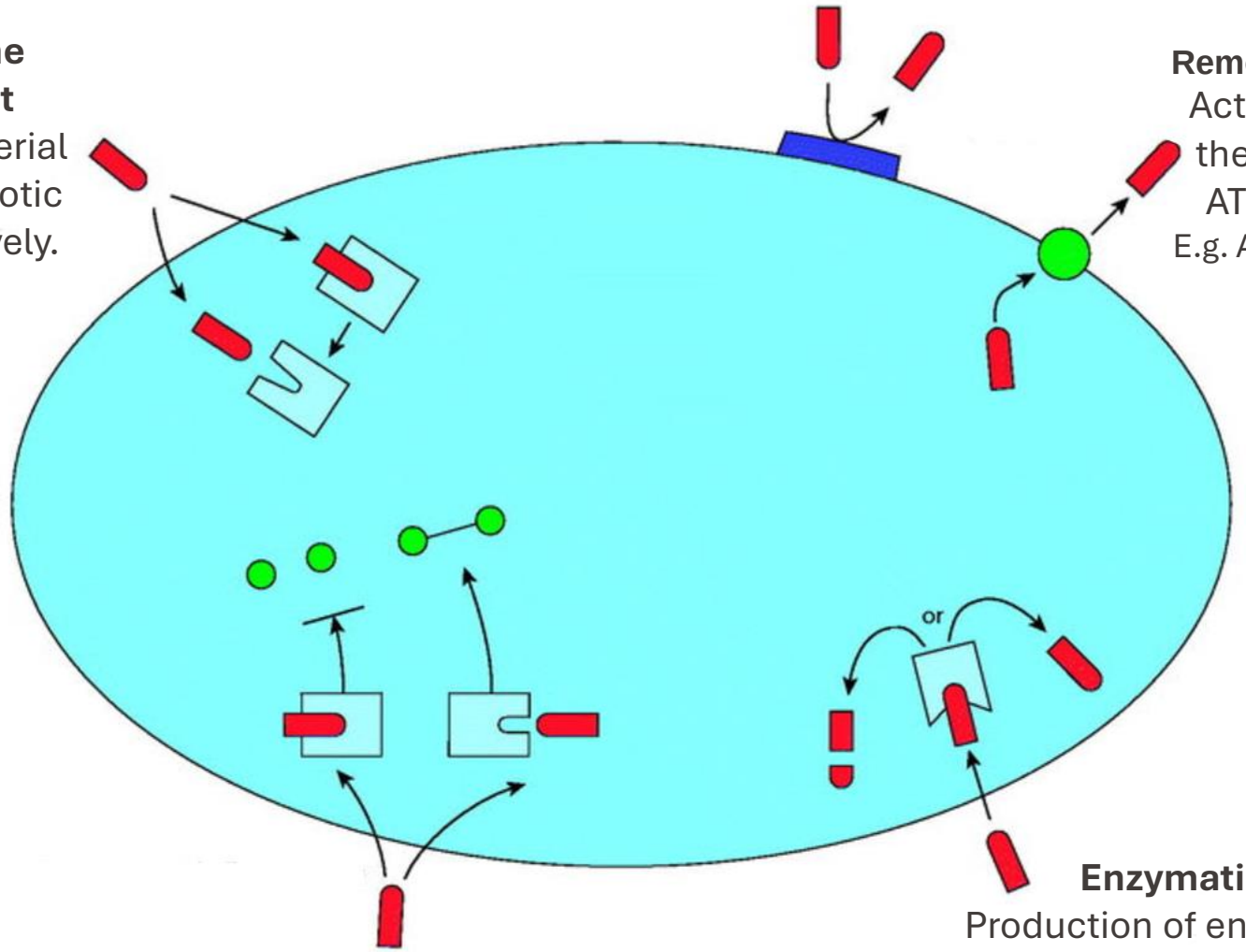
Conjugation, cell-to-cell contact using mobile genetic elements to share genetic information

Transduction, phage (virus) mediated

Antibiotic resistance – Mechanisms

Decreasing permeability → Decrease penetration

Modification of the antibiotic's target
Changes in the bacterial preventing the antibiotic from binding effectively.



Removal from the Cell via Efflux Pumps
Active transport of antibiotics out of the bacterial cell using energy from ATP hydrolysis or proton gradients
E.g. ABC pumps and proton gradient driven pump

Metabolic bypasses
Changes in the bacterial metabolic pathways to circumvent the inhibitory effects of the antibiotic.

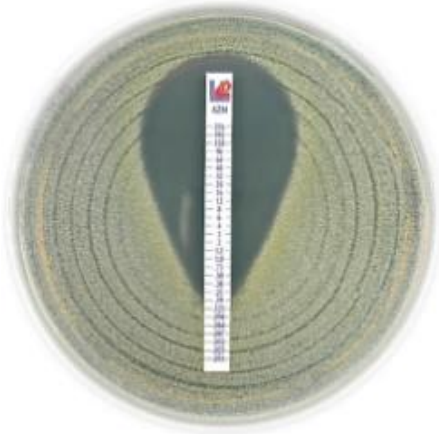
Enzymatic inactivation
Production of enzymes that modify or degrade the antibiotic, rendering it ineffective
E.g. Beta-lactamases (penicillins)

Antibiotic sensitivity screening

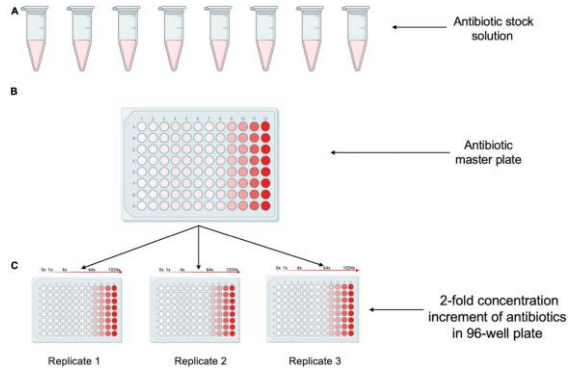
Several ways to test the efficacy of antibiotics against a bacterial isolate:

- **Disc diffusion**

- Antibiotic strip test



- Broth dilution



Disc diffusion test

Involves creating a lawn of bacteria on an agar plate and then placing an antibiotic disc or discs onto the lawn before incubation overnight.

4 main steps:

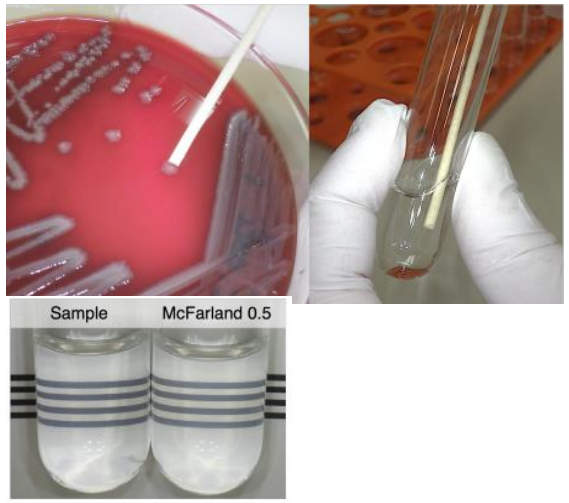
1. Aseptically picking a few bacterial colonies from a culture and suspend them in a sterile saline solution, giving a density equal to 0.5McFarland standard.

2. Dipping a cotton swab into the suspension, and then spread it to an agar plate

3. Using sterile forceps, apply antibiotic discs

4. Press the discs onto the agar, invert the plate and incubate them for 16-20h at 35°C

1



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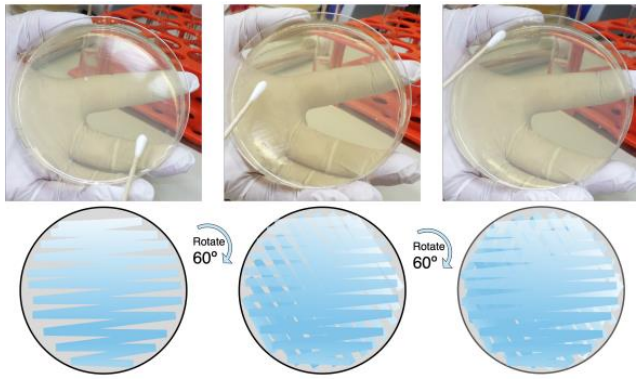

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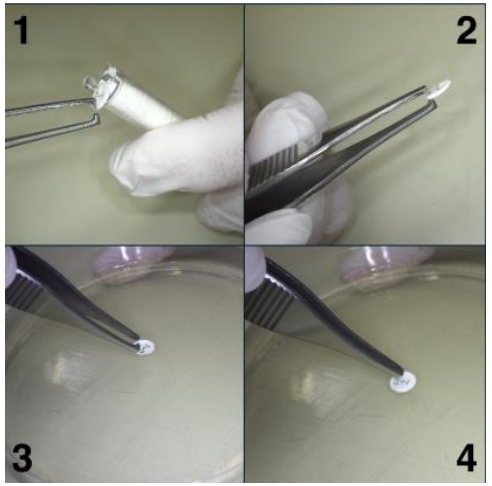

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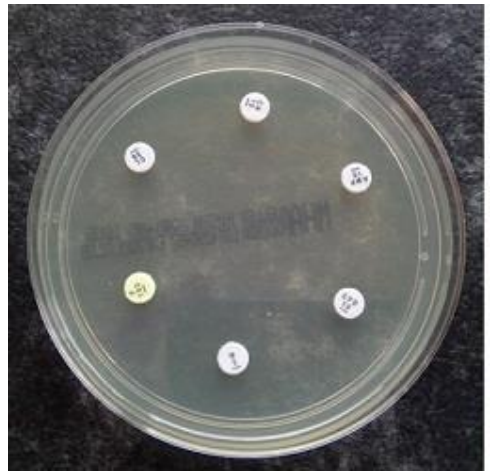
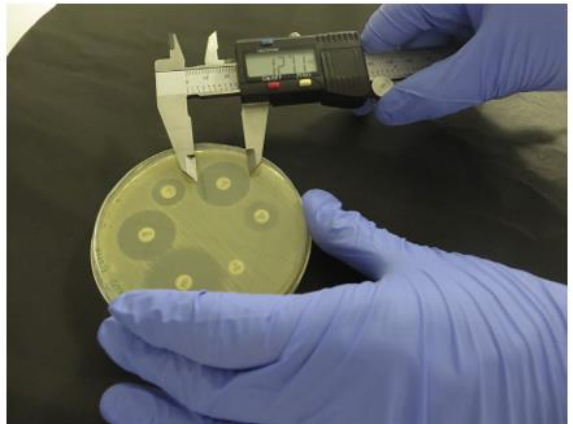


Results and analysis

1. After incubation, measure the zones of inhibition around the discs, using a caliper or a ruler.



2 . Compare the obtained values with ones published in breakpoint tables. Breakpoint is the size of the zone of inhibition below which a bacterium is resistant.

Penicillins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin								1. For information on how to implement the new aminopenicillin breakpoints, see https://www.eucast.org/eucastguidancedocuments/.
Ampicillin iv[1]	8	8		10	14[A]	14[A]		2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Ampicillin oral (uncomplicated UTI only)[1]	8	8		10	14[A]	14[A]		3(D). For information on how to use breakpoints in brackets, see https://www.eucast.org/eucastguidancedocuments/.
Ampicillin-sulbactam iv[2]	8[2]	8[2]		10-10	14[A]	14[A]		4. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Ampicillin-sulbactam oral (uncomplicated UTI only)[1]	8[2]	8[2]		10-10	14[A]	14[A]		5. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Amoxicillin iv[1]	8	8		-	None[B]	None[B]		6. Agar dilution is the reference method for mecillinam MIC determination.
Amoxicillin oral (infections originating from the urinary tract)[1]	0.001	8		-	None[C]	None[B]		A. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars.
Amoxicillin oral (uncomplicated UTI only)[1]	8	8		-	None[B]	None[B]		B. Susceptibility inferred from ampicillin (iv or oral).
Amoxicillin oral (other indications)[1]	(8)[2]	(8)[2]		-	None[A,C]	None[B,C]		C. Isolates susceptible to ampicillin (iv or oral) can be reported "susceptible, increased exposure" (I) to "amoxicillin oral (infections originating from the urinary tract)". Isolates resistant to ampicillin (iv or oral) can be reported resistant to "amoxicillin oral (infections originating from the urinary tract)".
Amoxicillin-clavulanic acid iv[2]	8[2]	8[2]		20-10	19[A]	19[A]	19-20	D. Infer from ampicillin oral, but the report should explain the meaning of breakpoints in brackets.
Amoxicillin-clavulanic acid oral (infections originating from the urinary tract)[1]	0.001[2]	8[2]		20-10	50[A]	19[A]	19-20	E. Infer from ampicillin oral.
Amoxicillin-clavulanic acid oral (uncomplicated UTI only)[1]	32[2]	32[2]		20-10	16[A]	16[A]		F. Ignore isolated colonies within the inhibition zone.
Amoxicillin-clavulanic acid oral (other indications)[1]	(8)[2,3]	(8)[2,3]		20-10	(19)[A,D]	(19)[A,D]	19-20	
Piperacillin	8	8		30	20	20		
Piperacillin-tazobactam	8[2]	8[2]	16	30-6	20	20	19	
Ticarcillin-clavulanic acid	8[2]	16[2]		75-10	23	20		
Temocillin (infections originating from the urinary tract), *E. coli, Klebsiella* spp. (except *K. aerogenes*) and *P. mirabilis*	0.001	16		30	50[F]	17[F]		
Phenoxymethylpenicillin	-	-			-	-		
Oxacillin	-	-			-	-		
Cloxacillin	-	-			-	-		
Dicloxacillin	-	-			-	-		
Flucloxacillin	-	-			-	-		
Mecillinam oral (pivmecillinam) (uncomplicated UTI only), *E. coli, Citrobacter* spp., *Klebsiella* spp., *Raoultella* spp., *Enterobacter* spp. and *P. mirabilis*	8[2]	8[2]		10	15[F]	15[F]		

3 . Plot the results in a bar graph, to visualize them quickly

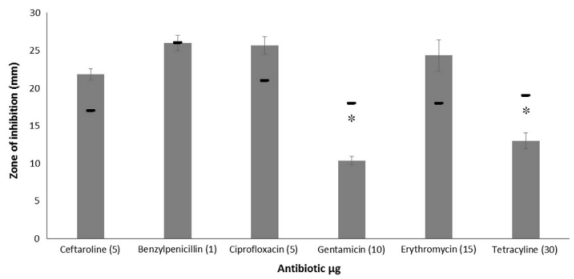
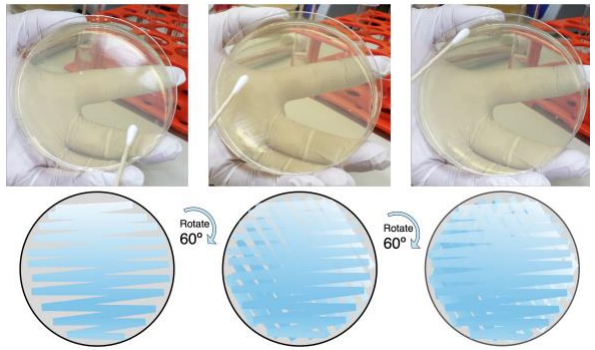


Figure 4.5 Zones of inhibition (in millimetres) generated by a range of antibiotics against one strain of *Staphylococcus aureus*. The *black horizontal lines* represent the breakpoint beneath which isolates are resistant (marked with *asterisks*). Error bars represent standard deviations of the mean.

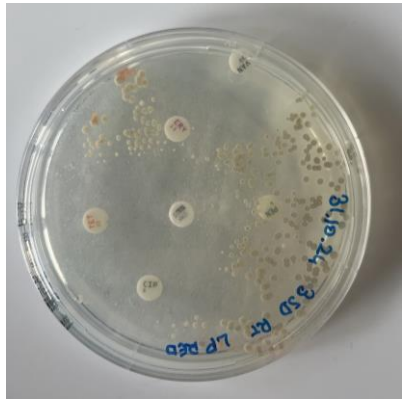
Common mistakes

- Not swabbing the whole of the plate with the inoculum → not getting an even lawn of bacteria



- Antibiotic discs falling from the agar before incubation → missing data

- Non sterile conditions → contamination, results may be not reliable



- Inhibition zone of an antibiotic disc influencing another inhibition zone → other options to measure the zone diameter

Scientific research and paper review

- *Antibiotic sensitivity screening methods* are most used in the field of clinical microbiology, pharmacology, and biotechnology → focus on combating antimicrobial resistance for health purposes.

- In environmental ecology and microbiology:
  - Environmental monitoring applications,
  - Investigations regarding wastewater as a source of antibiotic resistance

Seasonality impels the antibiotic resistance in Kelani River of the emerging economy of Sri Lanka

Manish Kumar[1] ✉, G. G. Tushara Chaminda[2] and Ryo Honda[3]

nature npj | clean water

Published: 13 April 2020

Antibiotic sensitivity screening application:

- Water samples are collected from various points along the Kelani River to isolate *E. coli* strains.
- Evaluation of resistance against six antibiotics: norfloxacin, ciprofloxacin, levofloxacin (fluoroquinolones), and kanamycin monosulfate, tetracycline, and sulfamethoxazole (non-fluoroquinolones).

Scientific research and paper review

Seasonality impels the antibiotic resistance in Kelani River of the emerging economy of Sri Lanka

Manish Kumar[1], G. G. Tushara Chaminda[2] and Ryo Honda[3]

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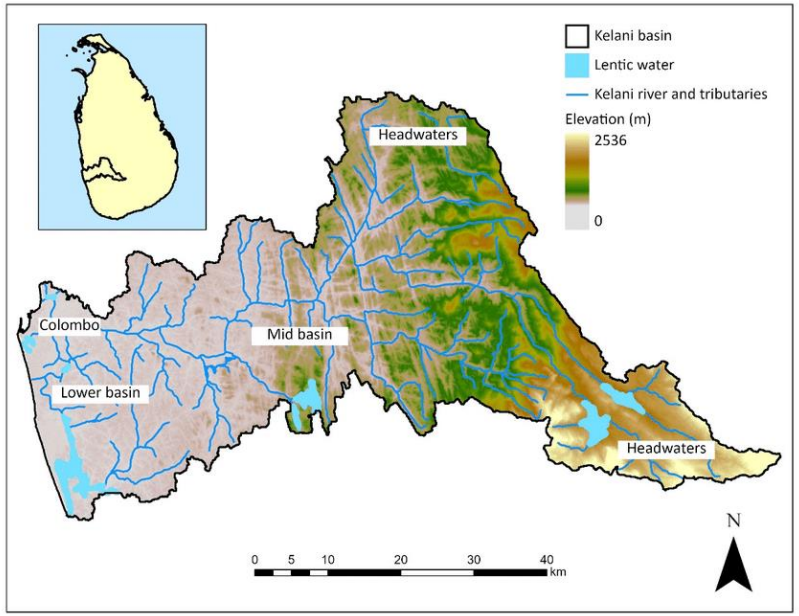
Context:

- Sri Lanka is an island country located in the Indian Ocean in South Asia. It shares maritime borders with India to the northwest and the Maldives to the southwest.
- The Kelani River flows through the capital city Colombo and provides 80% of its drinking water.
- The climate is marked by distinct wet and dry periods, without a winter season (tropical climate) and with a strongly variable rain distribution.
- Sri Lanka experiences two primary monsoon seasons:
  - Southwest Monsoon (Yala), from May to September, affects the southwest and western parts of the island, including Colombo and the south and west coasts,
  - Northeast Monsoon (Maha),from December to February, predominantly impacts the northeast and eastern parts of the island, including the dry zone.



Scientific research and paper review

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Background and previous researches:

- Rivers play an important role in the economic growth of an emerging country and often have social and religious significance.
- Rivers often serve as a disposal site for waste
  - → Rivers receive large amount of antibiotics and bacteria that are resistant to them, mainly through wastewater
- Previous studies have reported the increase of antibiotic-resistant genes (ARG) and bacteria (ARB) with the increase of antibiotics, nutrients, metals, and microplastics contaminating water bodies.
- In rivers, the transport of ARB and ARG is very dynamic, but the influence of seasonality has not yet been investigated → paper motivation

Hypothesis:

"Seasonality likely has significant influence on antibiotic resistance in rivers of monsoon-dominated tropical countries with emerging countries"

Objective 1: Quantification of seasonal variations between wet and dry season by using *E.Coli* ARB and AGR

Objective 2: Understand the underlying factors governing the variation in the relation between in situ parameter, metal, E.Coli prevalence and antibiotic resistance through multivariate statistical techniques

Scientific research and paper review

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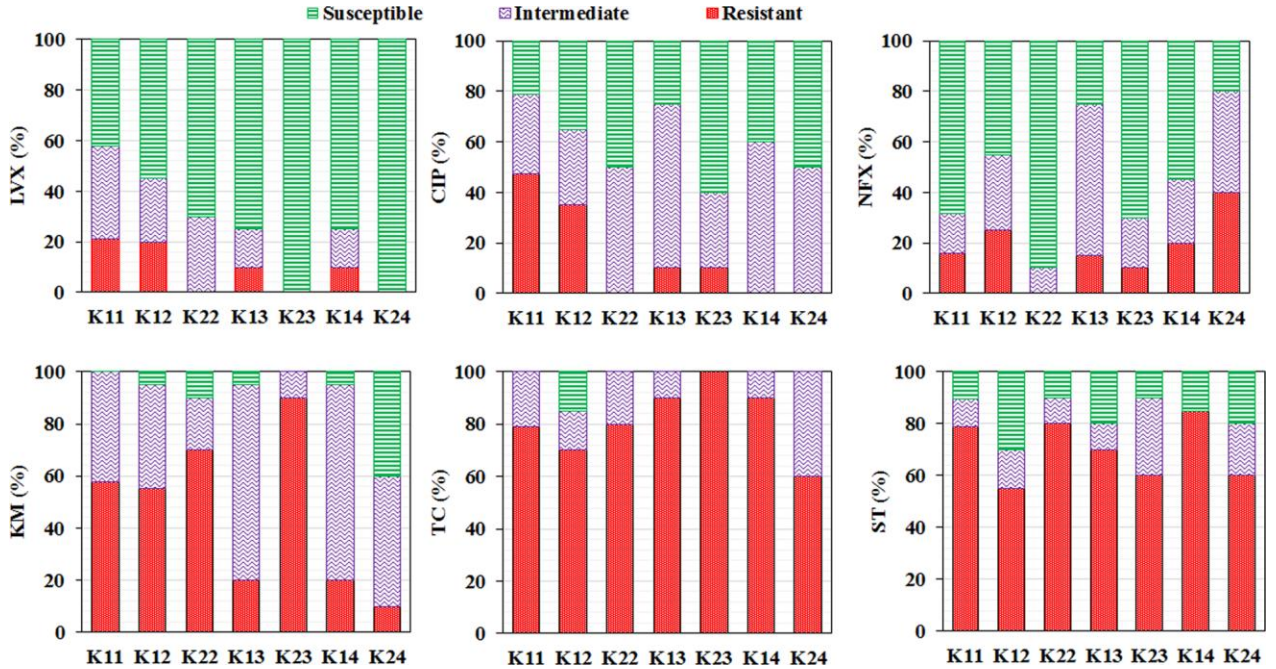
Presentation of results

- The study found that E. coli isolates in the Kelani River exhibited high levels of antibiotic resistance → The prevalence of E. coli ranged from 10 to 27 colony-forming units (CFU) per milliliter, with most isolates resistant to more than one antibiotic.
- The resistance to older antibiotics such as tetracycline (TC) and sulfamethoxazole (ST) was significantly higher compared to newer antibiotics like norfloxacin, ciprofloxacin, and levofloxacin.
  → Older antibiotics have been more extensively used, leading to higher resistance levels



Scientific research and paper review

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Presentation of results

- Seasonal changes had a significant impact on antibiotic resistance. The resistance to fluoroquinolones showed higher seasonal variation compared to non-fluoroquinolones. The effect was antagonistic, meaning that while resistance to some antibiotics increased in certain seasons, it decreased in others.
- The researchers detected genes that confer resistance to tetracyclines, sulfonamides, β-lactams, and fluoroquinolones. The number of antibiotic resistance genes (ARGs) copies increased during the dry season compared to the wet season.

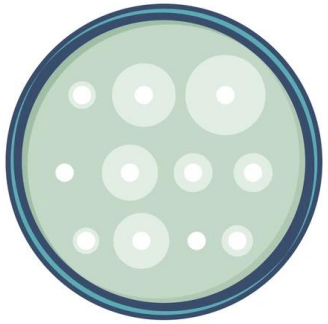
→ Given that the Kelani River is a crucial source of drinking water for a significant portion of the Sri Lankan population, the high levels of antibiotic-resistant bacteria pose a serious threat to public health.

EPFL

Scan to read the paper

Thank you for your attention

Questions?



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