



Food and Agriculture  
Organization of the  
United Nations

FMM/RAS/298: Strengthening capacities, policies and national action plans on  
prudent and responsible use of antimicrobials in fisheries Final Workshop  
in cooperation with AVA Singapore and INFOFISH  
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# Mechanisms and Pathways of AMR in the environment

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## Cell Wall Synthesis

### Beta Lactams

Penicillins  
Cephalosporins  
Carbapenems  
Monobactams

### Vancomycin

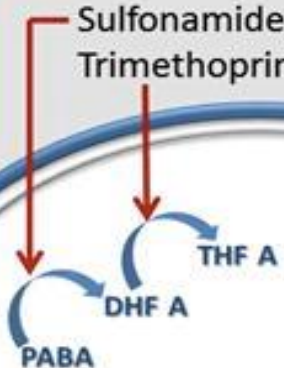
Bacitracin

### Cell Membrane

Polymyxins

## Folate synthesis

Sulfonamides  
Trimethoprim



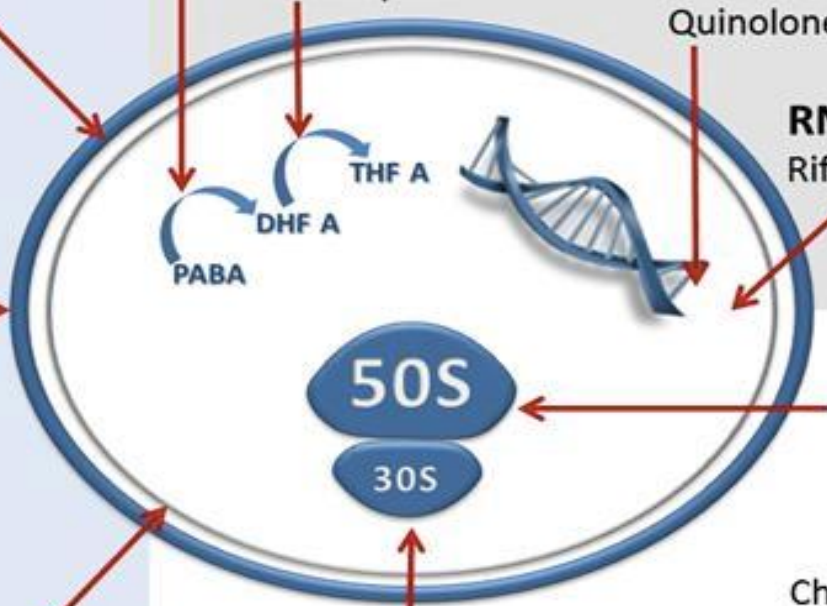
## Nucleic Acid Synthesis

### DNA Gyrase

Quinolones

### RNA Polymerase

Rifampin



### 50S subunit

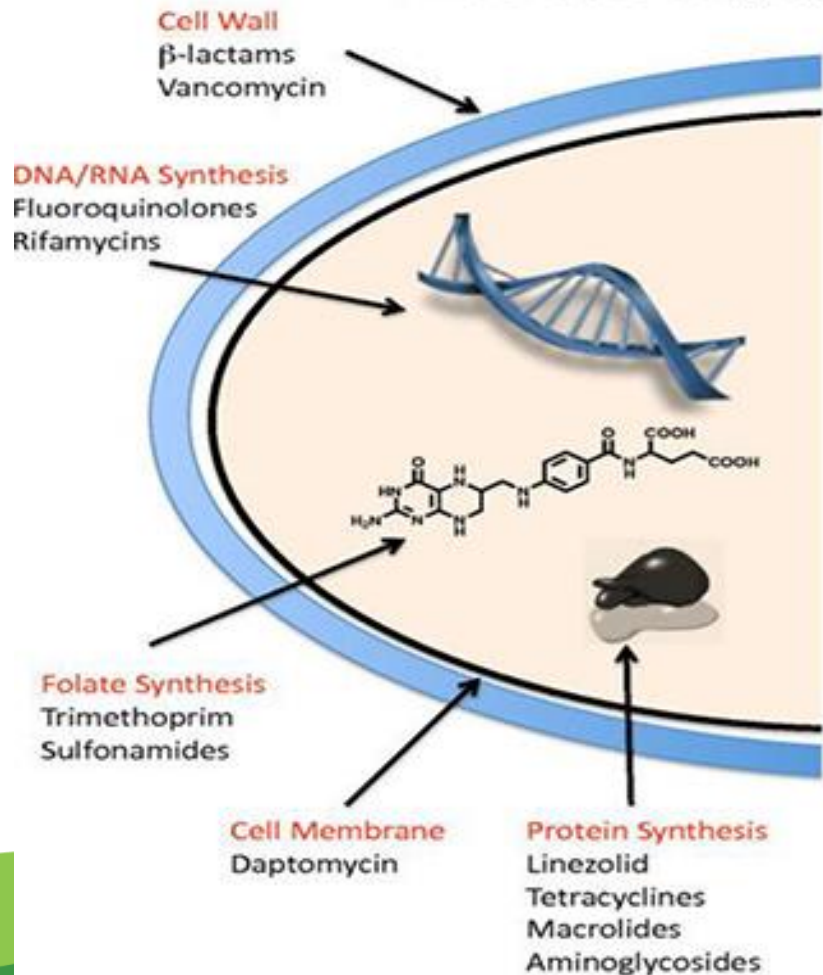
Macrolides  
Clindamycin  
Linezolid  
Chloramphenicol  
Streptogramins

### 30S subunit

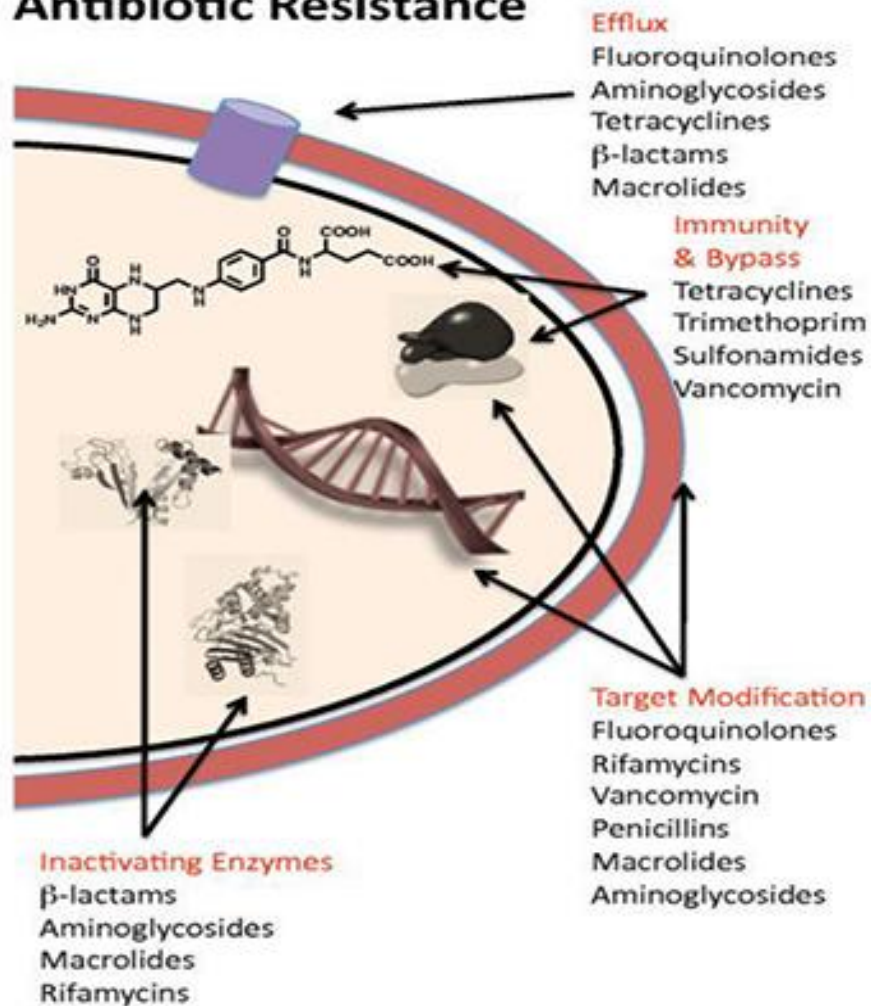
Tetracyclines  
Aminoglycosides

## Protein Synthesis

## Antibiotic Targets



## Antibiotic Resistance



# CONSEQUENCES OF OVERUSE OR MISUSE OF ANTIBIOTICS



## How Antibiotic Resistance Happens

1.

Lots of germs.  
A few are drug resistant.



2.

Antibiotics kill  
bacteria causing the illness,  
as well as good bacteria  
protecting the body from  
infection.



3.

The drug-resistant  
bacteria are now allowed to  
grow and take over.



4.

Some bacteria give  
their drug-resistance to  
other bacteria, causing  
more problems.



## Antimicrobial resistance is ancient, natural and is found in environments with no exposure to antibiotics

- Viable multidrug-resistant bacteria have been cultured from the Lechuguilla Cave in New Mexico, totally isolated for >4 million years (Bhullar et al., 2012).
- Antibiotic resistant marine bacteria have been found as far as 522KM offshore and in deep sea at depths of 8200m (Aminov, 2011).
- Evolution of antibiotic resistance genes predates evolution of Actinomycetes.
- Some of the antibiotic resistance genes have not evolved to protect against antibiotics but have other metabolic functions.



## Resistance genes have other functions in the cell

- ampC beta-lactamase is involved in maintaining normal morphology in *Escherichia coli*.
- Efflux pumps are involved in efflux of several compounds
- bla<sub>oxy</sub> beta-lactamase has metabolic function in *Klebsiella oxytoca*



## Resistance genes found in environmental bacteria without exposure to antibiotics

- *qnr* gene conferring resistance to quinolones are found in marine bacteria like *Shewanella algae* and *Vibrio* spp.
- CTX-M beta-lactamase is present in environmental bacteria like *Kluyvera*



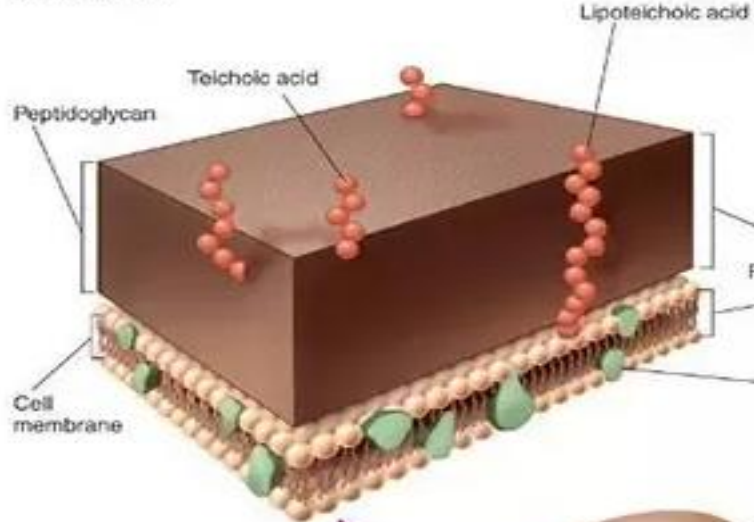
# Some bacteria have intrinsic resistance

- The intrinsic resistance of a bacterial species to a particular antibiotic is the ability to resist the action of that antibiotic as a result of inherent structural or functional characteristics.
- For example, the glycopeptide antibiotic vancomycin inhibits peptidoglycan crosslinking by binding to target d-Ala-d-Ala peptides, but in Gram-negative organisms, it cannot cross the outer membrane and access these peptides in the periplasm.
- *Aeromonas* has intrinsic beta-lactamases which makes them resistant to ampicillin.

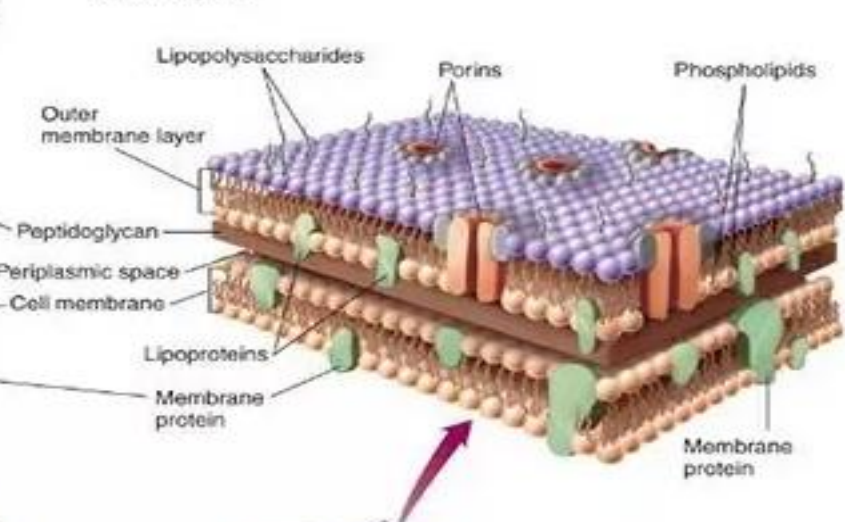




**Gram-Positive**



**Gram-Negative**



# Acquired antimicrobial resistance

- When microorganisms once sensitive to an antimicrobial agent become resistance to that particular antibiotic, the resistance is acquired.
- The acquired resistance could be due to genetic changes such as mutations or acquisition of genes contributing to resistance through horizontal gene transfer.

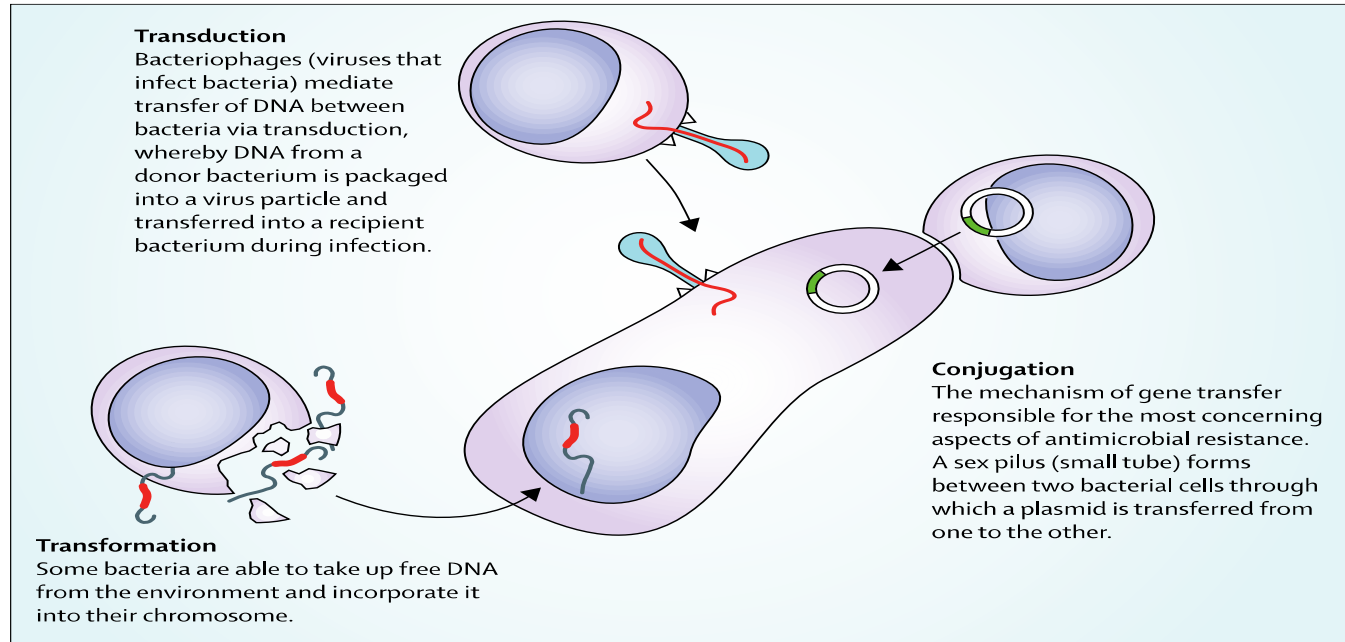


**TABLE 1.** Types of resistance observed in bacteria

	Intrinsic resistance	Acquired resistance
<b>Definition</b>	<ul style="list-style-type: none"><li>• Natural traits</li><li>• Species or genus specific</li></ul>	<ul style="list-style-type: none"><li>• A strain that develops resistance to an antimicrobial to which it was previously susceptible</li><li>• Present only in certain strains of a species or genus</li></ul>
<b>Mechanisms of resistance acquisition</b>	<ul style="list-style-type: none"><li>• Inherent structural or functional characteristics of the bacteria that allow it to tolerate or be insensitive to an antimicrobial substance or class</li></ul>	<p>Vertical transmission</p> <ul style="list-style-type: none"><li>• Spontaneous gene mutation</li><li>• Induced gene mutation</li></ul> <p>Horizontal gene mutation</p> <ul style="list-style-type: none"><li>• Bacterial transformation</li><li>• Bacterial transduction</li><li>• Bacterial conjugation</li></ul>

Source: Boerlin and White, 2013





**Figure 1: Transmission of genetic material between microorganisms**

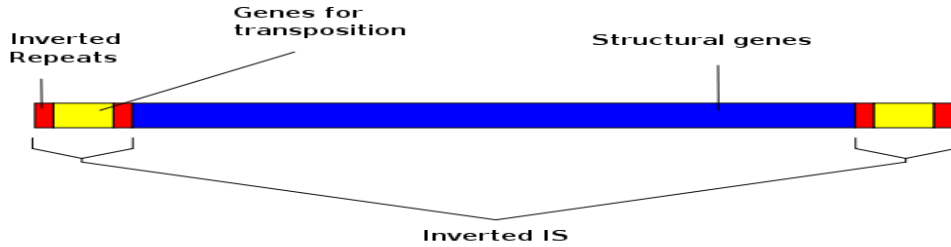
Genetic material is transferred between microorganisms through three main routes: (i) transformation—examples include recombination of foreign DNA from *Streptococcus mitis* to *Streptococcus pneumoniae*, conferring penicillin resistance via the formation of mosaic genes, and *Neisseria gonorrhoeae* where a mosaic *penA* gene is associated with ceftriaxone resistance;<sup>38</sup> (ii) transduction—where antimicrobial resistance genetic material has been identified in phage DNA isolated from waste water treatment,<sup>36</sup> and both extended-spectrum  $\beta$ -lactamase genes and *mecA* genes (the latter responsible for methicillin resistance in *Staphylococcus aureus*) have been identified in bacteriophage extracted from faecal samples at farms and abattoirs;<sup>37</sup> and (iii) conjugation—with plasmids being responsible for example for the global dissemination of genes encoding carbapenemases such as New Delhi metallo- $\beta$ -lactamase,<sup>15</sup> as well as ES $\beta$ Ls.<sup>39</sup> Furthermore integrative chromosomal elements (ICEs) can transfer these resistance genes between plasmids and the bacterial host chromosome in a range of Gram-negative species and streptococci.<sup>40</sup>

## Acquired antimicrobial resistance

- Antibiotic resistance genes may be transferred through in mobile genetic elements such as plasmids, transposons, bacteriophages, genomic islands or integrons.
- Though integrons are not self-mobile, they contain gene cassettes that are mobile.

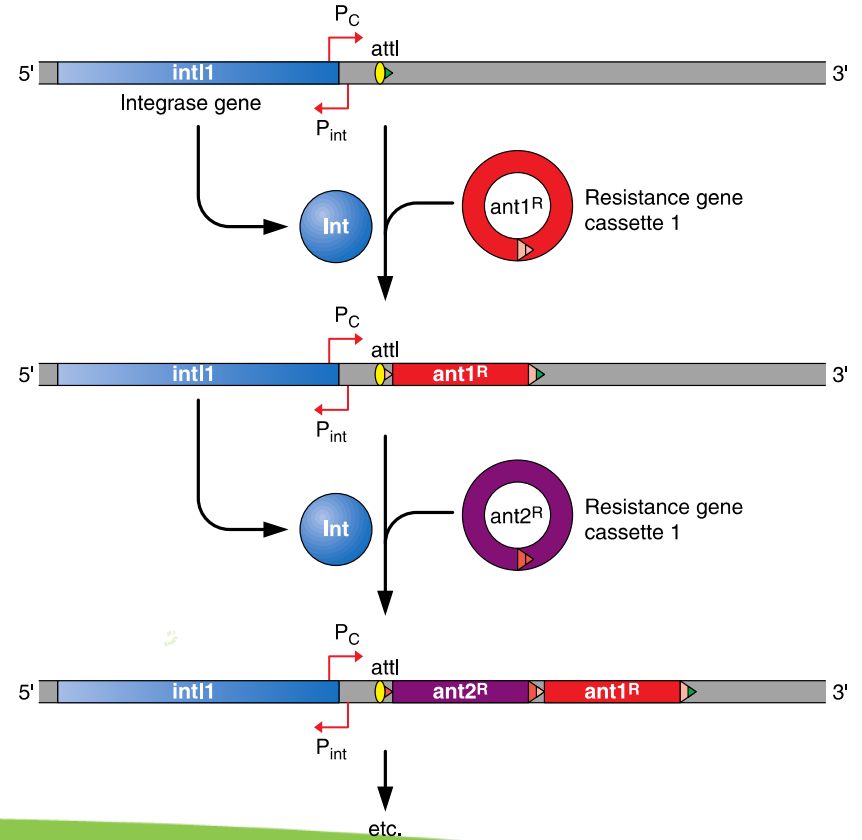
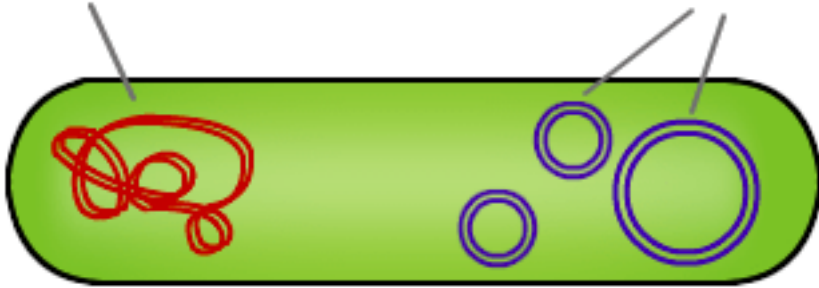


### Bacterial composite transposon



### Bacterial DNA

### Plasmids



## Phenotypic resistance and mechanisms of resistance

- When same phenotypic resistance is detected in two isolates eg one from aquatic environment and another from a clinical case, the two isolates may have different resistance genes.
- Eg tetracycline resistance could be due to (a) over production of efflux proteins or (b) production of ribosomal protection proteins or (c) production of tetracycline inactivating proteins



# Limitations of phenotypic methods

- Isolation of bacteria 24-48h
- Susceptibility testing 24h
- Some antibiotic genes could be silent, but could be a source for gene spread





# Genotypic methods

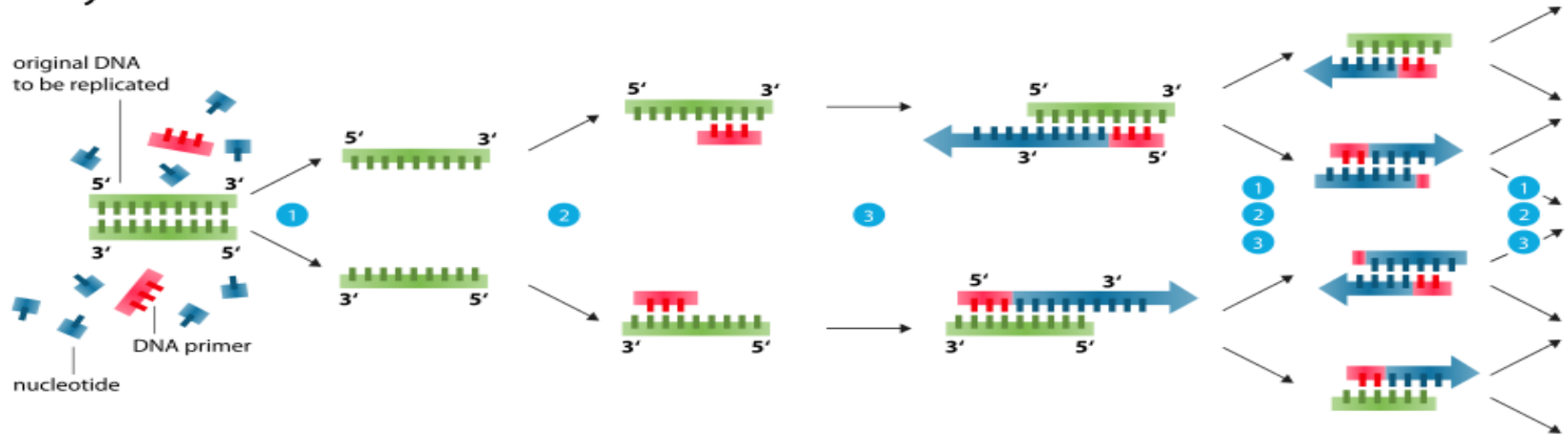
- Use modern molecular biology techniques to detect and characterise genes encoding antibiotic resistance
- No need for bacterial growth- rapid could be within 4 hrs
- Sensitive and point of care



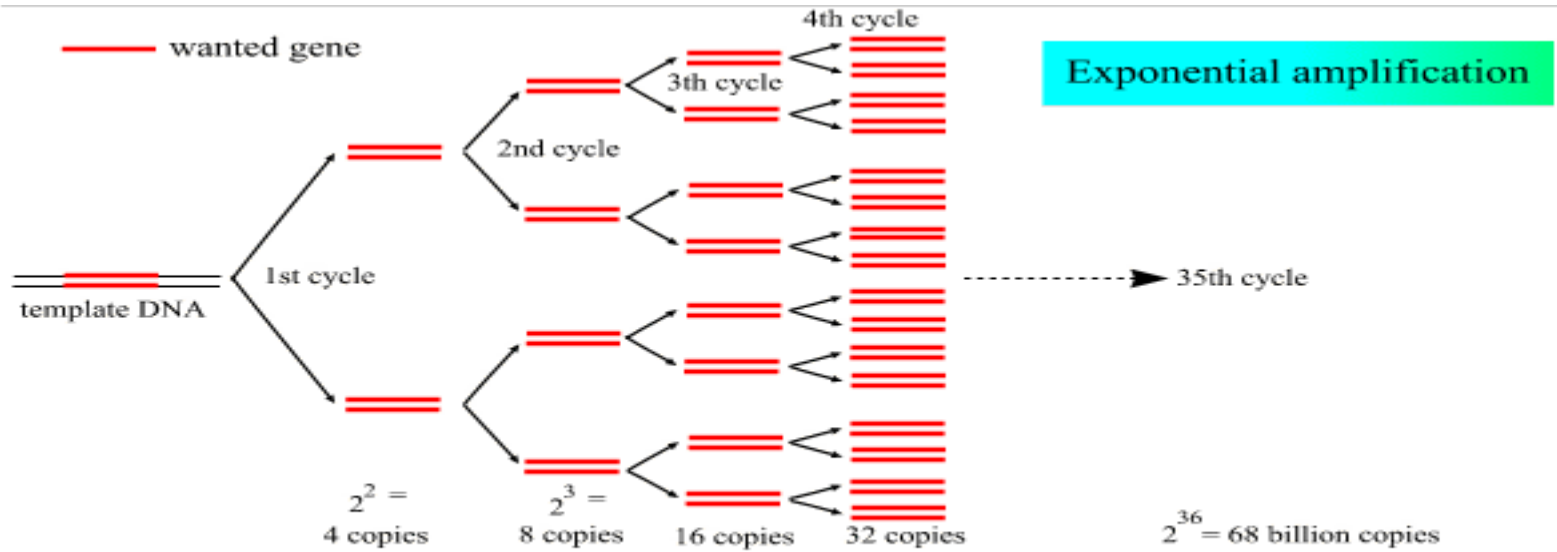
# Polymerase chain reaction (PCR)

○ In vitro nucleic acid amplification method

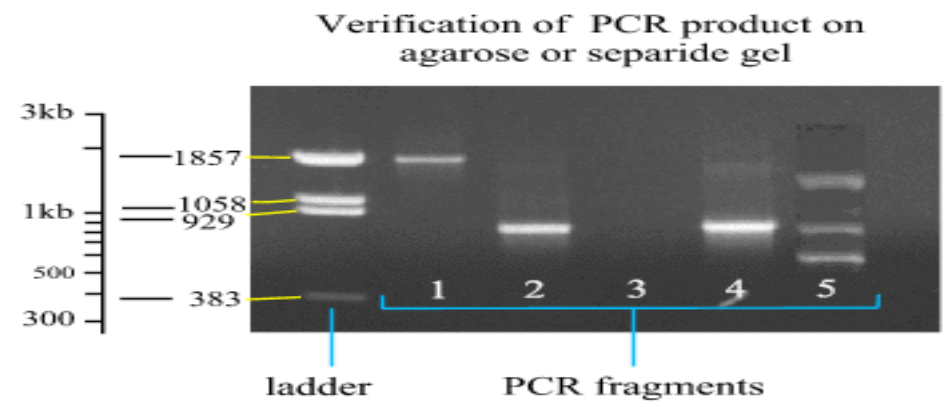
## Polymerase chain reaction - PCR



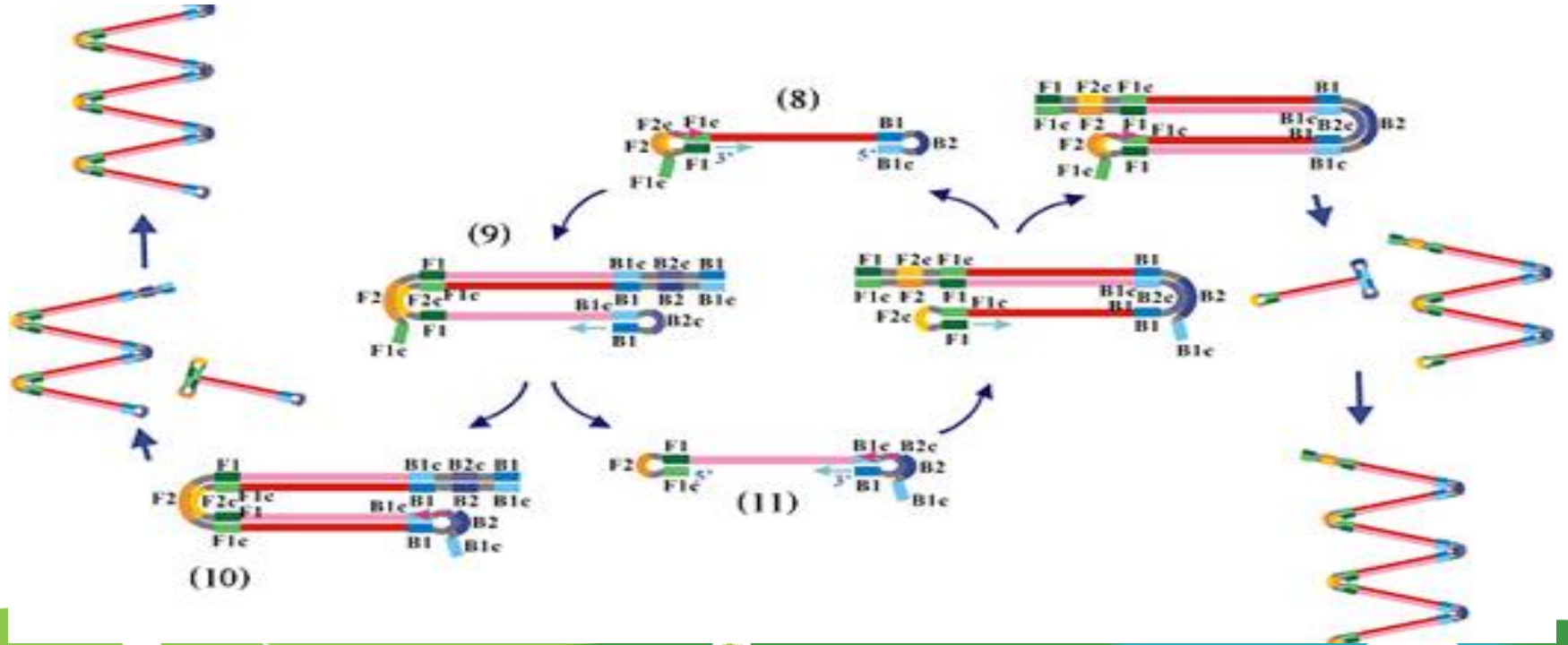
- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C



*The exponential amplification of the gene in PCR.*

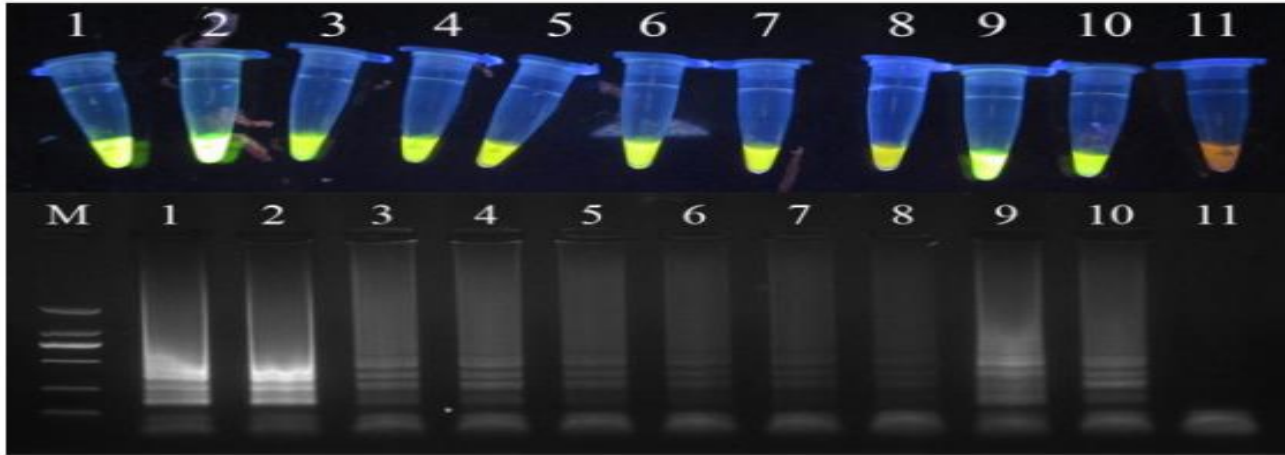


# Loop-mediated Isothermal amplification (LAMP)

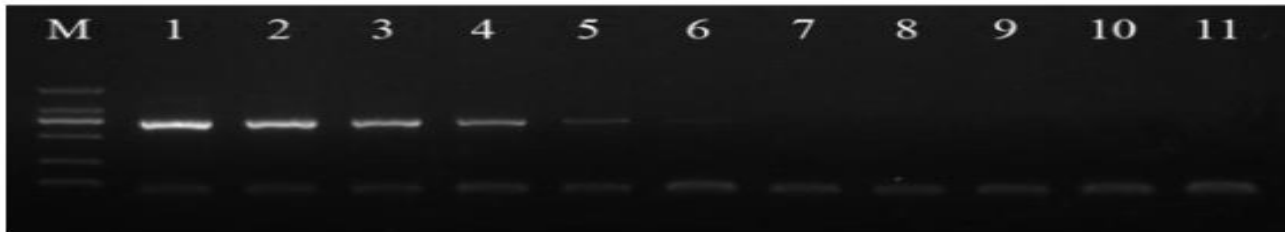


# LAMP- sensitivity and advantages

A



B







Food and Agriculture  
Organization of the  
United Nations

## TECHNICAL BACKGROUND PAPER

# Applications of Whole Genome Sequencing in food safety management

Food and Agriculture Organization of the United Nations  
in collaboration with the World Health Organization



# Whole genome sequencing

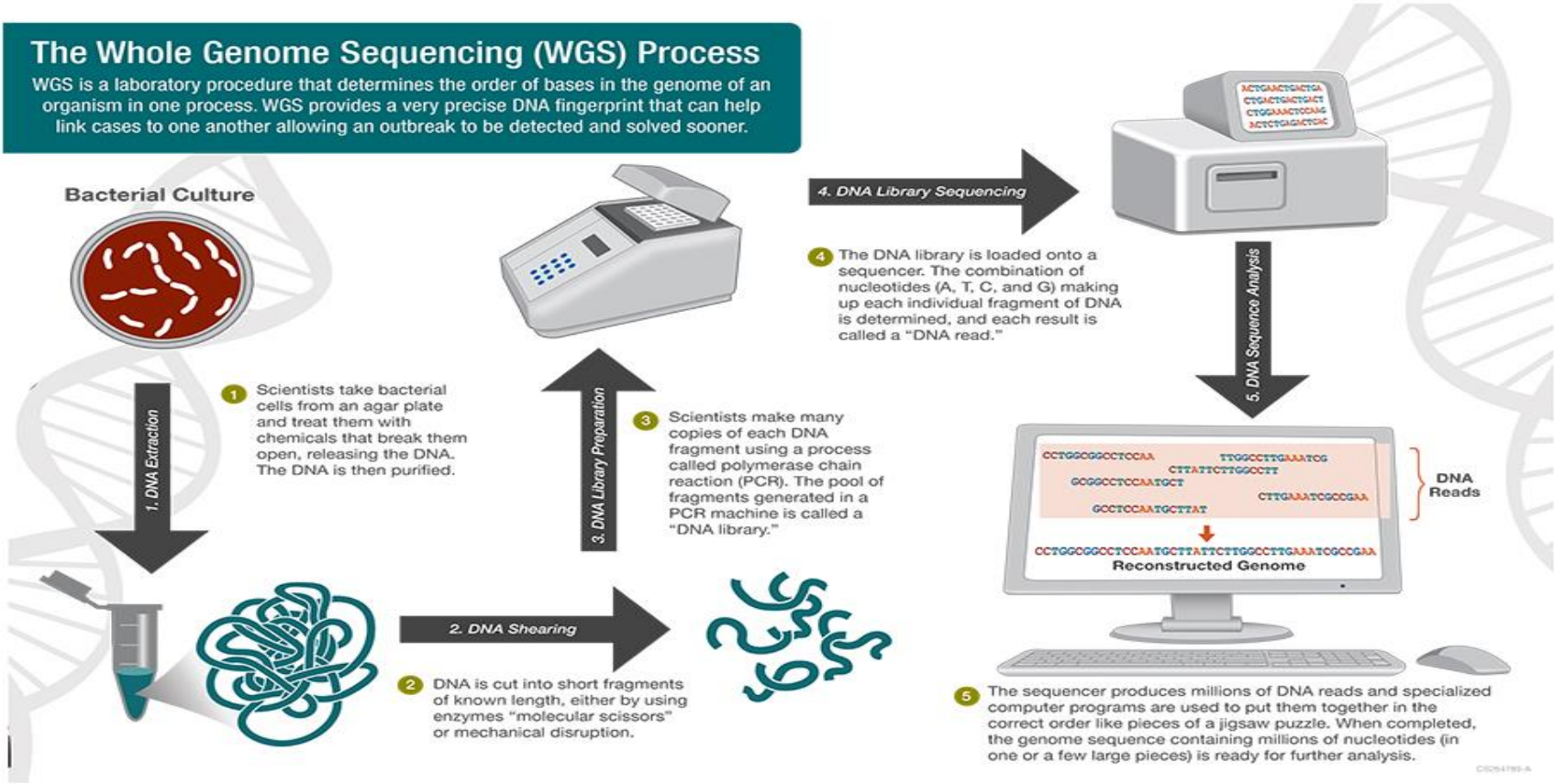
- The human genome project cost US\$ 1 billion and took 13 years to complete.
- Today sequencing human genome costs \$3000 and can be completed in two days.





# The Whole Genome Sequencing (WGS) Process

**The Whole Genome Sequencing (WGS) Process**  
WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.

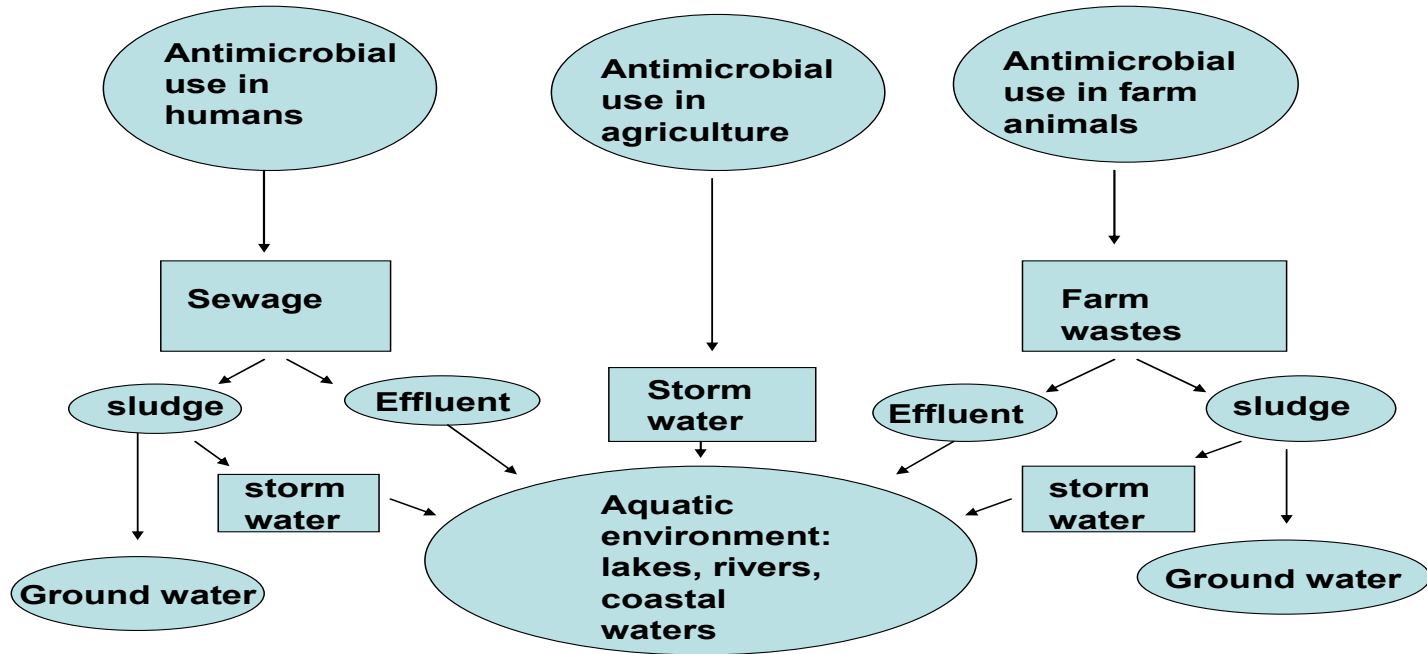


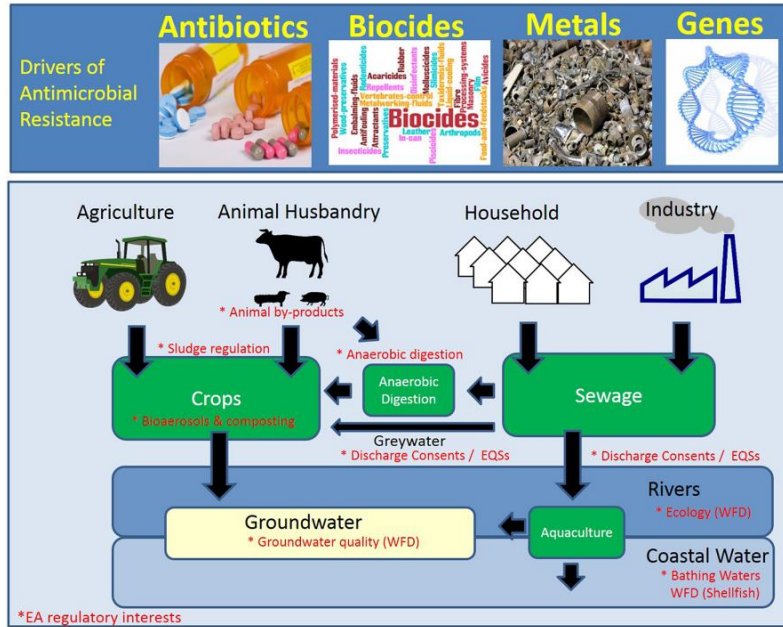
# Commercial availability

- Panels for genome testing available for clinical microbiology laboratories
- Cost coming down and technology becoming more accessible.



# RESISTANT MICROORGANISMS SELECTED IN OTHER SECTORS COULD REACH WATER SOURCE USED IN AQUACULTURE





Singer et al.,  
2016



Table 1. Details for the 50 Most Prevalent Resistance Classes Found in All Metagenomes

Resistance Class	Mechanism of Resistance	Antibiotic Specificity
mexef, ceo, mexvw, acr, mexhi, mexcd, mexab, mdtnop, amr, adeabc, smeabc, smedef, mdtef, mexxy, mdtk	RND class transporter	multidrug resistance efflux
macab	RND class transporter: macrolide	multidrug resistance efflux
bcr, bcr_mfs	ABC class transporter system: bacitracin	multidrug resistance efflux
mls_abc	ABC class transporter: macrolide	multidrug resistance efflux
mls_mfs, mls_hdr	MFS class transporter: macrolide	multidrug resistance efflux
cml	MFS class transporter: chloramphenicol	multidrug resistance efflux
rosab	potassium antiporter system	multidrug resistance efflux
mepa, norm	MATE transporter	multidrug resistance efflux
tcma, mdr, qac	MFS transporter	multidrug resistance efflux
vana, vanb, vanc, vand, vane, vang	vancomycin resistance operon genes (vanH, vanS, vanR, vanX, and vanY) for each vancomycin resistance operon: VanA, VanB, VanC, VanD, VanE, and VanG types	vancomycin
tet_rpp	tetracycline ribosomal protection protein	tetracycline
tet_efflux	tetracycline-specific efflux pump	tetracycline
tet_flavo	flavoproteins resistance to tetracycline	tetracycline
bla_a, bla_b	class A and class B $\beta$ -lactamases	$\beta$ -lactams
pbp	penicillin-binding protein	$\beta$ -lactams
Baca	bacitracin resistance	bacitracin
cata	chloramphenicol acetyltransferase	chloramphenicol
ksga	kasugamycin resistance	kasugamycin
arna	polymixin resistance	polymixin
pur8	puromycin resistance	puromycin
vat	virginiamycin resistance	streptogramin
sul	sulfonamide resistance	sulfonamide
dfra	trimethoprim resistance	trimethoprim

Source: Nesme et al., 2014



## Aspects not considered in many publications on AMR associated with aquaculture

- Intrinsic resistance in many aquatic bacteria – *Aeromonas* to ampicillin.
- Selection of antibiotic resistant bacteria due to exposure to chemical pollutants, heavy metals.
- AMR introduced into aquaculture environment from other sectors



## AMR not related to use of antibiotics in aquaculture

- Culture-independent studies in the Baltic sea show presence of resistance genes encoding resistance to sulphonamides, trimethoprim, tetracycline, aminoglycoside, chloramphenicol and also genes encoding multidrug efflux pumps in sediments below fish farms, though some antibiotics like tetracyclines, aminoglycosides and chloramphenicol are not used in this area (Muziasari et al., 2017).
- Most *Vibrio vulnificus* strains isolated from Dutch eel farms showed resistance to cefoxitin, though this antibiotic was not used in eel aquaculture (Haenen et al., 2014).



## AMR data Using microorganisms isolated from fish at retail level

- Changes in microflora during handling and processing
- It may not be possible to pick up indicators that represents aquaculture environment at this point.
- Large amount of data on aquaculture products at retail level





## Linking resistome in environment and clinic

- Environmental resistome highly diverse
- Resistome in clinical pathogens very small in diversity
- What maintains environmental resistome?
- What are transfer bottlenecks?



## Summary

- Antibiotic resistant bacteria can be found in many aquaculture systems.
- Some of them may be intrinsic resistance, some may be selected due to antibiotic use, some of them may be derived from antibiotic use in other sectors.
- It is difficult to trace the source of AMR found in aquatic bacteria
- Tracing the source of resistance determinants is very complex.



# THANK YOU







